

# Authentic acid blood mordanted the madder-dyed Shroud of Turin pinkish red before image formation – Jesus was dead

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## ABSTRACT

The chemical and physical properties of the Shroud of Turin, both of the non-image background, the image areas, the pinkish red bloodstains and the separate serum, especially their absorbance, fluorescence and FTIR spectra, are described and shown to be evidence for the presence of a pinkish red heme-madder complex, formed by the chemical interaction of authentic acid postmortem blood with yellow madder dye on the cloth before image formation. Besides the many normal blood characteristics, the lack of potassium of the red bloodstains indicates that the blood was hyperkalemic: that potassium had moved out of the red blood cells into the plasma, which inevitably occurs in acidemic blood. The absorbance spectra of the bloodstains indeed show the typical charge transfer band of acid blood at 630 nm. The lack of red blood cells, the lack of a large Soret band in the bloodstains' absorbance spectra (at ca. 400 nm), and the peculiar pinkish-red color of the bloodstains indicate that, after a draining away of the potassium-rich plasma/serum, the red blood cells hemolysed (broke open) and their hemoglobin denatured further to acid heme dimers – which only have a small Soret band – and that these acid heme dimers mordanted the yellow madder dye on the Shroud pinkish red. The FTIR spectra of Shroud bloodglobes are similar to those of madder lake. Also the reddish color of the bloodstain fluorescence corresponds to the reddish fluorescence of madder lake, and the shape of the mean UV-vis reflectance spectrum of four bloodstains is compatible with a combination of madder dye, madder lake and heme.

The presence of a thin coating of (acid) retrograded starch and acid madder dye on the superficial fibers of the Shroud is evidenced by the strippable coating in all Shroud areas, the erasing of superficial scorches and background color by hot firefighting water, leaving blue fluorescent areas, and the similar FTIR spectra of the main Shroud's background and those of the madder-and-starch-containing radiocarbon-dating corner, compatible with those of linen, starch and madder dye. Also the visible pinkish hue, the acidichromism, reflectance spectra, fluorescence spectra, and yellow-green fluorescence of the background, the weaker image fluorescence, the blue-fluorescent 'no-print' areas, and the microscopic observations are evidence for the presence of madder dye on the Shroud. Madder dye is soluble in xylene and in toluene, the solvents that were used to wash the adhesive from Shroud fibers stuck to sticky tape samples of the main Shroud. This may explain why no positive wet-chemistry evidence of dye on fibers from the main Shroud was reported, but, much later, madder was easily found by wet chemistry on whole thread samples from the radiocarbon-dating corner, which already has been shown to be no repair. To this can be added that FTIR spectroscopy found pectin but no protein of gum Arabic on both the main Shroud and radiocarbon-dating corner. The starch-and-madder coating served as many agents in the Shroud's history. It was probably originally applied as a fluorescing fabric brightener for an authentic Jewish temple mantle, and it was a waterresistant filter separating the potassium-poor red blood cells and the potassium-rich serum of acid hyperkalemic postmortem blood, a saponin layer hemolysing the red blood cells, a denaturing and adsorbing substrate for pinkish heme-madder lake formation, an acid catalyst and easily oxidizable substrate for acid oxidation in image formation in only this ultra-thin and strippable fiber coating, an acid coating that allowed the formation of pure iron oxide particles on the outer edges of the large waterstains from cellulose-bound iron, and finally a potent preservative for serum and heme and cloth. A coating with Saponaria (soapweed) residues is shown to be

incompatible with almost all properties of the Shroud and bloodstains.

A realistic mechanism of formation of the separate serum coated fibers and different types of bloodstains – moist drying-clot imprints especially on the ventral half of the Shroud, blood rivulets across the dorsal half of the Shroud, and later imprints of fibrinolysed scourge wound clots on both halves of the Shroud – is described and complies with the events that led to the death and burial of Jesus, as recounted in the Gospels: scourging and crowning with thorns before midday, carrying the cross uphill, crucifixion and then death in the middle of the afternoon, a stabbing in the side, and then the deposition, wrapping in linen and transferral to the tomb – allowing the re-opening of crucifixion and head wounds, the formation by wet bloodstained hands of bloodsmears outside the body contours and a bloodsmear possibly on the edge of an oval first-century Jewish ornament (the *petalon* of a Sanhedrin member such as Joseph of Arimathea) that was laid on the chest of the body, and the running of irregular blood rivulets across the dorsal half of the waterresistant Shroud on a shoulder-carried water-absorbing wooden burial bier –, and finally the laying down of the body (plus bier) in the tomb at the end of the afternoon. The strenuous exercise and the crucifixion of several hours would have implied severe cramping, respiratory failure, dehydration and thus severe antemortem acidemia that inevitably increased after death and then apparently started a hyperfibrinolysis process (a remoistening of blood clots on the wounds, from within the body), as is evidenced by the pattern of unsmeared dumbbell-shaped imprints of scourge wound clots. Hyperfibrinolysis only starts up at an acid blood pH < 6.8, which blood pH is incompatible with life but compatible with the absorbance spectra of the Shroud's bloodstains.

As the image fibers have lost their surface of easily oxidizable madder dye in the oxidative image formation process, evidenced by the image color and fluorescence, and as the bloodstains in image areas are all the same pinkish-red color and their reflectance spectra have the same shape, independent of the concentration of image fibers that would have been present in the bloodstain locations, the blood must have formed pinkish heme-madder lake before the start of image formation. This, and the realistic appearance of the bloodstains and the presence of separate serum, as evidenced by FTIR spectra, precludes that the bloodstains were painted on a pre-existent, e.g. artificial, body image. Some well known red-blood hypotheses, such as those based on an ultra-high bilirubin level in the blood, UV-irradiation of the blood, a Saponaria soap residue on the cloth, a nitric oxide ligand in a blood paint, and a red ochre paint are analysed and shown to be unable to explain all the Shroud's bloodstain characteristics consistently. Experiments showed that blood can form pinkish stains on starched and madder-dyed linen.

That there is a pinkish red bloodstain on the image of probably a first-century Jewish *petalon*, fits the high-priestly burial of Jesus Christ in the Shroud of Turin by Joseph of Arimathea. That the Shroud shows no signs of putrefaction of the dead body, which would have started about 40 hours after death, and that the dumbbell-shaped scourge marks on both halves of the Shroud are not smeared, means that the dead body and its image-bearing Shroud separated in an extremely delicate way that did not smear the moist fibrinolysed blood clots on the scourge wounds, which were still dry when the dead body was wrapped into the Shroud. This fits the bodily Resurrection of Jesus Christ. •

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## 1. INTRODUCTION

The Shroud of Turin is a 4.4 x 1.1 m linen cloth bearing the ventral and dorsal image of a man and apparent bloodstains in the areas of the hands, feet, side, head, and small of back, and also burn holes and scorch marks and waterstains (fig. 1). It is kept in the cathedral of Turin, Italy, and believed by many to be the burial cloth of Jesus Christ.



Fig. 1. The Shroud of Turin<sup>1</sup>

### 1.1. Normal blood features

It has been reported that the apparent bloodstains of the Shroud show a number of normal blood and serum features, as evidenced by several techniques, such as the following:

- red blood cells (29 of 500 particles) – microscopy, SEM, X-ray microfluorescence – Lucotte<sup>2</sup>
- a human red blood corpuscle with the characteristic elements of blood – SEM and X-ray microspectrometry – Baima Bollone<sup>3</sup>
- some cell-like structures, iron, antigen B, adult hemoglobin – Wright's technique, Prussian blue test, immunohisto-chemical tests – Garza-Valdez<sup>4</sup>
- hemoglobin – Raman analysis – Baraldi<sup>5</sup>
- hemochromagen-like color and cyanmethemoglobin type color producible – Heller and Adler<sup>6</sup>
- heme/porphyrins – Dotzauer and Keding method, Teichman crystals and hematine chlorohydrate producible – Baima Bollone<sup>7</sup>
- porphyrin fluorescence producible – after displacement of iron by treatment with hydrazine and formic acid vapor – Heller and Adler<sup>8</sup>
- iron concentration consistent with bloodstains – X-ray fluorescence – Morris, Schwalbe and London<sup>9</sup>
- the elements Mg, Al, Si, S, Cl, K, Ca and Fe – EDS - Baima Bollone<sup>10</sup>
- proteins – protease test and fluorescamine test– Heller and Adler<sup>11</sup>
- shiney honey yellow colored fibers, a deeper color than the image – microscopy – Schwalbe and Rogers<sup>12</sup>

<sup>1</sup> Unofficial photo <http://wpmedia.life.nationalpost.com/2013/03/shroud-of-turin.jpg> from <http://life.nationalpost.com/2013/03/29/shroud-of-turin-not-a-medieval-forgery-researchers-say/>; see also <https://www.shroud.com/examine.htm> and <http://www.sindonology.org/shroudScope/shroudScope.shtml>

<sup>2</sup> Lucotte, Optical and chemical..., 2012, p. 2547

<sup>3</sup> Baima Bollone, The Forensic Characteristics..., Proceedings Turin 2000, p. 213

<sup>4</sup> Garza Valdez, The DNA of GOD?, 2001 p. 112-113

<sup>5</sup> in Fanti and Gaeta, Il mistero della Sindone, 2013, p. 180

<sup>6</sup> Heller and Adler, A Chemical..., 1981, TOM 39

<sup>7</sup> Baima Bollone, The Forensic Characteristics..., Proceedings Turin 2000, p. 212

<sup>8</sup> Heller and Adler, Blood on the Shroud of Turin..., 1980, The Orphaned Manuscript (TOM) p. 31

<sup>9</sup> Morris, Schwalbe and London, X-Ray Fluorescence..., 1980, p. 40-42

<sup>10</sup> Baima Bollone, The Forensic Characteristics..., Proceedings Turin 2000, p. 210 (plot of his EDS spectrum is online in Kearse and Heimburger, The Shroud Blood Science..., 2013, p. 4)

<sup>11</sup> Heller and Adler, A Chemical... TOM 40-41 and table 4; Adler, The Nature of..., 1999, TOM 106-107

<sup>12</sup> Schwalbe and Rogers, Physics and Chemistry..., 1982, p. 37

- albumin – Bromocresol Green test – Heller and Adler<sup>13</sup>
- primate immunoglobulin – immuno-chemical tests – Heller and Adler<sup>14</sup>
- blue fluorescing parts (of serum?) of vacuumed loose red particles - Fanti<sup>15</sup>
- human immunoglobulin and human serum – direct and indirect immunological method – Baima Bollone<sup>16</sup>
- erythrocyte antigens A and B – immunohistochemical technique conjugating antisera with raphanin peroxidase – Baima Bollone<sup>17</sup>
- human X- and Y-chromosomes – advanced DNA technologies – Tryon<sup>18</sup>
- human blood – Canale 1995<sup>19</sup>
- realistic appearance: the borders of the forehead stains and of stains on the hair image are more coloured than their centres – Baima Bollone<sup>20</sup>; this is typical for blood clots formed on the skin – Barbet<sup>21</sup>.

## 1.2. Special features of the bloodstains

Remarkable features of the apparent bloodstains on the Turin Shroud are:

- the presence of both many pinkish red bloodstains and a few brown bloodstains (see 5.4)
- the absence of a detectable Soret absorbance band (see 2.1)
- the absence of a detectable XRF potassium signal from bloodstains on the Shroud (see 2.3)
- few, almost no, cells in the bloodstain surfaces (see 2.4)
- the resistance of the bloodstains and serum haloes to microbial attacks of  $\geq 650$  years (see 3)
- the presence of blood stains that were not in a body-sheet contact zone, if the Shroud was only loosely draped over a supine body that was lying on one half of the Shroud (see 5.6)
- the presence of a pattern of many pinkish red stains in a well-defined dumbbell form (see 5.5.4)
- the presence of hydroxyproline in red spots on the ‘Zina-thread’ from a blood area on a heel image (see 2.5)
- relatively high Na and Cl levels in organic red particles vacuumed from the reverse side of the Shroud (see 2.6)

## 1.3. Analysis in this paper

In this paper a consistent explanation of both normal and special features is presented (chapter 2 and 3 and 5), which is based on already published evidence and on results of new experiments, and which is also in congruence and evidenced by the properties of the background and image of the Shroud (chapter 4), and in congruence with the earlier proposed identification of the Shroud as a madder-dyed Jewish temple mantle with Pharisaic border without corner repair.<sup>22</sup> Other hypotheses regarding the redness of the bloodstains are also analysed, but dismissed as incongruent with the Shroud’s properties (chapter 6). Also new evidence for the Shroud’s presence in an authentic Jewish burial procession for the burial of Jesus is presented (chapter 7).

<sup>13</sup> Heller and Adler, *A Chemical...*, 1981, TOM 40, 36, 50 table 2

<sup>14</sup> Heller, *Report on...*, 1983, p. 188

<sup>15</sup> Fanti, *Statistical analysis...*, 2008, fig. 10,

<sup>16</sup> Baima Bollone, *The Forensic Characteristics...*, Proceedings Turin 2000, p. 212; discussed in Kears, *Blood on the Shroud of Turin: An Immunological Review*, 2012

<sup>17</sup> Baima Bollone, *The Forensic Characteristics...*, Proceedings Turin 2000, p. 212

<sup>18</sup> in Wilson, *Science Fiction to Science Fact?*, 1996

<sup>19</sup> “the presence of human blood was subsequently confirmed by Canale in 1995 before conducting DNA research on some threads I gave him”, Baima Bollone, *The Forensic Characteristics...*, Proceedings Turin 2000, p. 214

<sup>20</sup> Proceedings Turin 2000, p. 215-216

<sup>21</sup> in Brillante, Fanti and Marinelli, *Bloodstain characteristics...*, 2002, p. 5, 8 referring to Barbet

<sup>22</sup> Hoeven, *The seam and missing corners...*, 2011-2013; Hoeven, *Internal selvedge...*, 2012

## 2. COHERENCE OF SPECIAL BLOOD FEATURES

### 2.1. Red color but no Soret band

Adler reported on the Shroud blood: “It all looks perfectly acceptable for blood except for one thing – it is too red for blood that is supposed to be some 600 to 2000 years old. Everyone knows that blood changes color when exposed to the air; it changes to a methemoglobin which gives it a brown color. So we need to explain why the centuries-old blood on the Shroud is still so red.”<sup>23</sup> Baima Bollone reported the same problem: “Another open question is that of the colour of the bloodstains on the Shroud. People have always been astonished by their bright red colour while it is known that bloodstains become brown with the gradual passing of time.”<sup>24</sup> For an impression of the color, see fig. 2.1, from Shroud Scope.

The following analysis was given by biochemist Berry:

“Scientifically speaking, one should ask if the stains are consistent with their being extensively-degraded human blood (EDHB). But then one has to agree on what the markers are for EDHB. What would be the state of the original haemoglobin in the red blood cells, for example?

Intact haemoglobin – improbable, even as (oxidized) methaemoglobin with iron(III), or free haems, i.e. non-proteinaceous iron-complexed porphyrins (more likely, but with what ligand? Jackson’s CO? Pull the other one) or iron-free porphyrins (even more likely), end-stage iron oxide, Fe<sub>2</sub>O<sub>3</sub> or Fe<sub>3</sub>O<sub>4</sub> (possibly).”<sup>25</sup>

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<sup>23</sup> Adler, The origin and nature..., 1986, TOM 60

<sup>24</sup> Baima Bollone, The Forensic Characteristics..., Proceedings Turin 2000, p. 217

<sup>25</sup> Berry, Shroudie-Alert: Day 9. Is it real human blood on the Shroud? A reply to Richard Savage, aka Jabba., “Casting a critical eye on that Shroud of Turin”, Feb 27, 2013, <http://shroudofturinwithoutallthehype.wordpress.com/2013/02/27/shoudie-alert-day-9/> (line breaks added here for easier reading)



Fig. 2.1. Blood in area of the soles of the feet, screenshot of Shroud Scope, Durante 2002<sup>26</sup>

### 2.1.1. Acid heme dimers

#### 2.1.1.1. Acid heme

Three kinds of original UV-Vis absorbance data from Shroud blood material are available:

- 1) a “brownish red translucent crystal” (“named biltong”) that in transmission showed a Soret absorption band at 405-410 nm and probably more bands – as it was identified as an acid methemoglobin crystal – but these are not specified or shown;<sup>27</sup>
- 2) a “garnet red” stained fibril that in transmission showed an absorption band at ca. 450 nm, with a shoulder at ca. 530 nm (Adler mentions 520 nm as one of “the observed peaks”<sup>28</sup>), but, according to Adler, these may have been shifted and distorted due to a high degree of scattering<sup>29</sup> (fig. 2.2.A);

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<sup>26</sup> Latendresse, who publishes [www.sindonology.org](http://www.sindonology.org), also offers Shroud Scope (<http://www.sindonology.org/shroudScope/shroudScope.shtml>) with the option of a permalink to any screenshot, e.g. this one <http://www.sindonology.org/shroudScope/shroudScope.shtml?zl=7&image=3&lon=1947.0&lat=1015.0>. Image used with permission from Latendresse.

<sup>27</sup> Heller and Adler, Blood on the Shroud..., 1980, TOM 31; Heller, in his book, described the appearance of a clear Soret band at 405-410 nm in the spectrum from the crystalline Shroud sample, measured in transmission, and identified as acid methemoglobin: Heller, Report on..., 1983, p. 144-147, read online at [http://books.google.fr/books?ei=dNPnUbiBLoL\\_PKergcAH&hl=fr&id=o9glK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&q=acid+methemoglobin](http://books.google.fr/books?ei=dNPnUbiBLoL_PKergcAH&hl=fr&id=o9glK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&q=acid+methemoglobin) and [http://books.google.fr/books?ei=dNPnUbiBLoL\\_PKergcAH&hl=fr&id=o9glK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&q=hit+400](http://books.google.fr/books?ei=dNPnUbiBLoL_PKergcAH&hl=fr&id=o9glK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&q=hit+400)

<sup>28</sup> Adler, Updating recent..., 1996, TOM 84 (see quote in 6.1.2.3)

3) pinkish red Shroud bloodstains that in relative reflectance show no distinguishable Soret absorption but a broad absorption band from ca. 340-525 nm and a clear narrow absorption band at 630 nm (fig. 2.3.), although, according to Adler, this spectrum is also distorted due to anomalous dispersion.<sup>30</sup>

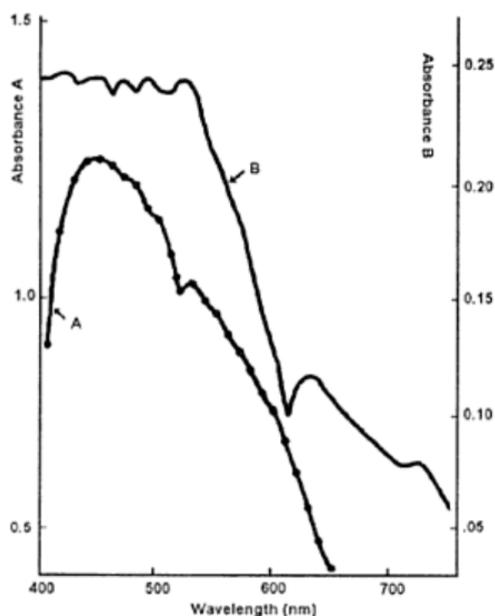


Fig. 2.2. Absorbance spectra: A of “a brownish red stained fibril from one of the blood areas of the Shroud”; “B, transmission spectrum obtained by transformation of the reflection spectrum of the blood areas of the Shroud” ©OSA<sup>31</sup>

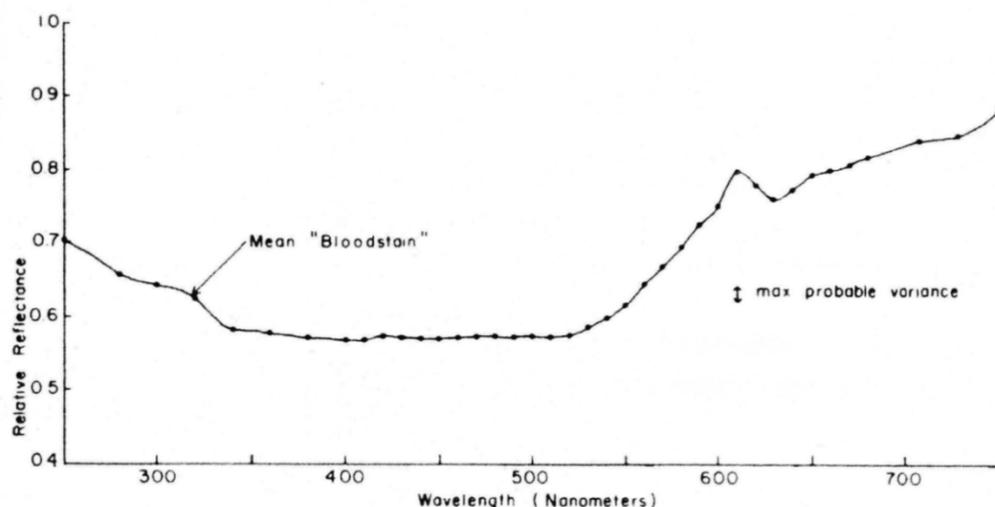


Fig. 2.3. Mean relative reflectance values of four big bloodstains on the Shroud ©OSA<sup>32</sup>

<sup>29</sup> The investigated Shroud fibers are called “a single microfiber ... garnet red” (Heller, Report on 126), and “seven microfibers with red stains on part of their length” (Heller, Report on..., 1983, 128) and “brownish red” (Heller and Adler, Blood on the Shroud..., 1980, TOM 30 and its fig.2 on TOM 31) by Heller and Adler, who saw these samples through the microscope, probably in transmitted light. See their article pages (TOM 30-31) with a plot of one of the transmission spectra showing a strong absorption band at 450 nm in fig. 2.2 A or through this link:

[http://books.google.nl/books?id=J2jBnDN3VxMC&pg=PA30&lpg=PA30&dq=spectral+data+of+the+Shroud+blood&source=bl&ots=zenDjCt69-&sig=WmPZiDBpkxQp61QSSpVy4ZjVddk&hl=en&sa=X&ei=F3djUc-uOYmi8gTLIoCIBw&redir\\_esc=y#v=onepage&q=spectral%20data%20of%20the%20Shroud%20blood&f=false](http://books.google.nl/books?id=J2jBnDN3VxMC&pg=PA30&lpg=PA30&dq=spectral+data+of+the+Shroud+blood&source=bl&ots=zenDjCt69-&sig=WmPZiDBpkxQp61QSSpVy4ZjVddk&hl=en&sa=X&ei=F3djUc-uOYmi8gTLIoCIBw&redir_esc=y#v=onepage&q=spectral%20data%20of%20the%20Shroud%20blood&f=false)

<sup>30</sup> Heller and Adler, Blood on the Shroud..., 1980, TOM 30-31

<sup>31</sup> Used from J.H. Heller and A.D. Adler, Blood on the Shroud of Turin, Applied Optics, 19 (16) 1980, pp. 2742-2744, <http://dx.doi.org/10.1364/AO.19.002742>, fig. 2, with permission from the Optical Society of America. (online at A.D. Adler, The Orphaned Manuscript (TOM) p. 31,

[http://books.google.nl/books?id=J2jBnDN3VxMC&pg=PA30&lpg=PA30&dq=spectral+data+of+the+Shroud+blood&source=bl&ots=zenDjCt69-&sig=WmPZiDBpkxQp61QSSpVy4ZjVddk&hl=en&sa=X&ei=F3djUc-uOYmi8gTLIoCIBw&redir\\_esc=y#v=onepage&q=spectral%20data%20of%20the%20Shroud%20blood&f=false](http://books.google.nl/books?id=J2jBnDN3VxMC&pg=PA30&lpg=PA30&dq=spectral+data+of+the+Shroud+blood&source=bl&ots=zenDjCt69-&sig=WmPZiDBpkxQp61QSSpVy4ZjVddk&hl=en&sa=X&ei=F3djUc-uOYmi8gTLIoCIBw&redir_esc=y#v=onepage&q=spectral%20data%20of%20the%20Shroud%20blood&f=false)

A characteristic of acid methemoglobin A (human methemoglobin = iron(III) protoporphyrin IX bound to globin, a protein) is the absorption peak at ca. 630 nm, the so-called charge transfer band.<sup>33</sup> It gradually disappears when the acid methemoglobin is neutralized or gets in alkaline conditions, giving a growing ca. 575 nm absorption peak in return: one of the Q twin bands  $\alpha$  and  $\beta$ .<sup>34</sup> In acid methemoglobin there is an actual peak at 630 nm at pH 1 to at least 5 (fig. 2.4.), and a peak at 500 nm, and the Soret peak is at 405/406 nm;<sup>35</sup> “an optical spectrum distinguished by relative intense maxima near 496 and 622 nm, which is a signature of high spin ferric heme”<sup>36</sup> corresponds to a brown heme color.<sup>37</sup> The 630 nm absorption peak also exists in both acid free heme monomer (fig. 2.5., part B) (heme = iron protoporphyrin IX) and in acid aqueous heme dimers<sup>38</sup> (heme dimer = two heme molecules/monomers bound to each other). The 630 nm band is present in the bloodstain spectra and probably in the crystal spectrum as well, for otherwise it would not have been specified by Adler, Cameron, and another specialist, as old/denatured acid methemoglobin, as reported by Heller.<sup>39</sup> In a drying solution, dissolved acid methemoglobin can form a (translucent) crystal, of which the crystal structure can be determined by X-ray crystallography.<sup>40</sup> More denotations of blood material yielding these various spectra are in Heller and Adler’s Applied Optics article: “Thermodynamically the latter fibrils would be expected to show the spectrum of a fully oxidized denatured met-hemoglobin, i.e., a so-called perturbed acid met-hemoglobin”<sup>41</sup>, and “In our opinion the spectral data taken in aggregate are positive in confirming the presence of perturbed acid met-hemoglobin species on the Shroud.”<sup>42</sup> Here, the meaning of “perturbed” is not specified, but it seems to have the notion of ‘denatured’ (applying to a deformed/unfolded protein or

<sup>32</sup> Used from R. Gilbert Jr. and M.M. Gilbert, Ultraviolet-visible reflectance and fluorescence spectra of the Shroud of Turin, Applied Optics, 19 (12) 1980, pp. 1930-1936, <http://dx.doi.org/10.1364/AO.19.001930>, fig. 14, with permission from the Optical Society of America (fair use). (The corresponding absorbance figure (Gilbert and Gilbert, 1989, fig. 15) is online on the OSA website at

<http://imagebank.osa.org/getImage.xqy?img=QC5sYXJnZSxhby0xOS0xMi0xOTMwLWcwMTU> )

<sup>33</sup> “Acid methemoglobin has a characteristic absorption peak at 630-631 nm” (A Mansouri, AA Lurie, Methemoglobinemia, American journal of hematology, 1993, <http://onlinelibrary.wiley.com/doi/10.1002/ajh.2830420104/abstract> , 5<sup>th</sup> find at <http://scholar.google.nl/scholar?hl=nl&q=absorption+acid+methemoglobin&lr>

<sup>34</sup> Austin & Drabkin, 1935, <http://www.jbc.org/content/112/1/67.short> p. 70 (p. 4 of pdf file)

<sup>35</sup> Wintrobe’s Clinical Hematology, Vol. 1, 12<sup>th</sup> edition, 2009, p. 147; “characterized ... by the extinction coefficients of the bands at 406, 500 and 630 m $\mu$ . [...] The extinction coefficients were E 406 mM = 141 to 150; E 500mM = 9.2; and E 630 mM = 3.7, to be compared with the values of 155, 9.5, and 4.1 (13) reported for ammonium sulfate-fractionated human Hb+.” Steinhardt and Hiremath, A Comparison of the Resistance of Human and Horse Ferrihemoglobin to Acid Denaturation, The Journal of Biological Chemistry, Vol. 242, no 6, 1967,

<http://www.jbc.org/content/242/6/1294.full.pdf> ; See for instance figure 48-2 (A = methemoglobin A) of

<http://www.drugswell.com/winow/+%20b19/+%20williams%20hematology-2007/V.%20The%20Erythrocyte/48.%20Methemoglobinemia%20and%20Other%20Causes%20of%20Cyanosis.htm> ; or

see methemoglobin at pH 7 at <http://www.pnas.org/content/97/7/2984/F1.expansion.html>

<sup>36</sup> Appleby et al., Leghemoglobin, 1976, <http://www.jbc.org/content/251/19/6090.full.pdf> (soybean leghemoglobin is a heme-containing protein comparable to human methemoglobin)

<sup>37</sup> cf. Adler, The origin and nature..., 1986, TOM 60-61; also one of the authors of “Crystal structure and ligand binding properties of the truncated hemoglobin from Geobacillus stearothermophilus”, 2007,

<http://www.sciencedirect.com/science/article/pii/S0003986106003560>, wrote in a personal communication, July 25,

2013, that a brown color is typical of high spin hexacoordinate heme; “acid haematin” is called brown in Meldrum, 1931,

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1260779/pdf/biochemj01118-0060.pdf>

<sup>38</sup> T.J. Egan (co-author of Villiers et al, Speciation and structure..., 2007), personal communication, 25-7-2013

<sup>39</sup> “Adler got on the phone right away and read the coordinates to his chosen specialist. The answer – old acid methemoglobin. Then we called Bruce Cameron ... “... It’s old acid methemoglobin. ...”” Heller, Report on..., 1983, p. 147

<sup>40</sup> “Although met- and oxyhemoglobin were reported to have similar conformations in the crystalline state (10), the present result clearly shows that the conformations of these two hemoglobins in dissolved state are different [...] x-ray crystallography which showed that the structure of acid methemoglobin is similar to that of oxyhemoglobin (10). 10= Perutz and Matthews, 1966, J. Mol. Biol. 21, 199-202” Heme-Spin Label Studies of Hemoglobin II. SPIN-LABELED OXY-AND DEOXYHEMOGLOBINS, Asakura and Tamura, 1974 <http://www.jbc.org/content/249/14/4504.full.pdf>

<sup>41</sup> Note 7 = B.F. Cameron and P. George, Biochimica et Biophysica Acta, 194 (1969): 16, now online at

<http://www.sciencedirect.com/science/article/pii/0005279569901743>

<sup>42</sup> Heller and Adler, Blood on the Shroud..., 1980, TOM p. 30-31

even a dissociated/lost protein and a remaining heme) and it is reminiscent of ‘turbidity’ (applying to the aggregation state of the heme compounds in solution).

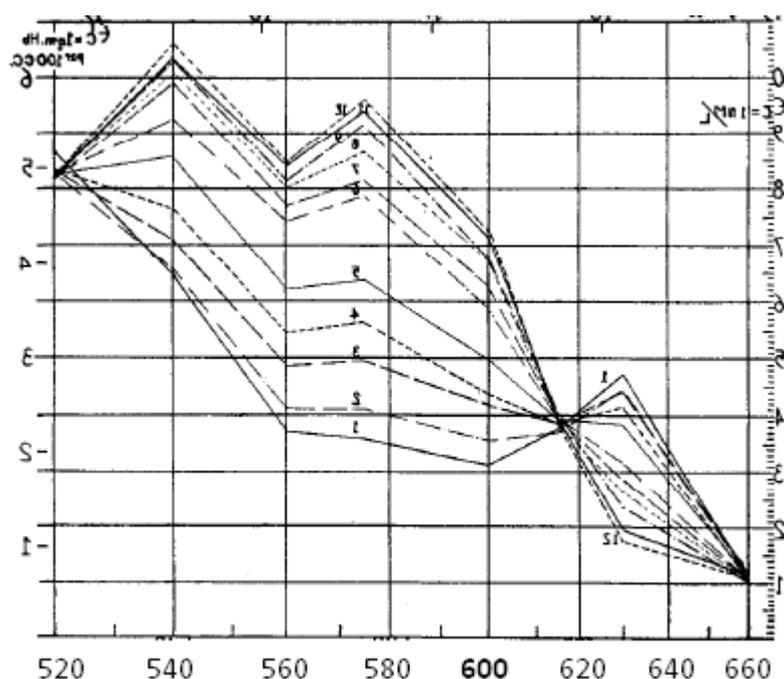


Fig. 2.4. Absorbance of methemoglobin at pH 1 to 12 (mirrored image) ©the American Society for Biochemistry and Molecular Biology<sup>43</sup>

### 2.1.1.2. Heme dimerization

The polymerisation/aggregation state of heme determines the presence or (nearly) absence of the Soret band. Both a heme bound to its globin protein and a single free heme shows the Soret band (at 405 nm if the heme is H<sub>2</sub>O-ligated<sup>44</sup>) although a 370 nm ‘denatured’ band appears when the globin dissociates from its acid methemoglobin: the ratio of the height of the Soret peak to the Q and charge transfer peaks stays the same in these acid species; only when two hemes form a dimer, the Soret peak disappears,<sup>45</sup> relative to the other bands: the ratio of the band intensities changes (fig. 2.5).<sup>46</sup>

<sup>43</sup> This research was originally published in *Journal of Biological Chemistry*. J.H. Austin and D.L. Drabkin. Spectrophotometric Studies: III. Methemoglobin. *Journal of Biological Chemistry*, 1935; 112:67-88. © the American Society for Biochemistry and Molecular Biology. <http://www.jbc.org/content/112/1/67.short> p. 70: fig. 1 (p. 4 of pdf file); According to Cameron and George (1969) an absorbance spectrum of methemoglobin at pH 7.0 seems not entirely reliable if there was “exposure even briefly to a pH below 6.0”, and therefore “The common practice of reporting a methemoglobin spectrum at pH 7.0 or 7.4 is to be discouraged.” Cameron and George, 1969, <http://www.sciencedirect.com/science/article/pii/0005279569901743>

<sup>44</sup> methemoglobin: Wintrobe’s *Clinical Hematology*, Vol I, 12<sup>th</sup> edition, 2009, p. 147; heme monomer: Cf. “To our surprise, the heme complex exhibited a sharp Soret band at 405 nm in benzene;” here, there is a surfactant-heme complex made from a pH 7.0 aqueous buffer solution that would contain an equilibrium of monomer heme and weakly bound pi-pi dimers. (the “surfactant-heme complex” can not be “decomposed”); in the pH 7.0 aqueous solution the species yielding the 393 nm Soret peak is not the assumed mu-oxo dimer (assumed because of Brown et al., 1970) but probably the – in 1997 still unknown - aqueous heme pi-pi dimer in equilibrium with the heme monomer, which yields the 405 nm peak. (Kamiya et al., Peroxidase activity and stability of surfactant-heme complex in nonaqueous media, 1997, <http://link.springer.com/article/10.1023/A:1018403518763>). Cf. Villiers et al., *Speciation and structure...*, 2007, p. 102, describing the assumption on the mu-oxo dimer, also in relation to the article of Brown et al. 1970; Villiers et al. also used “80% aqueous methanol, where Fe(III)PPIX is monomeric” p. 108.; “Spectra of aqueous Fe(III)PPIX [...] show that the Soret band (393 nm) decreases with increasing concentration as expected.” p. 107 (Fig. S3A, for pH 8.06, shows the isobestic point at 347 nm which later is also used for other pH values; however, the peak shifts to the blue with increasing concentration, so the 393 nm value is not the identical value for monomer and dimer, and probably is an intermediate value of the mix).

<sup>45</sup> “The fast measurements at low pH make it possible to distinguish between several steps in the spectral changes: A-> B, a small exceedingly fast reaction, accompanied by a red-shift of the Soret band; B -> C, expulsion of the heme from the protein (blue shift of the Soret band); C -> D, the dimerization reaction of the free heme (disappearance of the Soret

The Soret peak in heme solutions containing an increasing fraction of dimers becomes blunter and, as also a 340 nm band increases, takes the shape of a plateau from ca. 340 to 400 nm.<sup>47</sup> In (acid) aqueous solution, (acid) aqueous heme dimers are spontaneously formed from monomer heme, this formation being greatly enhanced by higher heme concentrations, such that “even at the lowest experimental concentrations of Fe(III)PPIX, the fraction of monomeric species is relatively low. [...] the dimer is easily studied in aqueous solutions because it is the overwhelmingly dominant species at relatively high concentrations (more than 92% dimerized above  $10^{-5}$  M)”<sup>48</sup> Aqueous heme dimers are dimers of two hemes of which the iron ions are directly  $\pi$ - $\pi$  bound to each other, and each iron is also bound/ligated on its other, outward, axial side to  $-H_2O$  or  $-OH$ . In acid solution, both ligands of the dimer can be  $H_2O$ , while in neutral solution a heterodimer exists ( $pK_a$  of first deprotonisation = 6.2), and in alkaline solution both ligands can be  $-OH$  ( $pK_a$  of second deprotonisation = 8.5).

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band). ... with human Hb+, it can be easily demonstrated that the disappearance of the Soret band at 406 m $\mu$  is a separate process from the appearance of the “denatured” band at 370 m $\mu$  (Steinhardt and Hiremath, A Comparison of..., 1967 <http://www.jbc.org/content/242/6/1294.full.pdf>.)” Polet and Steinhardt, Sequential stages in the acid denaturation of horse and human ferrihemoglobins, 1969, <http://pubs.acs.org/doi/abs/10.1021/bi00831a014>; at pH 0 the max of Soret band is at 365 nm (table at p. 602), “Hence the blue shift observed at low pH values of methemoglobin solution reflected the rupture of the heme- globin linkage” (p. 604), Journal of general chemistry of the U.S.S.R. in English translation - Volume 48, Pagina's 1-870, 1978, [http://books.google.co.uk/books?ei=P\\_94UYe5F46qOrWFgQg&hl=nl&id=HIMxAQAIAAJ&dq=porphyrin+methemoglobin+low+pH&q=soret+band](http://books.google.co.uk/books?ei=P_94UYe5F46qOrWFgQg&hl=nl&id=HIMxAQAIAAJ&dq=porphyrin+methemoglobin+low+pH&q=soret+band) )

<sup>46</sup> Compare fig. 2.5, part B (monomer in 80% aqueous methanol) and part D (aqueous solution of monomers and dimers) (= fig. 4 of Villiers et al., Speciation and structure..., 2007). In the acid monomer, the ratio height of Soret peak : height of 500 nm peak : height of 630 nm peak is about the same as in acid methemoglobin, viz. 179 : 10.0 : 4.4 (Wintrobe's Clinical Hematology, 12<sup>th</sup> edition, 2009, p. 147). In the acid aqueous heme solution where a certain fraction of dimers is present, the Soret band is significantly lower compared to the 500 and 630 nm bands (ratios ca. 53 : 7.0 : 3.6).

<sup>47</sup> Fig S3a of Supplementary material to Villiers et al., 2007, Speciation and structure..., 2007,

[http://link.springer.com/content/esm/art:10.1007/s00775-006-0170-1/file/MediaObjects/775\\_2006\\_170\\_MOESM1\\_ESM.doc](http://link.springer.com/content/esm/art:10.1007/s00775-006-0170-1/file/MediaObjects/775_2006_170_MOESM1_ESM.doc) .

<sup>48</sup> Fig S4 of Supplementary material [http://link.springer.com/content/esm/art:10.1007/s00775-006-0170-1/file/MediaObjects/775\\_2006\\_170\\_MOESM1\\_ESM.doc](http://link.springer.com/content/esm/art:10.1007/s00775-006-0170-1/file/MediaObjects/775_2006_170_MOESM1_ESM.doc) and Fig. 3 of Villiers et al., Speciation and structure..., 2007

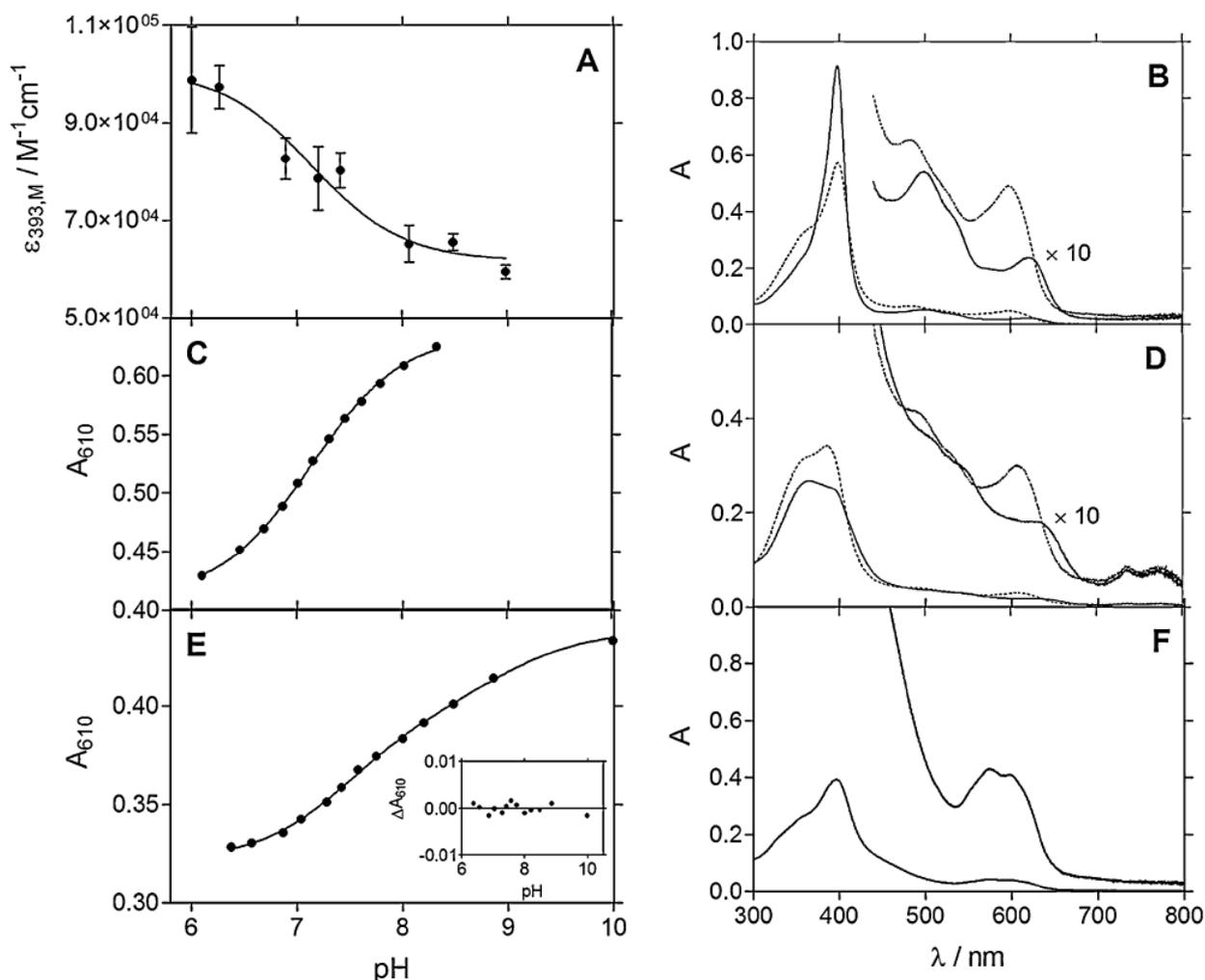


Fig. 2.5. B: Absorbance spectra of a buffered 1:4 water/methanol solution of heme monomer at pH 5.536 (solid line) and pH 8.862 (dotted line), and D: of a buffered aqueous solution of heme (dimer + a small monomer fraction) at pH 6.029 (solid line) and pH 9.669 (dotted line) ©Springer SBIC<sup>49</sup>

In acid solutions, dimers also tend to aggregate to amorphous insoluble clusters,<sup>50</sup> giving the solution they are in a turbid aspect - this does not take place when heme is in pure aqueous buffer solutions,<sup>51</sup> but takes place when aggregation catalysts (e.g. lipoids or proteins) are present in the solution.<sup>52</sup>

<sup>49</sup> With kind permission from Springer Science+Business Media: *Journal of Biological Inorganic Chemistry*, Speciation and structure of ferritroporphyrin IX in aqueous solution: spectroscopic and diffusion measurements demonstrate dimerization, but not  $\mu$ -oxo dimer formation, Volume 12, 2007, pp 101-117, K.A. de Villiers, C.H. Kaschula, T.J. Egan, H.M. Marques, figure 4, © SBIC 2006. DOI: 10.1007/s00775-006-0170-1, <http://link.springer.com/article/10.1007%2Fs00775-006-0170-1>

<sup>50</sup> "dimerized heme is insoluble at acid pH." Allis et al., 1970, Acid denaturation of carbonylhemoglobin. Protein unfolding without heme detachment, <http://pubs.acs.org/doi/abs/10.1021/bi00813a010>; "higher aggregates of heme, which cannot dissociate into dimers/monomers", Kuželová et al., 1997, Kinetics of heme interaction with heme-binding proteins: the effect of heme aggregation state, <http://www.sciencedirect.com/science/article/pii/S0304416597000627>; "the thermodynamically limiting step of spontaneous heme crystallization is the solubility of heme from its acid amorphous aggregate [26], [27]. Different chemical and physical factors, such as the degree of hydrophobicity of alcohols and lipids, their ability to solubilize acid heme aggregates *in vitro*, the reduction of solution surface tension and even an increase of physical contact between heme aggregates by stirring, suggest that increased dissolution of insoluble heme aggregates is a key parameter that would modulate  $\beta$ H formation [24]." Stiebler et al., 2010, Increase on the Initial Soluble Heme Levels in Acidic Conditions Is an Important Mechanism for Spontaneous Heme Crystallization *In Vitro*, <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0012694>

<sup>51</sup> "As can be seen, the data conform to the function expected for a dimerization process, with no evidence of higher aggregate formation over the entire concentration range in which satisfactory accurate absorbance measurements are feasible [...]" Villiers et al., Speciation and structure..., 2007, p. 107-08;

<sup>52</sup> "The method employed should estimate the concentration of all the forms of the haem-pigment circulating in the blood. [...] Samples of blood are frequently encountered in which the colour is not comparable with the standard. ...

Heme aggregation contributes to the broadening and disappearance of the Soret band of the solution.<sup>53</sup> The dimers and aggregates can also precipitate at the bottom and walls of the container, dependent on the material of the container: used glass or quartz cuvettes that have not been meticulously cleaned with concentrated NaOH and acid, and also plastic cuvettes, induce adsorption/precipitation.<sup>54</sup> Aggregation and/or precipitation of the dimer (or aggregate) would make the monomer-dimer equilibrium in the solution shift to the right, thus further decreasing the remaining monomer fraction, and thus also shifting the hemoglobin denaturation equilibrium toward more denaturation (more heme, less intact methemoglobin).

The extinction coefficient  $\epsilon$  at 393 nm of the heme monomer at pH 6.00 and ionic strength 0.154 M is  $9.9 \pm 1.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  and that of the aqueous acid heme dimer in the same conditions is only  $3.6 \pm 0.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ , both expressed as per mole of ferriprotoporphyrin IX (FP).<sup>55</sup> For comparison, the extinction coefficient at 405 nm of acid methemoglobin is  $17.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ , that of oxyhemoglobin at 415 nm is  $12.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ , and that of hemichrome at 411 nm is  $10.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ .<sup>56</sup> These numbers show that, compared with acid methemoglobin, the Soret absorption of acid heme dimers at pH 6.0 is a factor 5 smaller, and compared with hemichrome it is a factor 3 smaller. By comparing the height of the Soret peak ( $\epsilon_{393} = 3.6 \pm 0.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ) and that of the 500 nm band in the dimer's absorbance spectrum at pH 6.02,<sup>57</sup> the extinction coefficient  $\epsilon_{500}$  of the 500 nm band may be estimated to be  $0.6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ .

As the dimer's Soret extinction coefficient decreases with pH,<sup>58</sup> at lower pH than 6.0, such as at pH 5 and lower – where there is an actual 630 nm peak and 610 nm dip in the absorbance,<sup>59</sup> as would comply with the Shroud bloodstain reflectance dip at 630 nm and peak at 610 nm –, the dimer's

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There are several acid haematin methods in use, of which the most familiar are the Sahli and Newcomer. Several disadvantages are common to them all. The colour developed by the test does not quickly reach a maximum; while most of it develops in the first 30 or 40 minutes, the greatest optical density is not reached for from 24 to 36 hours, and the velocity of the colour reaction is not constant. [...] Wu (1922) has shown that the colour developed depends upon the concentration of protein and lipid in the test; and, since acid haematin is insoluble and exists as a colloid suspension protected by the protein present, there is often turbidity or actual precipitation." Clegg et al., Estimation of haemoglobin by the alkaline haematin method, 1942 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2164191/pdf/brmedj04008-0003.pdf>

<sup>53</sup> "As can be seen, the data conform to the function expected for a dimerization process, with no evidence of higher aggregate formation over the entire concentration range in which satisfactory accurate absorbance measurements are feasible [...]" Speciation and structure, p. 107-08; "The loss of Soret band intensity may result from the formation of  $\pi$ - $\pi$  complexes (25) or aggregation and precipitation of heme (33). To investigate both possibilities, a dilution test was performed with a solution containing C5 and heme in a molar ratio of 10 to 1. Dilution of this sample was found to obey the Beer-Lambert law in the concentration range of 1 to 50  $\mu\text{M}$  heme. This observation indicates that further heme aggregation and/or precipitation does not occur under our test conditions." Kelly et al., 2002, <http://aac.asm.org/content/46/1/144.long>; "AH was present in concentration lower than 2  $\mu\text{M}$ . In this case a shift of spectra in the Soret region, leading to the increase of the O.D. 400/385 nm ratio, suggested a transition toward a less aggregated state." (AH is alpha-hematin, i.e. OH-ligated human heme; O.D. = optical density, a measure correlated to absorbance), Omodeo-Salè et al., 2001, <http://www.sciencedirect.com/science/article/pii/S0006295201005585>

<sup>54</sup> Villiers et al., Speciation and structure..., 2007, p. 106-107

<sup>55</sup> Villiers et al., Speciation and structure..., 2007, Table 1.

<sup>56</sup> Wintrobe's Clinical Hematology, 12<sup>th</sup> edition, 2009, p. 147

[http://books.google.nl/books?id=68enzUD7BVgC&pg=PA147&lpg=PA147&dq=acid+methemoglobin&source=bl&ots=fEiUkJVcVt&sig=FtY3PTf6nZfxhR\\_qmWICsyoDRY&hl=nl&sa=X&ei=aEqCUZbYF8i-O5f3gJAD&redir\\_esc=y#v=onepage&q=acid%20methemoglobin&f=false](http://books.google.nl/books?id=68enzUD7BVgC&pg=PA147&lpg=PA147&dq=acid+methemoglobin&source=bl&ots=fEiUkJVcVt&sig=FtY3PTf6nZfxhR_qmWICsyoDRY&hl=nl&sa=X&ei=aEqCUZbYF8i-O5f3gJAD&redir_esc=y#v=onepage&q=acid%20methemoglobin&f=false). The standard unit for the millimolar extinction coefficient being  $\text{M}^{-1} \text{ cm}^{-1}$  ("Thus,  $D = E \cdot cd$ , where  $D$  = optical density (2 log T%)  $c$  = concentration in mmoles/l,  $d$  = layer thickness in cm,  $e$  = millimolar extinction coefficient; i.e.  $e = D$  when  $c = 1$  mmole/l and  $d = 1.000$  cm." Zijlstra et al., Standardization of hemoglobinometry: I. the extinction coefficient of hemoglobincyanide at  $\lambda = 540$  m $\mu$ :  $\epsilon_{540}\text{HiCN}$ , Clinica Chimica Acta, 1960, <http://www.sciencedirect.com/science/article/pii/0009898160900140>; The values 175, 125 and  $105 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ , are also expressed as per mole of ferriprotoporphyrin, for the extinction coefficient of oxyhemoglobin at 415 nm per mole of hemoglobin, containing four ferriprotoporphyrin subunits per hemoglobin molecule, is  $500 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  (Natural Phenomena Simulation Group, University of Waterloo, <http://www.npsg.uwaterloo.ca/data/blood.php>)

<sup>57</sup> "spectrum [...] of the dimer formed spontaneously in aqueous solution (Fig. 4, panel D)." Villiers et al., Speciation and structure..., 2007, p. 111 and fig 4 on p. 109 (here fig. 2.5 above)

<sup>58</sup> Villiers et al., Speciation and structure..., 2007, Table 1

<sup>59</sup> Austin and Drabkin, 1935, <http://www.jbc.org/content/112/1/67.short> p. 70 (p. 4 of pdf file)

Soret extinction coefficient might be 2 or even  $1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ; this pH-dependence of the extinction coefficient is represented in fig. 2.6, showing the values given in table 1 of Villiers et al., 2007.<sup>60</sup> This means that the extinction coefficients – and thus also the absorbance and reflectance values – of the Soret band and the 500 nm band of acid heme dimers in solution could be very close to each other at a low pH (<5). When aggregated or precipitated, the difference could be even less.

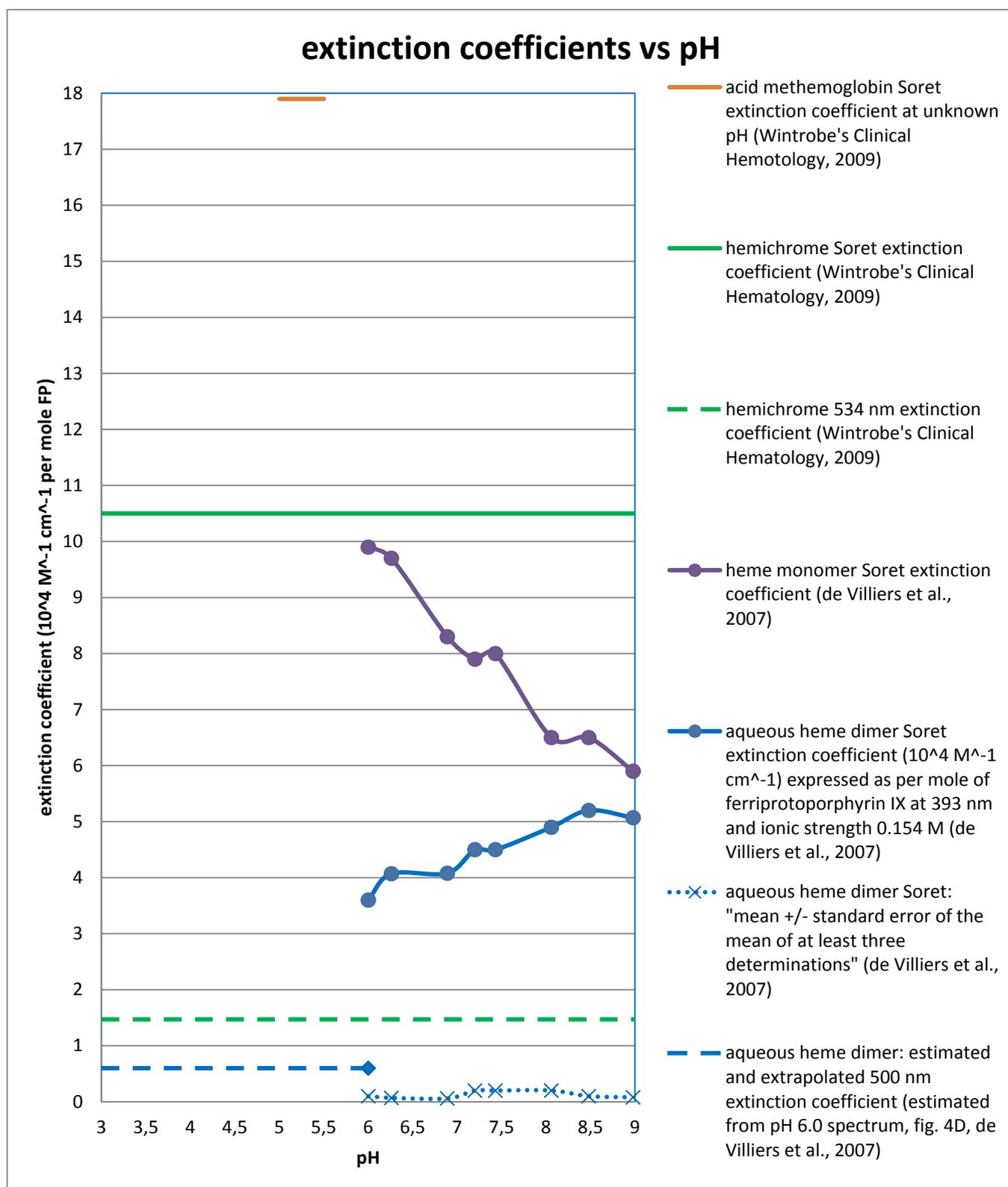


Fig. 2.6. Extinction coefficients of various heme products, dependent on pH ©AvdH

<sup>60</sup> Villiers et al., Speciation and structure..., 2007, <http://link.springer.com/article/10.1007%2Fs00775-006-0170-1>

So, one may indeed wonder why, in discussing the spectra of the Shroud blood, the presence of acid heme dimers has not been explicitly hypothesized before. Perhaps one of the reasons is that the spontaneous formation of the described aqueous  $\pi$ - $\pi$  heme dimers in acidic solutions was not known before 2006/2007,<sup>61</sup> although acid denaturation of hemoglobin – as causing disappearance of the Soret band – was known.<sup>62</sup> It seems that, until that time, one only knew of two kinds of human heme dimers: 1) the so-called “ $\mu$ -oxo dimer”, which has an oxygen as a bridge between the two iron and does not show the characteristic 630 nm absorbance peak, but shows a peak at ca. 610 nm<sup>63</sup>; 2) the dimer bound by two bridges from a carboxyl group of a heme’s side chain to the central iron of the other heme, formed inside the digestive vacuole of the malaria parasite, where it is crystallized to the so-called hemozoin crystal (also called  $\beta$ -hematin). Also this dimer/crystal does not show the required 630 nm band, but shows a band at ca. 600 nm.<sup>64</sup>

### 2.1.1.3. Loss of contrast of Soret band

There may be four reasons for the missing Soret band in relative reflectance spectra from Shroud bloodstains:

1) Acid heme dimerization and aggregation

If a large fraction of aggregated acid heme dimers, having largely lost the Soret band but still having the 500 and 630 nm bands, exists in the brown bloodstains of the Shroud, it diminishes the contrast of the Soret band compared to the 500 nm and 630 nm peaks formed by the dimers themselves and by any monomer hemes and intact methemoglobin, possibly still present in the stain.

2) Measurement and plotting in relative reflectance

Measurement in reflectance diminishes the contrast of the Soret band even further, as shown by Pellicori’s spectral measurements on a whole blood smear on glass, performed both in transmission and in reflectance mode (fig. 2.7). Also a whole blood smear on linen was examined, of course only in reflectance mode.

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<sup>61</sup> “Our study overturns the belief widely held for more than 35 years that Fe(III)PPIX exists as a  $\mu$ -oxo dimer in aqueous solution”. Villiers et al., *Speciation and structure...*, 2007, p. 115

<sup>62</sup> e.g. by Steinhardt and Hiremath, in 1967, who determined denaturation by the loss of the Soret band. (Steinhardt and Hiremath, *A Comparison of...*, 1967, <http://www.jbc.org/content/242/6/1294.full.pdf>

<sup>63</sup> “the solid precipitated from alkaline solution upon addition of solid NaOH or from alkaline dimethyl sulfoxide (DMSO) is a  $\mu$ -oxo dimer [2].” p. 102; Fig. 2.5. (Villiers et al., *Speciation and structure...*, 2007, Fig. 4).B: The solid line is the UV/Vis absorbance of heme monomer at pH 5.5, showing a Soret and 500 nm and 630 nm band; Fig. 2.5. (Villiers et al., *Speciation and structure...*, 2007, fig. 4) F : UV/Vis absorbance of heme  $\mu$ -oxo dimer, induced in a aqueous pyridine in 0.1 M NaOH, showing a ca. 610 nm band

<sup>64</sup> See curve 3 in Graphical abstract <http://ars.els-cdn.com/content/image/1-s2.0-S0166685110001994-fx1.jpg> of Mather et al., 2010, <http://www.sciencedirect.com/science/article/pii/S0166685110001994>

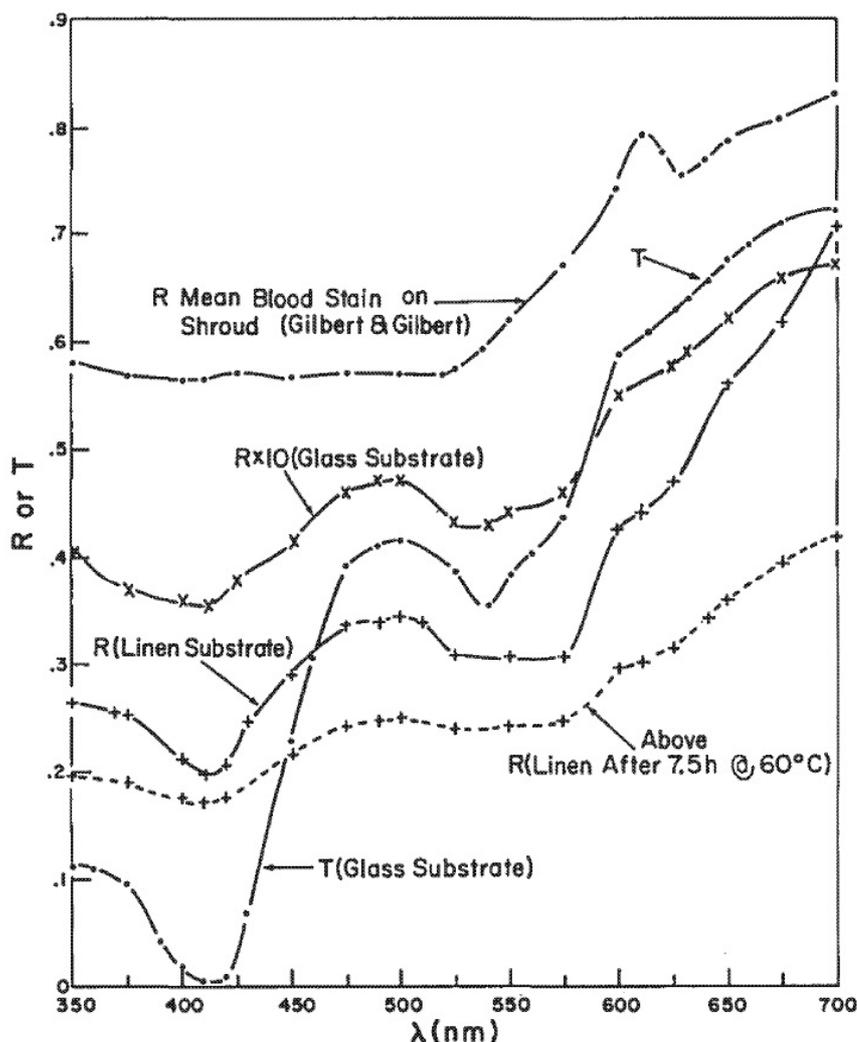


Fig. 2.7. Integrated reflectance and (one) transmission curve for laboratory blood preparations, including Shroud blood reflectance values (Pellicori) ©OSA<sup>65</sup>

“Figure [...] shows several blood reflectance spectra and one transmittance spectrum. The transmission curve shows greater band contrast than the reflection curve of the same sample, especially in the Soret 410-nm band”; the caption of the plot of the spectra reads “Laboratory blood is 4 days old and in one case was artificially aged by baking. The large difference in contrast of the Soret and  $\alpha$  and  $\beta$  bands occurs when the same sample (on glass slide) is measured in reflection as compared to transmission. Baking also reduces contrast of bands. [...] Instrument bandpasses are 4 nm for the laboratory and 5 nm for Shroud measurements at 410 nm”, and the text reads “It is appropriate to make comparisons with samples that have areal coverage more closely reproducing that of the Shroud blood stains, i.e., ratioed to clear substrate (linen), are presented.”<sup>66</sup> So, Pellicori calculated the relative reflectance spectra of the linen laboratory sample in the same way as Gilbert and Gilbert did for the Shroud bloodstains, viz. by dividing each measured stain reflectance value by the corresponding value of the measured clear background reflectance.<sup>67</sup>

<sup>65</sup> Used from S.F. Pellicori, Spectral properties of the Shroud of Turin, Applied Optics, Vol. 19, Issue 12, pp. 1913-1920 (1980) <http://dx.doi.org/10.1364/AO.19.001913> (fig. 4), with permission from the Optical Society of America.

<sup>66</sup> Pellicori, Spectral properties..., 1980, p. 1916

<sup>67</sup> “The raw response of these single-beam reflectance curves was read and digitized. Those of the clear areas were divided by the response from the magnesium oxide reference surface and replotted as absolute spectral reflectance assuming the reflectivity of magnesium oxide to be 1.0. Relative spectral reflectances of the various stains were determined by dividing the response from the stain area by the response of a mean of five clear areas of the Shroud and replotting the result. ... The absolute spectral reflectances of two clear (unstained) areas of the Shroud along with the

Pellicori's two spectra of a blood smear on linen look as the spectrum of either alkaline methemoglobin (methemoglobin that has its iron ion OH-ligated at a physiological pH 7.25 – 7.4) and/or of hemichrome (methemoglobin without external ligand but that has its iron ion ligated to the hemoglobin's own deformed protein at both axial sides and therefore is less influenced by pH), for it shows a shallow Soret band at ca. 410 nm, an even shallower Q band (= combined  $\alpha$  and  $\beta$  bands) in the 530-580 nm range, and only a very weak shoulder at ca. 600 nm. Maričić et al., 1964, published a transmission spectrum of the Q-region of hemichrome and called this methemoglobin species parahaemoglobin when they discovered its second formation path via the oxygen-free drying and then oxidation of hemoglobin, beside the already known path of the drying of methemoglobin.<sup>68</sup> In a study for forensic purposes, a non-linear least squares fit of reflectance spectra to determine the fractions of three different heme compounds present in a 7-days-old bloodstain, resulted in 49% hemichrome, 16% methemoglobin, and 35% oxyhemoglobin; further ageing made the hemichrome fraction increase at the expense of oxyhemoglobin.<sup>69</sup> Also because of this result, one may assume that the blood smear that had been artificially aged by Pellicori by baking it in air for 7.5 hours at 60°C<sup>70</sup>, would largely have lost the iron's aqueous ligand and consisted predominantly of hemichrome. Hemichrome's characteristic values are as follows (table 1.):

Wavelength $\lambda$ (nm)	Extinction coefficient $\epsilon$ per mole FP in hemichrome ( $\epsilon$ unit = $10^4 \text{ M}^{-1} \text{ cm}^{-1}$ )	Observed depth of dips beneath 500 nm-value in Pellicori's plot (relative reflectance units)
411 (reflectance dip)	10.5 (Wintrobe's)	0.079
500 (reflectance peak)	0.72 (estimated from Maričić et al. <sup>71</sup> )	-
534 (reflectance dip)	1.47 (Wintrobe's)	0.009
565 (reflectance shoulder/twin dip)	1.25 (Wintrobe's)	0.004

Table 1. Reference data of hemichrome and Pellicori's artificially aged blood smear on linen.

mean of five clear areas are shown in Fig. 6. This mean has formed the basis for the calculation of the relative spectral reflectances described below.” (Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, p. 1932-33).

<sup>68</sup> “We found experimentally this last shape to be almost identical with the spectrum obtained when a paste of methaemoglobin (used for the electrical measurements) was dried *in vacuo* ( $2 \cdot 10^{-2}$  mm Hg) for more than seven minutes and up to one and a half hour. It is the spectrum characteristic of parahaematin compounds, and was observed by Keilin and Hartree [8] in drying methaemoglobin. Thus, we would rather call this form “parahaemoglobin” for the sake of brevity.” S. Maričić, Greta Pifat, V. Pravdić, Hydration of Haemoglobin and its Reversible Oxygenation, *Berichte der Bunsengesellschaft für physikalische Chemie* Volume 68, Issue 8-9, pages 787–793, Oktober 1964, p. 787-793, p.790, <http://onlinelibrary.wiley.com/doi/10.1002/bbpc.19640680827/abstract> ; for other, more recent, hemichrome spectra see curves 2 and 3 in the following figure of Liu et al., 2005, <http://ars.els-cdn.com/content/image/1-s2.0-S0141813005002448-gr2.gif>, noting that “CTAB monomer can convert methemoglobin (metHb) to hemichrome” <http://www.sciencedirect.com/science/article/pii/S0141813005002448>; also see myoglobin's hemichrome spectrum in the dotted curve in <http://ars.els-cdn.com/content/image/1-s2.0-S0891584999002403-gr5.gif> of Skibsted et al., 2000, [http://dx.doi.org/10.1016/S0891-5849\(99\)00240-3](http://dx.doi.org/10.1016/S0891-5849(99)00240-3) and the spectrum of hemichrome of the earthworm *Glossoscolex paulistus* <http://ars.els-cdn.com/content/image/1-s2.0-S0300962996004483-gr3.gif> in Agostinho et al., 1997, <http://www.sciencedirect.com/science/article/pii/S0300962996004483> .

<sup>69</sup> Bremmer and Aalders, <http://www.slideshare.net/rolfok/ageing-bloodstains> , slide 16; cf. Bremmer et al., Age estimation of blood stains by hemoglobin derivative determination using reflectance spectroscopy, 2011, <http://www.sciencedirect.com/science/article/pii/S037907381000383X>

<sup>70</sup> Pellicori, Spectral properties..., 1980, legenda inside its fig. 4

<sup>71</sup> fig. 6 of Maričić et al., 1964, <http://onlinelibrary.wiley.com/doi/10.1002/bbpc.19640680827/abstract>

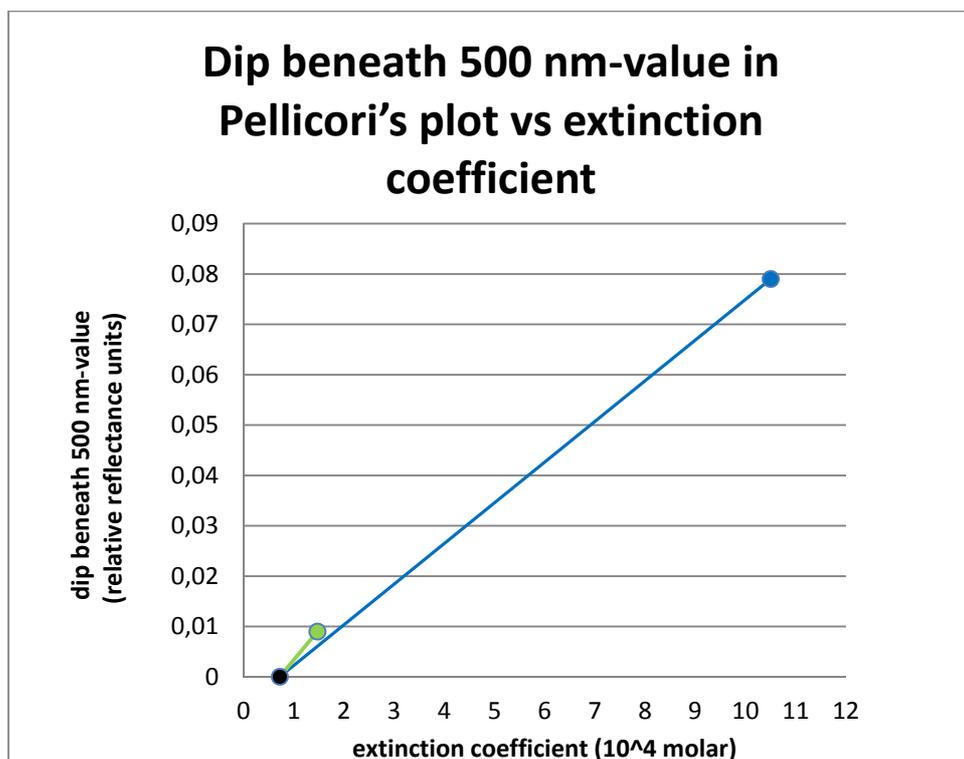


Fig. 2.8. Plot of reflectance difference (dip) vs extinction coefficient of reference data ©AvdH

In Pellicori's plot the depth of this 'hemichrome' Soret dip beneath the 500 nm value – at 500 nm the peak between the Soret dip and the 534 nm Q dip is located – is only ca. 0.079 relative reflectance units; the depth of the 534 nm dip beneath the 500 nm peak is 0.009 relative reflectance units and it represents an  $\epsilon$  increase of 0.75, viz. from 0.72 to  $1.47 \cdot 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  (cf. fig. 2.8). A linear approximation in this last short  $\epsilon$  range (in the green wavelengths region), is that the relative reflectance value drops 0.012 reflectance units per 1.0  $\epsilon$  unit increase. If this linear approximation is extrapolated to other possible small  $\epsilon$  increases, the depth of the dimer's Soret dip beneath its 500 nm value (the value which corresponds to an estimated  $\epsilon_{500}$  of 0.6  $\epsilon$  units<sup>72</sup>) may be estimated, if its spectrum was in Pellicori's figure (fig. 2.7. above). The acid dimer's Soret dip would be only 0.036 relative reflectance units deep if at pH 6.0 with  $\epsilon_{\text{Soret}} = 3.6$ , and it would be even smaller if at pH<5 (table 2).

When a linear approximation between 0.72 and 10.5  $\epsilon$  units (viz. a dip: $\epsilon$  ratio of 0.008) is applied, as appropriate for the blue (Soret) region where the greatest loss of contrast takes place, the estimated dimer's Soret dip would be only about 0.024 relative reflectance units deep if at pH 6.0 with  $\epsilon_{\text{Soret}} = 3.6$ , and again even smaller if at pH<5 (fig. 2.9).

Wavelength $\lambda$ (nm)	Extinction coefficient $\epsilon$ per mole FP ( $\epsilon$ unit = $10^4 \text{ M}^{-1} \text{ cm}^{-1}$ )	Estimated depth of Soret dip beneath 500 nm-value (relative reflectance units)
393 (pH 6.0)	3.6 (Villiers et al. <sup>73</sup> )	<b>0.036</b>
393 (pH<5)	2.0 (assumption)	<b>0.017</b>
393 (pH<5)	1.0 (assumption)	<b>0.005</b>
500 (pH 6.0)	0.6 (estimated from fig. 2.5.D)	0

Table 2. Calculated estimation of Soret dip of aqueous heme dimer beneath its 500 nm reflectance value, at different pHs and Soret extinction coefficients

<sup>72</sup> Value from Villiers et al., Speciation and structure..., 2007, fig. 4D (fig. 2.5. D above)

<sup>73</sup> Villiers et al., Speciation and structure..., 2007, table 1

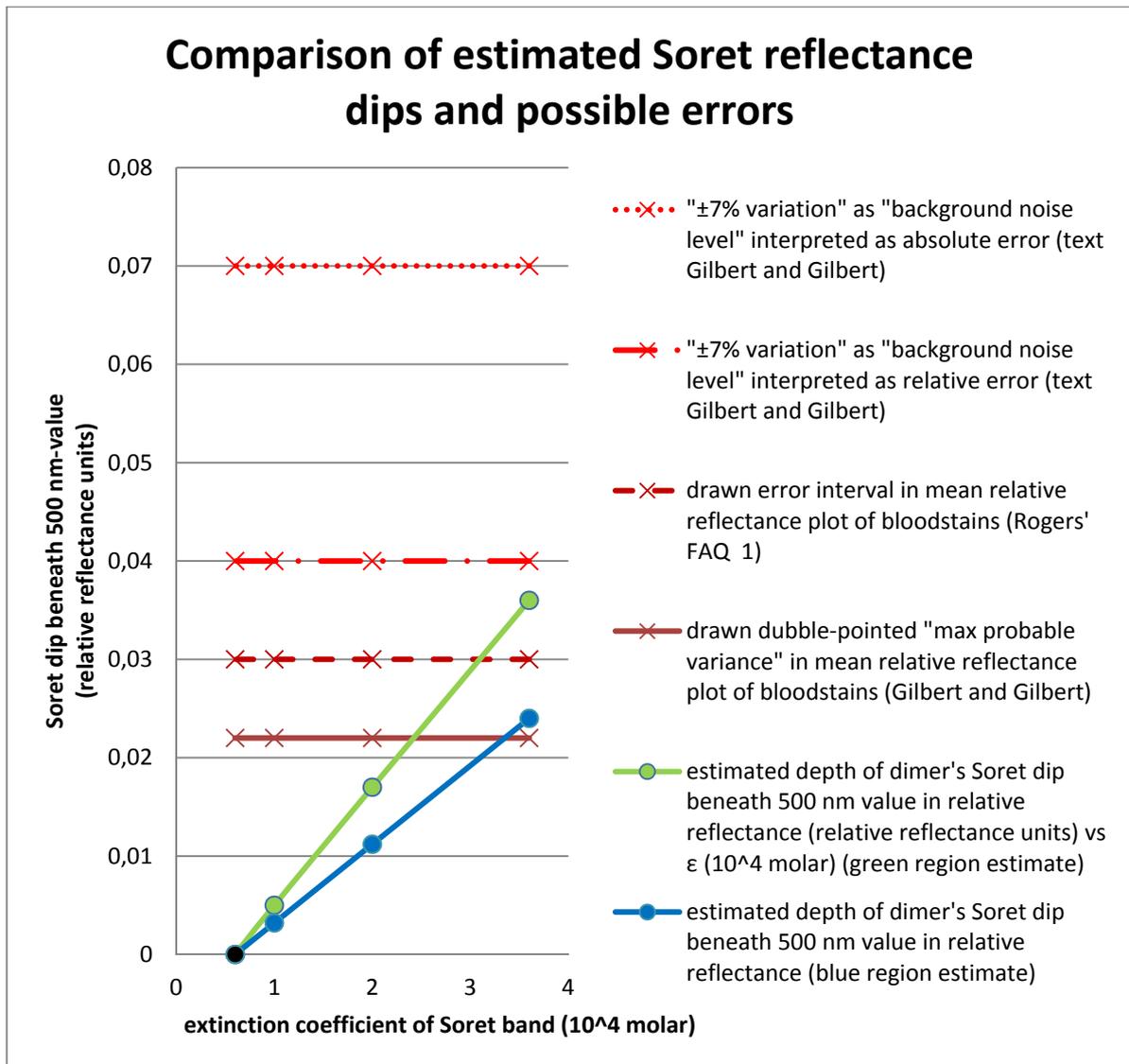


Fig. 2.9. Comparison of estimated depths of Soret dip of acid heme dimer (at various extinction coefficients) and 'variations' ©AvdH

### 3) Cloth noise

"The variation in spectral reflectance from a particular clear area to the mean clear referred to above was generally between  $\pm 3$  and  $\pm 7\%$  across the entire spectrum. This variation represents a background noise level that should be considered when evaluating the reflectance spectra of the image, scorched, and bloodstained points. The effect of the instrument noise and the background noise on the Shroud has been reduced by averaging several areas of a similar type."<sup>74</sup> A  $\pm 7\%$  variation either means a possible error of  $\pm 0.07$  relative reflectance units, or, in the 400-500 nm region, where the Shroud bloodstain relative reflectance is ca. 0.57, it corresponds to a variation of  $\pm 0.04$  relative reflectance units. Over and above this general "background noise level", there is also a double-pointed arrow with the text "max probable variance" in the mean relative reflectance plot of the Shroud bloodstains, and it is about 0.022 relative reflectance units large.<sup>75</sup> In the plot of the mean relative reflectance of the bloodstains in Roger's FAQ 15, an error interval of 0.030 is drawn beside the actual curve and datapoints. These possible errors/error intervals (especially when added to a background

<sup>74</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, p. 1933

<sup>75</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, fig. 14 (fig. 2.3 above) ; in Fig. 4 of Pellicori, Spectral properties..., 1980 (fig. 2.7 above), the ordinate ranges from 0 to 0.9 relative reflectance: it correctly interprets figure 14 of the Gilberts, which ordinate scale ranges from 0.4 to 1.0 units, the decimal dot being only visible after magnification of the plot).

noise level of 7%) would probably be enough to explain the invisibility of a heme dimer's Soret band when compared to the 500 nm band, if present in the relative reflectance of Shroud bloodstains. See fig. 2.9 to compare the possible errors/error intervals and the estimated depths of the assumed Soret dip. Also Pellicori wrote that "The Shroud curve shows ... no suggestion of a Soret band. The latter was probably obscured by the poor SNR of the measurement. [...] The spectrum suggests denatured met-hemoglobin." (SNR = signal-to-noise ratio)<sup>76</sup>

#### 4) Bound hydroxyanthraquinones

One may wonder whether or not the reflectance ramp, as large as 0.04, visible between 320 and 340 nm in the Shroud bloodstain reflectance, represents the end of the heme dimer's Soret band at 340 nm;<sup>77</sup> also the ramp as large as 0.04, visible between 725 and 750 nm, might perhaps correspond to the heme dimer's small absorption band at 725 nm (cf. fig. 2.3 and fig. 2.5 D),<sup>78</sup> where the loss of contrast is smallest. If they do, one might wonder if it is per chance that there is no or only a minimal suggestion of a peak between the Shroud's bloodstain Soret band and its 500 nm band in relative reflectance, and that the 500 nm heme band seems to have been extended to 525 nm (fig. 2.3). The question is whether a certain colored substance was complexed to the acid heme and added its own spectral features to the spectrum of the Shroud's bloodstains.

In a certain enzyme that contains a ferric heme and is bound to flavins, "The absorbance shoulders at 450–485 nm indicate the presence of bound flavins and are unusually prominent with respect to the Soret absorbance".<sup>79</sup> As can be seen in the absorbance spectrum of this flavin-ferric heme compound,<sup>80</sup> these shoulders virtually fill the gap between its Soret band and its 500 nm band. Also a complex of ferric iron and tannins exists.<sup>81</sup> The tannins gallic acid and tannic acid, when eaten by horses in the form of red maple leaves, even form brownish red complexes with hemoglobin and thus cause methemoglobinemia.<sup>82</sup> At least some root saponins form insoluble complexes with iron.<sup>83</sup> Tannins and saponins are phenols, and phenols form strongly colored complexes with ferric iron,<sup>84</sup> and are even known to oxidize human hemoglobin A to methemoglobin and become its ligand, and make it denature and precipitate.<sup>85</sup> The penolic dye brompenol blue<sup>86</sup> increases the rate of

<sup>76</sup> Pellicori, Spectral properties..., 1980, p. 1916

<sup>77</sup> Villiers et al., Speciation and structure..., 2007, Supplementary material, fig. 3S

[http://link.springer.com/content/esm/art:10.1007/s00775-006-0170-1/file/MediaObjects/775\\_2006\\_170\\_MOESM1\\_ESM.doc](http://link.springer.com/content/esm/art:10.1007/s00775-006-0170-1/file/MediaObjects/775_2006_170_MOESM1_ESM.doc)

<sup>78</sup> Villiers et al., Speciation and structure..., 2007, fig. 4D, p. 109, "spectrum [...] of the dimer formed spontaneously in aqueous solution (Fig. 4, panel D)." p. 111 <http://link.springer.com/article/10.1007%2Fs00775-006-0170-1> ;

<sup>79</sup> Uma Siddhanta et al., Domain Swapping in Inducible Nitric-oxide Synthase – Electron transfer occurs between flavin and heme groups located on adjacent subunits in the dimer, 1998, <http://www.jbc.org/content/273/30/18950.long>

<sup>80</sup> Uma Siddhanta et al., 1998, fig. 1, <http://www.jbc.org/content/273/30/18950/F1.expansion.html>

<sup>81</sup> RM Reeve, Histochemical tests for polyphenols in plant tissues, Biotechnic & Histochemistry, 1951, "[in] all cases the red color of the Hoepfner-Vorsatz reaction was observed in areas where iron-tannin complexes were obtained with ferric chloride." <http://informahealthcare.com/doi/abs/10.3109/10520295109113187>

<sup>82</sup> College of Veterinary Medicine, Cornell University, <http://ahdc.vet.cornell.edu/Sects/Toxic/redmaple.cfm>

<sup>83</sup> "Alfalfa root saponins and ammoniated glycyrrhizin, but not digitonin, quillaja and alfalfa leaf saponins, complexed zinc and iron, but not magnesium. It is proposed that one mechanism of action whereby saponins suppress growth is by the formation of insoluble saponin-mineral complexes. Such complexes could reduce the availability of minerals necessary for growth." West, 1978, <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1978.tb15309.x/abstract> \

<sup>84</sup> "If the phenol is water soluble, add a few drops of 2.5% aqueous ferric chloride solution to a 3% aqueous solution of the phenol. A deep red, green, or blue color is positive." Chickos, Garin and D'Souza, University of Missouri-St. Louis, <http://www.umsl.edu/~orglab/experiments/UNKEXP.html> on <http://www.umsl.edu/~orglab/> ; cf. Heller and Adler, A Chemical..., 1981, Table 7, TOM 54: "Tests employed .... Species: phenols – Method: a) nitrous acid b) ferric ion"

<sup>85</sup> "The spectral changes upon reaction of fluoride (500 mM) with 50  $\mu\text{M}$  HbAO<sub>2</sub> at pH 5.7 (0.1 M sodium maleate buffer) and 25 °C are shown in Fig. 2; the oxy spectrum is replaced by the spectrum of MetHb fluoride with  $\lambda_{\text{max}}$  ( $\epsilon$ ) = 606 nm (7.42 M<sup>-1</sup> cm<sup>-1</sup>) and 490 nm (8.99 M<sup>-1</sup> cm<sup>-1</sup>) and isobestic points at 587 and 520 nm (30). Analogous spectral changes were observed with other anions such as cyanide and thiocyanate (Fig. 3) as well as cyanate, chloride, and bromide (not shown). Phenols and nitrite were also found to promote the oxidation of HbAO<sub>2</sub>." p. 4967 ; "Kinetic studies of reactions with phenols were made difficult by precipitation (denaturation) phenomena that invariably occurred.

denaturation of carbonylhemoglobin in acid solution in air, and “gives a corresponding shift in the equilibrium-pH function to higher pH values”, which probably is the result of the binding of the dye to the heme in this acid condition.<sup>87</sup> A similar pH-shift is observed in certain antimalarial drugs, such as chloroquine, which make human ferric heme (ferriprotoporphyrin IX, FPIX) precipitate at higher pH values (viz. at pHs in the range 5.5 – 6.0) in acid environment, “with the net shift proportional to the relative affinity of the drug for FPIX”.<sup>88</sup> The mechanism of the antimalarial activity of chloroquine is thought to be the binding of the drug to heme inside the digestive vacuole of the malaria parasite, which has the acidic pH 4.8–5.4.<sup>89</sup> The potent antimalarial drug rufigallol (1,2,3,5,6,7-hexahydroxy-9,10-anthraquinone) – which is a polyhydroxyanthraquinone exactly similar to alizarin and purpurin (1,2-dihydroxy-9,10-anthraquinone and 1,2,4-trihydroxy-9,10-anthraquinone, respectively) except that it has more hydroxyl groups – has both “redox-cycling capacity” (which means it produces hydrogen peroxide in the presence of oxygen and H<sup>+</sup> ions of an acid environment) and “iron-chelating ability”.<sup>90</sup> In 1995, the antimalarial activity of a series of hydroxy- and polyhydroxyanthraquinones was reported; among these anthraquinones was not alizarin, but purpurin, which yielded a poor, but nevertheless measurable, activity against *Plasmodium falciparum*.<sup>91</sup>

The root of the madder plant (*Rubia tinctorum*) contains at least the following phenolics: saponins, tannins, and hydroxyanthraquinone dyestuffs such as alizarin and purpurin.<sup>92</sup>

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However, precipitation was usually slow enough to allow the reaction to be followed through the first half-life, and in some instances, through two half-lives. Within this limited kinetic framework, the reactions exhibited first order dependence upon Hb concentration (Fig. 6). The first order portions of the reactions also exhibited linear dependence upon the phenol concentration.” ... “The  $k_2$  values are strongly sensitive to the nature of the ligand. Reactivity decreases in the order nitrite > azide > thiocyanate > cyanate > cyanide > phenol > fluoride > chloride > bromide (Tables I and II). This order suggests that effectiveness in promoting autooxidation is related to nucleophilicity as a ligand except that cyanide seems out of order in terms of relative nucleophilicity.” (p. 4969): This research was originally published in The Journal of Biological Chemistry. W.J. Wallace, R.A. Houtchens, J.C. Maxwell and W.S. Caughey. Mechanism of autooxidation for hemoglobins and myoglobins. Promotion of superoxide production by protons and anions. The *Journal of Biological Chemistry*. 1982; 257:4966-4977. © the American Society for Biochemistry and Molecular Biology.

<http://www.jbc.org/content/257/9/4966.full.pdf>

<sup>86</sup> [http://en.wikipedia.org/wiki/Bromophenol\\_blue](http://en.wikipedia.org/wiki/Bromophenol_blue)

<sup>87</sup> “The equilibrium results obtained ... with the dye are included in Fig. 6.” Steinhardt, Polet and Moezie, Acid Denaturation of Horse Carbonylhemoglobin in the Absence of Oxygen, *The Journal of Biological Chemistry*, Vol. 241, No. 17, Sept 1966, pp. 3988-3996, <http://www.jbc.org/content/241/17/3988.full.pdf>. This 1966 article still assumes that the phenol binds to the protein of hemoglobin, but in 1975 and 1982 it was said that phenol autooxidize hemoglobin and binds to it, as a “ligand” (Wallace et al., 1975, Mechanism for the autooxidation of hemoglobin by phenols, nitrite, and “oxidant” drugs. Peroxide formation by one electron donation to bound dioxygen, *Biochem. Biophys. Res. Commun.* 62,561-567; Wallace et al., 1982 Mechanism of autooxidation for hemoglobins and myoglobins,

<http://www.jbc.org/content/257/9/4966.full.pdf>).

<sup>88</sup> See Ursos et al., 2001, <http://www.sciencedirect.com/science/article/pii/S016668510000342X>, fig. 1 for the shift of the FPIX solubility-pH curves by antimalarial drugs.

<sup>89</sup> Villiers et al., Speciation and structure..., 2007, p. 102; “It is therefore probable that CQ-hematin  $\mu$ -oxo dimer binding affinity does not diminish at the pH range (4.8–5.4) of the parasite food vacuole. ... A stoichiometry ( $n$ ) of 1:4 in the pH range 6.5–9.0 indicates that CQ binds to two hematin  $\mu$ -oxo dimers. At pH 5.6, a stoichiometry of 1:8 suggests that CQ binds to an aggregate of four hematin  $\mu$ -oxo dimers” (Portela et al., 2003,

<http://www.sciencedirect.com/science/article/pii/S0014579303006926>). Note that the heme would not be present in the aqueous solution in the form of hematin  $\mu$ -oxo dimers but in the form of (aggregates of) heme  $\pi$ - $\pi$  dimers – a discovery that was only made in 2007 by Villiers et al., Speciation and structure of ferriprotoporphyrin IX in aqueous solution: spectroscopic and diffusion measurements demonstrate dimerization, but not  $\mu$ -oxo dimer formation, 2007, <http://link.springer.com/article/10.1007%2Fs00775-006-0170-1>.

<sup>90</sup> Winter et al., Potentiation of the antimalarial activity of rufigallol, 1996, <http://aac.asm.org/content/40/6/1408.full.pdf> p. 1408, 1410

<sup>91</sup> Winter et al., 1995, Hydroxy-anthraquinones as antimalarial agents,

<http://www.sciencedirect.com/science/article/pii/S0960894X95003260>

<sup>92</sup> “Madder is a perennial plant with horizontal rhizome, numerous fruits, sharply pointed leaves sitting together in clusters and greenish-yellow flowers. The rhizome and roots are collected and dried at a temperature of 40 degrees. The dried stuff has a typical odor and contains inter alia organic acids, tannins, saponins, glycosides and alizarin.” (translation of “De meekrap is een meerjarige plant met horizontale wortelstok, talrijke vruchten, scherppuntige bladeren die in bosjes bij elkaar zitten en groengele bloempjes. De wortelstok en de wortels worden verzameld en bij een

Chromatography was used to identify alizarin and purpurin in a red 7-9<sup>th</sup> century AD Coptic textile, in which iron was the sole, and apparently stable, mordant of these madder dyestuffs.<sup>93</sup> Just as aluminum ( $\text{Al}^{3+}$ ) is a mordant of madder dye and forms red madder lake (used in the paint called Rose Madder), also iron (probably  $\text{Fe}^{3+}$ ) is a mordant and can form madder lakes (pigment particles) and thereby paints of red and pink colors.<sup>94</sup> When mixed in a binder, in a diluted form, Rose Madder pigments constitute a pink paint, but in a concentrated form, they may constitute a dull magenta-red paint.<sup>95</sup> McCrone called the “rose madder particles” he had seen on the Shroud “red particles”<sup>96</sup> (cf. fig. 2.10).

Therefore, the presence of a reddish heme-hydroxyanthraquinone compound (a red madder lake) on the Shroud, formed when acid heme was adsorbed to the Shroud’s coating of acid madder dye on starch, might be hypothesized.



Fig. 2.10 Natural madder lake swatches ©L.L. Bispo<sup>97</sup>

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temperatuur van 40 graden gedroogd. De drogerij heeft een typische geur en bevat o.a. organische zuren, looistoffen, saponinen, glycosiden en alizarin.”)

<http://www.e-gezondheid.be/meekrap-rubia-tinctorum/gids/981#paragrafe1> ; <http://en.wikipedia.org/wiki/1,2,4-Trihydroxyanthraquinone>

<sup>93</sup> Orska-Gawrýs, 2003, sample #5 of Fig. 5, which at sample #10 says “no mordant”, implying Fe and Al are mordants <http://ars.els-cdn.com/content/image/1-s2.0-S0021967303000839-gr5.gif> of

<http://www.sciencedirect.com/science/article/pii/S0021967303000839>

<sup>94</sup> “Aluminum lakes of dye extracted from the madder root or from a madder derivative were popular artists' pigments in the 19th century: for example rose madder were used as artists pigments. Madder forms a bright red color when precipitated on an amorphous hydrated alumina substrate, such as alumina trihydrate. Tin, chromium, and iron mordants can produce purple, brown, and pink colors, respectively.” (online encyclopedia of the Museum of Fine Arts, Boston, <http://cameo.mfa.org/wiki/Madder> )

<sup>95</sup> “Rose madder” is the commercial name of madder paint, i.e. a madder lake pigment, which is pink or violet-red in diluted form (medium solutions), but its undiluted color (its “masstone color”) is dull magenta red. “Rose madder ... moderately dull violet red pigment in tints and medum solutions, darkening to an impermanent, dull magenta red in masstone.” [https://en.wikipedia.org/wiki/Rose\\_madder](https://en.wikipedia.org/wiki/Rose_madder) ; “Masstone -The undiluted color of a pigment or a pigmented paint coating. Also known as mass color.” <http://encyclopedia2.thefreedictionary.com/masstone> ; rose madder color swatch: <http://memolo.blogspot.nl/2012/10/kings-highway-pt-38-rose-madder.html>, and <http://www.oldholland.com/oil/b172.html> , cf. madder lake color swatch <http://www.oldholland.com/oil/c28.html>; and rose madder color <http://colors.findthebest.com/1/197/Rose-Madder>

<sup>96</sup> McCrone, Judgement Day...., 1999, p. 166

<sup>97</sup> Used from the post ‘Madder from the Garden’, <http://sunsikell.wordpress.com/2011/03/06/madder-lake-from-the-garden/>, of March 6, 2011, on the blog Sunsikell <http://sunsikell.wordpress.com/about/> by L.L. Bispo, with permission from L.L. Bispo

The presence of acid madder dye on the Shroud is discussed in chapter 4. How both acid methemoglobin in crystalline form and acid heme dimers in a film/stain form may have got on the Shroud is discussed in chapter 5.

## 2.1.2. Heme-madder lake

### 2.1.2.1 Transmission curve of red-stained fibril - madder dye and lake

Adler's writings show that he interpreted the strong 450 nm band in the transmission curve of a brownish red stained Shroud fibril (fig. 2.2.A), as either a shifted and distorted (originally 410-nm) Soret band and/or a not-shifted not-distorted (450 nm<sup>98</sup>) bilirubin band.<sup>99</sup> There is, however, a much more simple interpretation: the presence of madder.

On unmordanted wool, a madder dye, containing protonated alizarin and deprotonated purpurin (red di-anion form) in a ratio of about 1:9 (alizarin:purpurin, having absorbance maxima at 430 and 547 nm, respectively<sup>100</sup>), shows, when measured in reflectance, a broad flat absorbance band from ca. 350 to 500 nm with a shallow maximum at ca. 430-450 nm; the wool mainly contributes to the absorbance in the 250-450 nm region.<sup>101</sup>

An acidic dye containing both protonated purpurin (absorbance maximum at 480 nm) and protonated alizarin (absorbance maximum at 430 nm) – and especially when alizarin is present in a higher, origin- and treatment-dependent, fraction<sup>102</sup> which only is increased by the ageing-degradation of purpurin, which is faster than that of alizarin<sup>103</sup> –, would in reflectance show a much higher peak in the 450 nm region, between the absorbance maxima of these two protonated madder constituents. The extinction coefficient of the 430 nm absorbance of protonated alizarin is  $0,47 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  and that of the 455 nm and 480 nm absorbance bands of protonated purpurin is 0,8 and  $0,90 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ , respectively.<sup>104</sup> When measured in transmission (and on a linen fiber) we may expect the fully protonated madder band to have even more contrast. Wool has an absorbance band at ca. 280 nm with a shoulder at ca. 375 nm<sup>105</sup>; lignin has just a single band at 280 nm with little absorbance >400 nm.<sup>106</sup>

Note that the linen fibrils that were investigated in transmission by Heller and Adler, only had “red stains on part of their length”, and “surely were not coated with iron oxide”.<sup>107</sup> Red iron oxide

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<sup>98</sup> absorbance spectrum of bilirubin: <http://www.biolab.cn/uploads/1/Image/20090606031136124.jpg> or [http://www.npsg.uwaterloo.ca/data/skin/bilirubin\\_extinction.png](http://www.npsg.uwaterloo.ca/data/skin/bilirubin_extinction.png)

<sup>99</sup> cf. “All these fibrils showed intense Soret (400-450 nm) absorption” (Heller and Adler, *Blood on the Shroud...*, 1980, TOM 30-31) and “There is an extraordinarily high bilirubin count, almost as high as the methemoglobin. ... In fact, we have been able to simulate this spectrum in the laboratory by the process described above.” (Adler, *The origin and nature*, 1986, TOM p. 61) and “Increasing the amount of bilirubin in this simulacrum will also improve the fit as with the FTIR data.” (Adler, *Updating recent*, 1996 TOM 84.). See notes in 6.1.2. Bilirubin, for links to spectra plots.

<sup>100</sup> Miliani et al, *Acidichromic effects...*, 2000, Table 1 and 3

<sup>101</sup> Fig. 5c <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr5.jpg>, and “the neutral form of alizarin (probably present in the absence of mordant)” (p. 49), “purpurin ... Due to its intensity and position, the emission is most probably due to the molecule in de di-anion form” (p. 49-50), and fig. 1 <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr1.jpg> and fig. 7 <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr7.jpg>, of Clementi et al., *A spectrometric...*, 2007, <http://www.sciencedirect.com/science/article/pii/S0003267007009464>; cf. purpurin absorbance

<sup>102</sup> “in madder lakes, obtained from natural sources, the ratio between purpurin and alizarin is dependent on the species and methods of preparation.” Claro et al., *The use of...*, 2008, p. 924

<sup>103</sup> Clementi et al., *A spectrometric...*, 2007, p. 51-53

<sup>104</sup> Miliani et al., *Acidichromic effects...*, 2000, Table 1 and 3

<sup>105</sup> Clementi et al, *A spectrometric...*, 2007, fig. 1, <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr1.jpg>

<sup>106</sup> UV-vis of lignin of sal leaves, fig. 3 [http://article.sapub.org/image/10.5923.j.ajps.20120201.03\\_003.gif](http://article.sapub.org/image/10.5923.j.ajps.20120201.03_003.gif) of Singh et al., <http://article.sapub.org/10.5923.j.ajps.20120201.03.html>; also Fig. 4 (solid line: absorbance spectrum of water-soluble products of kraft pulp lignin degraded by ligninases) of Fang et al., 1999, <http://ars.els-cdn.com/content/image/1-s2.0-S0032959299000163-gr4.gif> in <http://www.sciencedirect.com/science/article/pii/S0032959299000163>, and <http://cool.conservation-us.org/coolaic/sg/bpg/annual/v06/bp06-04.html> fig. 3

<sup>107</sup> Heller, *Report on...*, 1983, p. 128 and 140

particles are birefringent,<sup>108</sup> but blood/heme particles and/or madder lake of non-birefringent mordant particles are not: a description of a third-fourth century AD Egyptian painting on canvas reads “Madder lake Pinkish-red, clear isotropic masses”.<sup>109</sup> A fibril that is coated with madder dye all around and that is only partly “stained” with some kind of red on top of it, would in transmission yield a spectrum with a contribution of the madder dye absorbance. So, the 450 nm band, observed in the transmittance of “a brownish red stained fibril” from the Shroud (fig. 2.2.A), may simply result from the fibril’s madder dye coating. As Heller put it, when talking about the measurement of the transmittance of a free translucent crystal: “At least here we don’t have a fiber in the way.”<sup>110</sup> The microspot that was focussed on the fiber in transmission was only “about five microns” (5 µm) large<sup>111</sup>; “Most of the red particulates ranged from submicron to about 3 µm, and the birefringent red particulates from 0.7 µm to about 1 µm diameter”; “The majority of the linen fibrils ranged from 10-15 µm in diameter”.<sup>112</sup> The class of “Red coated fibrils” on the Shroud got the description: “Coating varies from smooth to fractured to particulate appearance”.<sup>113</sup> These numbers show that the spectrometric microspot, which was probably focussed on a red part of the fiber, may have contained a substantial madder dye coating underneath and/or next to that red part.

### Not linen/lignin

The linen of the fiber could not have produced the 450 nm transmittance peak, for the mean absolute reflectance of the Shroud cloth in background areas (normalized to the reflectance of MgO) only shows a band at ca. 280 nm, from where it ascends as an almost straight line to 750 nm.<sup>114</sup> Here, only the linen absorbance seems visible: fresh lignin, for example, has a ca. 280 nm absorbance band<sup>115</sup>. A 430-450 nm madder dye band would not only have been much shallower than in the fiber transmission because of measurement in reflectance mode, but would contribute to the total reflectance/absorbance of the only superficially dyed cloth (Rogers found no yellow madder coating inside the Raes threads<sup>116</sup>) to a much smaller extent than on a single dyed surface fibril. As the Shroud’s weave is very tight and does “not readily absorb water”<sup>117</sup> – even though a feature of linen fabric is that it absorbs water very easily<sup>118</sup> – and probably had a polished insoluble starch coating (see chapter 4), the madder dye may indeed have been extremely superficial and not contributing much to the cloth’s reflectance. Also in artwork, an aged yellow dye on cloth could hardly be determined by reflectance spectrometry (figures 4.23 and 4.24). On a single surface fiber, however, the madder dye could have been much more dominant, also because lignin may have been minimally present in this particular investigated fiber. The lignin content of a single linen fibril may be quite variable, which may be reflected in the apparent banding in the colour of the Shroud.

<sup>108</sup> “the birefringent red particles are Fe<sub>2</sub>O<sub>3</sub> by both chemical and microscopic tests.” Heller and Adler, *A Chemical...*, 1981, TOM 42.

<sup>109</sup> SP Sack, FC Tahk, T Peters Jr., *A technical examination of an ancient Egyptian painting on canvas*, Studies in Conservation, 1981, *JSTOR*,

<http://www.jstor.org/discover/10.2307/1505817?uid=3738736&uid=2&uid=4&sid=21102611584517>

<sup>110</sup> Heller, Report on..., 19863, p. 144

<sup>111</sup> Heller, Report on..., 1983, p. 144-145

<sup>112</sup> both Heller and Adler, *A Chemical...*, 1981, TOM 37

<sup>113</sup> Heller and Adler, *A Chemical...*, 1981, Table 2, TOM 50

<sup>114</sup> Gilbert and Gilber, *Ultraviolet-visible reflectance...*, 1980, fig. 6,

<http://imagebank.osa.org/getImage.xqy?img=dTcqLmxhcmdlLGFvLTE5LTEyLTE5MzAtZzAwNg>

<sup>115</sup> UV-vis of lignin of sal leaves, fig. 3 [http://article.sapub.org/image/10.5923.j.ajps.20120201.03\\_003.gif](http://article.sapub.org/image/10.5923.j.ajps.20120201.03_003.gif) of Singh et al.,

<http://article.sapub.org/10.5923.j.ajps.20120201.03.html> ; also Fig. 4 (solid line: absorbance spectrum of water-soluble

products of kraft pulp lignin degraded by ligninases) of Fang et al., 1999, <http://ars.els-cdn.com/content/image/1-s2.0-S0032959299000163-gr4.gif> in <http://www.sciencedirect.com/science/article/pii/S0032959299000163> , and

<http://cool.conservation-us.org/coolaic/sg/bpg/annual/v06/bp06-04.html> fig. 3

<sup>116</sup> “I could not detect any significant amount of dye on fibers from the insides of threads.” (Rogers and Arnoldi, *Scientific Method...*, 2002 , p. 20)

<sup>117</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, p. 32

<sup>118</sup> <http://textiles4u.wikispaces.com/Natural+Fibres> ;

<http://lib.ndsu.nodak.edu/repository/bitstream/handle/10365/17070/A-406-1963.PDF?sequence=1>

### Heme or madder lake or both?

As already shown, the extinction coefficient of an acid heme dimer's 393 nm Soret band is  $3,6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at pH 6.0, and perhaps only  $1,0 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at pH 4.5. Whether or not also a shoulder resulting from an acid heme dimer transmittance was visible near 393 nm, or as far as 340 nm, in the stained fibril's curve, we don't know, for, in the published article, the curve is only plotted from 410 to 750 nm, probably because measurement below 410 nm was impossible because of the poor measurement conditions (glass slide, sticky tape and fibril in the way), which might also have shifted the peaks.<sup>119</sup> Anyway, there is no suggestion of a 630 nm band, so perhaps the red on the fiber was not (only) heme. Perhaps there was (also) a red madder lake (= a madder-mordant complex), as there is a peak at ca. 530 nm and a pink/red madder lake's absorbance maximum is in the 520-530 nm range<sup>120</sup> (fig. 2.14). See also the effect of mordanting on alizarin on wool in fig. 2.11.

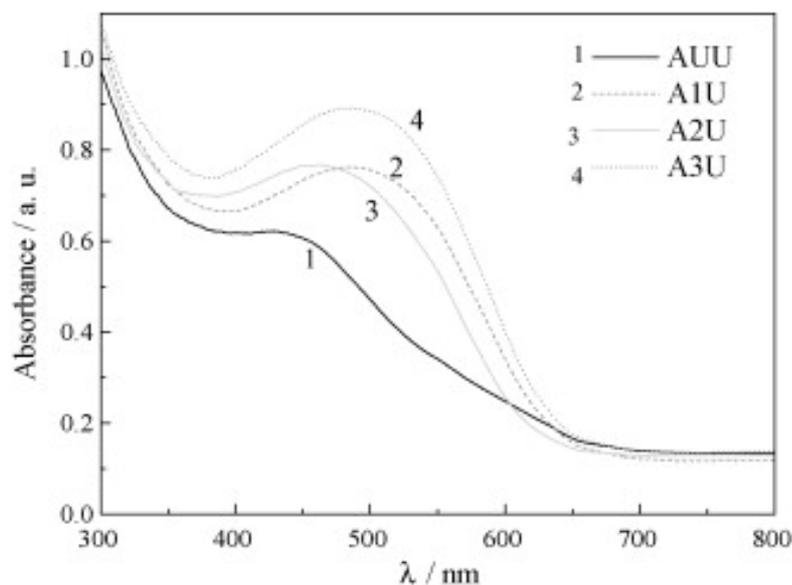


Fig. 2.11. Absorbance of wool dyed with (probably neutral form of) alizarin without mordant (1) and mordanted red with three different mordants (2,3,4) ©Elsevier<sup>121</sup>

Heller said “My seven microfibrils may not have held blood, but they surely were not coated with iron oxide”.<sup>122</sup> So, any madder lake present on these fibers probably was not formed by iron-oxide particles.

<sup>119</sup> cf. Heller, Report on..., 1983, 144-145; Heller and Adler, Blood on the Shroud..., 1980, TOM 30

<sup>120</sup> Red aluminum<sup>3+</sup>-alizarin and -purpurin complexes in methanol/water solution at pH 3.7 have absorbance maxima at 500 and 531 nm, respectively (Claro et al., The use of..., 2008, table 1, <http://www.sciencedirect.com/science/article/pii/S0039914007005309>), and the fluorescence excitation maximum of alizarin on alum mordanted wool is at 520-530 nm (fig. 3 in Clementi et al, A spectrometric..., 2007, <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr3.jpg> in <http://www.sciencedirect.com/science/article/pii/S0003267007009464>); cf. the absorbance spectra of pink madder lake in fig. 2.14 above: “Fig. 7. FORS spectra from a pink area from *Vienna Dioskurides* and comparison with standard paint: (a) pink area from f. 2v (blue line), standard madder (black line). of Aceto et al., 2012, that says “Pink – Madder lake” (Table 1) and “The presence of madder on both manuscripts was nonetheless suggested by FTOR analysis on pink areas in reason of the two minima around 515 and 540 nm (Fig. 7); the inflection point, located around 590 nm, is as well diagnostic [18].” (p. 241, [18] C. Bisulca, M. Picollo, M. Bacci, D. Kunzelman, in: Proceedings of ART2008 – 9th International Conference on Non-destructive investigations and Microanalysis for the Diagnostics and Conservation of Cultural and Environmental Heritage, Jerusalem, 2008) <http://www.sciencedirect.com/science/article/pii/S1386142512004568>

<sup>121</sup> Used from C. Clementi, W. Nowik, A. Romani, F. Cibir, G. Favaro, A spectrometric and chromatographic approach to the study of ageing of madder (*Rubia tinctorum* L.) dyestuff on wool, *Analytica Chimica Acta*, Vol. 596 Issue 1, July 2007, pp. 46-54, DOI: 10.1016/j.aca.2007.05.036, <http://www.sciencedirect.com/science/article/pii/S0003267007009464> (fig. 2 <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr2.jpg>) © 2007 Elsevier, with permission from Elsevier

<sup>122</sup> Heller, Report on..., 1983, p. 140

Some spots of red fluorescence were produced by some material on the sticky tape containing the fibers, after its treatment with vapour of hydrazine (very strong reductant and weak base<sup>123</sup>) and then formic acid (“strong reducing acid”).<sup>124</sup> The red fluorescence may have been that of madder lake. The fluorescence maximum of red madder lake is in the red, at ca. 620 nm<sup>125</sup> (fig. 2.26). On the other hand, if there was heme-madder lake, its two complexing components probably would have been dissociated by the pre-treatment. Hydrazine reduces the ligating iron to its ferrous state<sup>126</sup> and, as a weak base, could have dissolved the heme: (fresh) acid heme dimer aggregates dissolve in alkaline solutions (such as in the alkaline pyridine method and in NaOH solutions)<sup>127</sup>, and Heller and Adler reported that “A number of tests on the Spanish linen fibrils showed that 97% hydrazine (N<sub>2</sub>H<sub>4</sub>) served as the best reductant with the additional convenient property of solubilizing even strongly denatured or aged samples”<sup>128</sup>. Then the formic acid would have converted the free heme and alkaline madder to separate porphyrin and yellow acid madder, respectively.

<sup>123</sup> [http://en.wikipedia.org/wiki/Hydrazine#Reducing\\_agent](http://en.wikipedia.org/wiki/Hydrazine#Reducing_agent); “Hydrazine is a chemist’s sledgehammer – not what you need in a physiological milieu. ... The deployment of ferocious agents like hydrazine was frankly incomprehensible. One simply does not do that in biochemistry and physiology.” Berry,

<http://shroudofturinwithoutallthehype.wordpress.com/2013/11/11/heres-what-alan-d-adler-could-should-have-done-to-investigate-the-porphyrins-of-those-anomalous-shroud-bloodstains/>

“Adler had to resort to heroic measures simply to get the stuff into solution, i.e. using hydrazine” Berry,

<http://shroudofturinwithoutallthehype.wordpress.com/2012/07/20/no-dear-yannick-adler-and-heller-did-not-prove-it-was-real-blood-on-the-shroud-of-turin-even-if-some-real-blood-is-now-present/>; Hydrazine has pK<sub>b</sub> 5.9

<http://en.wikipedia.org/wiki/Hydrazine>

<sup>124</sup> Heller and Adler, *Blood on the Shroud*..., 1980, TOM 31 “We therefore peeled back the sticky tape from the glass slide and exposed the Shroud fibrils to, first, hydrazine vapor and then formic acid vapor. Irradiation with longwave UV then showed several red fluorescent spots indicative of the presence of a porphyrin species on the Shroud fibrils.”

<sup>125</sup> Claro et al., *The use of...*, 2008, Fig. 5 <http://www.sciencedirect.com/science/article/pii/S0039914007005309>; cf.

Clementi et al., *A spectrometric...*, 2007 fig. 3.b for alizarin lake <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr3.jpg> in <http://www.sciencedirect.com/science/article/pii/S0003267007009464>; “The fluorescence emission that we registered from the specimen of madder lake paint in egg tempera, has two peaks, one around 420 nm and the other around 620 nm (see. fig. 6b). The fluorescence emission of the corresponding specimen of genuine madder lake paint in linseed oil, has also two peaks, where the first peak is shifted of about 20 nm to higher wavelength range. We believe that the first peak be actually linked to the binder, (and in fact a peak in the 420-440 nm is common for almost all specimens), while the second one, around 620 nm, would be the one typical of natural *Madder*.”

Pelagotti et al., *A Study of UV Fluorescence Emission of Painting Materials*, 2005,

[http://www.ino.it/home/lella/pdf/Art2005\\_A%20study%20of%20UV%20fluorescence%20emission%20of%20painting%20ma%85.pdf](http://www.ino.it/home/lella/pdf/Art2005_A%20study%20of%20UV%20fluorescence%20emission%20of%20painting%20ma%85.pdf) p. 7-8

<sup>126</sup> “treating the heme material with a strong reductant to reduce the iron to its ferrous state and then treating it with a strong acid to displace the iron.” Heller and Adler, *A Chemical...*, 1981, TOM 31.

<sup>127</sup> cf. “the heme absorption at 418.5 nm of the reduced heme in alkaline pyridine” Srivastava et al., 2005,

[www.ncbi.nlm.nih.gov/pmc/articles/PMC1151790/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151790/); “The stock solution of 0.1 n NaOH used to dissolve heme is diluted 10-fold” Varfaj et al., 2012, [www.jbc.org/content/287/42/35181.full](http://www.jbc.org/content/287/42/35181.full); cf. fig. 1 of Ursoset al., 2001,

<http://www.sciencedirect.com/science/article/pii/S016668510000342X> and fig. 5

<http://aac.asm.org/content/57/1/365/F5.expansion.html> of Gorka et al., 2013, <http://aac.asm.org/content/57/1/365.long>

<sup>128</sup> Heller and Adler, *A Chemical...*, 1981, TOM 31; Note that the team of Frache (G. Frache, E. Mari Rizzati, E. Mari, *Relazione conclusiva sulle indagini d'ordine ematologico praticate su materiale prelevato dalla Sindone*, in *La S. Sindone. Ricerche e studi della Commissione di Esperti nominata dall'Arcivescovo di Torino*, Card. Michele Pellegrino, nel 1969, suppl. *Rivista diocesana Torinese* (1976)

[http://it.cathopedia.org/wiki/Studi\\_scientifici\\_sulla\\_Sindone#cite\\_note-19](http://it.cathopedia.org/wiki/Studi_scientifici_sulla_Sindone#cite_note-19)), which had examined a bloodstained thread taken from the Shroud in 1973, reported in 1976 that “the pigmented encrustations did not pass into solution in the solvents, acids and the alkalis we used.” (English quote in McCrone, *Judgement Day...*, 1999, p. 11). According to McCrone they used “sulphuric acid” before looking for porphyrin fluorescence, “sodium carbonate solution” before looking for a hemochromagen spectrum, and – McCrone quoting a translation of the report – “extractive incubation in alkaline solution” before performing thin-layer chromatography with “a solvent system made up of a mixture of methanol, acetic acid and water (proportions 90:3:7)”, all giving negative results (no fluorescence, hemochromagen color, or fluorescence/benzidine color/hydrogen peroxide color, respectively) (McCrone, *Judgement day...*, 1999, p. 10). This apparent inability to dissolve the red encrustations might mean that had tried to dissolve heme-madder lake. The sulphuric acid probably would have dissociated the madder from the heme (cf. Kiel et al, 1963, *Metal Complexes of Alizarin I—The Structure of the Calcium–Aluminium Lake of Alizarin*, wrote “In water of pH less than 4, it decomposes with precipitation of alizarin”, <http://onlinelibrary.wiley.com/doi/10.1111/j.1478-4408.1963.tb02507.x/abstract>), but then the heme would have formed insoluble aggregates (see 2.1.1).

If this dissociation took place, the retrieved acid yellow madder would have fluoresced green-yellow<sup>129</sup>, just as the clear parts of the fiber already were doing, and the observed spots of red fluorescence would not have been due to madder lake, and the original red material may indeed have contained a porphyrin species (heme) that had been made red-fluorescent by the reducing base-acid treatment, removing the iron from the heme.

The combination of the data of the transmission spectrum and the induced fluorescence color suggests that there was both madder dye (and heme) and a heme-madder complex that behaved as madder lake in reflectance and transmission. Because of the lack of a definitive sign of heme in the fiber's transmission spectrum, the fiber probably was just a madder-dyed fibril, partly stained by a reddish brown heme-madder complex with just a very small amount of heme left on top of it. Most of the heme had probably abraded from the madder-heme interface layer consisting of madder-heme complexes. If this was indeed the composition of the stains, there would have to have been madder dye on the fiber surface where and when the heme got onto the fiber.

#### No image under the blood?

“No dye was found on any image fibers”,<sup>130</sup> so the image formation process probably transformed any madder it encountered. This will be discussed further in 2.1.3. For madder dye to have contributed to the 450 nm and 530 nm transmission/absorbance of the “brownish red stained fibril”, the fiber would have to have been a non-image fiber or else an image fiber that had no image where and when the heme touched it. Also after the stain had formed, there would still have to have been madder dye under and/or next to the red material. As the heme may not have covered the whole circumference of this particular fibril, the yellow madder dye may simply have been on the opposite, clear side of the red stained part and contributing to the total measured transmittance from there. The yellow madder dye may also only have been present between the fiber and the madder-heme complex, as the madder dye that was just beneath the madder dye surface, when the heme got onto the cloth.

Heller and Adler found that “the red and blue silk fibers appear to be from backing cloths and borders and are present in almost every sample. This illustrates that there has been transposition of materials from one area of the cloth to another, as it was folded and unfolded numerous times through the centuries (as first noted by John Jackson)”<sup>131</sup>, and this may have been the reason why Heller and Adler called the investigated fiber “a brownish red stained fibril from one of the blood areas” in the caption of their absorbance plot<sup>132</sup> even though Heller found it on a slide labelled “Nonimage”.<sup>133</sup> Yet, as the configuration of any actual image parts on the fiber is not known, no conclusions can be drawn yet about the presence or lack of image under the red stained parts.

#### **2.1.2.2. Reflectance curve of bloodstain areas - madder lake and dye**

In the relative reflectance spectrum of a bloodstain, the madder dye reflectance of the background would have disappeared entirely because of the arithmetic division of the raw bloodstain data by the background data in order to find their relative spectrum. When the relative reflectance of the bloodstains is multiplied by the ‘absolute’ clear background reflectance, the ‘absolute’ bloodstain reflectance is regained (‘absolute’ here meaning normalized against “the response from the magnesium oxide reference surface”<sup>134</sup>): their curves are plotted in fig. 2.12., and these ‘absolute’ bloodstain values can then be used to make a different comparison to the ‘absolute’ background values.

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<sup>129</sup> Miliani et al., *Acidichromic effects...*, 2000; see also 4.2.3 and 4.2.4 below

<sup>130</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, p. 18

<sup>131</sup> Heller and Adler, *A Chemical...*, 1981, TOM 37

<sup>132</sup> Heller and Adler, *Blood on the Shroud...*, 1980, TOM 31 fig. 2

<sup>133</sup> Heller, *Report on...*, 1983, p. 125

<sup>134</sup> Gilbert and Gilbert, *Ultraviolet-visible reflectance...*, 1980, p. 1932-33

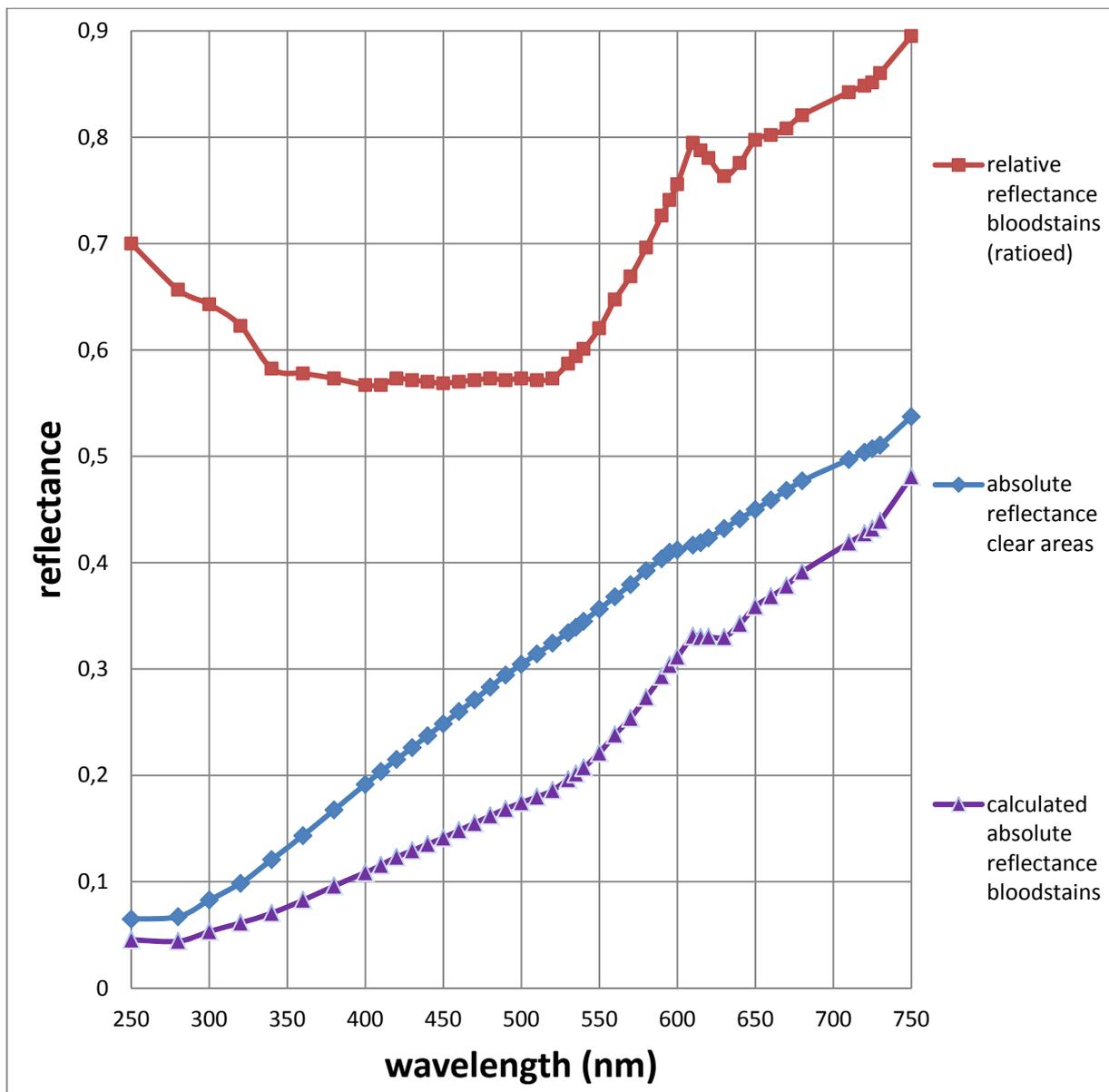


Fig. 2.12. Measured relative reflectance of bloodstains and absolute reflectance of clear area and calculated absolute reflectance of bloodstains ©AvdH

In comparing colors of pulp and paper before and after ageing or bleaching, “There are thus many ways that one might report the yellowness of paper. None is the “best” or “correct” way.”: “the reporting of differences in optical density [ $\log(I_0/I)$ ] or in K/S curves can indicate the character of the colored materials removed or generated in the treatment of pulp and paper.”; “Here, the Kubelka-Munk K/S value is  $(1-R)^2/2R$  and R is the reflectance of an “infinitely thick” stack of papers (...). The reflectance of a stack of six papers is customarily used for this purpose. The difference before (zero) and after accelerated aging (time, t) multiplied by 100, i.e.  $100[(K/S)_t - (K/S)_0]$ , has been designated the “post-color number””; “The latter authors used the designation PRO for the “percent relative post-color number”, or  $100[(K/S)_t - (K/S)_0] / (K/S)_0$  in carrying out their subtractions.”<sup>135</sup> See fig. 2.13 for the calculated PRO plot of the Shroud’s bloodstains when compared to the clear areas by means of their respective ‘absolute’ reflectance values.

<sup>135</sup> <http://cool.conservation-us.org/coolaic/sg/bpg/annual/v06/bp06-04.html>

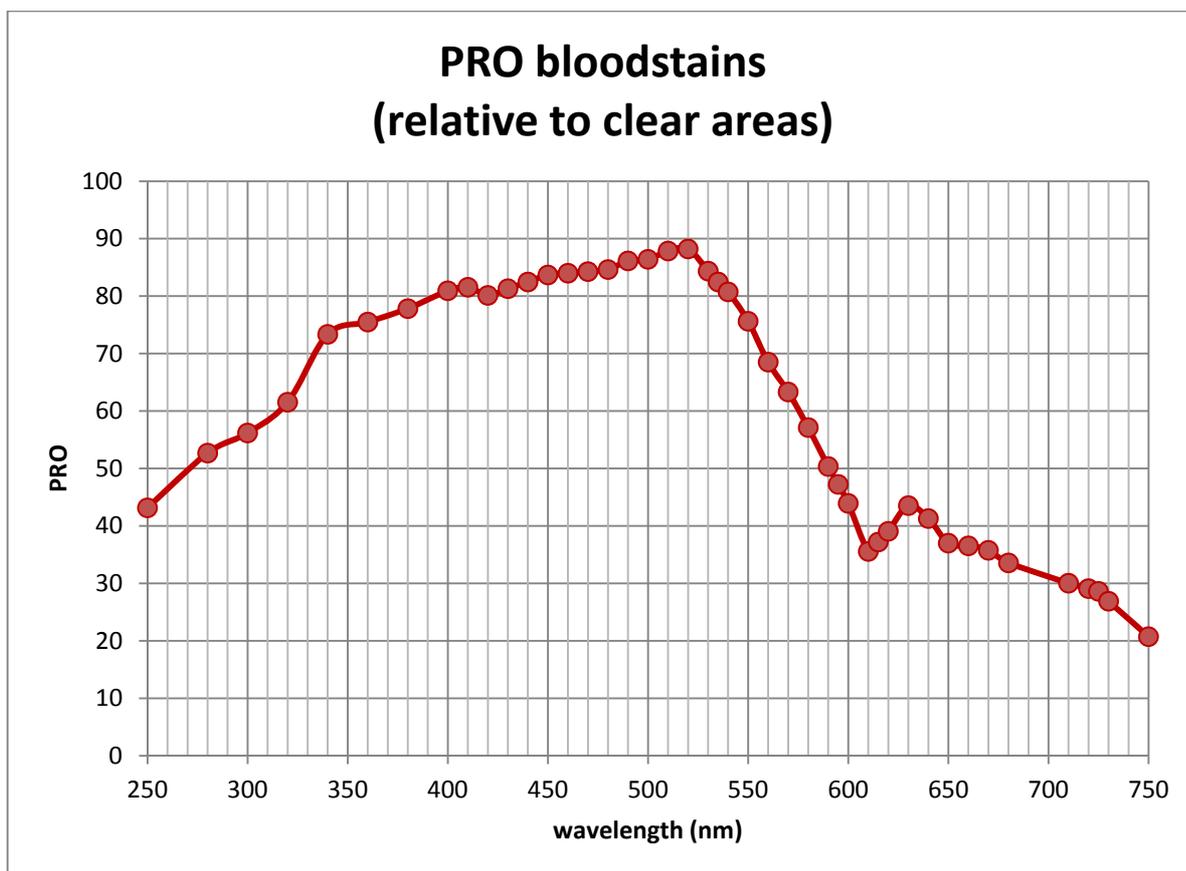


Fig. 2.13. Calculated PRO value, based on *k/s* values, representing optical difference of mean of bloodstains with background ©AvdH

Here, where the linen and madder dye of the background do not interfere, the ca. 525 nm band is dominantly present. As (red) aluminum<sup>3+</sup>-alizarin and -purpurin complexes in methanol/water solution at pH 3.7 have absorbance maxima at 500 and 531 nm, respectively,<sup>136</sup> and mordanted alizarin on wool has an absorbance peak at ca. 525 nm (fig. 2.11), and the absorbance peak of pink madder lake on a 6<sup>th</sup> century AD Byzantine manuscript is located at ca. 525 nm and its other peak is at ca. 280 nm (fig. 2.14),<sup>137</sup> and the K/S absorption spectra of various red Al(OH)<sub>3</sub>-madder lakes have a peak at 507 nm, with a shoulder at 540 nm, and a band at ca. 280 nm (fig. 2.16),<sup>138</sup> the bloodstains' 525 nm band and smaller 280 nm band may very well result from red madder lake: heme-madder lake formed by acid heme dimers – these dimers probably produce the 340-410 (Soret)

<sup>136</sup> Claro et al., The use of..., 2008, table 1, <http://www.sciencedirect.com/science/article/pii/S0039914007005309>

<sup>137</sup> Note that the madder of fig. 2.14 (fig. 7 of the article of Aceto et al., First analytical..., 2012

<http://www.sciencedirect.com/science/article/pii/S1386142512004568>) is madder lake, as “The presence of madder on both manuscripts was nonetheless suggested by FORS analysis on pink areas in reason of the two minima around 515 and 540 nm (Fig. 7); the inflexion point, located around 590 nm, is as well diagnostic [18]” and as a) the spectrum of the pink area is compared to the spectrum of a standard madder paint, and b) The “Pink” hue in the Vienna Dioskurides was identified as “Madder lake” by FORS (table 1), and the violet of the manuscript is described and identified by FORS as a mixture of indigo and madder lake: “Violet - Indigo/madder lake” (table 1), and c) the spectrum of a violet area is compared to the spectra of the pink area and standard indigo paint in “Fig. 8. FORS spectra from a violet area from *Vienna Dioskurides* and comparison with standard paint: pink area from f. 2v (blue line), standard indigo (black line), violet area from f. 147v (red line).”

<http://www.sciencedirect.com/science/article/pii/S1386142512004568>; these peaks (525 and 280 nm) are also present in the absorbance of madder (85% purpurin) in an acetonitrile solution (Miliani et al., A spectrophotometric..., 1998, fig. 1a, <http://ars.els-cdn.com/content/image/1-s2.0-S1386142597002400-gr1.gif> of <http://www.sciencedirect.com/science/article/pii/S1386142597002400>), so the 280 nm peak is probably not completely the result of the substrate.

<sup>138</sup> Clementi et al, Vibrational and electronic properties of painting lakes, *Applied Physics A*, July 2008, Vol. 92 Issue 1, pp. 25-33, fig. 4, <http://link.springer.com/article/10.1007%2Fs00339-008-4474-6>

band, a 500 nm band(Q β band, linked to and lifted by the 525 nm lake band), the 630 nm band (charge transfer) band, and perhaps the small 725 nm band as well.

If the Shroud's 500 nm heme band is indeed greatly lifted by a 525 nm madder lake band – even to the level of the heme's Soret band –, the original heme without madder lake could have had a Soret band that was considerably higher than its 500 nm band. This means that the heme dimer needn't have had an extremely small Soret extinction coefficient (less than 2,5-3, cf. fig. 2.9) to lose the contrast of its Soret band in relative reflectance, but it may have been near the value, which Villiers et al. measured for the aqueous heme dimers without (higher) aggregation at pH 6.0, viz.  $3.6 \cdot 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  (cf. fig. 2.6).

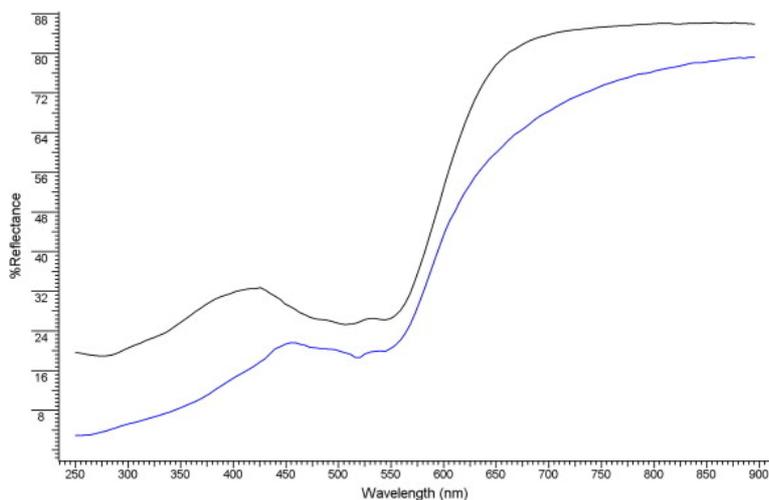


Fig. 2.14. Reflectance spectra “from a pink area from Vienna Dioskurides and comparison with standard paint: (a) pink area from f. 2v (blue line), standard madder (black line).” (black line = upper line; the Vienna Dioskurides f. 2v is shown in fig. 2.15 below) ©Elsevier<sup>139</sup>

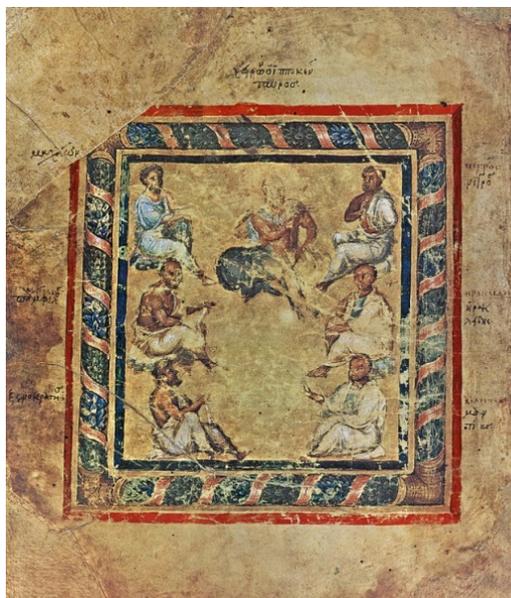


Fig. 2.15. Folio 2v of the 6th century AD manuscript Vienna Dioskurides, Group of physicians with Chiron, f. 2v, pink (not violet): madder lake (public domain)<sup>140</sup>

<sup>139</sup> Reprinted from *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, Vol 95, September 2012, M. Aceto, A. Agostino, G. Fenoglio, P. Baraldi, P. Zannini, C. Hofmann, E. Gamillscheg, First analytical evidences of precious colourants on Mediterranean illuminated manuscripts, Pages 235–245, Copyright 2012, with permission from Elsevier. (Fig. 7 of <http://dx.doi.org/10.1016/j.saa.2012.04.103> = <http://www.sciencedirect.com/science/article/pii/S1386142512004568>)

<sup>140</sup> [http://commons.wikimedia.org/wiki/File:Cheirongruppe\\_\(Wiener\\_Dioskurides\).jpg](http://commons.wikimedia.org/wiki/File:Cheirongruppe_(Wiener_Dioskurides).jpg)

The remaining question is whether or not the near flatness of the 340-525 nm plateau can be attributed to the mere overlap of the heme dimer's Soret band and its lifted 500 nm band. It is also possible that another distinct band is present at 450 nm, which fills the gap between the two heme bands. It is possible that this 450 nm band is simply a band of yellow madder dye that is more dominantly present under the heme-madder lake film than on ordinary background fibers that were never covered with heme. Perhaps the lake and the original heme and methemoglobin deposits on bloodstained fibers have for a long time somewhat prevented the photo- and oxygen-degradation of the yellow madder dye beneath the deposits and the lake. Madder dye degradation heavily depends on the presence of oxygen: "The use of an inert environment noticeably reduces the sample bleaching preserving the dye from photodegradation. ... In an inert atmosphere, the light induced fading of purpurin on wool was not completely inhibited, but the photodegradation of the dye and, at a lesser extent, the deterioration of the fibre were noticeably reduced. ... Oxygen has a relevant effect on ageing, accelerating the degradation."<sup>141</sup> Even anaerobic heat does not greatly affect madder dye, as is observed in the intact madder coating found in the lightly scorched Raes corner (where dirt deposits may also have prevented sublimation of the dye).<sup>142</sup> On the other hand, the formation of a madder lake film from madder dye would have reduced the 450 nm band of the madder dye a little. As the 3 x 6 mm datapoints for the relative reflectance measurements of the bloodstains are all in image areas (only in the B3C small of back datapoint there is no or hardly any image), also the presence of at least some not-bloodstained image fibers – that don't have any madder dye - in these datapoints would reduce the madder dye content (450 nm absorbance) of the datapoints relative to the (imageless and madder containing) background areas. Yet, the reflectance of the bloodstain datapoints seems to have a larger madder dye content than the background (less 450 nm reflectance = more 450 nm absorbance, as for calculating the relative bloodstain reflectance, the bloodstain reflectance was ratioed to the background reflectance (fig. 2.3)). This means that the madder dye content of the bloodstained fibers in the datapoints must more than compensate the loss of madder dye on the image fibers in these points, and thus must be higher than the madder dye content of the background fibers. (The presence of madder dye in the background will be discussed in chapter 4.) This means that the original blood deposits protected the madder dye beneath it from image formation and ageing degradation and sublimation. This remaining layer of madder dye under a thin madder lake surface (with hardly any remaining heme left now) would also explain the dominant peak at 450 nm in the microspot-transmission of the red-stained fibril. If the 450 nm absorbance in the bloodstain spectrum is (also) due to preserved madder dye, the entire bloodstain spectrum could be explained as in the following figure (fig. 2.17):

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<sup>141</sup> Clementi et al., A spectrometric..., 2007

<sup>142</sup> Most madder on non-image fibers may have degraded or sublimated (= evaporated from the solid phase) for instance in the heat of the 1532 AD fire. The sublimation temperature of alizarin is only 368-498 K = 95-225 degrees C (NIST alizarin <http://webbook.nist.gov/cgi/cbook.cgi?ID=C72480&Units=SI&Mask=4#Thermo-Phase>). Yet, a nearly invisible madder layer still can have very noticeable fluorescence: compare, for instance, the statues in normal light and in UV light on [http://cameo.mfa.org/wiki/Rose\\_madder](http://cameo.mfa.org/wiki/Rose_madder). Heller and Adler reported on "the fibrils and particles found on the Shroud samples" that "There are (a) the clear to pale yellow background fibrils (non-image) of the cloth which bear no image or blood stains." (Heller and Adler, A Chemical..., 1981, TOM 36). So, some non-image fibers from the surface of the main Shroud looked clear – not yellow.

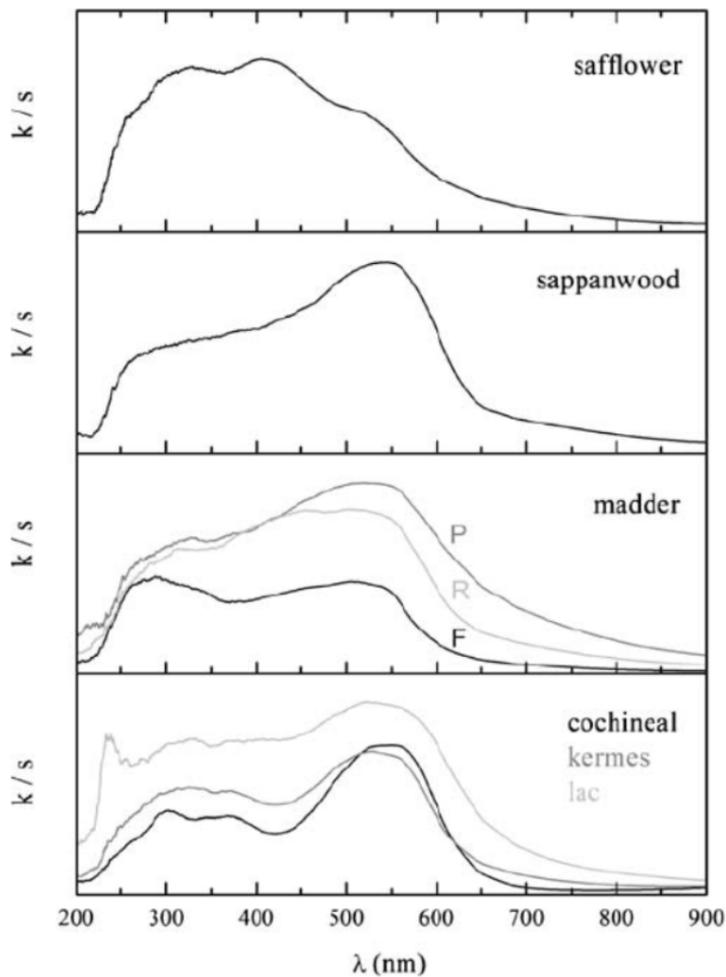


Fig. 2.16 Absorbance spectra of red lakes in Kubelka-Munk ( $k/s$ ) value; madder P = prepared from plant root extract, R and F = prepared from extract of dyed wool samples ©Springer Science+Business Media<sup>143</sup>

<sup>143</sup> With kind permission from Springer Science+Business Media: *Applied Physics A: Materials Science & Processing*, Vibrational and electronic properties of painting lakes, Vol. 92, 2008, pp. 25-33, C.Clementi, B. Doherty, P.L. Gentili, C. Miliani, A. Romani, B.G. Brunetti, A. Sgamellotti, figure 4. doi: 10.1007/s00339-008-4474-6, <http://link.springer.com/article/10.1007%2Fs00339-008-4474-6>

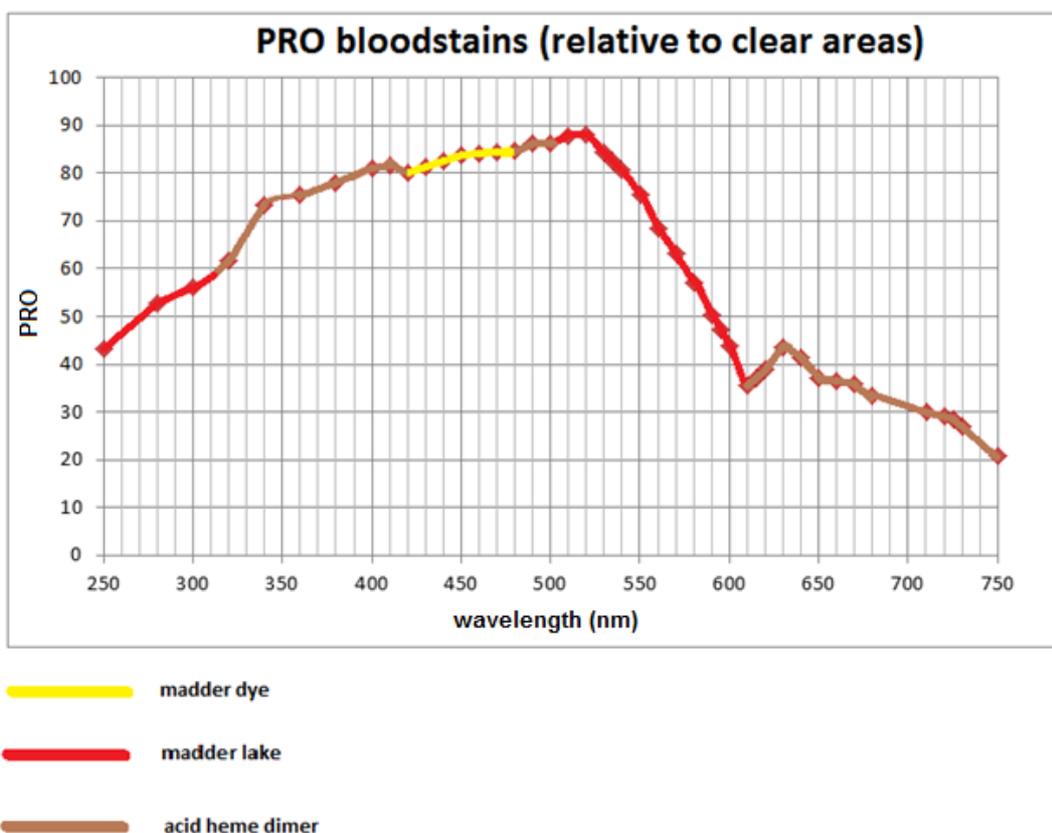
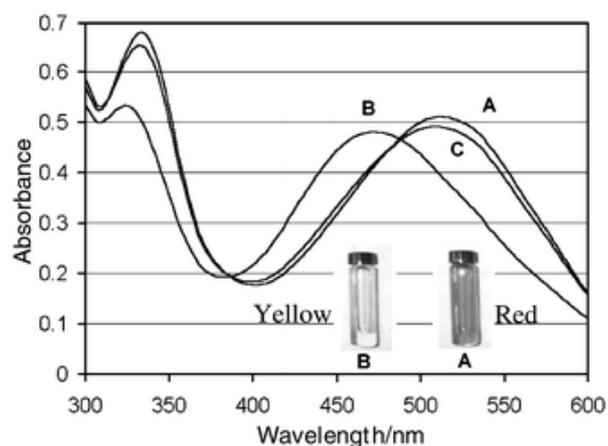


Fig. 2.17. Possible composition of the PRO spectrum (based on  $k/s$  values) of Shroud bloodstains ©AvdH

Just as an illustration, see the visible color change, which accompanies the absorbance peak shift induced by the binding of boronic acid to alizarin red S<sup>144</sup>, a synthetic, sulphonated form of alizarin<sup>145</sup> in fig. 2.18.



Reproduced by permission of The Royal Society of Chemistry

Fig. 2.18. An example of how a change in absorbance spectrum (from B to A/C, in this case of forms of the synthetic dye 'Alizarin Red S.') represents a change in color from yellow to red ©The Royal Society of Chemistry<sup>146</sup>

<sup>144</sup> <http://www.rsc.org/ej/CC/2001/b104895n/b104895n-f2.gif> in Springsteen et al., 2001, Alizarin Red S. as a general optical reporter for studying the binding of boronic acids with carbohydrates, *Chemical Communications*, Issue 17, "Abstract Alizarin Red S. displays a dramatic change in fluorescence intensity and color in response to the binding of a boronic acid and can be used as a general reporter for studying carbohydrate–boronic acid interactions, both quantitatively and qualitatively.", <http://pubs.rsc.org/en/content/articlelanding/2001/cc/b104895n#divAbstract>

<sup>145</sup> <http://stainsfile.info/StainsFile/dyes/58005.htm>

## Image-independent visible color = no image under bloodstains

This interpretation of the bloodstain absorbance spectrum as a combination of heme and madder lake and madder dye spectra has important implications in combination with the observation that the visible color of the bloodstains in image areas is not obviously browner than in the non-image or less-image areas. One would expect a browner color in more dense image areas, where nothing but unligated heme would be present on bloodstained fibers where no heme-madder lake could have formed, i.e., on the madderless image fibers. Yet, the areal density of the image that would have been present in the bloodstain, has no noticeable effect on the color of the bloodstain (for instance, the blood in the dense image of the forehead looks just as red/pink as the blood in the imageless small of the back area (fig. 2.19). Also the four relative reflectance spectra of individual bloodstains – Foot (B1A), Forehead - “3” mark (F8C), Wrist (F3E), “Lance wound” (F6B) – have about the same overall shape and therefore represent about the same color, and only have different overall intensities (fig. 2.20)

In the heme-madder lake hypothesis, all reddish bloodstained fiber parts must have a madder lake coating, and therefore must have had a madder dye coating before the blood got onto it; in other words, image formation did not remove the madder dye before the blood got onto it, so, there was and is no image under the reddish bloodstained fiber parts. If blood got onto an image area after image formation, the madder-coated non-image fibers in the image would have colored red, but the madderless image fibers in the area would have colored brown or black, determining the brownishness of the overall color of the bloodstain, proportional to the percentage of image fibers – the local image density – in the area. Nothing of such an image-density-dependent brown is seen in bloodstains. This means that all fibers that got blood on them probably were non-image fibers: the blood was present before the image was formed.

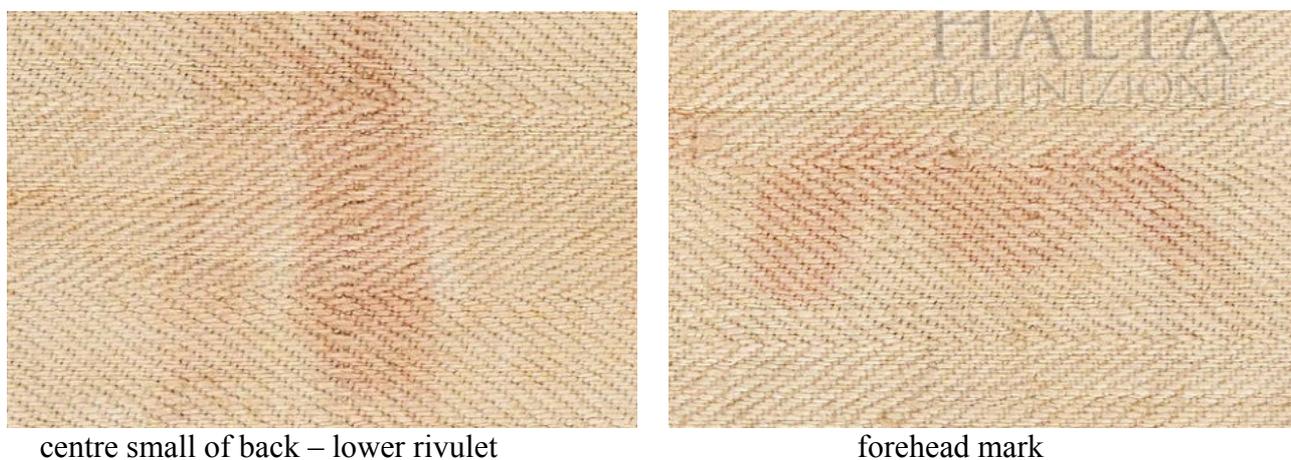


Fig. 2.19. Comparison of bloodstains in non-image (small of back) and image (forehead) areas. Photos by HALTADEFINIZIONE® IMAGE BANK - Copyright Arcidiocesi di Torino<sup>147</sup>

<sup>146</sup> Used from G. Springsteen and B. Wang, Alizarin Red S. as a general optical reporter for studying the binding of boronic acids with carbohydrates, *Chemical Communications*, Issue 17, 2001, pp. 1608-1609, <http://dx.doi.org/10.1039/B104895N> © Royal Society of Chemistry

<sup>147</sup> Used, with permission, from the Shroud 2.0 application made by Haltadefinizione, downloadable from <http://www.haltadefinizione.com/en/the-shroud>. Note that for the Haltadefinizione photography “The lighting system was especially designed to filter any harmful radiation to the cloth, but most of all special attention was given to the calibration of the spectrum, which helped faithfully reproduce the actual color and fabric of the Shroud image.” <http://www.haltadefinizione.com/en/the-shroud>

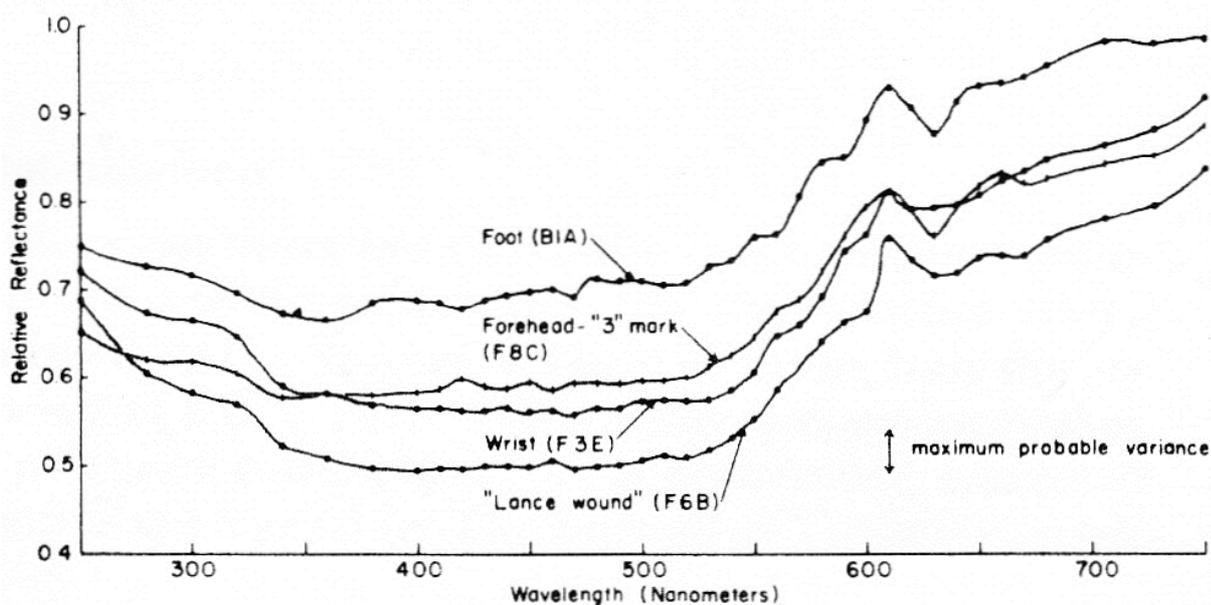


Fig. 2.20. Relative reflectance spectra of four bloodstains ©OSA <sup>148</sup>

### 2.1.2.3. FTIR spectra of blood globs match FTIR spectra of madder lake (plus heme)

The FTIR spectra (= Fourier Transform Infra-Red spectra) of Shroud “blood globs”<sup>149</sup> are qualitatively a very good match with the FTIR spectra of (reddish) madder lakes (see fig. 2.21 and 2.22). The madder FTIR spectrum of the IRUG database,<sup>150</sup> shown here, in blue at the top and in red at the bottom, is called “Natural Red 8”, which is the description of a madder lake.<sup>151</sup> The plot in the middle here, is fig. S8<sup>152</sup> from the supplementary material to a scientific article of Brosseau et al., in which the curve labelled a) is explicitly denoted as “Madder lake”.<sup>153</sup> The centre curve of the Shroud FTIR spectra shows not only the same shapes and positions of the peaks as in the madder lake plots, but also approximately the same relative heights of the three low wavenumber peaks (right part of plot) as in curve “a) Madder lake” of Brosseau’s plot. All blood glob FTIR spectra show the very shallow shoulder at ca. 1700 cm<sup>-1</sup> in the 1600 cm<sup>-1</sup> peak, which is clearly visible in the ‘rocket-shape’ of this peak in the IRUG’s FTIR spectrum of madder, but also as a very low peak/shoulder at ca. 1700 cm<sup>-1</sup> in Brosseau’s FTIR spectra.

<sup>148</sup> Used from R. Gilbert Jr. and M.M. Gilbert, Ultraviolet-visible reflectance and fluorescence spectra of the Shroud of Turin, *Applied Optics*, Vol. 19 Issue 12 (1980), pp. 1930-1936, <http://dx.doi.org/10.1364/AO.19.001930>, fig. 16, with permission from the Optical Society of America.

<sup>149</sup> Alder, Selzer and DeBlase, *Further Spectroscopic...*, 2002, TOM 101, fig. 9, <http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMlcq90QWto4GAAw&ved=0CDEQ6AEwAA#v=snippet&q=clot%20controls&f=false>

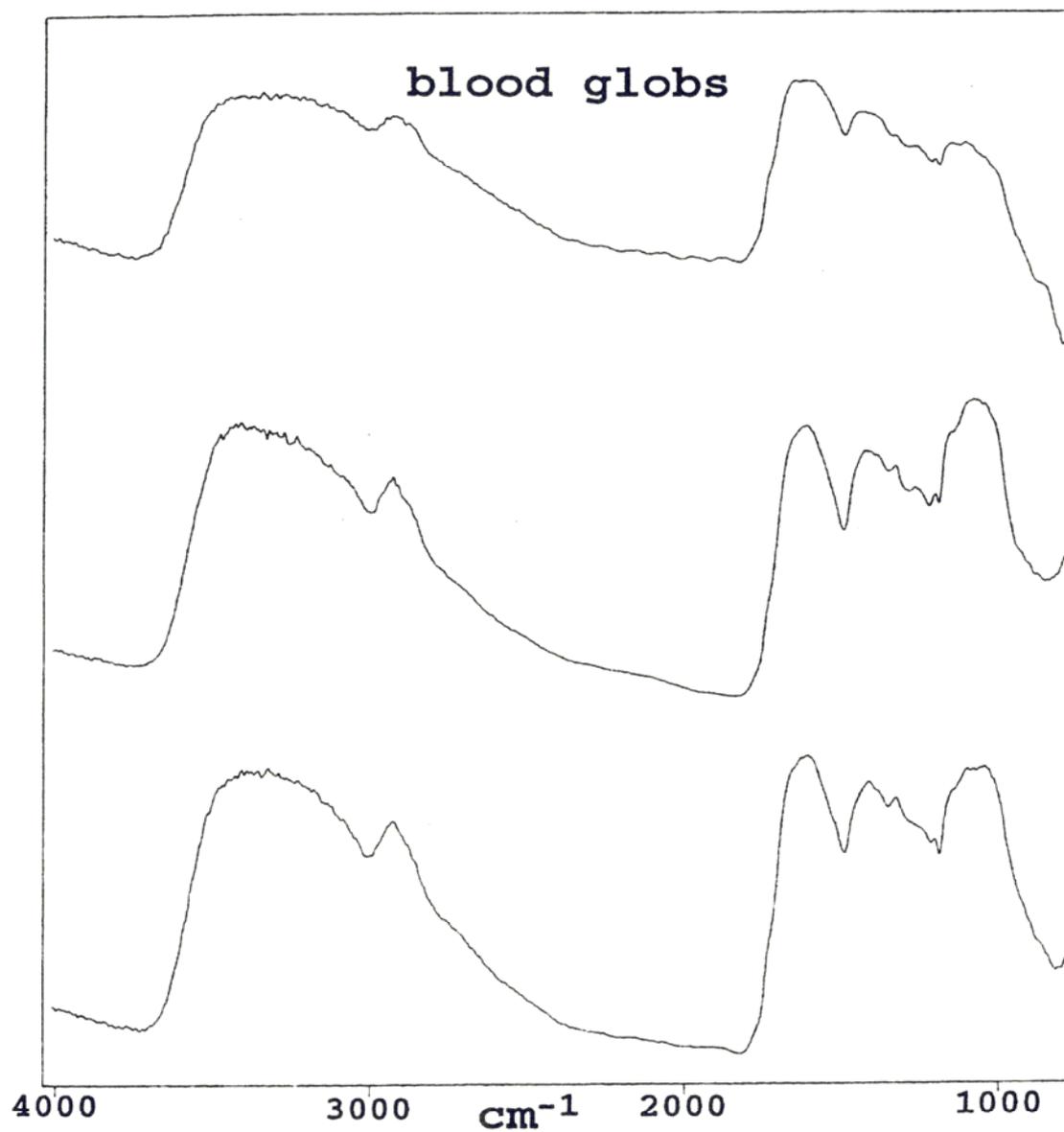
<sup>150</sup> <http://www.irug.org/ed2k/spectra.asp?file=IOD00201.DX> (accessed mid-September 2013), since the reconstruction of the IRUG database site in Oct 2013, it is interactively online at <http://www.irug.org/jcamp-details?id=176>;

<sup>151</sup> <http://www.artiscreation.com/red.html#NR8> on <http://www.artiscreation.com/red.html#.U9ZZEmZhVct>; cf. Kremer pigments “Madder Lake Coral - Chemical description: Natural Red 8” <http://www.kremer-pigmente.com/en/product.html?info=1824>

<sup>152</sup> Brosseau et al., 2011, Supplementary material [http://onlinelibrary.wiley.com/store/10.1002/jrs.2877/asset/supinfo/jrs\\_2877\\_FigureS8VanduyneSERS.tif?v=1&s=a43115ef595a88b27ecd4f780c91149cd1b1a3ed](http://onlinelibrary.wiley.com/store/10.1002/jrs.2877/asset/supinfo/jrs_2877_FigureS8VanduyneSERS.tif?v=1&s=a43115ef595a88b27ecd4f780c91149cd1b1a3ed) of [http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011\\_Brosseau.pdf](http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011_Brosseau.pdf)

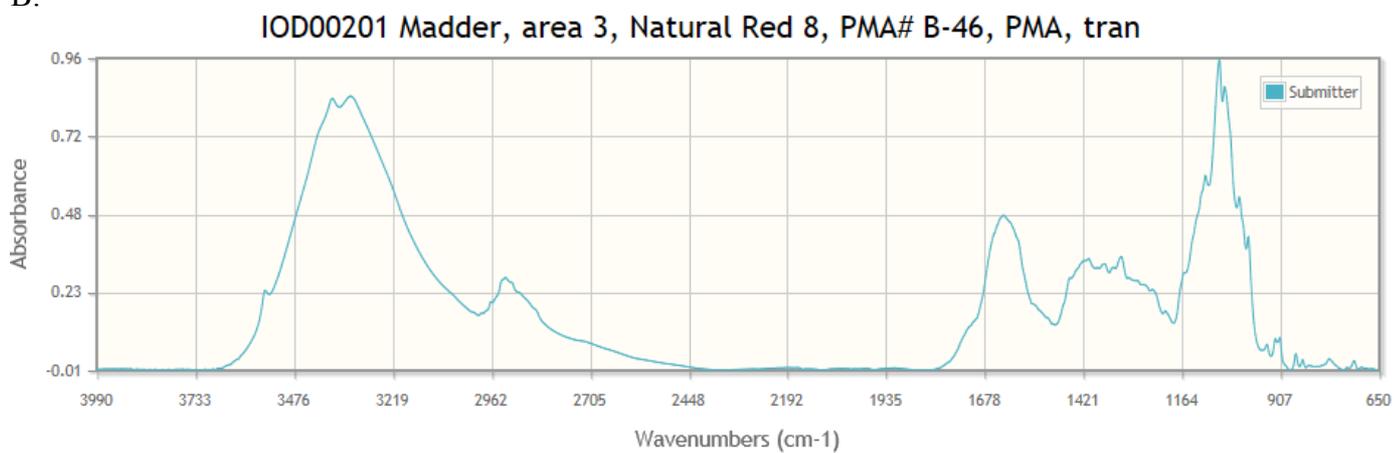
<sup>153</sup> Brosseau et al., Revealing the invisible: using surface-enhanced Raman spectroscopy to identify minute remnants of color in Winslow Homer’s colorless skies, *Journal of Raman Spectroscopy* 2011, 42, 1305–1310, DOI 10.1002/jrs.2877 at <http://onlinelibrary.wiley.com/doi/10.1002/jrs.2877/supinfo>, full text free online at [http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011\\_Brosseau.pdf](http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011_Brosseau.pdf), and Supplementary material also free online at [http://onlinelibrary.wiley.com/store/10.1002/jrs.2877/asset/supinfo/jrs\\_2877\\_supplementarymaterial.doc?v=1&s=5db71ae1821f4c2f99876bcb11f8d9bcc232c35e](http://onlinelibrary.wiley.com/store/10.1002/jrs.2877/asset/supinfo/jrs_2877_supplementarymaterial.doc?v=1&s=5db71ae1821f4c2f99876bcb11f8d9bcc232c35e)

A:

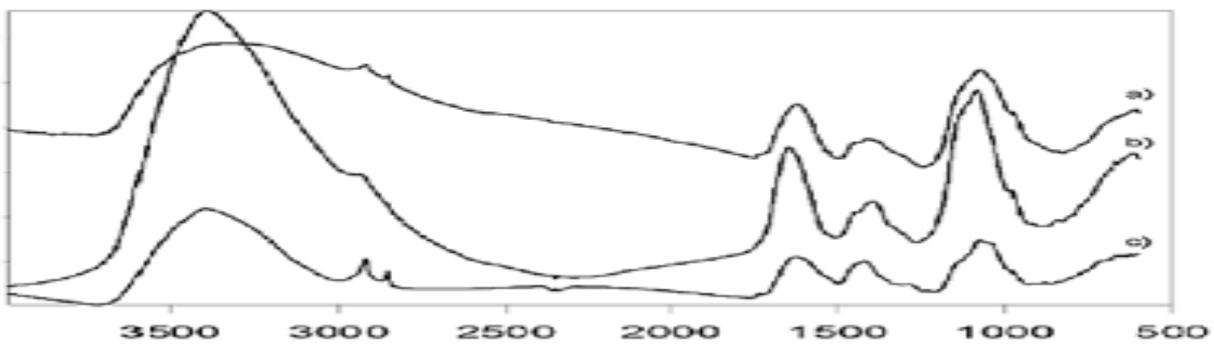
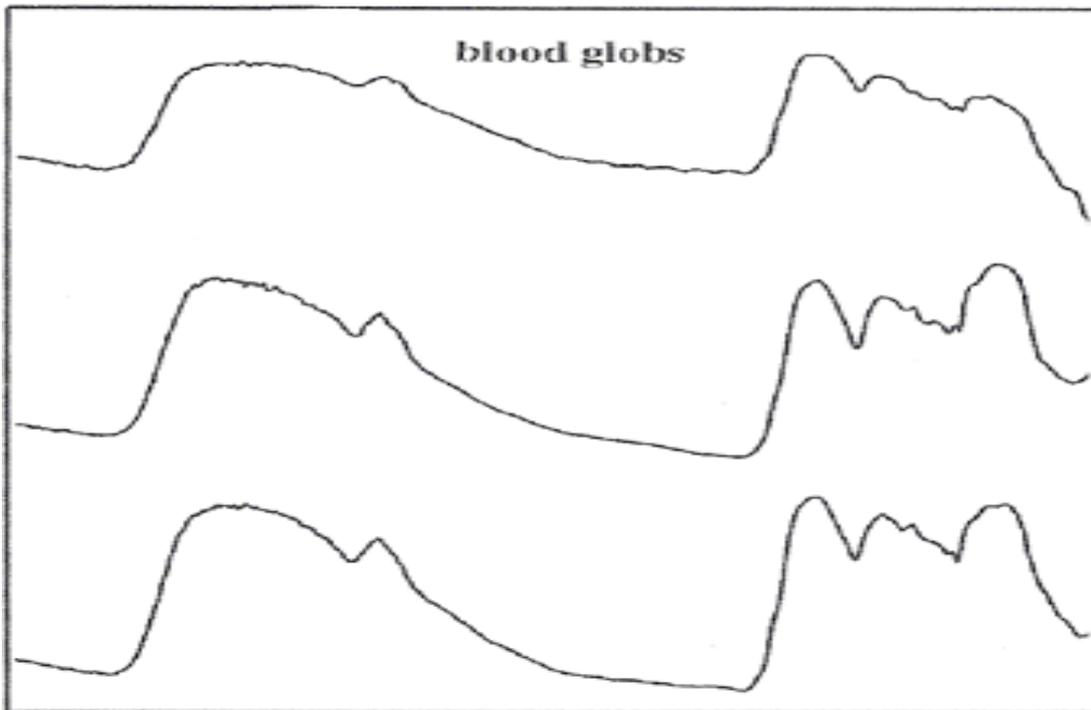


*Fig. 9 Typical FTIR absorbance patterns of blood globs*

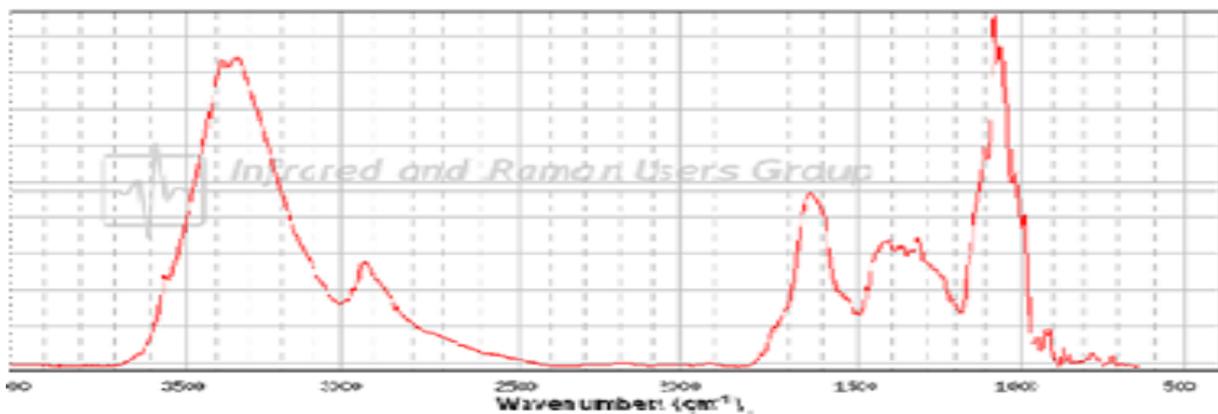
B:



C:



a) Madder lake b) Pink madder c) Purple madder (Brosseau, 2011, Fig. S8)



IOD00201 Madder, area 3 (IRUG)

Fig. 2.21. FTIR spectra of Shroud blood globs, and madder lakes (adapted horizontally for scale alignment).

A: FTIR spectrum of Shroud blood globs ©A.D. Adler, R. Selzer, and F. DeBlase<sup>154</sup>

B: FTIR spectrum of madder lake, online interactive version ©2014 IRUG<sup>155</sup>

C: Top: FTIRs of Shroud blood globs (adapted for alignment) ©A.D. Adler, R. Selzer, and F. DeBlase<sup>156</sup>. Centre: FTIRs of a) madder lake b) pink madder c) purple madder (adapted for alignment) ©John Wiley and Sons, Ltd.<sup>157</sup>. Bottom: same FTIR spectrum of madder lake as in B (older version of plot and adapted for alignment) ©IRUG<sup>158</sup>

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<sup>154</sup> Used from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, *The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium*, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-181, © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer

<sup>155</sup> Used from Philadelphia Museum of Art. "IOD00201 Madder, area 3, Natural Red 8,PMA# B-46, tran". Infrared and Raman Users Group Spectral Database. Ed. Beth Price and Boris Pretzel. <http://www.irug.org/jcamp-details?id=176> ©IRUG, with permission from IRUG. Natural Red 8 is a madder lake: see <http://www.artiscreation.com/red.html#NR8> and Kremer pigments "Madder Lake Coral - Chemical description: Natural Red 8" <http://www.kremer-pigmente.com/en/product.html?info=1824>

<sup>156</sup> Adapted from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, *The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium*, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-181, © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer

<sup>157</sup> Adapted from Christa L. Brosseau, Francesca Casadio, Richard P. Van Duyne, Revealing the invisible: using surface-enhanced Raman spectroscopy to identify minute remnants of color in Winslow Homer's colorless skies, *Journal of Raman Spectroscopy*, 11 Feb 2011, 42, 1305–1310, <http://dx.doi.org/10.1002/jrs.2877> full text: [http://sites.northwestern.edu/vanduyne/files/2012/10/2011\\_Brosseau.pdf](http://sites.northwestern.edu/vanduyne/files/2012/10/2011_Brosseau.pdf) and supplement: [http://onlinelibrary.wiley.com/store/10.1002/jrs.2877/asset/supinfo/jrs\\_2877\\_supplementarymaterial.doc?v=1&s=5db71ae1821f4c2f99876bcb11f8d9bcc232c35e](http://onlinelibrary.wiley.com/store/10.1002/jrs.2877/asset/supinfo/jrs_2877_supplementarymaterial.doc?v=1&s=5db71ae1821f4c2f99876bcb11f8d9bcc232c35e) fig. S8, with permission from John Wiley and Sons, Ltd.

<sup>158</sup> Adapted from the Philadelphia Museum of Art. "IOD00201 Madder, area 3, Natural Red 8,PMA# B-46, tran". Infrared and Raman Users Group Spectral Database. Ed. Beth Price and Boris Pretzel (a much more resolved figure of this red curve was online at <http://www.irug.org/ed2k/spectra.asp?file=IOD00201.DX> before the online IRUG database was reconstructed in 2013-2014 and the spectrum became interactive and moved to <http://www.irug.org/jcamp-details?id=176>), © IRUG, with permission from IRUG. Natural Red 8 is a madder lake: see <http://www.artiscreation.com/red.html#NR8> and Kremer pigments "Madder Lake Coral - Chemical description: Natural Red 8" <http://www.kremer-pigmente.com/en/product.html?info=1824>

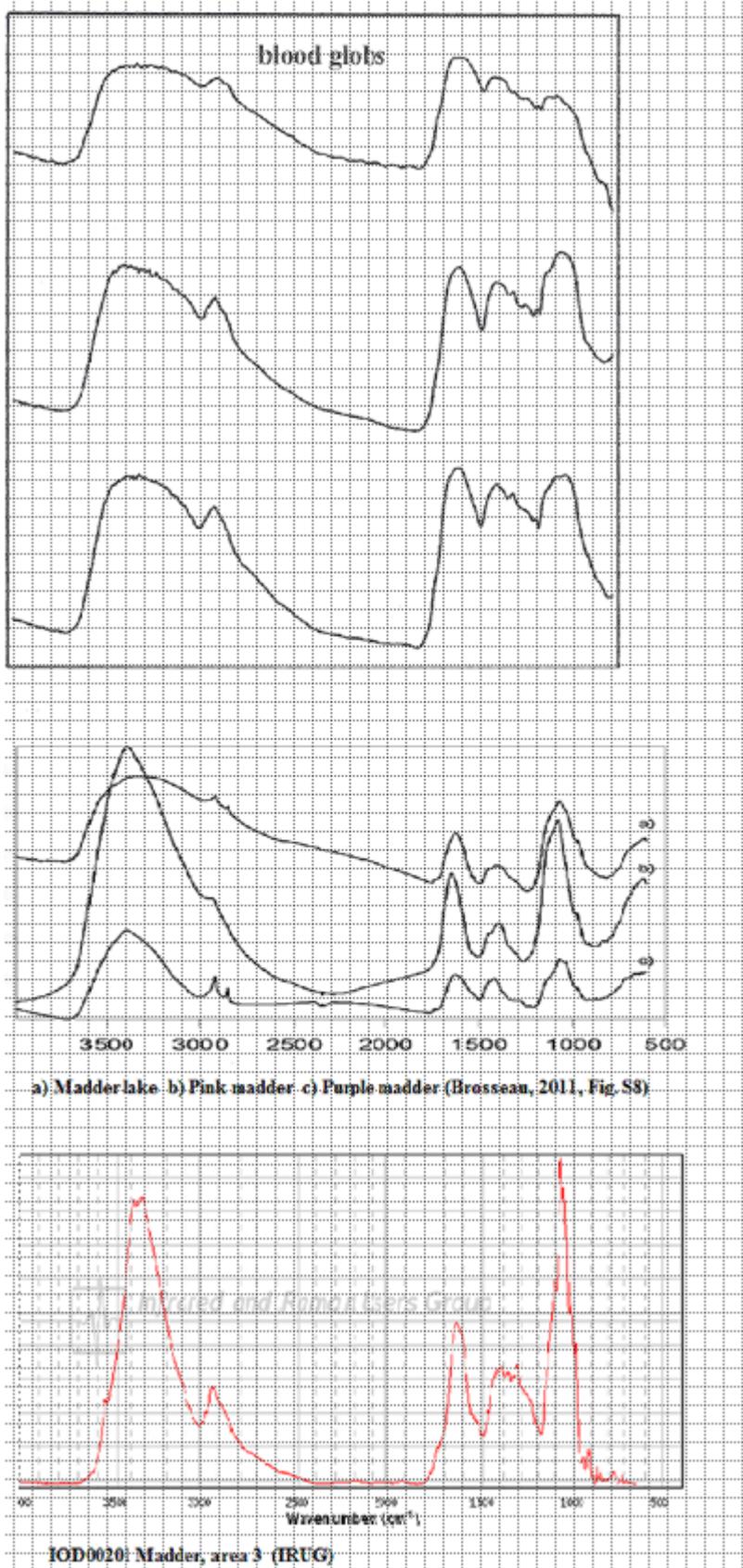


Fig. 2.22. Same as fig. 2.21 above: FTIR spectra of Shroud blood globs (Adler, Selzer and DeBlase) and madder lakes (Brosseau and IRUG), adapted for alignment, but with lath ©A.D. Adler, R. Selzer and F. DeBlase/John Wiley and Sons, Ltd./IRUG/AvdH<sup>159</sup>

<sup>159</sup> See copyright notices of previous figure

In these figures, the scale marks that were present on the horizontal axis of the FTIR of the blood globs in The Orphaned Manuscript p. 101 have been removed, as they were not spaced linearly.<sup>160</sup> Fortunately, linear horizontal scale marks are present in Adler's Figures 1 and 2 of The Orphaned Manuscript p. 83 (see fig. 2.23), in which one of the depicted curves is a blood glob curve: the same as the top curve of the three blood globs curves of TOM 101. More fortunately, most of the FTIR plots in the 2002 book of the proceedings of the 1998 Dallas Symposium have linearly spaced scale marks. Using this linear scale for all three blood globs curves, also a numerical comparison of the wavenumbers of the FTIR peaks of the blood globs and those of madder could be made (see fig. 2.23 and fig. 2.22). The results are shown in table 3, and can be considered a good match, taking into account that there is some variation in the madder FTIR's, and of course an aligning and reading error in the blood glob values, besides the variation in the blood glob curves themselves.

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<sup>160</sup> See p. 101 of A.D. Adler, The Orphaned Manuscript, <http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMIcq90QWto4GAAw&ved=0CDEQ6AEwAA#v=snippet&q=clot%20controls&f=false>

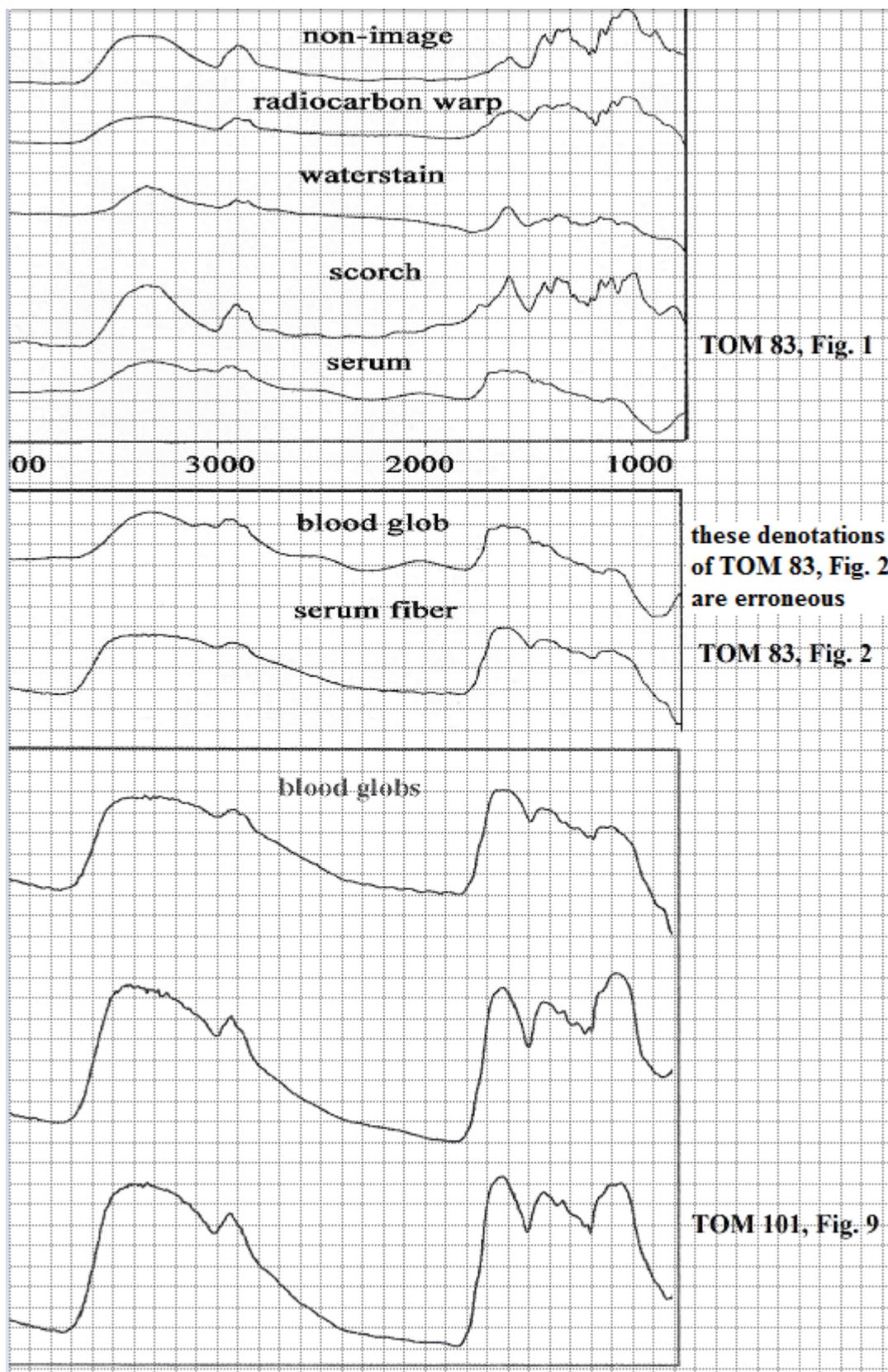


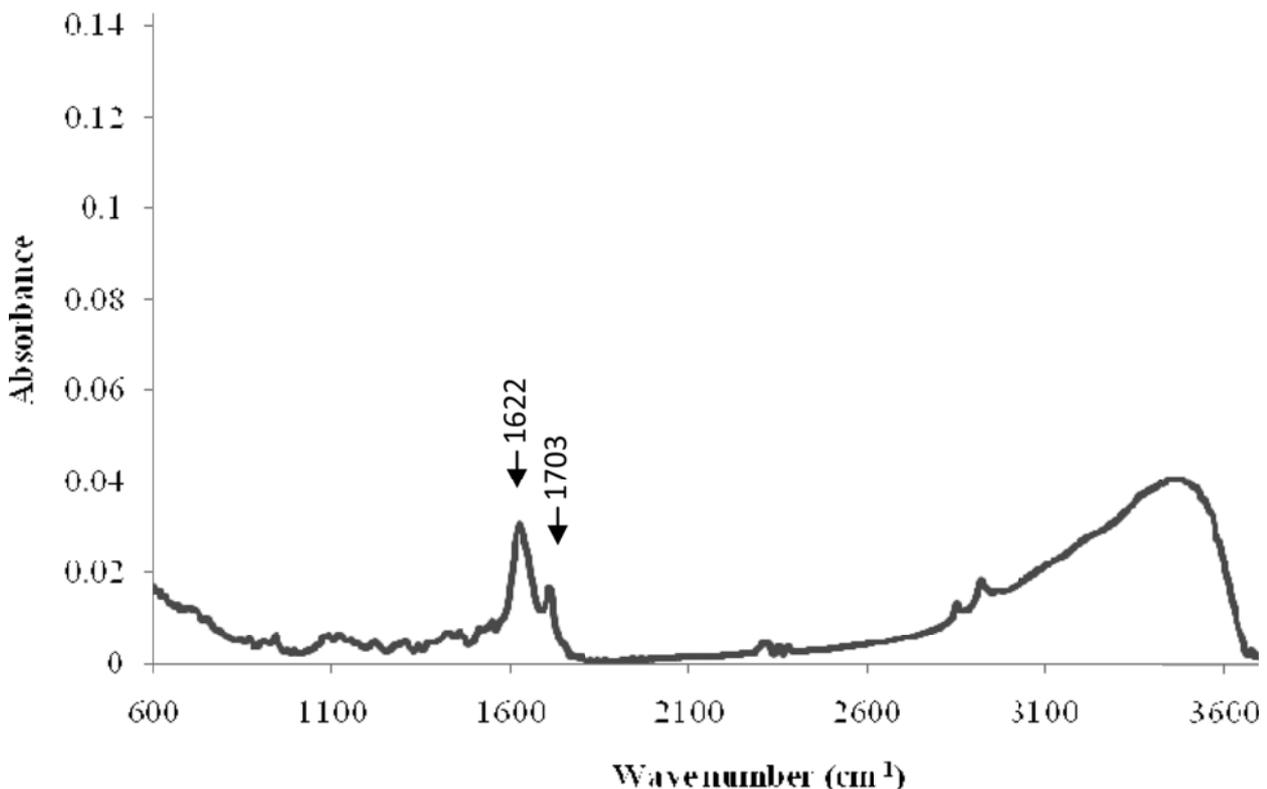
Fig. 2.23. Aligned FTIR spectra, covered with lath. Top and centre: FTIR spectra of various Shroud samples ©ACS<sup>161</sup> Note: in the centre plot the denotations “blood glob” and “serum fiber” should be

<sup>161</sup> Adapted with permission from A.D. Adler, Updating Recent Studies on the Shroud of Turin, Archeological Chemistry, chapter 17, pp. 223-228, *ACS Symposium Series*, Vol. 625, May 1996, Chapter DOI: 10.1021/bk-1996-0625.ch017, ISBN13: 9780841233959 eISBN: 9780841215740, <http://pubs.acs.org/doi/abs/10.1021/bk-1996-0625.ch017?prevSearch=Updating%2Brecent%2Bstudies%2Bon%2Bthe%2BShroud%2Bof%2BTurin&searchHistoryKe>

*interchanged* (cf. bottom plot = TOM 101 fig. 9, and fig. 2.40.A and 2.40.B=TOM 100 fig. 8);  
Bottom: FTIRs of Shroud blood globs ©A.D. Adler, R. Selzer, F. DeBlase<sup>162</sup>

Brosseau et al. wrote that “The spectrum is in fact a combination of bands characteristic of the chromophores of madder (with key band assignments, characteristic of anthraquinones including  $3401\text{ cm}^{-1} = \nu(\text{O-H})$ ,  $2919\text{ cm}^{-1} = \nu(\text{C-H})_{\text{ring}}$ ,  $1620\text{ cm}^{-1} = \nu(\text{C=C})_{\text{ring}}$ ,  $\nu(\text{C=O})$ ,  $1423\text{ cm}^{-1} = \nu(\text{OH})$ ,  $\nu(\text{CH})_{\text{ring}}$ ,  $1075\text{ cm}^{-1} = \nu(\text{C-OH})$ )[41] as well as broad features related to the use of amorphous hydrated alumina as lake substrate. In fact, as discussed in the literature, broad peaks in the O–H stretching region ( $3400\text{ cm}^{-1}$ ) and deformation (centered at  $1650\text{ cm}^{-1}$ ) can be related to the presence of an amorphous aluminum hydroxide substrate.[42]”<sup>163</sup>

Also a heme contribution could be present in the Shroud blood globs FTIRs. The heme FTIR is shown in figure 2.24, in which the second plot is a mirrored version of the first one, in order to show the low wavenumbers on the right side, as in the madder and blood globs FTIR plots. The heme appears to show hardly any low wavenumber vibrations, and therefore probably does not interfere much with the madder FTIR in this part of the curve. The broad high wavenumber peak in heme almost coincides with that of madder lake, although the heme peak value is at ca.  $3477\text{ cm}^{-1}$ , a bit higher value than the ca.  $3400\text{ cm}^{-1}$  peak value of the madder lake and the blood globs (see table 3). This may mean that the three investigated blood globs contain very little heme that is not bound to madder.



© Copyright © 1996 American Chemical Society. (fig. 1 and fig. 2, also in The Orphaned Manuscript (TOM) p. 83 and online at <http://www.sindone.info/ADLER.PDF>).

<sup>162</sup> Adapted from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-18, © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer.

<sup>163</sup> Brosseau et al., Revealing the invisible..., 2011,  
[http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011\\_Brosseau.pdf](http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011_Brosseau.pdf)

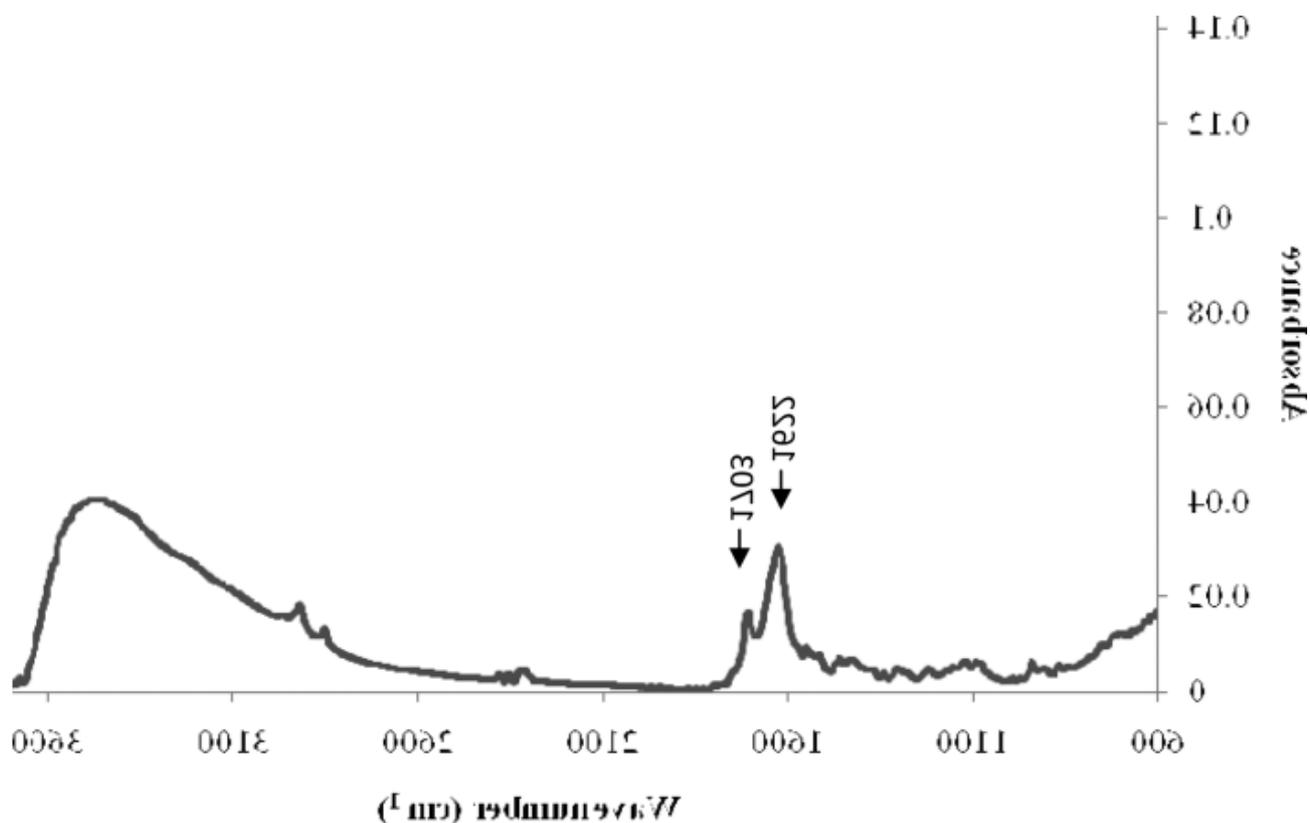


Fig. 2.24. FTIR spectrum of heme, and its mirrored plot ©ACS<sup>164</sup>

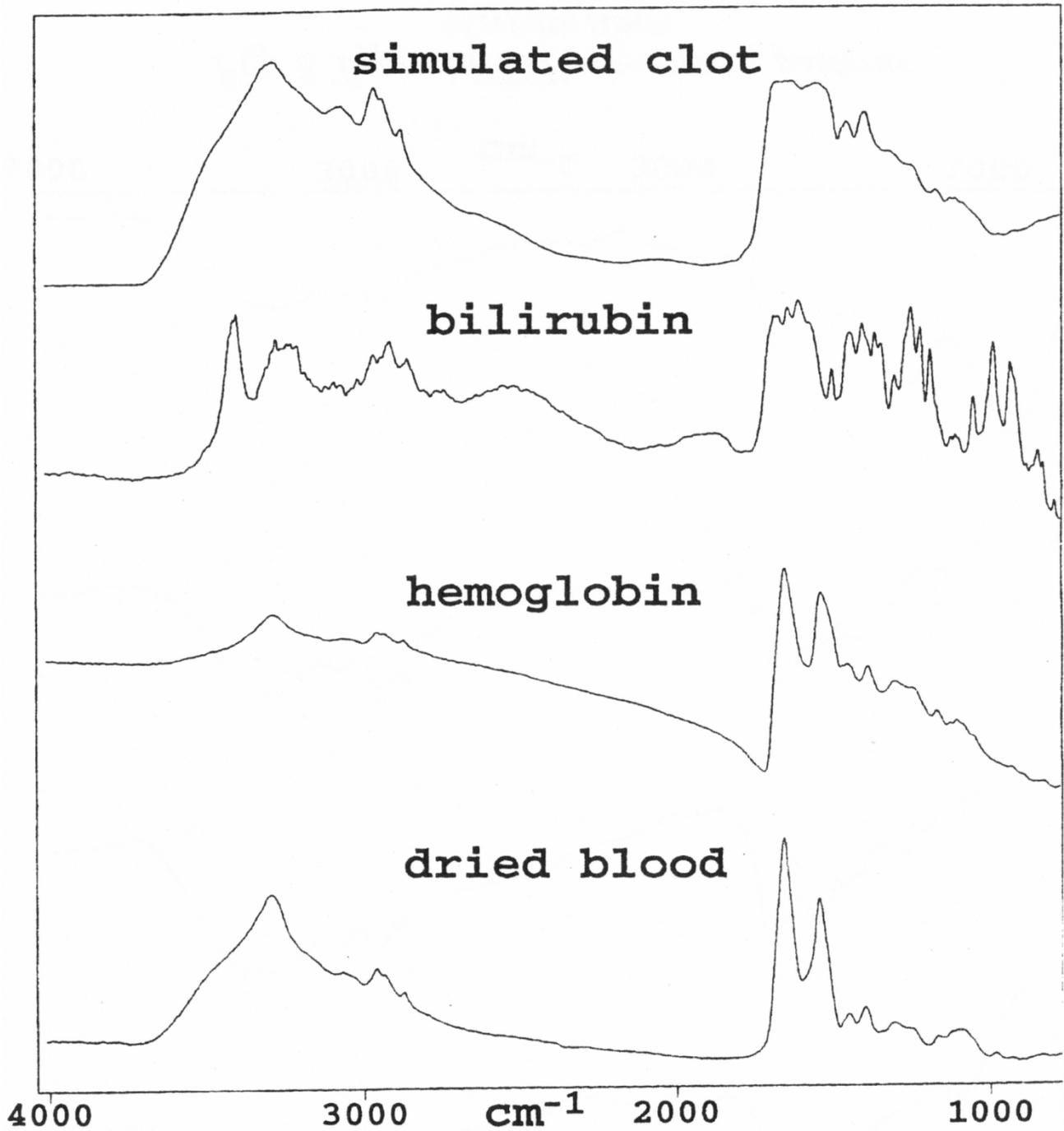
As can be seen below in the FTIR plot of a simulated traumatic blood clot exudate (three drops of whole blood mixed with three drops of a bilirubin/albumin solution<sup>165</sup>), called “simulated” in fig. 10 of Adler et al. 2002<sup>166</sup> (fig. 2.25) and its wavenumber values (table 3), the blood globs’ FTIRs are a much closer match to the FTIRs of madder lake – even without adding the broad round heme band above 3000  $\text{cm}^{-1}$  – than to the FTIR of the simulacrum.

<sup>164</sup> Reprinted and adapted with permission from Nafiseh Soltanizadeh and Mahdi Kadivar, Role of Globin Moiety in the Chemical Role of Globin Moiety in the Chemical Structure of Curing Pigment, *Journal of Agricultural and Food Chemistry*, May 1, 2012, 60. pp. 4718-4724, Copyright 2012 American Chemical Society, Fig. 2 [http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file\\_pubwdet/role\\_of\\_globin\\_moiety\\_in\\_the\\_chemical\\_structure\\_of\\_curing\\_pigment\\_0.pdf](http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file_pubwdet/role_of_globin_moiety_in_the_chemical_structure_of_curing_pigment_0.pdf)

<sup>165</sup> Adler, Selzer and DeBlase, *Further Spectroscopic...*, 2002, TOM 94

<sup>166</sup> Adler, Selzer and DeBlase, *Further Spectroscopic...*, 2002, TOM 101; the curve is called “simulated clot” in the book of the proceedings of Dallas 1998

A:



*Fig. 10 Typical FTIR absorbance patterns of simulated exudate blood clot controls*

B:

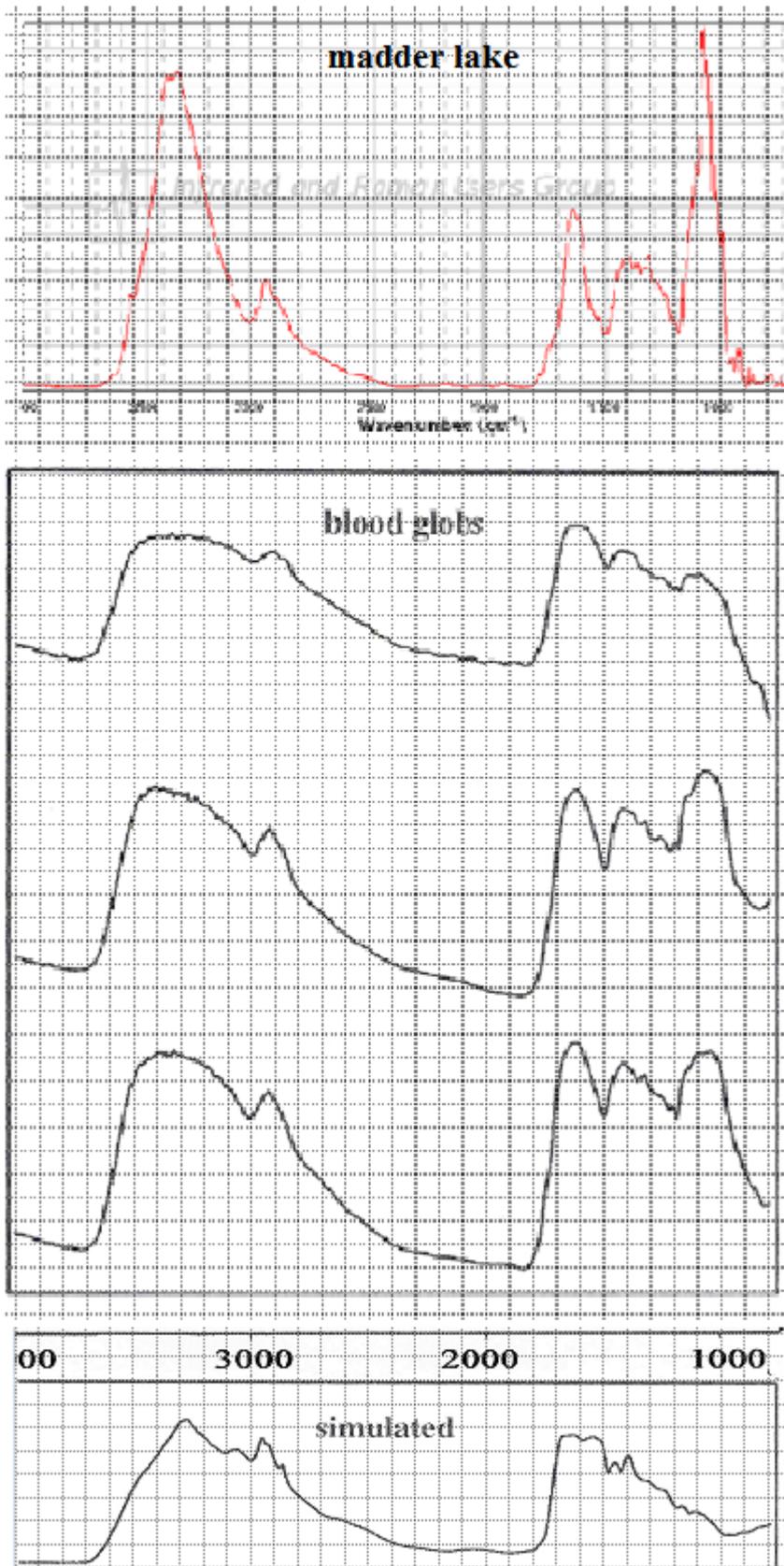


Fig. 2.25. A: FTIR spectra of “controls”, among which the “simulated clot” curve is perhaps a simulated spectrum composed of various reference patterns ©A.D. Adler, R. Selzer, F. DeBlase<sup>167</sup>

<sup>167</sup> Used from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, Compiled by

*B: Rough comparison of vertically aligned FTIR spectra. Top: FTIR spectrum of madder lake ©IRUG<sup>168</sup> Centre: FTIR spectra of Shroud blood globs ©A.D. Adler, R. Selzer, F. DeBlase<sup>169</sup> Bottom: Simulated FTIR spectrum composed of reference spectra or a measured FTIR spectrum of a clot made of a mixture of whole blood and albumin-bound bilirubin (label “simulated” as in *The Orphaned Manuscript*, fig. 10, p. 101) ©A.D. Adler, R. Selzer, F. DeBlase<sup>170</sup>*

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Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-18,1© A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer.

<sup>168</sup> Adapted from the Philadelphia Museum of Art. "IOD00201 Madder, area 3, Natural Red 8,PMA# B-46, tran". Infrared and Raman Users Group Spectral Database. Ed. Beth Price and Boris Pretzel (a much more resolved figure of this red curve was online at <http://www.irug.org/ed2k/spectra.asp?file=IOD00201.DX> before the online IRUG database was reconstructed in 2013-2014 and the spectrum became interactive and moved to <http://www.irug.org/jcamp-details?id=176>), © IRUG, with permission from IRUG; Natural Red 8 is a madder lake: see <http://www.artiscreation.com/red.html#NR8> and Kremer pigments “Madder Lake Coral - Chemical description: Natural Red 8” <http://www.kremer-pigmente.com/en/product.html?info=1824>

<sup>169</sup> Adapted from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, originally published in *The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium*, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-18,1© A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer.

<sup>170</sup> Adapted from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, originally published in *The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium*, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-18,1© A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer.

simulated clot Proc-1998 174-1	blood glob 1 Proc-1998	blood glob 2 Proc-1998	blood glob 3 Proc-1998	red madder lake IRUG area 1 <sup>171</sup> tran	red madder IRUG area 2 <sup>172</sup> tran	red madder lake IRUG area 3 <sup>173</sup> tran	red madder lake IRUG <sup>174</sup> refl	madder Bross. 2011 (madder lake/pink madder)	madder MFA – CAMEO (“Pink madder”)	red madder lake F Clem. 2008 <sup>175</sup>	red madder lake P Clem. 2008	red madder lake R Clem. 2008	heme	nitroso-heme <sup>176</sup>	photo-oxidized nitroso-heme <sup>177</sup>
	785	775	780	780		781	768 792	773/775							740
			805				826								
850	840		840		850	849	850		845						810
		870	860	868	865	868	876								
		895			910	910	896								
925	935	925		921	921 943	919 943					915				930
				994	992	991	976	975/975	975	975	975				
			1010	1018	1007 1014	1015	1012								
	1035	1035	1040	1053	1054	1054	1030 1046							1033	1055
	1075	1075	1065	1067	1067	1068	1064	1068/1078		1075		1080			1083
1115	1100		1095	1102 1126	1105 1116	1103	1091	-/1125	1118	1117	1105				
1135	1145	1145	1145	1153	1159	1158	1125 1163 1181	1145/-				1165		1141	
1180															
	1195	1195	1200	1204 1237	1206 1237	1206 1241	1199								
1255	1255	1260		1266		1261	1269			1240		1240		1261	1240
				1277	1279	1281	1295	1283/-			1290				
1325	1320	1320	1320	1321	1321	1322	1331	-/1325		1310					
		1375	1375	1372	1368 1386	1366	1397	-/1385	1375			1385			
1405	1430	1420	1410	1407	1425	1408	1437	1405/-	1440	1400	1420				1404
1460				1453	1458	1454	1455			1450	1450	1450			
1475				1515	1471	1515	1509			1495	1525				
1560				1544		1548				1540					
	1575						1589				1590				
		1605	1600	1608	1613						1605			1591	
			1625			1630	1631	1625/-					1622		1622
1635	1640	1655	1650	1639	1653		1653	-/1650 <sup>178</sup>	1645	1650	1640	1650			
1660							1667								
1690				1705		1702							1703		
	1725	1725	1725		1715		1719 1733	1735/-						1720	1720
2650									2500					1790	
	2860	2865		2845		2847	2854 2870			2870		2870			2860
2885					2890	2891									
		2905			2910	2911									
	2920	2920	2920	2918	2927	2926	2926	2918/2918	2920	2925		2930			2920
2940					2938 2967	2963	2950 3008			2960		2960			2960
2970					3248		3034 3070			3056		3070			
3075															
3295	3335	3405	3325	3327 3373	3332 3382 3408	3330 3376 3404	3364	3390/3325 3401 = anthra-quinones <sup>179</sup>	3375	3415	3430	3290 3425			3320
3475				3548	3556	3551							3477		

<sup>171</sup> Natural red 8 <http://www.irug.org/jcamp-details?id=821>; Natural Red 8 is a madder lake: see <http://www.artiscreation.com/red.html#NR8> and <http://www.kremer-pigmente.com/en/product.html?info=1824>

<sup>172</sup> <http://www.irug.org/jcamp-details?id=1470>

<sup>173</sup> Natural red 8 <http://www.irug.org/jcamp-details?id=176>

<sup>174</sup> Natural red 8, <http://www.irug.org/jcamp-details?id=742>

<sup>175</sup> Clementi et al, Vibrational and ..., 2008, fig. 2 <http://link.springer.com/article/10.1007%2Fs00339-008-4474-6>

<sup>176</sup> Fig. 6.2 (fig. 4 of Soltanizadeh 2012, <http://pubs.acs.org/doi/abs/10.1021/jf300023s>)

<sup>177</sup> Fig. 6.3 (fig. 6 of Soltanizadeh 2012, <http://pubs.acs.org/doi/abs/10.1021/jf300023s>)

<sup>178</sup> “broad features related to the use of amorphous hydrated alumina as lake substrate” Brosseau et al., Revealing the invisible..., 2011, [http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011\\_Brosseau.pdf](http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011_Brosseau.pdf)

<sup>179</sup> “broad features related to the use of amorphous hydrated alumina as lake substrate” Brosseau et al., Revealing the invisible..., 2011, [http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011\\_Brosseau.pdf](http://sites.weinberg.northwestern.edu/vanduyne/files/2012/10/2011_Brosseau.pdf)

Table 3. FTIR peak wavenumbers as estimated from the plots, plus a few published numbers.

#### 2.1.2.4. Fluoresce of bloodstains is redder than the background

##### Heme does not fluoresce above 390 nm

The fluorescence of methemoglobin seems to have its maximum at ca. 334 nm,<sup>180</sup> just as hemoglobin's maximum fluorescence is at ca. 330 nm,<sup>181</sup> which is beyond the range measured in the Shroud's fluorescence spectra for blood, with excitation at 365 nm and fluorescence emission measurement starting at 390 nm.<sup>182</sup> "Detection of blood on a crime scene is important, but blood is not fluorescent."<sup>183</sup> Here, it is probably meant that hemoglobin fluoresces at too short wavelengths to be detected in ordinary fluorimetry.<sup>184</sup>

##### Red madder lake fluoresces at ca. 620 nm, if under proper excitation

The fluorescence maximum of red madder lake is at ca. 620 nm<sup>185</sup> (fig. 2.26), and the absorption maximum, which is almost equal to the excitation maximum, is at ca. 525 nm (fig. 2.14).<sup>186</sup> The

<sup>180</sup> "Fig. 3. Fluorescence spectra of Hb (12.2  $\mu\text{M}$ /heme). From bottom to top, the concentration of CTAB: 0,  $2.0 \times 10^{-4}$ ,  $6.0 \times 10^{-4}$ ,  $1.0 \times 10^{-3}$ ,  $1.0 \times 10^{-2}$  mol/L. Inset in figure shows the fluorescence intensity of Hb (12.2  $\mu\text{M}$ /heme) ( $\lambda_{\text{em}} \approx 334$  nm) with CTAB. "CTAB monomer can convert methemoglobin (metHb) to hemichrome"

<http://www.sciencedirect.com/science/article/pii/S0141813005002448> ; <http://ars.els-cdn.com/content/image/1-s2.0-S0141813005002448-gr3.gif>

<sup>181</sup> "Fig. 2. UV-vis spectrum and fluorescence spectrum of Hb.  $c_{\text{Hb}}$  (mol L<sup>-1</sup>):  $5 \times 10^{-6}$ , (1) UV-vis spectrum of Hb, (2) fluorescence spectrum of Hb. (a) Hb/H<sub>2</sub>O system, (b) Hb/niosome system." <http://ars.els-cdn.com/content/image/1-s2.0-S0927775706005693-gr2.gif> ; "Fig. 1. Fluorescence studies: (a) Relative fluorescence of Hb as a function of TFE [♦].

Error bars represent the mean  $\pm$  SD ( $n = 3$ ). \*Significance  $p < 0.05$  with respect to control. (b) Intrinsic fluorescence emission spectra of native Hb in 20 mM sodium phosphate buffer, pH 7 (curve 1); in the presence of 15% (curve 2); 20% (curve 3); 25% (curve 4) and 45% TFE (curve 5). The excitation wavelength was 295 nm and emission wavelength was in the range 300–400 nm." Iram et al., 2013, Detection and analysis of protofibrils and fibrils of hemoglobin:

Implications for the pathogenesis and cure of heme loss related maladies,

<http://www.sciencedirect.com/science/article/pii/S0003986113000799>

<sup>182</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, p. 1933

<sup>183</sup> Bernard Valeur, Mario Nuno Berberan-Santos, Molecular Fluorescence: Principles and Applications, p. 513

[http://books.google.nl/books?id=jn98Ppv4HiwC&pg=PA96&lpg=PA96&dq=Soret+fluorescence&source=bl&ots=cEzdtSHH72&sig=JO2awd5lgg-GQzb-zf2juxr8\\_G8&hl=nl&sa=X&ei=MgbpUeCJNMzAa3rYCIBA&ved=0CGkQ6AEwBw#v=onepage&q=hemoglobin&f=false](http://books.google.nl/books?id=jn98Ppv4HiwC&pg=PA96&lpg=PA96&dq=Soret+fluorescence&source=bl&ots=cEzdtSHH72&sig=JO2awd5lgg-GQzb-zf2juxr8_G8&hl=nl&sa=X&ei=MgbpUeCJNMzAa3rYCIBA&ved=0CGkQ6AEwBw#v=onepage&q=hemoglobin&f=false)

<sup>184</sup> "Porphyrins also have intense red fluorescence, and a fluorescence detector set at excitation and emission wavelengths of 400 to 415 nm and 600 to 620 nm, respectively, provides a highly sensitive and specific method of detection. Heme is a nonfluorescence compound and cannot be detected fluorimetrically." Heme, Chlorophyll, and Bilins: Methods and Protocols, redacted by door Alison Smith, Michael Witty, p. 107,

[http://books.google.nl/books?id=UxonyKn2vdEC&pg=PA142&lpg=PA142&dq=Soret+fluorescence&source=bl&ots=zQbYbOmZAY&sig=Zub80tLloHUiXIO\\_Ev5QDihUkBU&hl=nl&sa=X&ei=MgbpUeCJNMzAa3rYCIBA&ved=0CGUQ6AEwBg#v=onepage&q=Soret%20fluorescence&f=false](http://books.google.nl/books?id=UxonyKn2vdEC&pg=PA142&lpg=PA142&dq=Soret+fluorescence&source=bl&ots=zQbYbOmZAY&sig=Zub80tLloHUiXIO_Ev5QDihUkBU&hl=nl&sa=X&ei=MgbpUeCJNMzAa3rYCIBA&ved=0CGUQ6AEwBg#v=onepage&q=Soret%20fluorescence&f=false)

<sup>185</sup> Claro et al., The use of..., 2008, Fig. 5 <http://www.sciencedirect.com/science/article/pii/S0039914007005309>; cf.

Clementi et. al, A spectrometric..., 2007 fig. 3.b for alizarin lake <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr3.jpg> in <http://www.sciencedirect.com/science/article/pii/S0003267007009464>; "The fluorescence emission that we registered from the specimen of madder lake paint in egg tempera, has two peaks, one around 420 nm and the other around 620 nm ... The fluorescence emission of the corresponding specimen of genuine madder lake paint in linseed oil, has also two peaks, where the first peak is shifted of about 20 nm to higher wavelength range. We believe that the first peak be actually linked to the binder, (and in fact a peak in the 420-440 nm is common for almost all specimens), while the second one, around 620 nm, would be the one typical of natural *Madder*." Pelagotti et al., A Study of UV Fluorescence Emission of Painting Materials, 2005,

[http://www.ino.it/home/lella/pdf/Art2005\\_A%20study%20of%20UV%20fluorescence%20emission%20of%20painting%20ma%85.pdf](http://www.ino.it/home/lella/pdf/Art2005_A%20study%20of%20UV%20fluorescence%20emission%20of%20painting%20ma%85.pdf) p. 7-8

<sup>186</sup> Aceto et al., First analytical..., 2012, "Pink – Madder lake" (Table 1), "Fig. 7. FORS spectra from a pink area from *Vienna Dioskurides* and comparison with standard paint: (a) pink area from f. 2v (blue line), standard madder (black line)." ("analysed with *in situ* non-invasive techniques. Raman spectroscopy, UV-Vis diffuse reflectance spectrophotometry with optic fibres") (in 6<sup>th</sup> century Byzantine manuscript)

<http://www.sciencedirect.com/science/article/pii/S1386142512004568> ; cf. Clementi et. al, A spectrometric..., 2007, fig 3.a for alizarin lake <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr3.jpg> <http://www.sciencedirect.com/science/article/pii/S0003267007009464>

excitation and absorption minimum of both alizarin lake and purpurin lake is at ca. 365 nm, between the two peaks at ca. 300 and 500 nm<sup>187</sup> (fig. 2.27). Excitation at 440 nm still yields at least alizarin lake fluorescence.<sup>188</sup> But the wavelengths of the excitation used in the fluorescence photography and fluorimetry of the Turin Shroud in 1978 are 335-375 nm and 365 nm, respectively,<sup>189</sup> and thus exactly in the excitation minimum for madder lake. A photo of the excitation-dependent fluorescence of madder lake pigments in linseed oil (fig. 2.28) shows that under 365 nm excitation hardly any red madder fluorescence is visible.<sup>190</sup>

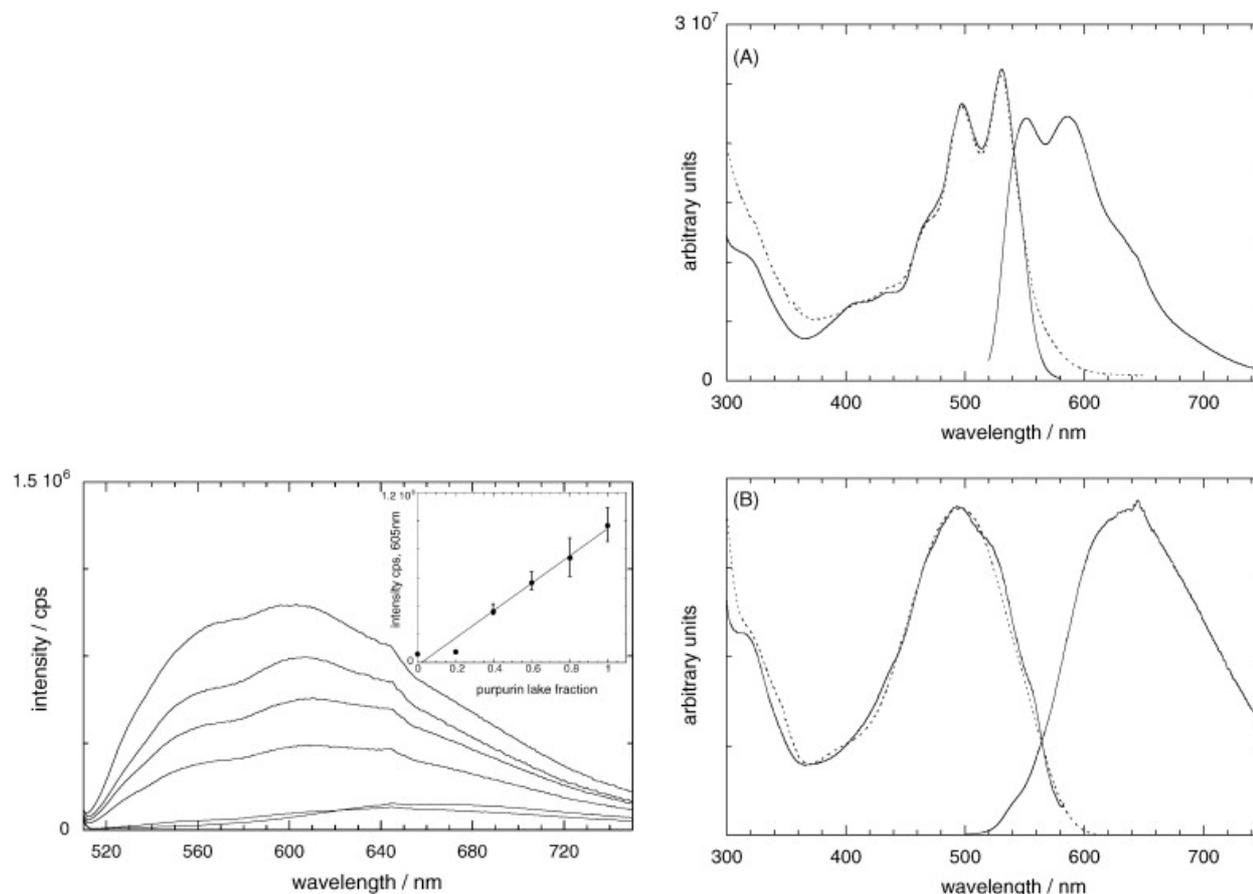


Fig. 2.26 (left). Fluorescence emission spectra of mixtures of purpurin lakes and alizarin lakes (bottom to top curves: 0:100 , 20:80 , 40:60 , 60:40, 80:20 and 100:0 purpurin:alizarin) ©Elsevier<sup>191</sup>

Fig. 2.27 (right). Fluorescence excitation and emission spectra of purpurin (A) and alizarin (B) aluminum complex (= lakes) in methanol/water solution (dotted line = absorption spectrum) ©Elsevier<sup>192</sup>

<sup>187</sup> Claro et al., The use of..., 2008, fig. 3; cf. Clementi et al, A spectrometric..., 2007, fig. 3.a for alizarin lake <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr3.jpg> in <http://www.sciencedirect.com/science/article/pii/S0003267007009464>

<sup>188</sup> Clementi et al., A spectrometric..., 2007, "Fig. 3. (a) Reflectance emission excitation ( $\lambda_{em} = 620$  nm) and (b) emission spectra ( $\lambda_{exc} = 440$  nm) of wool samples dyed with alizarin in different conditions." <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr3.jpg> in <http://www.sciencedirect.com/science/article/pii/S0003267007009464> p. 53

<sup>189</sup> Miller and Pellicori, Ultraviolet Fluorescence..., 1981, p. 71; Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, p. 1933

<sup>190</sup> Antonino Cosentino, Multispectral Imaging of Madder Lake, March 6, 2013,

<http://chsopensource.wordpress.com/2013/03/06/madder-lake-multispectral-imaging/>

<sup>191</sup> Used from A. Claro, M.J. Melo, S. Schäfer, J. Sérgio Seixas de Melo, F. Pina, K.J. van den Berg, A. Burnstock, The use of microspectrofluorimetry for the characterization of lake pigments, *Talanta* Vol. 74 Issue 5, Jan. 2008, pp. 922-929, DOI: 10.1016/j.talanta.2007.07.036, <http://www.sciencedirect.com/science/article/pii/S0039914007005309> (fig. 5 <http://ars.els-cdn.com/content/image/1-s2.0-S0039914007005309-gr5.jpg>) © 2008 Elsevier, with permission from Elsevier.



Fig. 2.28. Appearance in white light and UV-fluorescence of various swatches of madder lakes (Kremer Pigment) painted across non-fluorescing red ochre ©Antonino Cosentino, Cultural Heritage Science Open Source, [chsopensesource.org](http://chsopensesource.org)<sup>193</sup>

#### The bloodstains fluoresce redder than background and image

The blood stains are described as non-fluorescent relative to the background: Miller and Pellicori wrote on the fluorescence of the bloodstains, as seen in photographs: “no color” (p. 75), “Blood is red visually, but neutral to black (absorbing) fluorescently” (p.76), “distinctly absorbant against the fluorescent background linen” (p. 79), and “The blood and body image are similar in the fluorescence photos; i.e. grey and non-emitting” (p. 82), and “Laboratory data for whole blood displayed total absorption, which is in agreement with the Shroud data.” (p. 84). On the other hand: “Some of the densest marks within the blood flow area might be scorches” (p. 79 on back blood)<sup>194</sup>, and Heimburger’s “slightly enhanced” fluorescence photo of a part of the small of the back area (fig. 2.29) shows reddish brown fluorescence of the (dense) blood rivulets and the pink fluorescence of a very faint scourge mark (dumbbell stain in upper left of photo), which probably has very little heme on top of the madder lake, and the orange red fluorescence of a scorch mark.<sup>195</sup> Also the online UV fluorescence photo of the wrist shows a reddish fluorescence of the bloodstain (fig. 2.30).<sup>196</sup> An enlarged part of a fluorescence photo from a photocopy of Miller and Pellicori’s article of two blood rivulets in the small of the back area (fig. 2.31) shows that, while the cloth itself is green yellow, these rivulets are a bit pinkish red in fluorescence but the densest blood at the tip of one rivulet is dark brown – probably because at the tip more heme is on top of a pink fluorescing madder lake –

<sup>192</sup> Used from A. Claro, M.J. Melo, S. Schäfer, J. Sérgio Seixas de Melo, F. Pina, K.J. van den Berg, A. Burnstock, The use of microspectrofluorimetry for the characterization of lake pigments, *Talanta* Vol. 74 Issue 5, Jan. 2008, pp. 922-929, DOI: 10.1016/j.talanta.2007.07.036, <http://www.sciencedirect.com/science/article/pii/S0039914007005309> (fig. 3 <http://ars.els-cdn.com/content/image/1-s2.0-S0039914007005309-gr3.jpg>) © 2008 Elsevier, with permission from Elsevier.

<sup>193</sup> Used from the weblog of Antonino Cosentino, Cultural Heritage Science Open Source, [chsopensesource.org](http://chsopensesource.org) with permission; cf. <http://kremer-pigmente.de/en/blog/multispectral-imaging-of-madder-lake>

<sup>194</sup> Miller and Pellicori, *Ultraviolet fluorescence...*, 1981

<sup>195</sup> Heimburger, A detailed..., 2008, p. 18 <http://www.shroud.com/pdfs/thibault%20final%2001.pdf>

<sup>196</sup> cf. Rogers and Arnoldi, *Scientific Method...*, 2002, p. 5, fig. 3, <http://shroudstory.files.wordpress.com/2012/02/i8.jpg>

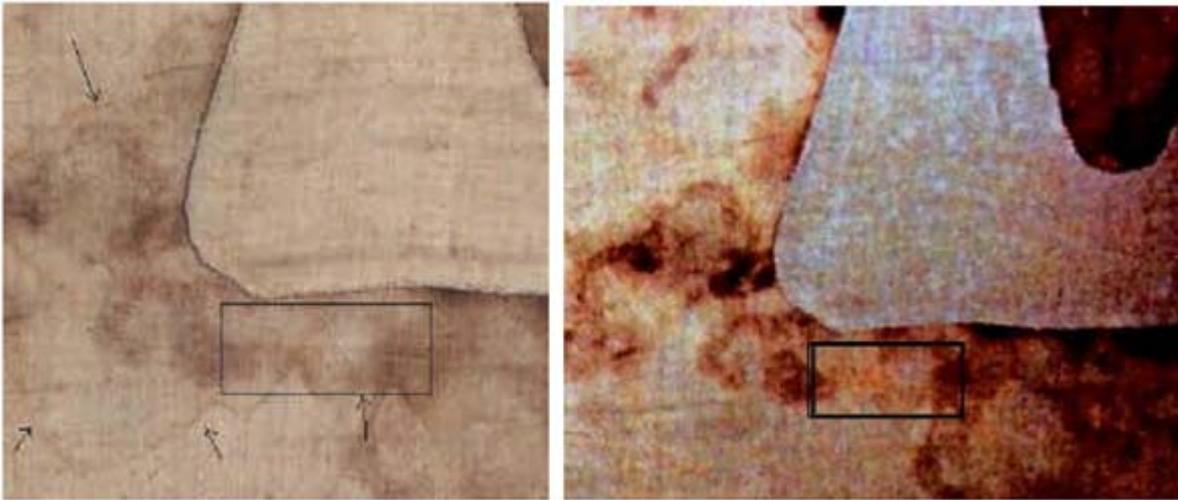


Fig. 2.29. A part of the small of the back area of the Shroud in visible light (left) and UV light, showing fluorescence “slightly enhanced” (right) ©T. Heimburger<sup>197</sup>



Fig. 2.30. Ultraviolet-fluorescence photograph of the image of the hands ©2005 Raymond N. Rogers Collection, STERA, Inc.<sup>198</sup>

<sup>197</sup> Used from T. Heimburger, A detailed critical review of the chemical studies on the Turin Shroud: Facts and Interpretations, 2008, <http://www.shroud.com/pdfs/thibault%20final%2001.pdf>, fig. on p. 18, with permission from T. Heimburger.

<sup>198</sup> Used from Rogers, A Chemist’s Perspective on the Shroud of Turin, 2008, fig. IV-2, p. 19, [http://books.google.fr/books?id=p-n84sc4ayYC&printsec=frontcover&hl=fr&source=gbg\\_summary\\_r&cad=0#v=onepage&q&f=false](http://books.google.fr/books?id=p-n84sc4ayYC&printsec=frontcover&hl=fr&source=gbg_summary_r&cad=0#v=onepage&q&f=false) with permission from STERA, Inc.



Fig. 2.31. UV-fluorescence photo (cropped) of thin and dense bloodflows in small of back area of the Shroud ©Biocommunications Association, Inc.<sup>199</sup>

The ratio of the intensities of the fluorescence at 450 nm ( $F_{450}$ , the datapoint with the highest intensity) and at 600 nm ( $F_{600}$ , the datapoint closest to 620 nm, the fluorescence maximum of madder lake) can be calculated from the fluorescence spectra of background, image, bloodstains, and scorches, which were published in spectra showing datapoints at 50 nm intervals (the experimental set-up had a 17 nm-resolution) (e.g. fig. 2.32 and 2.33). The smaller the ratio, the redder the visible color of the total fluorescence. The values derived from the spectra published in the article of the Gilberts<sup>200</sup> are presented in table 4 and figure 2.34.

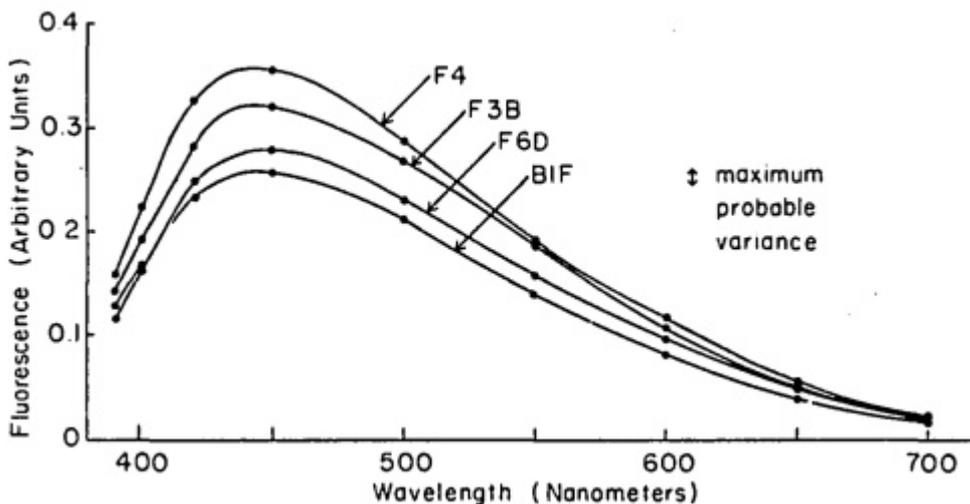


Fig. 2.32. Smoothed fluorescence curves of four clear areas of the Shroud ©OSA<sup>201</sup>

<sup>199</sup> Image reprinted (mirrored) from Miller & Pellicori, *Journal of Biological Photography*, Vol 49, No 3, 1981, p. 71-85, with permission from the publisher, the Biocommunications Association, Inc.

<sup>200</sup> Gilbert and Gilbert, *Ultraviolet-visible reflectance...*, 1980, figures 7, 11, 13, 17; their fig. 11, fluorescence of four image areas, is at <http://imagebank.osa.org/getImage.xqy?img=M3cubGFyZ2UsYW8tMTktMTItMTkzMC1nMDEx>

<sup>201</sup> Used from R. Gilbert Jr. and M.M. Gilbert, *Ultraviolet-visible reflectance and fluorescence spectra of the Shroud of Turin*, *Applied Optics*, 19 (12) 1980, pp. 1930-1936, <http://dx.doi.org/10.1364/AO.19.001930>, fig. 7, with permission from the Optical Society of America.

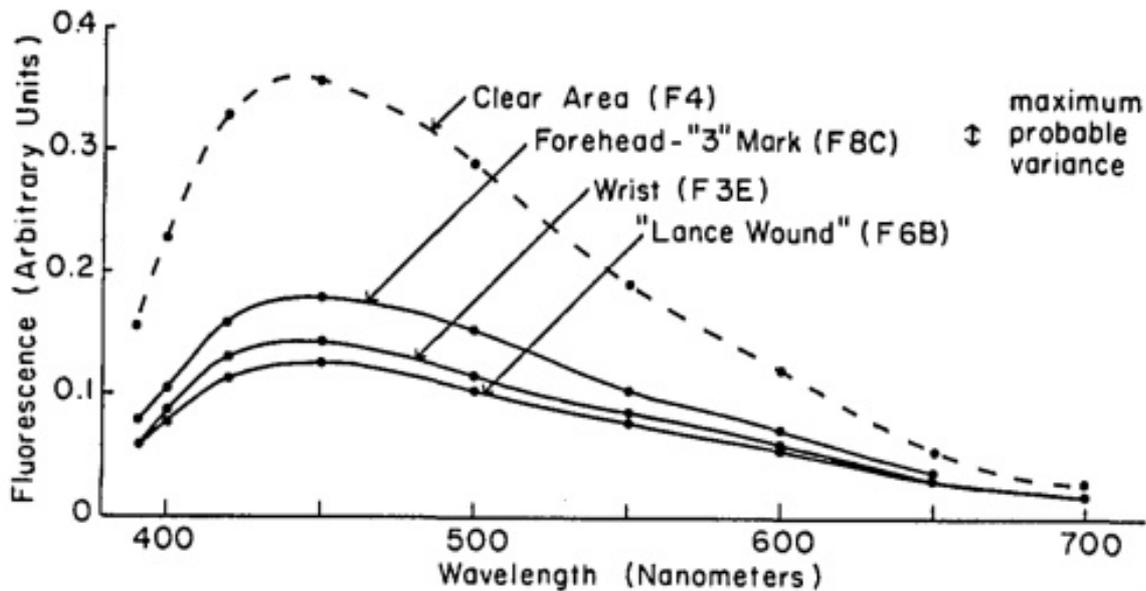


Fig. 2.33. Smoothed fluorescence curves of three bloodstains of the Shroud ©OSA<sup>202</sup>

area	location (as labelled in Gilbert and Gilbert)	F <sub>450</sub>	F <sub>600</sub>	ratio F <sub>450</sub> /F <sub>600</sub>	mean ratio F <sub>450</sub> /F <sub>600</sub>
background	F4	0.354	0.118	3.00	2.98
	F3B	0.318	0.107	2.97	
	F6D	0.277	0.097	2.86	
	B1F	0.255	0.082	3.11	
image	Calf B1E	0.282	0.104	2.71	2.55
	Neck B6A	0.247	0.100	2.47	
	Nose F8F	0.195	0.087	2.24	
	Heel B1D	0.165	0.059	2.80	
bloodstain	Forehead – “3” mark F8C	0.178	0.075	2.37	2.29
	Wrist F3E	0.142	0.063	2.27	
	“Lance Wound” F6B	0.125	0.056	2.23	
scorch	B3E	0.217	0.117	1.85	1.64
	F8H	0.203	0.094	2.16	
	F3C	0.150	0.078	1.92	
	B1C	0.106	0.096	1.10	
	F3D	0.056	0.049	1.14	

Table 4. Fluorescence values of various areas of the Shroud, derived from Gilbert and Gilbert, 1980, fig. 7, 11, 13, and 17 (F<sub>450</sub> = fluorescence intensity at 450 nm, F<sub>600</sub> = fluorescence intensity at 600 nm, in arbitrary units of a scale variable)

<sup>202</sup> Used from R. Gilbert Jr. and M.M. Gilbert, Ultraviolet-visible reflectance and fluorescence spectra of the Shroud of Turin, *Applied Optics*, 19 (12) 1980, pp. 1930-1936, <http://dx.doi.org/10.1364/AO.19.001930>, fig. 17, with permission from the Optical Society of America.

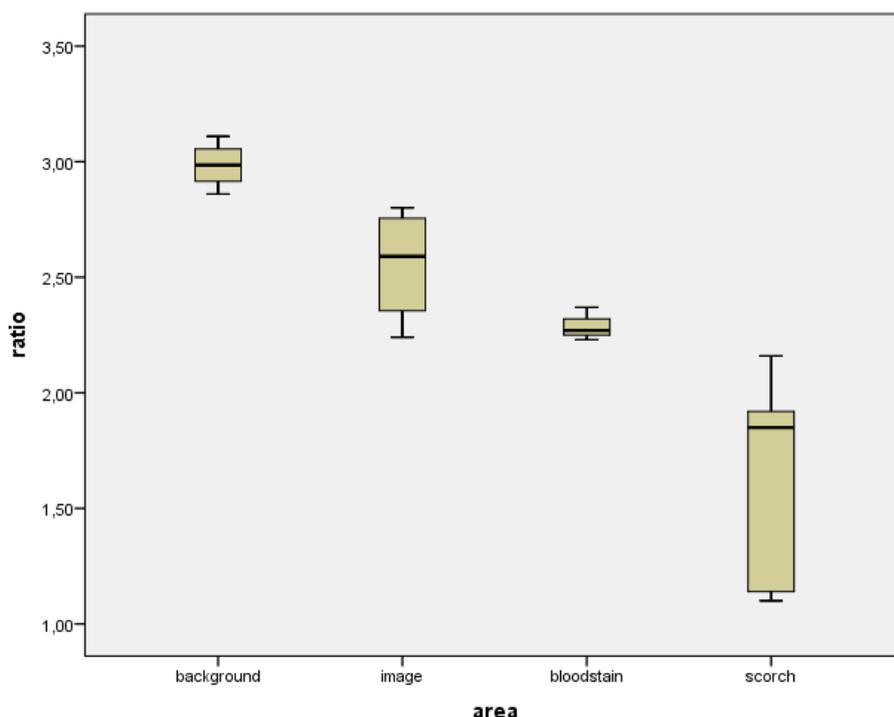


Fig. 2.34. Distribution of  $F_{450}/F_{600}$  ratios of fluorescence of Shroud areas, derived from Gilbert and Gilbert, figures 7, 11, 13, and 17 (clear area and bloodstains here in fig. 2.32, 2.33) ©AvdH

They show that the mean  $F_{450}/F_{600}$  ratio of the bloodstains is smaller than that of the image and much smaller than that of the background. Of the image it is said that “as the fluorescence level is reduced, the peak shifts slightly to longer wavelengths” and “these stains [image and scorch] seem to exhibit a low level fluorescence of their own in the 600-700 nm region. This lower level fluorescence is somewhat more pronounced with the scorched areas than the body image areas”.<sup>203</sup> Looking at the mean  $F_{450}/F_{600}$  values above, it can certainly be said that the bloodstains, although weaker in overall fluorescence, also seem to have a low level fluorescence of their own in the 600-700 nm region, even in more pronounced way than the image areas. Although Gilbert and Gilbert say about the bloodstains that “There is little evidence of shifting of the peak fluorescence to longer wavelengths”<sup>204</sup>, this doesn’t preclude that there is a red fluorescence contribution that pulls the balance to the red. This red shift, that may also be partly due to the fluorescence quenching effect of brown heme deposits (absorbing more in the blue and green than in the red), might also be due to the presence of weakly red fluorescing madder lake. The fluorescence of faint bloodstains looks redder than the fluorescence of dense bloodstains (cf. fig. 2.31), perhaps because the intrinsic red fluorescence of madder lake has more reddening power than the absorbance of heme deposits. A very thin heme deposit alone probably would not have turned the relatively strong green yellow background fluorescence red, but just a bit weaker.

#### Bloodstains are redder in daylight

In 2000, Pellicori stated that “It appears that in the photoelectric measurements, the fluorescence of the linen dominates and obscures signals that might be characteristic of the other types of stains. It is believed that diagnostic information might be present in short wave and fluorescent responses, but that different measurement parameters should be implemented to extract this information. For example, excitation at shorter wavelengths than 365 nm might cause different components to fluoresce. Specific wavelengths might excite emission for specific species.”<sup>205</sup>

<sup>203</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, p. 1934-1935

<sup>204</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, p. 1935

<sup>205</sup> Pellicori, Spectrophotometry of the Shroud: A Critical Review, Proceedings International Scientific Symposium, Turin 2000, p. 108

Sunlight contains a wide spectrum from about 100 nm to 1 mm,<sup>206</sup> and thus includes wavelengths shorter than 365 nm, which would cause madder lake to fluoresce stronger. It has been reported by Goldoni, that Legrand, when comparing his observations made in 1931 and after 1969, noticed that the bloodstains of the Turin Shroud are a brighter red when shown outside in sunlight – that contains both UV and visible excitation for madder lake – than when shown inside the Turin cathedral, where only blue to green visible light (ca. 400-550 nm) could cause madder lake to fluoresce; Goldoni also reported that Barbet, in the first half of the last century, and another observer in a private exposition in 2000, stressed the red color of the bloodstains when seen in daylight without protective glass.<sup>207</sup> A Dutch scientist typified madder as: “Red, redder, madder”<sup>208</sup>.

### **Survey of madder absorbance and fluorescence peak wavelengths**

Some spectral data for madder, in its various forms, are presented in various figures (fig. 2.35 to 2.39) and listed and compared to Shroud data in table 5. Shroud absorbance and fluorescence maxima/shoulders appear to correspond to madder maxima (which are located between the corresponding alizarin and purpurin maxima).

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<sup>206</sup> <http://en.wikipedia.org/wiki/Sunlight>

<sup>207</sup> Goldoni, The Shroud of Turin and ..., 2008, <http://www.ohioshroudconference.com/papers/p04.pdf>

<sup>208</sup> This is the first part of the title of the scientific publication: Red, redder, madder. Analysis and isolation of anthraquinones from madder roots (*Rubia tinctorum*), G.C.H. Derksen, Nutrient Cycling in Agroecosystems, 2001 <http://academic.research.microsoft.com/Publication/59525769/red-redder-madder-analysis-and-isolation-of-anthraquinones-from-madder-roots-rubia-tinctorum>

	alizarin	purpurin	madder	Shroud
<b>dye absorbance</b>	neutral, protonated molecule: <b>430 nm*</b>	neutral, protonated molecule: (455 and) <b>480 nm*</b>	aged and unaged madder on unmordanted wool (containing protonated alizarin and di-anion of purpurin): <b>ca. 450 nm<sup>209</sup></b>	red-stained fiber in transmission: <b>450 nm<sup>210</sup></b>
<b>dye fluorescence</b>	neutral, protonated molecule: <b>485 nm*</b>	neutral, protonated molecule: <b>575 nm*</b>	(no plot or peak wavelengths found)	raw fluorescence scan background: <b>540 nm shoulder<sup>o</sup></b>
<b>dye, anion form absorbance</b>	mono-anion: <b>535 nm</b> di-anion: <b>573 nm*</b>	mono-anion: <b>515 nm</b> di-anion: (513 and) <b>547nm*</b>	madder dye (85% purpurin), probably in anion form, in interaction with linseed oil on canvas: <b>ca. 525 nm<sup>211</sup></b>	
<b>dye, anion form fluorescence</b>	monoanion: <b>625 nm</b> di-anion: <b>682 nm*</b>	mono-anion: <b>585 nm</b> di-anion: <b>600 nm*</b>	- madder dye (85% purpurin), probably in anion form, in interaction with linseed oil on canvas: <b>ca. 625 nm<sup>212</sup></b> - unmordanted red madder dye (= mono-anion or di-anion as in lakes): from 650 to <b>610 nm</b> in ageing <sup>213</sup>	
<b>lake absorbance</b>	- Al <sup>3+</sup> -lake at pH 3.7: <b>500 nm<sup>#</sup></b> - fluorescence excitation maximum of Al <sup>3+</sup> -lake on wool: <b>520-530 nm<sup>□</sup></b>	Al <sup>3+</sup> -lake at pH 3.7: <b>531 nm<sup>#</sup></b>	pink madder lake on 6 <sup>th</sup> century manuscript: <b>ca. 525 nm<sup>214</sup></b>	relative reflectance bloodstains: <b>ca. 525 nm ‘shoulder’<sup>o</sup></b>
<b>lake fluorescence</b>	- Al <sup>3+</sup> -lake at pH 3.7: <b>640 nm<sup>#</sup></b> - Al <sup>3+</sup> -lake on wool: <b>ca. 640 nm<sup>□</sup></b>	Al <sup>3+</sup> -lake at pH 3.7: <b>595 nm<sup>#</sup></b>	- madder lake fluorescence: red-orange <sup>215</sup> - various mixtures of purpurin and alizarin lakes (100% purpurin to 100% alizarin fractions): <b>605 to 640 nm<sup>216</sup></b> - madder lake (both in egg tempera and linseed oil): <b>ca. 620 nm<sup>217</sup></b>	“slightly enhanced” fluorescence photo bloodstains: reddish brown <sup>218</sup> - 50 nm-resolution fluorescence spectra of bloodstains: smaller F <sub>450</sub> /F <sub>600</sub> ratio (= redder color) than in background <sup>o</sup> - raw fluorescence scan of background: tiny <b>620 nm</b> shoulder <sup>o</sup>

Table 5. Absorbance and fluorescence maxima of alizarin, purpurin, madder and Shroud areas.

sources: \*<sup>219</sup> □<sup>220</sup> #<sup>221</sup> □<sup>222</sup>

<sup>209</sup> Clementi et al., 2007, fig. 7 <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr7.jpg>

<sup>210</sup> Heller and Adler, Blood on the Shroud..., 1980, TOM 31

<sup>211</sup> Miliani et al., 1998, Fig. 1 <http://ars.els-cdn.com/content/image/1-s2.0-S1386142597002400-gr1.gif> ;

<sup>212</sup> Miliani et al., A spectrophotometric..., 1998, fig. 4 <http://ars.els-cdn.com/content/image/1-s2.0-S1386142597002400-gr4.gif> ; 1 = purpurin, 2 = alizarin, 3 = madder (85% purpurin)

<sup>213</sup> Clementi et al., , fig. 8 <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr8.jpg>

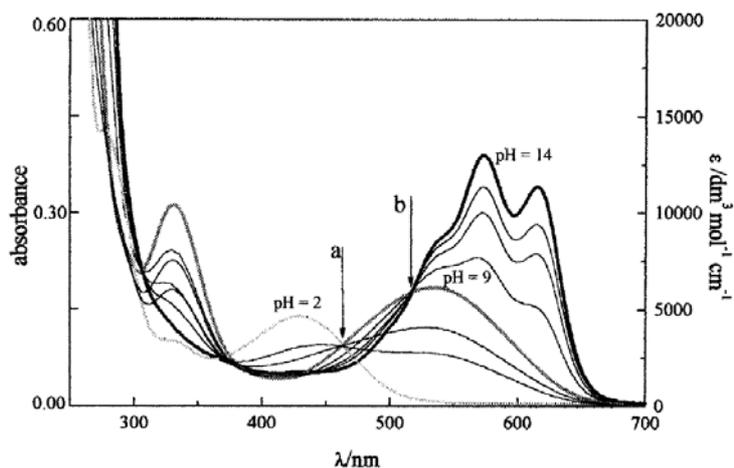
<sup>214</sup> Aceto et al., First analytical..., 2012, Fig. 7. <http://ars.els-cdn.com/content/image/1-s2.0-S1386142512004568-gr7.sml>

<sup>215</sup> “Among them genuine madder lake fluoresces a fiery red- orange distinguishing it from the synthetic alizarin” Keck, 1969, <http://www.opticsinfobase.org/ao/abstract.cfm?id=15620>

<sup>216</sup> Claro et al., The use of..., 2008, Fig. 5

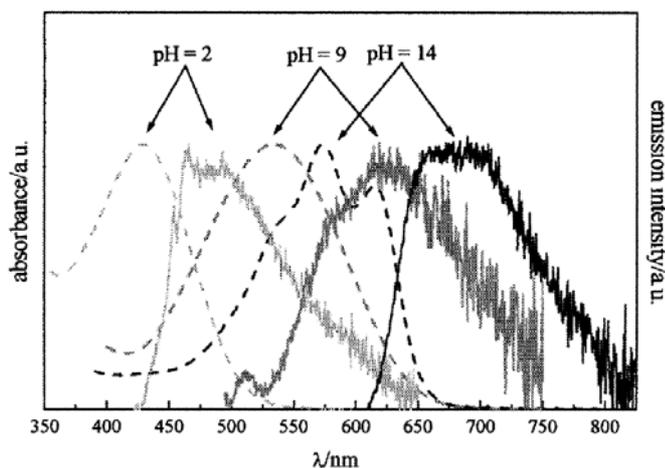
<sup>217</sup> Pelagotti et al., A Study of UV Fluorescence Emission of Painting Materials, 2005, Proc. of Art’05 8th Int. Conf. on "Non-Destructive Testing and Microanalysis for the Diagnostics and Conservation of the Cultural and Environmental Heritage", [http://www.ino.it/home/lella/pdf/Art2005\\_A%20study%20of%20UV%20fluorescence%20emission%20of%20painting%20ma%85.pdf](http://www.ino.it/home/lella/pdf/Art2005_A%20study%20of%20UV%20fluorescence%20emission%20of%20painting%20ma%85.pdf) p. 7-8

<sup>218</sup> Heimburger, A detailed..., 2008, photo p. 18



**Figure 1.** Evolution of the absorption spectrum of 1,2-HAQ ( $3.0 \times 10^{-5} \text{ mol dm}^{-3}$ ) in water-dioxane solutions (2:1, v/v) as a function of pH. Light grey, pH = 2 (neutral form); grey, pH = 9 (mono-anion); black, pH = 14 (di-anion). Arrows indicate isosbestic points between the neutral form and mono-anion (a, 463 nm) and between the mono-anion and di-anion (b, 516 nm)

*Fig. 2.35. Absorption spectra of alizarin (1,2-HAQ) at various pHs, used from Miliani et al., 2000 ©John Wiley and Sons, Ltd.<sup>223</sup>*



**Figure 2.** Absorption (dotted line) and fluorescence (full line) normalized spectra of the three species derived from 1,2-HAQ in water-dioxane solutions (2:1, v/v). Light grey, pH = 2 (neutral form); grey, pH = 9 (mono-anion); black, pH = 14 (di-anion)

*Fig. 2.36. Absorption and fluorescence spectra of alizarin (1,2-HAQ) at various pHs, used from Miliani et al., 2000 ©John Wiley and Sons, Ltd.<sup>224</sup>*

<sup>219</sup> Miliani et al., Acidichromic effects..., 2000; they used 377 nm excitation (see caption of their fig. 4)

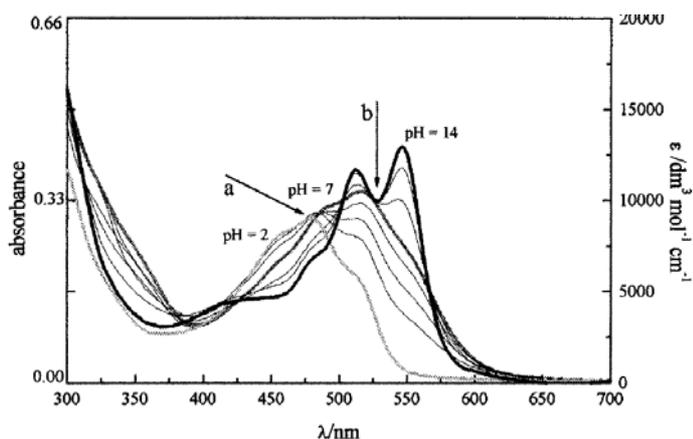
<sup>220</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980

<sup>221</sup> Claro et al., The use of..., 2008, Table 1

<sup>222</sup> Clementi et al., A spectrometric..., 2007, fig. 3 (1 and 2 = Alum mordant, 3 = Alum plus cream of tartar mordant) <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr3.jpg>; Cream of tartar is "Potassium bitartrate, also known as potassium hydrogen tartrate, with formula  $\text{KC}_4\text{H}_5\text{O}_6$ " [http://en.wikipedia.org/wiki/Cream\\_of\\_tartar](http://en.wikipedia.org/wiki/Cream_of_tartar)

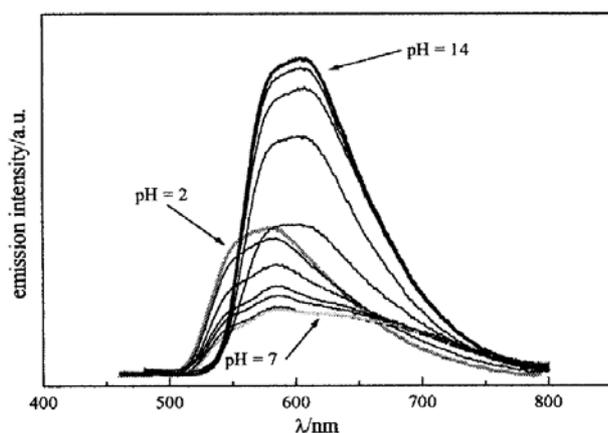
<sup>223</sup> Used from C. Miliani, A. Romani, G Favaro, *Journal of Physical Organic Chemistry*, Acidichromic effects in 1,2-di- and 1,2,4-tri- hydroxyanthraquinones. A spectrophotometric and fluorimetric study, Vol. 13 Issue 3 pp. 141-150, March 2000, DOI: 10.1002/(SICI)1099-1395(200003)13:3<141::AID-POC220>3.0.CO;2-J, [http://onlinelibrary.wiley.com/doi/10.1002/\(SICI\)1099-1395\(200003\)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract](http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1099-1395(200003)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract) © 2000 John Wiley & Sons, Ltd., with permission from John Wiley & Sons, Ltd.

<sup>224</sup> Used from C. Miliani, A. Romani, G Favaro, *Journal of Physical Organic Chemistry*, Acidichromic effects in 1,2-di- and 1,2,4-tri- hydroxyanthraquinones. A spectrophotometric and fluorimetric study, Vol. 13 Issue 3 pp. 141-150, March



**Figure 3.** Evolution of the absorption spectrum of 1,2,4-HAQ ( $3.3 \times 10^{-5} \text{ mol dm}^{-3}$ ) in water–dioxane solutions (2:1, v/v) as a function of pH. Light grey, pH = 2 (neutral form); grey, pH = 7 (mono-anion); black, pH = 14 (di-anion). Arrows indicate isosbestic points between the neutral form and mono-anion (a, 480 nm) and between the mono-anion and di-anion (b, 528 nm)

Fig. 2.37. Absorption spectra of purpurin (1,2,4-HAQ) at various pHs, used from Miliani et al., 2000 ©John Wiley and Sons, Ltd.<sup>225</sup>



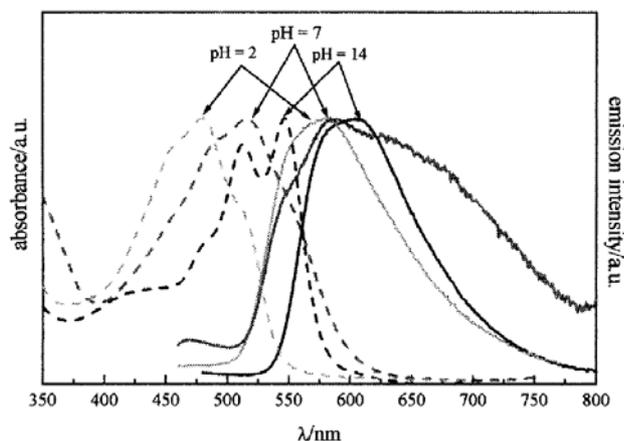
**Figure 4.** Emission changes of 1,2,4-HAQ ( $2.3 \times 10^{-5} \text{ mol dm}^{-3}$ ) in water–dioxane (2:1 v/v) solutions as a function of pH;  $\lambda_{\text{exc}} = 377 \text{ nm}$ . Light grey, pH = 2 (neutral form); grey, pH = 7 (mono-anion); black, pH = 14 (di-anion)

Fig. 2.38. Fluorescence spectra of purpurin (1,2,4-HAQ) at various pHs, used from Miliani et al., 2000 ©John Wiley and Sons, Ltd.<sup>226</sup>

2000, DOI: 10.1002/(SICI)1099-1395(200003)13:3<141::AID-POC220>3.0.CO;2-J,  
[http://onlinelibrary.wiley.com/doi/10.1002/\(SICI\)1099-1395\(200003\)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract](http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1099-1395(200003)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract) © 2000 John Wiley & Sons, Ltd., with permission from John Wiley & Sons, Ltd.

<sup>225</sup> Used from C. Miliani, A. Romani, G Favaro, *Journal of Physical Organic Chemistry*, Acidichromic effects in 1,2-di- and 1,2,4-tri- hydroxyanthraquinones. A spectrophotometric and fluorimetric study, Vol. 13 Issue 3 pp. 141-150, March 2000, DOI: 10.1002/(SICI)1099-1395(200003)13:3<141::AID-POC220>3.0.CO;2-J,  
[http://onlinelibrary.wiley.com/doi/10.1002/\(SICI\)1099-1395\(200003\)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract](http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1099-1395(200003)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract) © 2000 John Wiley & Sons, Ltd., with permission from John Wiley & Sons, Ltd.

<sup>226</sup> Used from C. Miliani, A. Romani, G Favaro, *Journal of Physical Organic Chemistry*, Acidichromic effects in 1,2-di- and 1,2,4-tri- hydroxyanthraquinones. A spectrophotometric and fluorimetric study, Vol. 13 Issue 3 pp. 141-150, March 2000, DOI: 10.1002/(SICI)1099-1395(200003)13:3<141::AID-POC220>3.0.CO;2-J,  
[http://onlinelibrary.wiley.com/doi/10.1002/\(SICI\)1099-1395\(200003\)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract](http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1099-1395(200003)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract) © 2000 John Wiley & Sons, Ltd., with permission from John Wiley & Sons, Ltd.



**Figure 5.** Absorption (dotted line) and fluorescence (full line) normalized spectra of the three species of 1,2,4-HAQ in water-dioxane solutions (2:1, v/v). Light grey, pH = 2 (neutral form); grey, pH = 7 (mono-anion); black pH = 14 (di-anion)

*Fig. 2.39. Absorption and fluorescence spectra of purpurin (1,2,4-HAQ) at various pHs, used from Miliani et al., 2000 ©John Wiley and Sons, Ltd.*<sup>227</sup>

### 2.1.2.5. Ehrlich's reagent detected (madder) phenols in blood samples

"In addition, we tested for heme breakdown products, i.e., bile pigments such as bilirubin, with Ehrlich's reagent using the method of Jendrassik. For these purposes we employed the commercially available American Monitor "525" bilirubin test kit. Using the same microspotting technique as described above, characteristic blue azobilirubin colors could be positively detected in reflected light on the surfaces of the olive colored shards, the orange globs, and, also, weakly on the more orange colored red coated fibrils. The test color was sensitive to acid, turning a paler purple, and discharged by 10 minutes of short wave UV light as is a characteristic of this color test."<sup>228</sup>

From this description of the Ehrlich's reagent test by Adler, it seems there was no positive test from "golden-yellow ("serum") coated fibers"<sup>229</sup>, either because the blue-coloring substance was not present on them or because these fibers were not tested at all, perhaps for lack of enough golden-yellow serum fibers. Also a positive test on more red colored red coated fibrils is not mentioned.

Adler's description shows that the blue colors of the diazo bilirubin test were obtained in alkaline solution, for these colors turned a paler purple when acid was added: if the first blueish mixture would have been acid, addition of another acid would not have turned the blue colors into a paler purple. Acid azobilirubin colors are red and such an acid mixture needs to be made alkaline in order to minimize interference of hemoglobin or carotene.<sup>230</sup> The procedure that is prescribed for the specific kit used by Adler, indeed ends with the addition of an alkaline reagent.<sup>231</sup> However, the

<sup>227</sup> Used from C. Miliani, A. Romani, G Favaro, *Journal of Physical Organic Chemistry*, Acidichromic effects in 1,2-di- and 1,2,4-tri- hydroxyanthraquinones. A spectrophotometric and fluorimetric study, Vol. 13 Issue 3 pp. 141-150, March 2000, DOI: 10.1002/(SICI)1099-1395(200003)13:3<141::AID-POC220>3.0.CO;2-J, [http://onlinelibrary.wiley.com/doi/10.1002/\(SICI\)1099-1395\(200003\)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract](http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1099-1395(200003)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract) © 2000 John Wiley & Sons, Ltd., with permission from John Wiley & Sons, Ltd.

<sup>228</sup> Heller and Adler, *A Chemical...*, 1981, TOM 39

<sup>229</sup> Heller and Adler, *A Chemical...*, 1981, TOM 40

<sup>230</sup> "Estimation of serum bilirubin (Jendrassik and Grof method) ... Conjugated (direct) bilirubin in serum is coupled with diazotized sulfanillic acid to form red-colored compound. Ascorbic acid is used to stop the coupling reaction and to eliminate interference by hemoglobin. Caffeine benzoate solution is used to split the unconjugated bilirubin protein complex releasing the bilirubin so that it can react with diazotised sulfanillic acid. The tartrate buffer makes the mixture alkaline and converts the red acid bilirubin to a green-colored compound which shows peak absorbance at 607 nm. At this wavelength the absorbance due to hemoglobin or carotene is minimal." Palit et al., *Journal of Basic and Clinical Pharmacy*, 2012, vol. 3, no. 4, pp 352-357, <http://www.jbclinpharm.org/article.asp?issn=0976-0105;year=2012;volume=3;issue=4;spage=352;epage=357;aualast=Palit>

<sup>231</sup> "Kit: D. Jendrassik Bilirubin Reagent System (American Monitor Corp., Indianapolis, IN 46268) – Conditions for azobilirubin formation: Reaction with *p*-diazobenzenesulfonic reagent at acidic pH in the presence of caffeine reagent followed by an alkaline reagent addition (Jendrassik-Grof method)"; "In the Jendrassik-Grof procedure, bilirubin reacts

alkaline diazo color is very unspecific: “Type A alkaline diazo reaction is likely to remain of little value as a biochemical test. The interpretation is still too uncertain, as the reagent is too unspecific and too easily interfered with to give reliable information even regarding iminazoles.” - “The writer has ... reviewed elsewhere the main substances which give a colour with the reagent [Hunter, 1922, 1]. These include phenols, iminazoles, certain purines, tyrosine, sulphides and ammonium salts. The reagent is thus very unspecific”<sup>232</sup>.

This means that phenols of madder dye (alizarin, purpurin, pseudopurpurin, xanthopurpurin, quinizarin, nordamnacanthal etc.) may have produced the observed blue test colors on the tested Shroud samples and that bilirubin needn't have been present at all.

#### 2.1.2.6. MOLE experiment and Cargille oil make red particles behave as an organic phase

Rogers first describes how “tapes, fibers from non-image areas, and fibers from image areas” were taken to the firm Instruments SA, in Metuchen, N.J.; then he continues, by writing that “Similar samples were analyzed by Mark Anderson, McCrone’s MOLE expert ... Anderson observed that most of the red flecks on the Shroud “bubbled up and turned black” when he hit them with the laser beam. This was an entirely different response than he got from authentic hematite crystals. He said it “acted like an organic phase”<sup>233</sup>. Anderson’s observation in the MOLE experiment with red flecks on Shroud samples is consistent with the presence of an organic substance such as heme, or heme-madder lake. Perhaps the laser beam of the MOLE experiment (MOLE is Laser Microprobe Raman Spectroscopy) made the Shroud’s red madder lake flecks bubble, separating the madder from its (heme) mordant, and then turned the iron of the heme into black iron-oxide and the madder and the organic part of the heme into black carbon. As Rogers wrote that Anderson observed that “most of the red flecks” on Anderson’s image and non-image fibers acted this way,<sup>234</sup> most of the red (possibly transposed) material on the Shroud may have been or have contained heme-madder lake.

Kohlbeck wrote a letter to Nelson on 27 august 1984: “Ray gave me permission to remove some of the fibers from the sticky tape. I remounted these fibers in a common inert mounting oil which I use for most microscopical work. [...] After three months I re-examined the slides again and much to my surprise there was a pronounced change in the once red particles on the fibers. The particles had blackened and exuded a yellow color which certainly could not be iron oxide. Heller no doubt is right; however, I am no blood expert but I know the particles are not iron oxide.”<sup>235</sup> Bracaglia specified that the “common inert mounting oil” was “cargille oil”, and published a few photomicrographs of Kohlbeck on the site of the Holy Shroud Guild,<sup>236</sup> and two others on the Facebook page of the Holy Shroud Guild.<sup>237</sup> Also these observations in red material from “the lance wound and other blood areas” of the Shroud<sup>238</sup> might be explained by the presence of red heme-madder lake that dissociated to form black heme and yellow madder dye. It would be interesting to know which solvent Kohlbeck had used to remove the fibers from the sticky-tape.

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to form a broad absorbance band from 400 to 700 nm with peak absorbance at 595 nm.” Poon et al., 1985,

<http://www.clinchem.org/content/31/1/92.full.pdf> p.93 = p.2 of pdf, Table 1; p. 93 (pdf p. 3) ;

[http://www.vernier.com/images/innovate/food\\_coloring\\_spectra.jpg](http://www.vernier.com/images/innovate/food_coloring_spectra.jpg) .

<sup>232</sup> Hunter, The diazo reaction in urine, 1924,

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1259545/pdf/biochemj01158-0039.pdf>

<sup>233</sup> Rogers, A Chemist’s..., 2008, p. 61

<sup>234</sup> Rogers, A Chemist’s..., 2008, p. 61

<sup>235</sup> <http://holyshroudguild.org/dr-nitowski-new.html> (first Scribd document, p. 3-4 = second letter)

<sup>236</sup> <http://holyshroudguild.org/dr-nitowski-new.html> and in the slide show on [http://holyshroudguild.org/dr-raes-problematic-threads\\_2.html](http://holyshroudguild.org/dr-raes-problematic-threads_2.html)

<sup>237</sup>

<https://www.facebook.com/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater#!/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater> and

<https://www.facebook.com/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater#!/photo.php?fbid=10201282418765168&set=oa.10151506022204822&type=1&theater>

<sup>238</sup> <http://holyshroudguild.org/dr-nitowski-new.html> (first Scribd document, p. 3-4 = second letter)

### 2.1.2.7. Proteases “dissolved” red particles leaving fibers as non-image fibers

Heller and Adler classified their Shroud samples and one of the classes was: “Designation: Red coated fibrils – Predominant Locations: Blood areas – Comments: Coating varies from smooth to fractured to particulate appearance, color varies from red to orange, coating not birefringent or pleochroic”<sup>239</sup> In their description of the proteases test they do not use the general designation “Red coated fibrils”, but go to great lengths to point out that the positive test was obtained from one specific subclass, viz. the red-particulate-coated fibers: “a fresh concentrated mixture of trypsin, chymotrypsin, carboxypeptidase and lysozyme in pH 8.4 buffer was employed. Within a half hour this solution completely “dissolved” the non-birefringent red particulate coated fibril coatings, leaving no particulate residues”<sup>240</sup>. In the rest of this or any other text by Heller or Adler, nothing is said about a protease test on the smooth and fractured red coatings. As the removal of the red particulates by proteases left fibers that “closely resemble the non-image fibrils when viewed under phase contrast”, it is possible that these fibers were non-image fibers to which dry acid methemoglobin particles, or perhaps dry heme particles, adhered after these particles had abraded from nearby bloodstains. Heller wrote on Jackson’s particle transposition hypothesis: “That is exactly what we found. The blood had abraded off in many places and was transposed everywhere. It was blood that accounted for most of the red dots on the body image fibrils.”<sup>241</sup> Likewise, also the non-image fibers in image and non-image areas would have received red particles from bloodstain areas. The “brownish red translucent crystal” – also called a “glob”<sup>242</sup> – that was subjected to UV-vis spectroscopy, is just a single example of such particles. The proteins on the surface of the dry methemoglobin crystal – the globin parts – would stick to the madder phenols on the surface of the dry non-image fiber (“phenols adsorb organic compounds”<sup>243</sup>). The dry crystal would not be denatured by the phenols as there is no liquid solution that allows movement of the hemes out of the globin proteins. Although heme is not a protein, the pH 8.4 buffer solution used in the protease test could have dissolved adhering acid heme aggregates, for these can dissolve in alkaline solution just as hemoglobin can.<sup>244</sup> Any pink heme-madder lake, which may have been present at the small interface between a fiber and an adhering transposed dry heme particle, would probably not be “dissolved” by the proteases, but it would not have had a particle shape, but a film shape, and thus is consistent with the described test result that no particulate residues remained, and is also consistent with the resemblance with less-corroded non-image fibers when viewed under phase contrast. Note that Heller and Adler did not write about the color of the test result, which probably was brownish because of the alkaline dissolved methemoglobin/hematin.<sup>245</sup>

“This protease treatment also removes the golden yellow coating from the golden yellow fibrils, corroborating their identification as “serum” coated fibrils. Interestingly, fibrils freed of their coatings using this technique closely resemble the non-image fibrils when viewed under phase contrast. The protease solution also dissolved the shards, the orange globs, and the brown globs

<sup>239</sup> Heller and Adler, *A Chemical...*, 1981, Table 2, TOM 50

<sup>240</sup> Heller and Adler, *A Chemical...*, 1981, TOM 40-41

<sup>241</sup> Heller, *Report on...*, 1983, p. 196; Heller checked the mechanism of transposal of red particles by folding and unfolding an old Spanish linen sample that had bloodstains.

<sup>242</sup> Heller and Adler, *Blood on the Shroud...*, 1980, TOM 30-31; Heller, *Report on* p. 128

<sup>243</sup> “phenol adsorbs organic compounds” <http://pharmabook.net/en/protivomikrobnnye--protivoparaz/antiseptiki-i-dezinficiruyusch/carbolic-acid.html>

<sup>244</sup> cf. “the heme absorption at 418.5 nm of the reduced heme in alkaline pyridine” Srivastava et al., 2005, [www.ncbi.nlm.nih.gov/pmc/articles/PMC1151790/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151790/); “The stock solution of 0.1 n NaOH used to dissolve heme is diluted 10-fold” Varfaj et al., 2012, [www.jbc.org/content/287/42/35181.full](http://www.jbc.org/content/287/42/35181.full); cf. fig. 1 of Ursos et al., 2001, <http://www.sciencedirect.com/science/article/pii/S016668510000342X> and fig. 5 <http://aac.asm.org/content/57/1/365/F5.expansion.html> of Gorke et al., 2013, <http://aac.asm.org/content/57/1/365.long>

<sup>245</sup> “When adult human blood 'is added to decinormal sodium hydroxide a brown colour is quickly developed which on spectroscopic examination contains the specific absorption band of alkaline haematin (607 m,M).” ... “the brown colours produced in sodium hydroxide from blood and from crystalline haemin” Clegg et al., 1942, *Estimation of haemoglobin by the alkaline haematin method*, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2164191/pdf/brmedj04008-0003.pdf>

(leaving the small dark embedded particulates, probably carbonized material, as residue).<sup>246</sup> Adler's 1999 article reads: "Sticky tape non-image, image, and serum coated fibers were [...] tested along with a number of fibers from the radiocarbon threads employed in the FTIR studies. The protease was only active against the serum coated fibers and as in the previous study revealed smooth, non-corroded fiber surfaces indicating that the blood images went onto the cloth before the image forming process and protected the underlying cloth."<sup>247</sup> This is consistent with the presence of serum proteins on the golden yellow fibrils and with the presence of methemoglobin or perhaps heme in the abraded shards and orange and brown globs.

"Proteases had absolutely no effect on the yellow (body) image or pale yellow non-image fibrils of the Shroud."<sup>248</sup> As the used proteases are themselves proteins<sup>249</sup> – yet, without containing mordanting metals such as iron or alum – they would be adsorbed to any remaining madder phenols on probably toluene-washed<sup>250</sup> non-image fibers (probably through hydrogen bonding with the protein<sup>251</sup>) without outstandingly changing the fiber's color.

### 2.1.3. Blood before image

Heller and Adler already reported that after proteases digested some red particulates from Shroud fibers, these fibers closely resembled non-image fibers "when viewed under phase-contrast"<sup>252</sup>, which microscopical mode does not necessarily show the actual visible color of the fiber<sup>253</sup>, but would have distinguished the non-image from image fibers by "the progressively corroded appearance of their surfaces under observation by phase contrast microscopy (cf. , Table 2)": "Pale yellow fibrils – Non-image areas – Surface appear slightly corroded under phase contrast" – "Yellow fibrils – Body image (non-blood) areas – Surfaces appear corroded under phase contrast".<sup>254</sup> But the digested particulates may have been dry acid methemoglobin particles or dry heme particles that got stuck to a non-image fiber after having been abraded and transposed from a nearby blood area. Heller reported that for this proteases test they used "some blood- and serum-covered fibrils from a body image area"<sup>255</sup>. But also a body image area contains non-image fibers, as the image is a so-called 'halftone' image, of which the color density in a certain area is determined by the areal ratio of intermixed image and non-image surface fibers.<sup>256</sup>

There is a stronger argument for the blood-before-image hypothesis. If it is correct that, in bloodstains, red madder lake was formed locally by acid blood mordanting a yellow madder dye, this yellow madder dye would have to have been present before the blood got unto it.

The question therefore is, whether it is correct that there is, and was, no dye on image fibers. Wet chemistry did not detect dyes or phenols on pure image fibers<sup>257</sup>, and in chapter 4 the evidence for the presence of dye on non-image fibers will be described. Yet, the dyestuffs alizarin and purpurin

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<sup>246</sup> Heller and Adler, A Chemical..., 1981, TOM 41

<sup>247</sup> Adler, The Nature of the Body Images..., 1999, TOM 106-107

<sup>248</sup> Heller and Adler, A Chemical..., 1981, TOM 41

<sup>249</sup> "Source of proteins, crystalline trypsin, crystalline chymotrypsin, and crystalline bovine albumin, Armour and Company, Chicago", and trypsin and chymotrypsin give a positive Folin reagent test for proteins (, Table 1, p 296, Lowry, Rosebrough, Farr and Randall, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 1951, 193:265-275, <http://www.jbc.org/content/193/1/265.full.pdf> )

<sup>250</sup> Heller and Adler, A Chemical..., 1981, TOM 37

<sup>251</sup> Cf. "Hydrogen bonding between phenolic hydroxyl and peptide carbonyl is a major force stabilizing proanthocyanidin" Hagerman and Butler, The specificity of proanthocyanidin-protein interactions, *J. Biol. Chem.* 1981, 256, 4494-4497, <http://www.jbc.org/content/256/9/4494.short>

<sup>252</sup> Heller and Adler, A Chemical..., 1981, TOM 40-41

<sup>253</sup> cf. figures 18, 20 and 26, 28, 30, 31, 32, 38 of Svensson, Light microscopy..., <http://www.ligklaedet.dk/images/Light%20microscopy%20of%20fibers%20by%20Niels%20Svensson.pdf>

<sup>254</sup> Heller and Adler, A Chemical..., 1981, TOM 43, 50

<sup>255</sup> Heller, Report on..., 1983, p. 203

<sup>256</sup> "the darker portions of the image were not due to a variation of the degree of yellowing of the fibrils, but rather to the presence of more yellowed fibrils per unit area" (Jumper et al., A Comprehensive Examination..., 1984, p. 451

<sup>257</sup> Heller, Report on..., 1983, p. 171-172; Heller and Adler, A Chemical..., 1981, TOM 43

are soluble in xylene and in toluene<sup>258</sup>, so they may have been washed off of image fibers when these were freed from the adhesive of the sticky-tapes by washing with xylene (by Rogers-Janney)<sup>259</sup> and toluene (by Heller and Adler)<sup>260</sup> (see 4.2.1.1.). Therefore, the wet chemistry tests that could have detected alizarin and purpurin (tests for dyes and phenols) on these washed fibers may have been negative because the alizarin and purpurin had already been washed off by the xylene/toluene, before the tests. Yet, their absence on image fibers can be made plausible in another way.

Alizarin and purpurin are antioxidants,<sup>261</sup> and “reducing agents”, which means that they easily get oxidized.<sup>262</sup> Alizarin and purpurin dusts even “may form an explosive mixture with air, and any source of ignition, i.e. flame or spark, will cause fire or explosion. ... Avoid contamination with oxidizing agents i.e. nitrates, oxidizing acids, chlorine bleaches, pool chlorine etc. as ignition may result. ... Storage incompatibility ... Avoid reaction with oxidizing agents.”<sup>263</sup> As the image formation process is assumed to have caused an “acid catalyzed oxidation” of the fiber surface – “it is seen that the image fibrils are simply more dehydratively oxidized than the non-image fibrils”<sup>264</sup>, any acid madder dye on top of a starch coating would probably have been oxidized before or with the starch and have become part of the insoluble straw yellow image layer. The straw yellow color of the image layer could not be altered or extracted from image fibers by methanol or ethanol,<sup>265</sup> in which alizarin is soluble.<sup>266</sup> If the oxidation products of yellow madder dye on the Shroud would have been mere mono- or di-anion forms of the dyestuffs alizarin and purpurin – which anion forms might perhaps still get mordanted by iron/heme but are already pink by themselves, without mordant – the image fibers, and therefore the appearance of the body image on the cloth, would have been pink, not yellow. So, the straw yellow color of the more oxidized image layer shows that there are no alizarin or purpurin dyestuffs on it that can be mordanted pink. If there was only ‘painted-on’ mordantable yellow madder dye on image fibers, and not on non-image fibers, the image areas would have fluoresced stronger than the background, instead of weaker. Besides, this ‘dyed’ image would have to have been present before the blood mordanted these dyed image fibers, but not its non-image fibers. Another indication for the absence of mordantable madder dye on image fibers on the Shroud

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<sup>258</sup> Alizarin datasheet <http://datasheets.scbt.com/sc-214519.pdf>; purpurin datasheet <http://datasheets.scbt.com/sc-205822.pdf>; R.W. Sabnis, Handbook of Biological Dyes and Stains – Synthesis and Industrial Applications, 2010, John Wiley & Sons, [http://samples.sainsburysebooks.co.uk/9780470586235\\_sample\\_382294.pdf#page=30](http://samples.sainsburysebooks.co.uk/9780470586235_sample_382294.pdf#page=30) (alizarin: book page 10, pdf page 32); A US patent states “An ink jet head was produced in the same manner as in Example 2 except that a xylene solution of 1,2-dihydroxyanthraquinone was applied as a material absorbing the i-line to the ink flow path pattern”, and specifies that “Examples of the compound absorbing the i-line and having one or more phenolic hydroxyl groups include ... anthraquinones such as 1-hydroxyanthraquinone, 1,2-dihydroxyanthraquinone (alizarin), 1,4-dihydroxyanthraquinone (quinizarin), 1,5-dihydroxy-anthraquinone (anthrarufin), 2,6-dihydroxyanthraquinone (anthraflavic acid) and 1,2,4-trihydroxyanthraquinone (purpurin) ... These may be used either singly or in any combination thereof.” <http://www.google.com/patents/US8304177>

<sup>259</sup> Rogers, A Chemist’s..., 2008, p. 37; cf. Rogers, Scientific Method..., 2002, p. 14

<sup>260</sup> Heller and Adler, A Chemical..., 1981, TOM 37

<sup>261</sup> “The structural and electronic properties of hydroxyanthraquinone derivatives, alizarin, purpurin, pseudopurpurin, and their radicals were investigated using density functional theory. It turned out that these three molecules appear to be good candidates for high antioxidant activity species, particularly for pseudopurpurin. ... Phenols are widely used as antioxidants in living organisms.” Jin et al., 2011, Theoretical Study on the Antioxidant Activity of Alizarin, Purpurin, and Pseudopurpurin, DOI: 10.4018/978-1-60960-064-8.ch012, <http://www.igi-global.com/chapter/theoretical-study-antioxidant-activity-alizarin/48371> and <http://www.irma-international.org/viewtitle/48371/>; “The antioxidant activity of purpurin was elucidated by its bond dissociation enthalpy and ionization potential.” Jeremić et al., 2012, Antioxidant and free radical scavenging activity of purpurin, DOI: 10.1007/s00706-011-0695-z, <http://link.springer.com/article/10.1007%2Fs00706-011-0695-z>

<sup>262</sup> “Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.” <https://en.wikipedia.org/wiki/Antioxidant>.

<sup>263</sup> Alizarin datasheet <http://datasheets.scbt.com/sc-214519.pdf>; purpurin datasheet <http://datasheets.scbt.com/sc-205822.pdf>

<sup>264</sup> “acid catalyzed oxidation” Interview of Heller by Case (Case, The Shroud of Turin..., 1996, p. 63, 83); Heller and Adler, A Chemical..., 1981, TOM 46, cf. “acid oxidizing conditions” Ibid.

<sup>265</sup> Heller and Adler, A Chemical..., 1981, TOM 43

<sup>266</sup> R.W. Sabnis, Handbook of Biological Dyes and Stains – Synthesis and Industrial Applications, 2010, John Wiley & Sons, [http://samples.sainsburysebooks.co.uk/9780470586235\\_sample\\_382294.pdf#page=30](http://samples.sainsburysebooks.co.uk/9780470586235_sample_382294.pdf#page=30) (book page 10, pdf page 32)

is that “The color of image fibers was often stripped off of their surfaces, leaving molds of the fibers in the adhesive. ... The color in the molds has the same chemical characteristics as the image color”<sup>267</sup>. “These colored “ghosts” still show all of the chemical properties of the complete image fibers.”<sup>268</sup>

So, as there is dye on non-image fibers (see chapter 4) but there are no mordantable dyes on pure image fibers in the Shroud and as the color and spectra of the Shroud’s bloodstains are independent of the concentration of image fibers that would have been present in the location of the stain, the blood must have got unto these locations when all fibers were still yellow madder-dye-coated non-image fibers. So, if the Shroud’s bloodstains are red because of the formation of a red heme-madder lake, the blood got unto the Shroud before the image. The blood deposits in the bloodstains then would have protected the remaining madder dye beneath them from degradation (e.g. by photooxidation) during the image formation process and from further photooxidation during the subsequent ageing. Eventually the blood deposits would have been abraded from the cloth. (This has already been described in more detail in 2.1.2.2.)

Even if the image formation process created a latent image that was still invisible initially, there must have been a chemical difference between image and non-image fibers, especially in their top-most molecules, e.g. a loss of –OH groups of madder dye molecules, which groups would have been the substrate for heme-madder lake formation. Besides, if right after the image formation process there was no visible image, a painter would not have been able to paint the bloodstains in the correct locations on the imageless cloth.

## 2.2. Separate serum - UV-fluorescence halo on wrist

### 2.2.1. Identification of separate plasma/serum

Separate plasma/serum has been identified on the Shroud by means of

- a. the golden yellow/honey yellow color of fibers from tape samples from blood areas including the narrow tip of the wrist bloodstain<sup>269</sup>
- b. cementation of these golden yellow fibers, which were also present under darker material and not confined to the first layer of fibrils: “where the darker material has been removed, one can see “yellow” fibrils that appear to be coated and cemented together. These coated yellow fibrils are also a deeper yellow color than those in the body-only areas (6), and are not confined to the first layer of fibrils as are those in the body-only areas.”<sup>270</sup>; c.f. “the “shiny honey-yellow” color that Frache et al. [59] reported for the interior fibers of the “blood” threads ... It would seem that the yellow material in the “blood” areas derives from blood serum directly.”<sup>271</sup>
- c. localized fluorescence on parts of reddish brown dust particles under microscope (dust vacuumed from reverse side of Shroud) as shown in online photomicrographs<sup>272</sup>
- d. positive fluorescamine test on honey-yellow fibers<sup>273</sup>

<sup>267</sup> Rogers , A Chemist’s..., 2008, p. 109

<sup>268</sup> Carreira, The Shroud of Turin, p. 30, citing an email of Rogers to the Shroud Science Group

<sup>269</sup> Schwalbe and Rogers, Physics and Chemistry..., 1982, p. 37; “golden yellow fibrils” Adler, The Nature of ..., 1999, TOM 106-107; The sticky-tape sample of the narrow tip of the wrist blood (<http://www.shroud.com/maptap2v.htm> sample 3EF) belonged to the tape set used for blood tests by Heller and Adler (A Chemical..., 1981, Table 1 TOM 49).

<sup>270</sup> “where the darker material has been removed, one can see “yellow” fibrils that appear to be coated and cemented together. These coated yellow fibrils are also a deeper yellow color than those in the body-only areas (6), and are not confined to the first layer of fibrils as are those in the body-only areas.” (6 =Pellicori, *Applied Optics*, 1980) Jumper et al., A Comprehensive..., 1984, p. 459:

<sup>271</sup> Schwalbe and Rogers, Physics and Chemistry..., 1982, p. 37

<sup>272</sup> Blood-like particles in dust from reverse of Shroud are brown in transmitted visible light, and show blue fluorescing parts (of “serum”) in UV light, “possible crust of blood and serum”: Fanti, Statistical analysis..., 2008, <http://www.ohioshroudconference.com/papers/p16.pdf> fig. 10

<sup>273</sup> Heller wrote “we picked a fibril from what we believed was a serum area ... Using a one-lambda pipette ... we filled it with fluorescamine and added it to the fibril. ... the erstwhile honey-yellow fibril glowed with a positive test like a

- e. positive proteases test and positive Bromcresol Green test for serum albumin on golden yellow fibers<sup>274</sup>
- f. positive immuno-chemical test on a golden yellow fiber<sup>275</sup>
- g. the FTIR wavenumbers of free human serum albumin: they appear to be present in the FTIR spectrum of a golden yellow “serum” fiber (see table 6, and fig. 2.40). Besides, the FTIR spectra of “serum” fibers show some likeness to the FTIR spectrum of a (simulation of?) a mixture of whole blood and albumin-plus-bilirubin (cf. fig. 2.25, “simulated” curve).<sup>276</sup>
- h. the position of lighter margins only in UV – not lighter in visible light – relative to some blood flows (e.g. wrist and chest)

At least one margin, viz. the one in the shape of a halo around the narrow tip of the wrist bloodmark, is lighter only in UV: the ordinary light Durante 2002 photo as in Shroud Scope (fig. 2.41), also when contrast enhanced (fig. 2.42), shows no lighter margin here, but the UV-fluorescence photo (fig. 2.43) does. This margin that is lighter only in UV, seems to be part of the red-orange stain as seen in ShroudScope (showing darker colours than the Shroud 2.0 app), and could have been formed by orange concentrated plasma/serum.

The background of the Shroud is “weakly fluorescent”, but serum on linen “does fluoresce moderately”<sup>277</sup>, e.g. its minimal constituent lipofuscin fluoresces blue: 402 nm under 366 nm<sup>278</sup>, and also albumin – a major constituent – is fluorescent<sup>279</sup>; Pellicori wrote that “The light outline for blood in the lance wound area fluoresced noticeably.<sup>17</sup>” (note 17 = “As do blood plasma stains on linen in the laboratory.”)<sup>280</sup> Besides adding its own fluorescence, serum/plasma, by covering the fluorescent Shroud, would also have prevented the fluorescence-quenching effect of image formation on the Shroud.

Serum, now at the tip of the wrist bloodmark, could have flowed to the tip of the blood outflow when this blood was clotting on a vertical cold (body) surface, before it got unto the cloth. “Recently, Gil Lavoie has done some experiments in which he studied the clotting of blood and its transfer onto a linen cloth. ... he did the experiment of letting the blood clots hang vertically, and found that a lot of the exuded serum dripped off.”<sup>281</sup> This was only observed when the vertical surface was relatively cold – serum draining did not occur when the blood was on the relatively warm skin of a living person: “It should be noted that very little serum drained from the clots in the vertical position since the normal skin temperature hastened serum drying.”<sup>282</sup> This means that the wrist bloodmark may and almost must have been a transfer of an authentic clot of post-mortem blood, as the deliberate and artificial addition of a dark orange, concentrated serum halo to the tip of the wrist mark – a halo that can only be noticed in pure UV light –, or the artificial production of a serum draining blood clot on a cold vertical surface in order to imprint it on the Shroud, is a very improbable scenario. The postmortem blood may have flowed from an

bright green fluorescent beacon. We repeated this again and again on one honey-yellow fibril after another ...” (Heller, Report on..., 1983, p. 182).

<sup>274</sup> Heller and Adler, A Chemical..., 1981, TOM 40-41

<sup>275</sup> Adler, The origin and nature..., 1986, TOM 60

<sup>276</sup> Adler, Selzer and DeBlase, Further spectroscopic..., 2002, TOM 100-101, fig. 8 and 10

<sup>277</sup> Rogers, A Chemist’s..., 2008, p. 51; “Miller and Pellicori [14] suggested that the non-pigmented fluid itself may be fluorescing; they demonstrated that blood serum on linen does fluoresce moderately. ... the non-pigmented flow may have eradicated an existing image or it may have reduced or altogether prevented image formation. .... In view of the conclusions that the “blood” is blood and that the image is a cellulose degradation effect, there seem to be no likely reactions capable of eradicating the image in any way resembling the spot tests with strong reductant mentioned earlier.” (Schwalbe and Rogers, Physics and Chemistry..., 1982, p. 40).

<sup>278</sup> Heqedus et al., 1987, <http://www.ncbi.nlm.nih.gov/pubmed/2452619>

<sup>279</sup> Human serum albumin (a) fluoresces ca. 365 nm (peak wavelength) with a fluorescence tail until ca. 450 nm, at  $\lambda_{ex} = 295$  nm (fig. 1 <http://ars.els-cdn.com/content/image/1-s2.0-S0927776512007187-gr1.jpg> in Li et al., 2013, <http://www.sciencedirect.com/science/article/pii/S0927776512007187>; in another study it fluoresces ca. 340 nm under  $\leq 295$  nm excitation, Naveenray et al., 2010, <http://www.sciencedirect.com/science/article/pii/S0731708510002207> fig. 3.(b)A

<sup>280</sup> Pellicori, Spectral properties..., 1980, p. 1919-20, text and note 17

<sup>281</sup> Adler, The origin and nature..., 1986, TOM 60

<sup>282</sup> Lavoie et al., Blood on the Shroud..., part II, 1983, <http://www.shroud.com/pdfs/ssi08part3.pdf> p. 6

authentic crucifixion nailwound at the wrist, during and after removal of the nail from the cross and the wrist.

How the Shroud's separate plasma/serum may have been preserved through the centuries is discussed below in chapter 3.

HSA <sup>283</sup>	HSA <sup>284</sup>	HSA <sup>285</sup>	HSA <sup>286</sup>	HSA <sup>287</sup>	Shroud “serum”(1)	Shroud “serum”(2)	Shroud “serum”(3)	Shroud “serum”(4)
						790	805	
					925	925		920
						965		
					1025	1035		
					1060	1070	1075	
					1100			
						1120	1105	1125
					1155	1165	1160	1170
					1225	1235		1245
		1317			1300	1305		
1400		1409	1420		1390	1400	1385	1390
1450		1463	1460		1440	1445	1440	1450
						1505	1495	1515
1546	1561.3	1552	1548		1545	1545		1560
					1600	1620	1625	1625
1652	1648.5	1656	1653		1665	1640		
						1685	1680	1680
					1725		1735	
					2025	1940	1945	1875
					2520			
					2835		2840	
					2860	2865	2860	2865
					2915	2920	2915	2920
				2950	2945	2950	2950	2955
				3060	3045	3055	3060	3055
						3180		
				3300	3305	3295	3315	3270
				3430				

Table 6. Comparison of FTIR wavenumbers ( $\text{cm}^{-1}$ ) of free human serum albumin (HSA) and Shroud “serum” fibers (numbers estimated from plots and published numbers)<sup>288</sup>

<sup>283</sup> “Fig. 4 FT-IR spectra for free HSA and the difference spectra of the MBI–HSA complexes at the following molar concentration ratios of MBI to HSA: (a) free HSA, (b) 1:1, (c) 2:1, and (d) 3:1. CHSA = 0.25 mM, pH 7.4, T = 298 K..”  
<http://ars.els-cdn.com/content/image/1-s2.0-S0927776512007187-gr4.jpg> in  
<http://www.sciencedirect.com/science/article/pii/S0927776512007187>

<sup>284</sup> “Fig. 7 FTIR spectra and difference spectra of HSA/BSA; the FTIR spectra of free HSA (a1)/BSA (b1) (subtracting the absorption of buffer solution from the absorption of protein solution) and the FTIR difference spectra of HSA (a2)/BSA (b2) (subtracting the absorption of ETH-free form from that of ETH–HSA/BSA bound form) in phosphate buffer;  $C_{\text{HSA/BSA}} = 5 \mu\text{M}$  and  $C_{\text{ETH}} = 5 \mu\text{M}$ .”  
<http://ars.els-cdn.com/content/image/1-s2.0-S0731708511005310-gr7.jpg> in Li et al., 2013,  
<http://www.sciencedirect.com/science/article/pii/S0731708511005310>

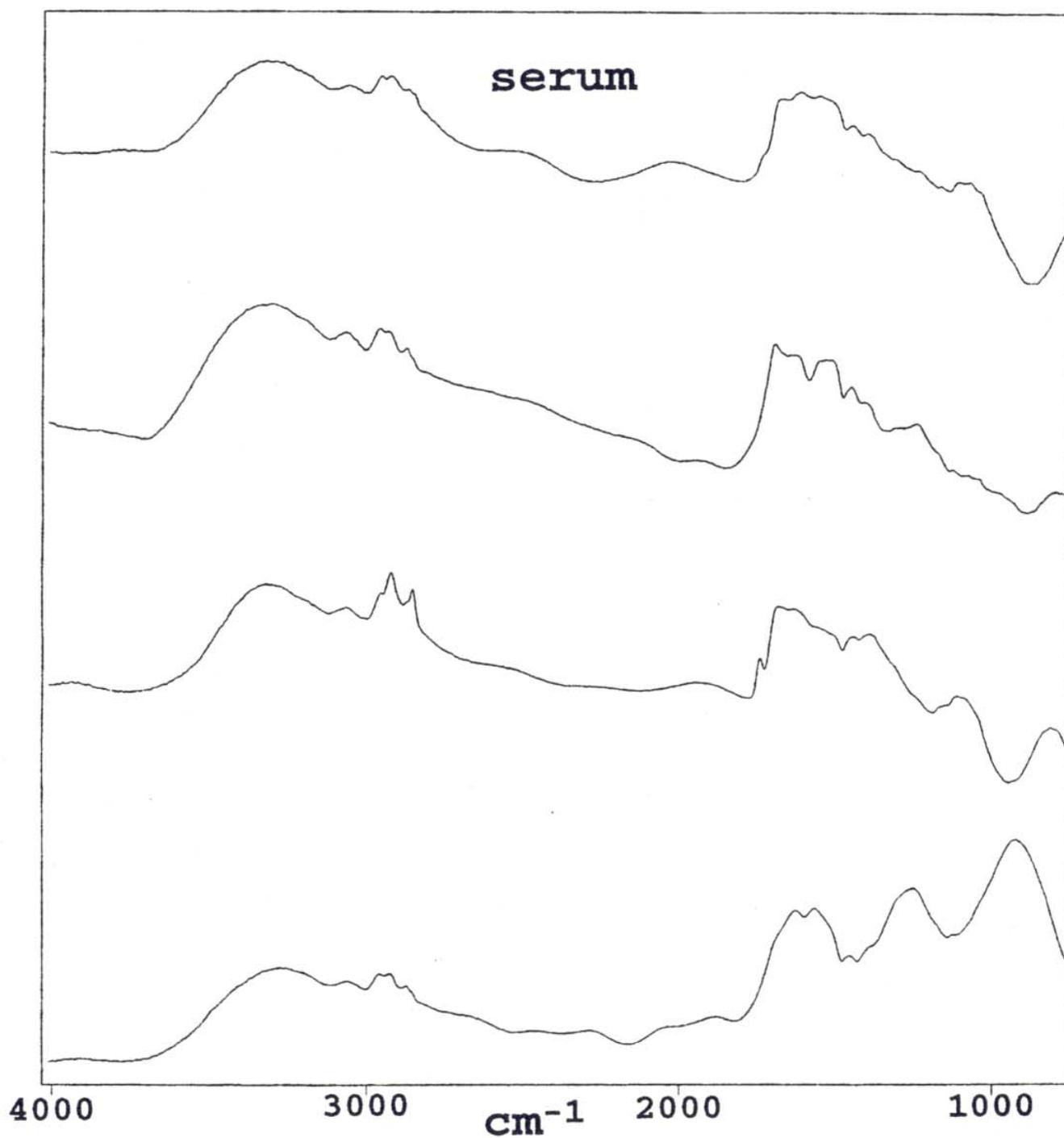
<sup>285</sup> “Fig. 1 FTIR spectra in the region of 1800–600  $\text{cm}^{-1}$  of hydrated films (pH 7.2), (A) for free HSA, free SFN (2 mM) and their HSA complexes; (B) free BSA (2 mM), free SFN (2 mM) and their BSA complexes with difference spectra (diff.) (bottom two curves) obtained at different drug concentrations (indicated on the figure).”  
<http://ars.els-cdn.com/content/image/1-s2.0-S1011134413000274-gr1.jpg> in Abassi et al., 2013,  
<http://www.sciencedirect.com/science/article/pii/S1011134413000274>

<sup>286</sup> “Fig. 6 FT-IR spectra and difference spectra [(HSA solution+TPNS solution)–(HSA solution)] of free HSA and its TPNS complexes in buffer solution with different drug concentrations (1–4) in the region of 1780–1380  $\text{cm}^{-1}$ . Difference spectra 1, 2, 3 and 4 corresponds to  $C_{\text{TPNS}}/C_{\text{HSA}}$  of  $0.282 \times 10^{-3}$ ,  $2.82 \times 10^{-3}$ ,  $28.2 \times 10^{-3}$  and  $141 \times 10^{-3}$ , respectively.”  
<http://origin-ars.els-cdn.com/content/image/1-s2.0-S1386142503000556-gr6.gif> in Liu et al., 2003,  
<http://www.sciencedirect.com/science/article/pii/S1386142503000556>

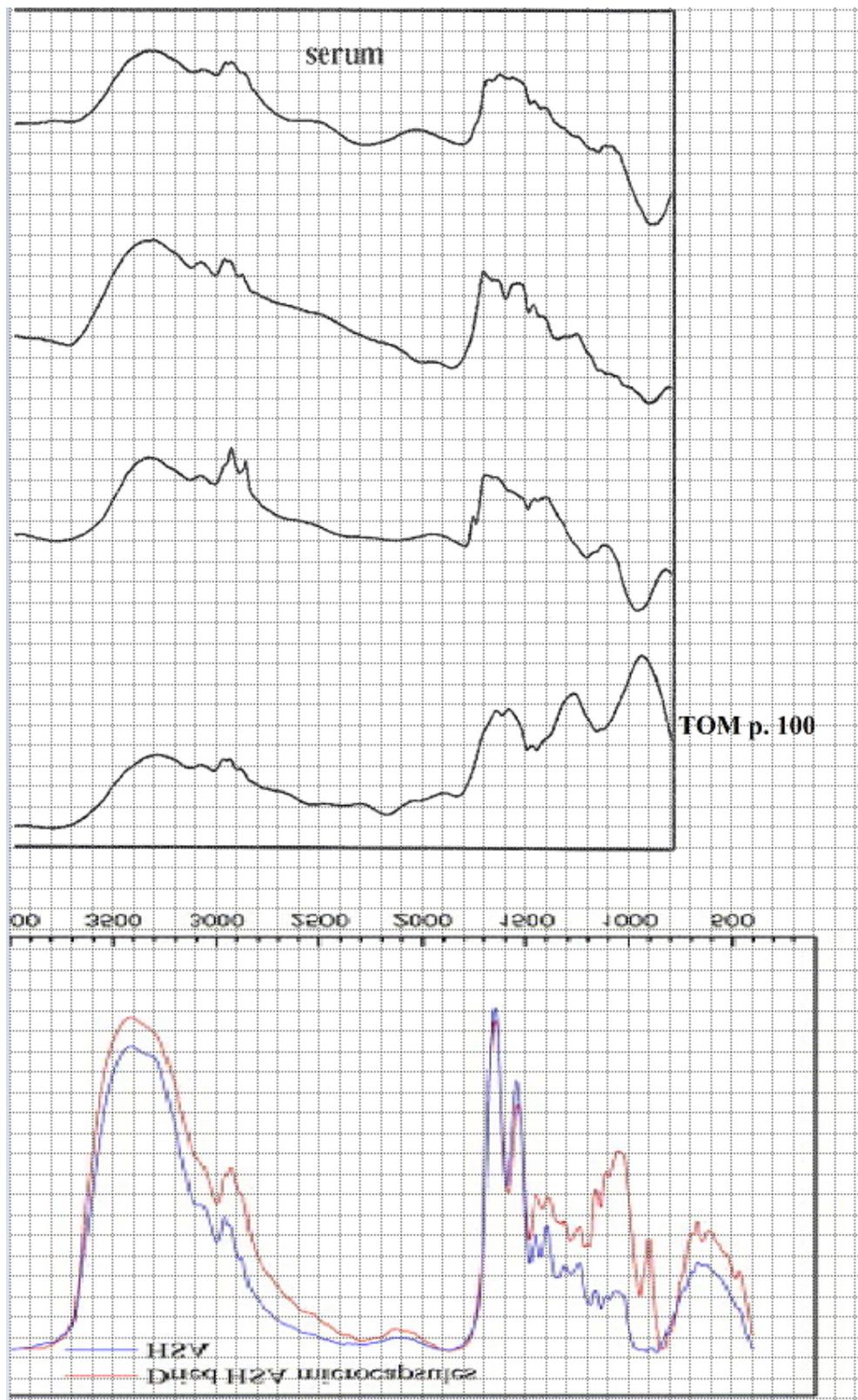
<sup>287</sup> “Fig. 4 (A) CD spectrum of HSA aqueous solution; (B) CD spectrum of the dried HSA microcapsules; and (C) FTIR spectra of HSA aqueous solution and the dried HSA microcapsules.”  
<http://ars.els-cdn.com/content/image/1-s2.0-S0006291X04000695-gr4.jpg> in Lu et al., 2004,  
<http://www.sciencedirect.com/science/article/pii/S0006291X04000695>

<sup>288</sup> Adler, Updating recent..., 1996, TOM 83 (and Adler, Selzer and DeBlase, Further spectroscopic..., 2002, TOM 100)

A:



*Fig. 8 Typical FTIR absorbance patterns of serum fibers*



B:

Fig. 2.40. A: FTIR spectra of Shroud "serum fibers" ©A.D. Adler, R. Selzer, F. DeBlase<sup>289</sup>  
 B: Comparison of FTIR spectra of Shroud "serum fibers" ©A.D. Adler, R. Selzer, F. DeBlase<sup>290</sup>, and FTIR spectra of Human Serum Albumin (blue = HSA in aqueous solution, red = dried HSA microcapsules) (mirrored figure) ©Elsevier<sup>291</sup>

<sup>289</sup> Used from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, *The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium*, Compiled by Michael Minor, Alexander Books, Alexander - North Carolina, 2002, pp. 166-181. (= fig. 8 on TOM 100), © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer.



Fig. 2.41. Wrist bloodstain in ordinary light ©Shroud Scope, Durante 2002<sup>292</sup>



Fig. 2.42. Wrist bloodstain in ordinary light; from Shroud Scope, contrast enhanced by Berry<sup>293</sup>

<sup>290</sup> Adapted from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, *The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium*, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-181. (= fig. 8 on TOM 100), © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer.

<sup>291</sup> HSA spectrum adapted (mirrored plot) from Gang Lu, Zhihua An, Junbai Li, Biogenic capsules made of proteins and lipids, *Biochemical and Biophysical Research Communications*, Vol. 315 Issue 1, Feb. 2004, pp. 224-227, DOI: 10.1016/j.bbrc.2004.01.042, <http://ars.els-cdn.com/content/image/1-s2.0-S0006291X04000695-gr4.jpg> in <http://www.sciencedirect.com/science/article/pii/S0006291X04000695> Copyright © 2004 Elsevier Inc. All rights reserved, with permission from Elsevier. (mirrored fig. C of fig. 4 “(A) CD spectrum of HSA aqueous solution; (B) CD spectrum of the dried HSA microcapsules; and (C) FTIR spectra of HSA aqueous solution and the dried HSA microcapsules.”)

<sup>292</sup> <http://www.sindonology.org/shroudScope/shroudScope.shtml?zl=11&image=3&lon=2952.0&lat=17595.0> on Shroud Scope <http://www.sindonology.org/shroudScope/shroudScope.shtml>; Latendresse, who offers Shroud Scope commented “The Durante 2002 photograph is a bit misleading because its coloration is not always accurate.” March 23, 2014 at 9:31 pm on <http://shroudstory.com/2014/03/23/baked-in-creases-really/>. Image used with permission from Latendresse.

<sup>293</sup> Used from C.S. Berry, Shroud Scope 10: my very own gallery of 20 close-up views of the Shroud – all lightly photo-edited for optimised colour-differentiation, <http://shroudofturinwithoutallthehype.wordpress.com/2012/06/25/shroud->



Fig. 2.43. Cropped UV-fluorescence photo of the wrist blood area ©2005 Raymond N. Rogers Collection, STERA, Inc.<sup>294</sup>

### 2.2.2. No fluorescent “serum” scratches but dark images of stripes

The photo published by Heimburger<sup>295</sup> that shows white scratches on a black-blue background on the calves is a negative of a UV-photo, not the original positive UV photo. In the positive UV photo, these scratches are dark, as in the corresponding ordinary white light photo.<sup>296</sup> Miller and Pellicori wrote: “Scourges – UV Fluorescent Characteristics : Highly absorbing and many resolve into scratch-like lines in parallel grouping of 3 or 4. – Visual Appearance : Reddish, diffuse in structure. Some dark spots seen within.”<sup>297</sup> So, these dark scratches certainly are not transferred serum, but rather transferred dark blood products or images of elevated stripes. These scratches are on the back of the ankles and calves, even in non-contact zones: their location is in the zone of back sample #5, labeled 1E, called “Image: Calf/Ankle (non-contact)”.<sup>298</sup> These dark stripes have more contrast in the original UV photo than in the corresponding visible white light photo, just as all body image has. Miller and Pellicori stated it literally: “Body Image UV: No color; prevents or absorbs the background fluorescence. Greater contrast with background here than in reflected visible; less gradation in shading across a body feature than contained in visible image”<sup>299</sup>; “Dorsal feet area ... Right foot ... The scourge marks have greater contrast in fluorescence than in white light and on the calf appear as lines rather than as dumbbell shapes as they do further up on the leg. Some scourges are not perceptible in the white light photo.”<sup>300</sup> Note that most of the scratches on the calves, as seen as white scratches in the negative of the UV photo, are indeed also visible as white scratches in the negative of the white light photo (see fig. 2.44). When speaking about the hands and ventral thighs, Miller and Pellicori also say that “Some scourge marks appear only in the fluorescence photos: examples are noted between the hands and the forearm areas”,<sup>301</sup> but perhaps they did not look for these scourge marks between hands and forearms in the negative of the ordinary photo.<sup>302</sup>

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[scope-10-my-very-own-gallery-of-20-close-up-views-of-the-shroud-all-lightly-photo-edited-for-optimised-colour-differentiation-25/](http://scope-10-my-very-own-gallery-of-20-close-up-views-of-the-shroud-all-lightly-photo-edited-for-optimised-colour-differentiation-25/), fig. 12, with permission from Berry

<sup>294</sup> Used from Rogers, A Chemist’s Perspective on the Shroud of Turin, 2008, fig. IV-2, p. 19,

[http://books.google.fr/books?id=p-n84sc4ayYC&printsec=frontcover&hl=fr&source=gbg\\_summary\\_r&cad=0#v=onepage&q&f=false](http://books.google.fr/books?id=p-n84sc4ayYC&printsec=frontcover&hl=fr&source=gbg_summary_r&cad=0#v=onepage&q&f=false) with permission from STERA, Inc.

<sup>295</sup> Heimburger, A Detailed..., 2008, p. 8, <http://www.shroud.com/pdfs/thibault%20final%2001.pdf>

<sup>296</sup> Miller and Pellicori, Ultraviolet fluorescence..., 1981

<sup>297</sup> Miller and Pellicori, Ultraviolet fluorescence..., 1981, Table I, p. 74

<sup>298</sup> Schwartz, Mapping of..., <http://www.shroud.com/maptap2d.htm>

<sup>299</sup> Miller and Pellicori, Ultraviolet Fluorescence..., 1981, Table 1, p.75);

<sup>300</sup> Miller and Pellicori, Ultraviolet Fluorescence..., 1981, p. 76

<sup>301</sup> Miller and Pellicori, Ultraviolet fluorescence..., 1981, p. 82 on Ventral hands and thighs

<sup>302</sup> In the digital photocopies of the printed UV photos in Miller and Pellicori’s JBP article – supplied by the publisher –, there are some dark (scourge) marks on the hips/thighs, between the hands and forearm regions, but these are also visible in the ordinary photo.



Fig. 2.44. Detail from the negative of an ordinary white light photo of the dorsal legs and ankles ©Shroud Scope, Durante 2002; white light photo, but all colors inverted and contrast-enhanced<sup>303</sup>

### 2.2.3. Some “serum” margins possibly a tenting effect around other (parts of) bloodmarks

It is important to note that fluorescent halos or margins weren't seen in Turin during the UV-fluorescence photography by the group of the Shroud of Turin Research Project (STURP), but only on the later developed fluorescence photographs. Schwalbe and Rogers say: “The u.v.-fluorescence photographs of Miller and Pellicori [14] reveal an interesting effect in several nominal image areas where “blood” flows are present: the dark “blood” regions are partially surrounded by margins that appear to fluoresce as intensely as do the non-image background areas. Weaver [3] has published four of these photographs along with drawings to illustrate the “light-colored” margin locations at the side wound, the “nail” wound in the wrist, and the “blood” flow at the right foot on the dorsal image. (At other “blood” locations, such as the flow across the back and the left dorsal foot region, no fluorescent margins are seen.) This unexpected effect is most clearly visible under the special conditions described above, and it is unfortunate that this observation was made only after the direct examination period.”<sup>304</sup> Here the sentence in parentheses, that explicitly negates the presence of fluorescent margins at other blood locations than parts of the blood on the side, wrist and right foot, belongs to the text of Rogers and Schwalbe that was published in *Analytica Chimica Acta* 135 (1982). Also Miller and Pellicori themselves state in 1981: “Blood UV: Highly absorbing. No color. Fluorescing borders apparent around some areas.”<sup>305</sup>

This is in sharp contrast to what Heller and Adler later said, in 1983, 1986 and 2000: “Miller had brought a large number of beautiful scientific photographs taken in Turin. Those taken by ultraviolet were most illuminating. At the margin of each scourge mark there was a pale white fluorescence that could not be seen in white light”<sup>306</sup>; “Also the border of every blood mark shows the typical

<sup>303</sup> settings -7, 100, 7 in MS Office Picture Manager. Image used with permission from Latendresse.

<sup>304</sup> Schwalbe and Rogers, *Physics and Chemistry...*, 1982, p. 39

<sup>305</sup> Miller and Pellicori, *Ultraviolet fluorescence...*, 1981, Table 1, p.75

<sup>306</sup> Heller, *Report on...*, 1983, p. 185

yellowish fluorescence of the serum exudate ring around scabs [...] (cf., Figure 2)” (Adler’s Fig. 2 = UV photo of upper part of frontal body image with side bloodstain)<sup>307</sup>; “The bloodmarks on the Shroud are all depressed in the centres, raised on the edges, and in the ultra-violet photography we can see around all of these a halo of the exuded serum.”<sup>308</sup> As Heller and Adler may have seen only a subset of the UV photos, and Miller and Pellicori are the ones who made, saw and published all of them, it is probable that Miller and Pellicori are correct, saying that “some” – not all – blood areas have fluorescing borders.

From comparison of unresolved photocopies of the published UV photos<sup>309</sup> and the Durante 2002 ShroudScope photos, it seems that many blood and scourge marks have no lighter border in any light. Comparison certainly makes clear that some marks have lighter margins in both UV and ordinary light. Miller and Pellicori mention them when describing the UV photos in their 1981 article, without pointing out that these margins are also lighter in visible light. When speaking about the dorsal midsection area, Miller and Pellicori say that “Many scourges have fluorescing bordering areas”, i.e. not all; on the dorsal feet area: “Left foot. ... A fluorescing border in the blood flow off the body image is seen. Right foot. A more distinct light border area is seen”; “The blood stains on the dorsal head area are bounded by brighter areas.”; “The lower left arm blood stains, B and C at 16, have light border areas.”; “The blood streaks in the hair are denser on the right side and have fluorescing boundaries, C and D at 13”; “On the right shoulder, the blood stains are in very sharp detail, with the lower stain broken into dots. Compare this area with some of the scourges on the right side. Circles of yellow-green fluorescence are associated with these wounds”; “Notice the clear fluorescing borders around the hand wound blood stains”<sup>310</sup> - at the bottom and around the broad tip, the wrist bloodmark indeed has lighter margins in both UV and visible light. These doubly lighter margins might have been formed by

- a) perhaps a very clear plasma/serum that prevented image formation and/or darkening of the cloth in ageing (in that case the plasma/serum would be pre-image, for a post-image painted-on ‘serum’ would not have created lighter margins in ordinary light). The presence of such a very clear plasma/serum on the Shroud seems improbable because of the dark orange, strongly fluorescent halo on the wrist.<sup>311</sup> However, if this halo is dark orange only because a lot of golden-yellow serum concentrated here during drying on the wrist, the lighter margins might perhaps be golden-yellow serum that is only present as a very thin film around the fibers, where it dispersed through the waterresistant Shroud and/or drained through it to an adsorbing submaterial.
- b) plasma/serum that flaked off (in that case the plasma either flaked off together with the less fluorescent image layer beneath it, or else the plasma had prevented image formation, and later flaked off, leaving the more fluorescent and lighter non-image layer beneath it intact on the cloth). This flaking off from the cloth would have to have taken place in a margin form around some stains. Such a margin form seems improbable for flaking off of blood material, but seems a possibility for a plasma/serum margin around the red blood material. A plasma/serum deposit would have other mechanical properties than a red blood cell/hemolysate deposit, and its chemical tie with the cloth would be different and, after abrasion, it could leave a lighter margin in visible light.
- c) a kind of ‘tenting effect’ in image formation (in that case the welt or elevated clot would be pre-image): electrostatic field lines ‘tent-away’ from concave surfaces such as those surrounding a welt or dry elevated blood clot, but concentrate on convex or pointed surfaces: photos of corona discharge experiments show that the corona discharge marks of concave hollows are light, and

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<sup>307</sup> Adler, *Chemical and Physical...*, 2002, TOM 14

<sup>308</sup> Adler, *The Origen and Nature...*, 1986, TOM 60

<sup>309</sup> Supplied by the copyright owner in 2012, *the Journal of Biocommunication*, <http://www.jbiocommunication.org/archives.html>

<sup>310</sup> Miller and Pellicori, *Ultraviolet fluorescence...*, 1981, p. 79; 76; 80; 81; 81; 81; 82, respectively

<sup>311</sup> The presence of a lot of concentrated clear/golden serum on top of red blood material at this tip on the cloth is improbable, for serum that is drying on top of a red blood clot on a body, would get into the cloth first and then be covered by the red blood material from the clot and thus would not be strongly fluorescent.

those of convex hills are dark with a light surrounding halo because of their concave margins<sup>312</sup>; for scorch tenting from a bas-relief, the artist would need to have made a welt or clot bas-relief, and to have painted its dark mark red later.

So, all of these three mechanisms seem possible explanations of the doubly lighter margins. As there are lighter borders in UV and visible light around bloodmarks next to the dorsal left foot, which are not in an image area, but in an area that looks a bit brownish-orange in Shroud Scope and is not strongly fluorescent (perhaps from some waterstain or scorch material), an image-tenting effect could not have been the cause of these off-image lighter margins. Here draining or abrasion of serum/plasma seems the best explanation. As the only sample taken from blood on a scourge mark (sticky-tape 1FB = "Image: Blood on Scourge Mark"<sup>313</sup>, taken from one of the calves) did not belong to the sample set that Heller and Adler had at their disposal for the testing of blood<sup>314</sup>, there is no chemical evidence for the presence or absence of serum/plasma on fibers adjacent to scourge marks.

### 2.3. No potassium signal in three X-ray fluorescence spectra of bloodstains

#### 2.3.1. Postmortem blood is hyperkalemic

Morris, Schwalbe and London<sup>315</sup> reported that X-ray fluorescence measurements were made of three 1.3 cm<sup>2</sup> blood areas of the Shroud: #1: red outer off-image blood stain next to dorsal foot, at the location of sticky tape sample 1AB; #18: red outer part of the 'question mark' blood stain in hair image, last sample point in a series of measurements (scan) from the tip of the nose toward the bloodstain; #23: a red part in the middle of the bloodstain at the side, along and to the left of the transverse weave fault across the middle of the blood area.<sup>316</sup> In datapoint #18, in the hair image, the iron concentration was higher than the background level, but not significantly. From the data published by Morris et al. can be concluded that, although the excess iron (Fe) concentration of the off-image bloodstain above background level was almost twice as high as the iron concentration they detected in whole blood soaked in Whatmann paper ("about 20 µg cm<sup>-2</sup> above background" in datapoints #1-7, vs about 12,5 µg cm<sup>-2</sup> in Whatmann paper<sup>317</sup>), no potassium (K) signal was observed in the Shroud data while they did observe a potassium signal from the blood in the Whatmann paper. Excess iron levels for the Shroud datapoint of the side may even be as high as 30-40 µg cm<sup>-2</sup>. The blood potassium signal being "typically at least an order of magnitude smaller" than that of iron, the article of Morris et al. says that "poor signal-to-noise ratios may preclude definite conclusions on this point".

Yet, a lack of potassium in a surplus or iron would be exactly consistent with the chemistry of red blood cells of post-mortem blood that had lost its plasma/serum. In normal whole blood, the potassium level in the red blood cells is about ten times as high as in the plasma.<sup>318</sup> When blood becomes acid in a living person (a condition called acidemia), the potassium level in the plasma rises with respect to the potassium level in the red blood cells, as if potassium to some degree moves out of the red blood cells into the plasma; the mechanism and strength of this effect depend on the cause

<sup>312</sup> Fig. 5 and especially fig. 13 of Fanti et al., Body image formation..., 2005, <http://www.dii.unipd.it/-giulio.fanti/research/Sindone/corona.pdf>

<sup>313</sup> Schwartz, Mapping of..., <http://www.shroud.com/maptap2d.htm>

<sup>314</sup> Heller and Adler, A Chemical..., 1981, Table I, TOM 49; the golden yellow coated fibrils, that were examined by Heller and Adler, and that revealed the presence of serum, were "(c) golden yellow coated fibrils adjacent to heavy blood flows" (Heller and Adler, A Chemical..., 1981, TOM 36)

<sup>315</sup> Morris et al., X-Ray Fluorescence..., 1980

<sup>316</sup> in this Shroud Scope view:

<http://www.dshroud.com/shroudScope/shroudScope.shtml?zl=11&image=4&lon=8341&lat=4068>

<sup>317</sup> "We measured whole blood iron concentrations to be about 0.5 µg mm<sup>-3</sup> and found that roughly 25 mm<sup>3</sup> saturated a 1 cm<sup>2</sup> area of 10 mg cm<sup>-2</sup> Whatmann 42 paper." (Morris et al., X-Ray Fluorescence..., 1980); this is roughly 25 x 0.5 µg per cm<sup>2</sup>.

<sup>318</sup> [http://en.wikipedia.org/wiki/List\\_of\\_human\\_blood\\_components](http://en.wikipedia.org/wiki/List_of_human_blood_components)

of the acidemia, and the effects are small but increase progressively with time.<sup>319</sup> When a person dies, the blood not only becomes more acid (see 5.1), but there are also big potassium movements immediately. “Upon death, potassium quickly begins to diffuse out of cells into the extracellular fluids.”<sup>320</sup> As to the chemistry of postmortem blood, “Jetter pointed out that within one hour after death there was a marked increase in potassium with values up to 18 mEq per liter followed by further although gradual increase in the levels.<sup>58</sup> It has been shown by extensive work in blood banks and elsewhere that the release of potassium from the cells occurs so rapidly after death as to make evaluation of potassium metabolism impossible by any known method.<sup>127</sup>”<sup>321</sup> As to the determination of the postmortem interval, a forensic standard work says that “potassium breaks down in the cells of the body, including blood, and rises precipitously following death thereby prohibiting serum levels from being used.”<sup>322</sup>

About 80% of the chloride (atomic number 17) that is present in whole blood “as NaCl”, resides in the plasma.<sup>323</sup> Furthermore, whole blood contains about ten times more chloride than iron, in relative mass, and about 17 times more, in molar ratio.<sup>324</sup> The X-ray fluorescence measurements on the Shroud seems to have shown no discernable chloride signal, as chloride is not mentioned in the text of the article. The atomic number of chloride is 17, and therefore should have been detectable by the X-ray fluorescence equipment, that “allowed detection of elements with atomic numbers greater than 16”.<sup>325</sup>

### Microscopic blood sample techniques

The techniques, used at other times by other investigators, which did detect a (too) small potassium signal from Shroud blood samples in some but not all cases, as described below, were all microscopic techniques and therefore do not replace or nullify the observation of STURP’s X-ray fluorescence technique on 1.3 cm<sup>2</sup> areas that did detect potassium in whole blood on Whatmann paper, but did not detect any potassium in the three Shroud bloodstain areas. STURP’s X-ray fluorescence technique showed that the overall potassium level is remarkably reduced in at least these three Shroud bloodstain areas.

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<sup>319</sup> “immediately following the induction of the acidemia, the change in the plasma potassium concentration per unit change in pH is very small, but it increases progressively with time” (Sterns et al., Internal potassium balance and the control of the plasma potassium concentration, 1981, <http://www.ncbi.nlm.nih.gov/pubmed/6268928>); “Acute respiratory acidosis in dogs causes hyperkalemia as a result of movement of potassium out of the cells.” (Scribner et al., The effect of acute respiratory acidosis on the internal equilibrium of potassium, 1955, p. 1281-1282, 1285 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC438696/pdf/jcinvest00626-0040.pdf>); “acute organic-acid acidosis is not associated with a significant change in plasma potassium concentration. ... Spontaneous recovery from grand mal seizure-induced lactic acidosis in man was not accompanied by a significant change in plasma potassium concentration. ... With few exceptions ..., the available studies clearly demonstrate that acute respiratory acidosis results in an increment in plasma potassium concentration.” (Adrogué and Madias, Changes in plasma potassium concentration during acute acid-base disturbances, 1981, [http://www.amjmed.com/article/0002-9343\(81\)90182-0/abstract](http://www.amjmed.com/article/0002-9343(81)90182-0/abstract)).

<sup>320</sup> Postmortem Chemistry, Taylor and Francis Group, 2012, [http://www.elliottlakeinquiry.ca/exhibits/pdf/09242-DMQ\\_E000003331.pdf](http://www.elliottlakeinquiry.ca/exhibits/pdf/09242-DMQ_E000003331.pdf), (p. 239/ 5 of pdf)

<sup>321</sup> Coe, J.I., Postmortem chemistry of blood, cerebrospinal fluid, and vitreous humor, pp. 1033-1060 in Forensic medicine : a study in trauma and environmental hazards / Tedeschi, Cesare George (ed.), 1977, (quote from p. 1040-1041, its references are: “58. Jetter, W.W., and McLean, R.: Biochemical changes in body fluids after death. *Am. J. Clin. Pathol.*, 13:178, 1943 ... 127. Walla, B.N.J.S., Sarin, G.W., Chandra, R.I., and Ghai, O.P.: Preterminal and postmortem changes in serum potassium of children. *Lancet*, 1:1187, 1963.”)

<sup>322</sup> Sturner WQ, 2006. Chemical considerations, in Medicolegal Investigation of Death, 4<sup>th</sup> ed., ed by W.U. Spitz, Springfield, IL, Charles C Thomas, pp. 128-148. <http://www.bol.com/nl/p/spitz-and-fisher-s-medicolegal-investigation-of-death-guidelines-for-the-application-of-pathology-to-crime-investigation/1001004002763529/> ( Sturner’s reference 1 is 1. Coe, J.I.: Postmortem chemistry of blood, cerebrospinal fluid and vitreous humor. In Wecht, C.H. (Ed.): Legal Medicine Annual 1976. New York: ACC, pp. 55-92, 1976.)

<sup>323</sup> [http://en.wikipedia.org/wiki/List\\_of\\_human\\_blood\\_components](http://en.wikipedia.org/wiki/List_of_human_blood_components)

<sup>324</sup> [http://en.wikipedia.org/wiki/List\\_of\\_human\\_blood\\_components](http://en.wikipedia.org/wiki/List_of_human_blood_components); relative atomic mass chloride = 35, relative atomic mass iron = 56

<sup>325</sup> Morris et al., X-Ray Fluorescence..., 1980, p. 40

### *Baima Bollone*

Kearse and Heimburger reported that Baima Bollone used EDS, analysing “a volume of several micrometers” of Shroud “blood threads”, among which a thread from the “sole of the left foot”, and found potassium (K).<sup>326</sup> Kearse and Heimburger reproduced the plot of the SEM-EDX spectra of Shroud blood and of whole blood on cloth<sup>327</sup>: both have K and Cl peaks, but the Shroud K and Cl peaks are much smaller than in the whole blood curve, and the Ca and Fe peaks are larger than in the whole blood curve. Kearse and Heimburger explain that “it is possible that the calcium (and iron) excess found in the “TS blood” spectrum is due to the high amount of calcium and iron bounded to the underlying fibers as shown by Heller and Adler.”<sup>328</sup>

### *Heller and Adler*

Heller and Adler studied microscopical “globs” and “fibrils” from blood, image, and non-image tape samples, using an Energy Dispersive Spectrometer (EDS): “The fibrils all show strong Ca and Fe signals. The globs all show Na, Mg, Al, Si, P, S, Cl, K, Ca and Fe. Some also show Cu and Zn.”<sup>329</sup>

### *Fanti, Calliari and Canovaro*

A red particle that seemed organic was studied by SEM and EDX (called EDS in the Italian book describing the tests<sup>330</sup>): “The spectra of Fig. 21 reveal the presence of Si, Mg, Na, Cl, Ca and Fe. It is to note that these spectra are very similar to those obtained by P.L. Baima Bollone.”<sup>331</sup> An important difference is that potassium (K) is missing in the spectra obtained by Fanti.

### *McCrone*

An EDS published by McCrone as “Figure 12. Energy dispersive spectrum for a single a blood-image sherd” shows a high S peak (labelled Hg/S by McCrone), and high Ca, Fe, Cl, and Si peaks, but only a very small K peak.<sup>332</sup> Sulfur is the highest peak in the EDS of wole blood on cloth published by Baima Bollone and reproduced by Kearse and Heimburger.<sup>333</sup>

## **2.3.2. Vertical serum draining**

The separation of potassium-rich plasma/serum from the potassium-poor red blood cells of postmortem blood is a simple explanation of the lack of detectable potassium in the iron-overloaded red Shroud bloodstains. “Heller has suggested that because potassium compounds in blood are quite soluble, they may have been dispersed in the presence of moisture”.<sup>334</sup> This moisture that took away the potassium from the red material, most probably was the postmortem plasma itself, as separate serum/plasma was found on the Shroud (see 2.2.). This separation of serum from red clotting blood may have taken place by vertical draining (cf. fig. 2.45), which, according to Lavoie’s experiments, hardly takes place on the skin of a warm living human body, but readily on a relatively cold surface (room temperature, “70-80°F”).<sup>335</sup>

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<sup>326</sup> Kearse and Heimburger, *The Shroud Blood Science...*, 2013, p. 3-5

<sup>327</sup> Kearse and Heimburger, *The Shroud Blood Science...*, 2013, p. 4

<sup>328</sup> Heller and Adler, *A Chemical...*, 1981, TOM 41, describe “relatively strong positive tests only for Ca and Fe ... probably a chelated covalent form”.

<sup>329</sup> Heller and Adler, *A Chemical...*, 1981, TOM 48

<sup>330</sup> Fanti and Gaeta, *Il mistero della Sindone*, p. 181

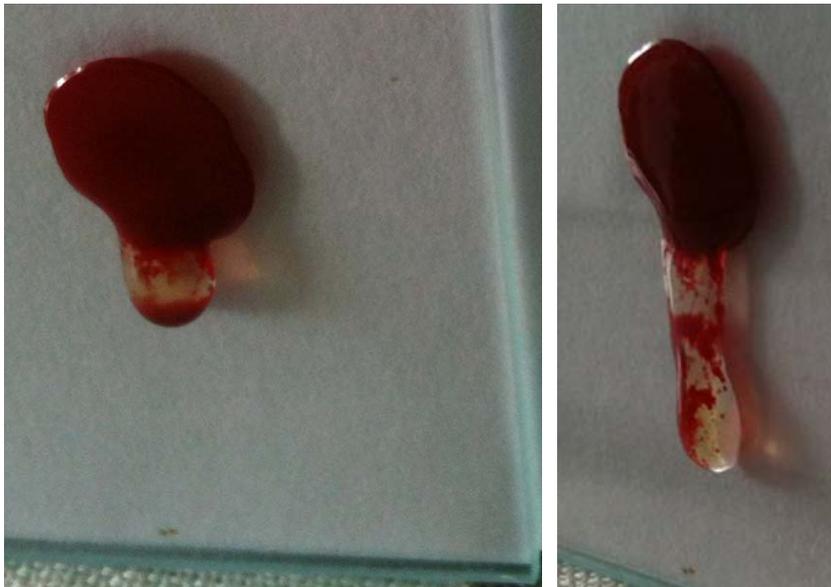
<sup>331</sup> Fanti et al., *Analysis of micro-particles...*, 2012, <http://www.dii.unipd.it/-giulio.fanti/research/Sindone/Paper%20Valencia.pdf> p. 16

<sup>332</sup> McCrone, *The Shroud of Turin: Blood or Artist’s Pigment?*, 1990

<sup>333</sup> Kearse and Heimburger, *The Shroud Blood Science...*, 2013, p. 4

<sup>334</sup> Schwalbe and Rogers, *Physics and chemistry...*, 1982, p. 38

<sup>335</sup> “One half hour after the clots were placed on the saran wrap, the specimens were placed vertically. ... Clear serum dripped down from these stable clots ... it continued to drip for another 40 minutes.”; “After ½ hour, the skin surface on which the blood had clotted was changed to the vertical position. ... It should be noted that very little serum drained from the clots in the vertical position since the normal skin temperature hastened serum drying.” Lavoie, *Blood on the Shroud of Turin: Part II*, <http://www.shroud.com/pdfs/ssi08part3.pdf>, procedure 1, p. 3-6



A:

B:

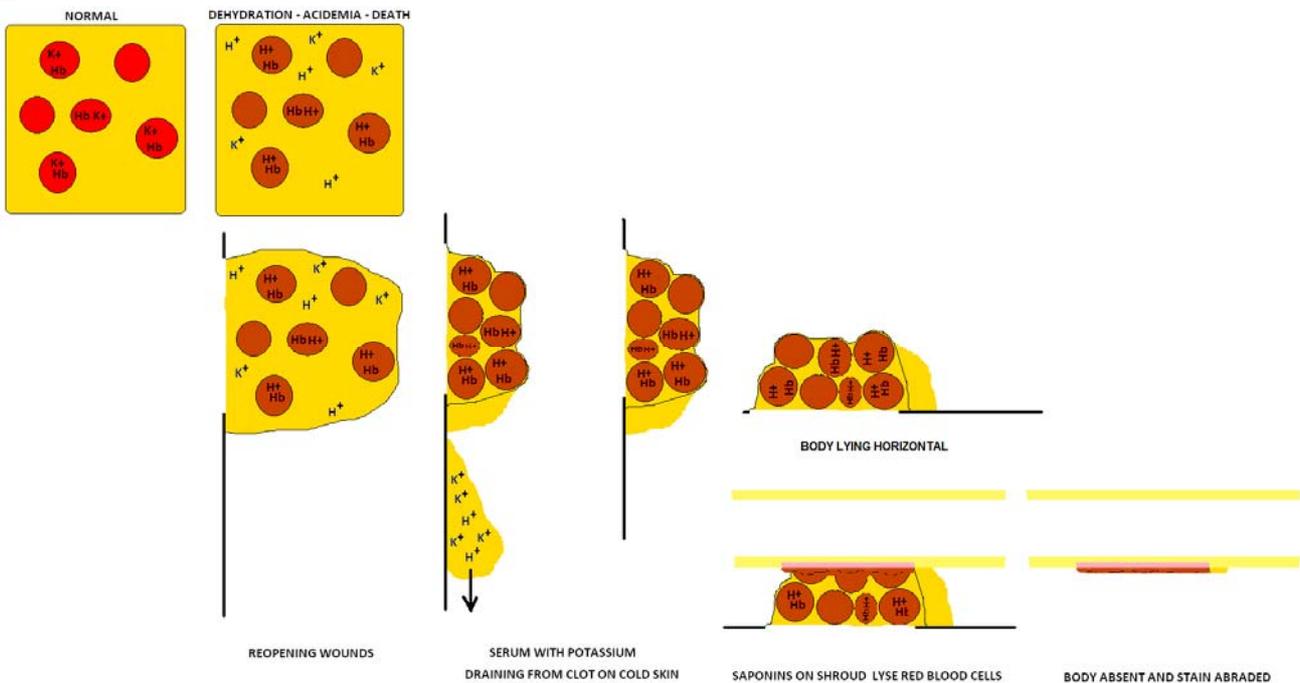


Fig. 2.45. A: Serum draining from a blood clot on a glass plate that was placed from horizontal to vertical 30 min after the fresh whole blood was dripped unto it and left to clot in a room at 18-19 °C, 60-70% relative humidity. B: Sketch of hypothesized vertical serum draining and hemolysis and lake formation on the Shroud. ©AvdH

If at some point during the clotting of the blood, determined by the lower water content<sup>336</sup> and the changing clotting-lysing equilibrium of postmortem blood<sup>337</sup> (see 5.1 and 5.5.) and also by the air

<sup>336</sup> “The water content of postmortem blood was observed to range from 60 to 90%”, G. Skopp, Preanalytic aspects in postmortem toxicology, *Forensic science international*, Volume 142, Issues 2–3, 10 June 2004, Pages 75–100, <http://www.sciencedirect.com/science/article/pii/S0379073804001021>

<sup>337</sup> “Regardless of cause of death, the lower the blood pH, the higher the serum FDP [Fibrinolysis Degradation Products] level increase. ... In the vascular perfusion system, the lower the pH of the perfusate, the higher the fibrinolytic activity.” (Fluidity of Cadaveric Blood After Sudden Death: Part III: Acid-Base Balance and Fibrinolysis, Takeichi, Sanae; Tokunaga, Itsuo; Hayakumo, Koji; Maeiwa, Michihiko, *American Journal of Forensic Medicine & Pathology* 7 (1), 1986 [http://journals.lww.com/amjforensicmedicine/abstract/1986/03000/fluidity\\_of\\_cadaveric\\_blood\\_after\\_sudden\\_death\\_7.a\\_spx](http://journals.lww.com/amjforensicmedicine/abstract/1986/03000/fluidity_of_cadaveric_blood_after_sudden_death_7.a_spx)

temperature and humidity, the red material of the clot was too dry for draining down inside the Shroud and for a complete direct transfer, the serum margin at the bottom of the clot may still have been wet enough for direct transfer. Lavoie noted that “As time passes, the upper portion of each clot dries faster than the lower, allowing only the lower portion of the clot to be imprinted.”<sup>338</sup> In such a condition, saponins on the Shroud could have lysed the drying red blood cells at the clot surface, allowing the imprint of both a larger part of the red clot in the form of a red dense hemolysate stain and the imprint of the serum margin at the bottom of it.

Also in the case of the blood on the side, its serum may have exuded and drained from clotting post-mortem blood on a cold dead body – the UV photo published and probably contrast enhanced by Weaver, shows that, although there is lighter fluorescence along other margins of the side bloodstain, it is most notable along the bottom of the stain.<sup>339</sup> In his presentation in Rome 1993, Jackson showed the, probably original, fluorescence photo of the side, as also published in the article by Miller and Pellicori, in which the top and upper anatomical-left margin of the bloodstain shows the lightest fluorescence.<sup>340</sup>

### 2.3.3. Horizontally and vertically imprinted serum halos

An experiment showed that a glob of three drops of fresh whole blood from a finger stick, fallen on a glass plate straight from the finger, in a relatively cold environment (18-24 °C), clots in such a way that when a piece of linen is dropped on it from 1 cm above it, about an hour and a half after the release of the blood, a serum halo is formed in the cloth (fig. 2.46). Also when a similar bloodclot on a horizontal glass plate is placed vertical, half an hour after blood release, and a piece of linen is taped on it about an hour later, it is still possible to obtain a serum halo around the imprint on the cloth (fig. 2.47).



bloodclots right before imprinting

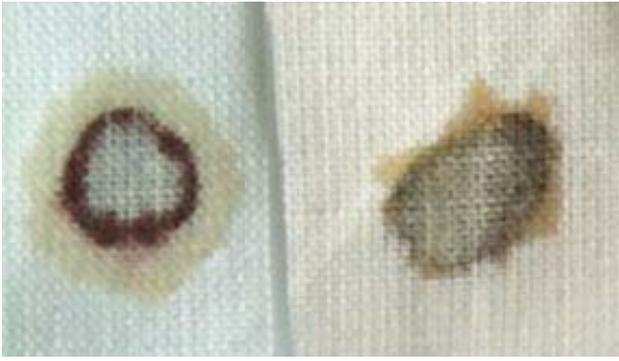


7 min after imprinting

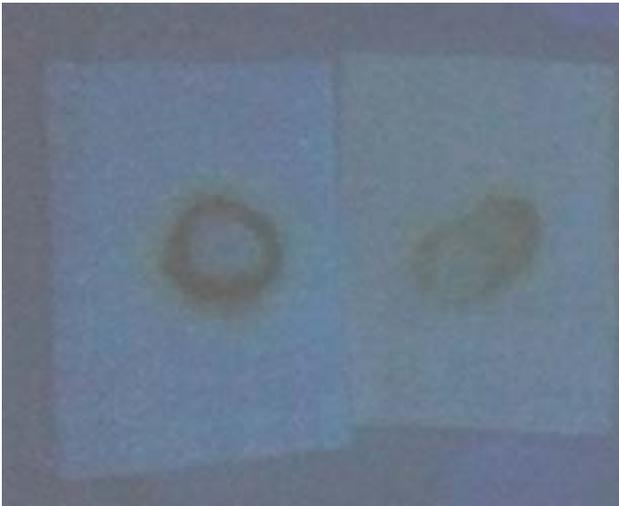
<sup>338</sup> Lavoie et al., Blood on the Shroud... Part II, p. 6

<sup>339</sup> Weaver, Science seeks to solve..., 1980. Also in Adler, Chemical and Physical..., 2000, fig. 2. TOM p. 14, and the UV photo is online in Fanti and Faccini, Sindone: la scienza spiega la fede, p. 11 <http://www.dii.unipd.it/-giulio.fanti/research/Sindone/Mostra%20Sindone.pdf>; Although the resolution of this UV photo is not high, it seems the fluorescence results from a clearer margin in visible light (cf. <http://www.dshroud.com/shroudScope/shroudScope.shtml?zl=10&image=3&lon=3410&lat=14378>) either from clear (diluted?) serum or perhaps from (orange) concentrated serum that largely abraded here after the UV photo was taken, leaving a light margin surrounded by the darker image of the side.

<sup>340</sup> Jackson, New Evidence that the Turin Shroud was the Mandyllion, presentation Rome 1993, <https://www.youtube.com/watch?v=WGCi-FALoDg> from 32:22; cf. Miller and Pellicori, Ultraviolet..., 1981, fig. 8



17 hours after imprinting



2 days, under UV lamp, Hoya L-42 UV filter in front of camera, no blue-light filter in front of Wood lamp (see blue specular reflection from plate), brightness-enhanced



8 days, contact side, most crusts removed



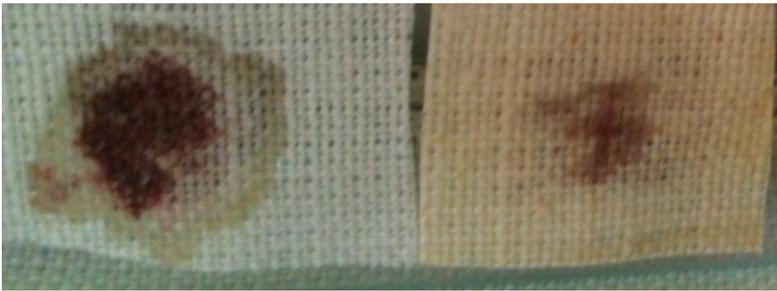
8 days, contact side, more crusts removed

*Fig. 2.46. Horizontal imprints in pure linen and starched-washed-and-madder-dyed linen, of blood clots formed during 1.5 hour of clotting of the same fresh whole human blood in  $\sim 23$  °C, 60-70%*

*relative humidity on a horizontal glass plate. Note that these new imprints of normal warm alkaline blood (pH ~7.4) on loosely woven linen are not directly comparable to old imprints of cold acidic post-mortem blood on the tight weave of the Shroud. ©AvdH*



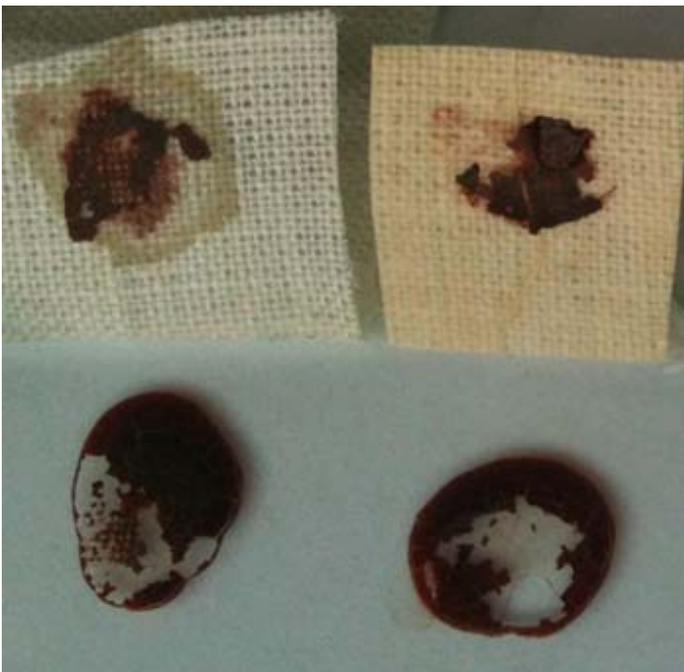
1.5 hours old blood clots



1 day old imprint, non-contact side



1 day old, contact side seen through glass plate



1 day old, contact side, just lifted upside down



15 days old, contact side, most crusts just removed



28 days old, contact side

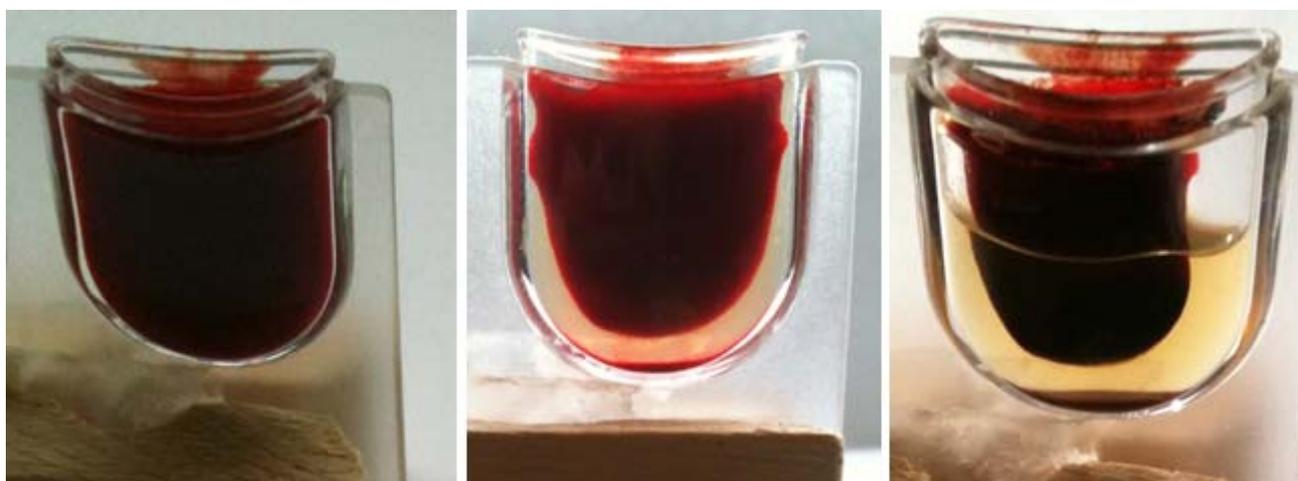


*Fig. 2.47. Vertical clot imprints on pure and starched-and-madder-dyed linen. Photos: 1.5 hour-old blood clots (placed vertical 30 min after blood was released from finger on horizontal glass plate in a room at 18-19 °C and 60-70% rel. humidity) right before imprinting; non-contact side, contact side seen through the reverse side of the glass plate, and contact side just lifted up from the blood clot, respectively, 24 hours after blood release. The red/orange flow away from the clot was caused by the manual turning around of the glass plate to see the reverse. Last photos: contact side, 15 days and 28 days after imprinting, and UV-fluorescence photo (Hoya L-42 UV filter, no blue-light filter), most loose crusts removed from the stain. ©AvdH*

### 2.3.4. Filter effect

In the case of the most lateral small bloodstain next to the dorsal right foot, a drop of cold post-mortem blood may have fallen on the dorsal half of the Shroud, of which the fibers had a coating of retrograded and thus waterresistant starch (this will be discussed in chapter 4) and therefore functioned as a kind of filter for the blood, allowing the plasma to drain down through the Shroud and get absorbed into a waterabsorbing substrate beneath it, for instance a burial bier of bare dry wood. This small red and brown stain is also visible on the reverse side of the Shroud, so, either red blood cells or red hemolysate (see 2.4.) or both soaked down through the Shroud as well. Note that no UV-vis reflectance spectrum was measured at this particular stain.

That the STURP team did not detect an in situ XRF potassium reflectance signal from the top layer of the observe side of the outer right stain beside the right dorsal foot, but Baima Bollone did find a EDS potassium signal from a cut-out thread (approachable from observe and reverse side) of the bloodstain at or next to the left dorsal foot, may be due, besides to the different locations of the stains, to the draining of the serum from the top of the waterresistant Shroud (observe side) to the back of the Shroud (reverse side) and the substrate beneath it, such as a wooden burial bier. That plasma/serum, in some circumstances, can drain down from clotting blood even inside a container is shown in fig. 2.48.



*Fig. 2.48. Fresh whole blood separating into serum and red material in a narrow plastic container, right after blood drawal, 1.5 hour after, and 16 hours after, respectively. ©AvdH*

An indication for the possibility of a filter effect of the Shroud, is that Frache, Rizzatti and Mari, who had threads that in 1973 were cut from the bloodstains of the dorsal feet and the “blood belt” in the small of the back region,<sup>341</sup> reported that the insides of the bloodstained threads are not red: “With a low power stereomicroscope, they see more intense coloration, a fairly uniform reddish color but only on the top fibers of the threads. They also report reddish coloration on all of the circumference of the top fibers in the thread.”<sup>342</sup> Baima Bollone, who also investigated these 1973 bloodstained fibers, reported on “the traces suspected of being “blood””: “under these traces the threads of the cloth appear to be “clean”.”<sup>343</sup>

<sup>341</sup> “in 1981 I centred research on the threads of the weft and warp taken in correspondence with the C9d area of the reference map (the so-called “belt of blood”), B12c (the sole of the left foot) and C9d (the sole of the right foot) of the feet of the Shroud.” Baima Bollone, *The Forensic Characteristics...*, Turin 2000 Proceedings, p. 212 (quoted in Kears and Heimburger, *The Shroud Blood Science...*, 2013); “immunofluorescence investigations on C12a (right foot), C8a (back) and D2c (white, with less response).” (Baima Bollone, *The Forensic Characteristics...*, Turin 2000 Proceedings, p. 213)

<sup>342</sup> McCrone, *Judgement Day...*, 1999, p. 209, [http://www.amazon.com/Judgment-Shroud-Turin-Walter-McCrone/dp/1573926795/ref=sr\\_1\\_1?ie=UTF8&qid=1372771620&sr=8-1&keywords=judgment+day+for+the+turin+shroud#reader\\_1573926795](http://www.amazon.com/Judgment-Shroud-Turin-Walter-McCrone/dp/1573926795/ref=sr_1_1?ie=UTF8&qid=1372771620&sr=8-1&keywords=judgment+day+for+the+turin+shroud#reader_1573926795) )

<sup>343</sup> Baima Bollone, *The Forensic Characteristics...*, Proceedings Turin 2000, p. 210

The filter effect is perhaps also reflected by the presence of deep yellow color (probably of plasma/serum) of some cemented fibers, observed by STURP directly on the cloth: “where the darker material has been removed, one can see “yellow” fibrils that appear to be coated and cemented together. These coated yellow fibrils are also a deeper yellow color than those in the body-only areas (6), and are not confined to the first layer of fibrils as are those in the body-only areas.”<sup>344</sup>

#### 2.4. Few cells – hemolysate stains

Very few red blood cells have been found on the Shroud. Heller and Adler examined sticky-tapes by optical microscopy – also Scanning Electron Microscopy (SEM) was used by Jackson and Ercoline – and reported “that there are very few, almost no cells in the blood”<sup>345</sup>. They did not find enough red blood cells to consider them a “class” or “type” to be analyzed chemically.<sup>346</sup> Also Filogamo and Zina, as reported by Schwalbe and Rogers,<sup>347</sup> found very few red blood cells. Lucotte found only 29 red blood cells among the first 500 particles examined on a tiny sticky tape sample from the forehead 3-mark bloodstain.<sup>348</sup>

Yet, there were many red particles. Heller and Adler reported that “Most of the red particulates ranged from submicron to about 3  $\mu\text{m}$ , and the birefringent red particulates from 0.7  $\mu\text{m}$  to about 1  $\mu\text{m}$  diameter. ... The globs vary in size from about 5  $\mu\text{m}$  to 50  $\mu\text{m}$  in linear dimensions.”<sup>349</sup> They also reported that the red coated fibrils from blood areas of the Shroud had various coatings: “Coating varies from smooth to fractured to particulate appearance, color varies from red to orange, coating not birefringent or pleochroic”.<sup>350</sup> Rogers’ posthumous book says “Many fibers from blood areas are a deep red” and it shows a photomicrograph of red colored fibers from sticky-tape 6AF, taken from the “lance wound” area (fig. 2.49). It shows three fibers that are completely (smoothly and unfractured) reddish, in shades varying from pink to orange and red-brown.

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<sup>344</sup> ((6) = Pellicori, *Applied Optics*, 1980) Jumper et al., *A Comprehensive...*, 1984, p. 459; Note that STURP did not cut threads but only removed material from the cloth (dirt, globs and fibers) by sticky tape.

<sup>345</sup> “Under SEM we see clearly that there are very few, almost no cells in the blood.” (Adler, *The origin and...*, 1986, TOM 60; “J. Jackson and W. Ercoline in their SEM studies” Heller and Adler, *A Chemical...*, 1981, TOM 48

<sup>346</sup> Heller and Adler, *A Chemical...*, 1981, TOM 37 and 50 Table 2

<sup>347</sup> Schwalbe and Rogers, *Physics and Chemistry...*, 1982, p. 36-37; the reported “red-orange” color (p. 37) on the photomicrographs published by Pellicori and Evans in 1981 as referred to by Schwalbe and Rogers (“8 S. F. Pellicori and M.S. Evans, *Archeology*, 34 (1981) 34.” (Schwalbe and Rogers, *Physics and Chemistry...*, 1982, references on p. 48), is not congruent with the pinkish red color of bloodstained fibers shown on a photomicrograph published in Rogers’ posthumous book in 2008 (Rogers, *A Chemist’s...*, 2008, Figure V-4: A fiber from sample tape 6AF) or with the pinkish red color of the Shroud’s bloodstains in the high definition photographs taken in 2008 with a lighting of a calibrated spectrum, as published by Haltadefinizione in 2012 in their Shroud 2.0 app (*The Shroud and Haltadefinizione: the Shooting of 2008*, [www.haltadefinizione.com/en/the-shroud](http://www.haltadefinizione.com/en/the-shroud)).

<sup>348</sup> Lucotte, *Optical and chemical...*, 2012, p. 2547

<sup>349</sup> Heller and Adler, *A Chemical...*, 1981, TOM 37

<sup>350</sup> Heller and Adler, *A Chemical...*, 1981, TOM 50 Table 2



Fig. 2.49. Fibers from sticky-tape 6AF, taken from the “lance wound” area of the Shroud, ventral image<sup>351</sup> ©2005 Raymond N. Rogers Collection, STERA, Inc.<sup>352</sup>

Note that red blood cells are 6-8  $\mu\text{m}$  in diameter<sup>353</sup> ( $\mu\text{m}$  = micrometer = micron), and “Both red ochre and vermilion are about one micron ( $1/25,00^{\text{th}}$  inch) in diameter”<sup>354</sup>, while the diameter of Shroud linen fibers is 10-20  $\mu\text{m}$ <sup>355</sup>, and the “majority of the linen fibrils ranged from 10-15  $\mu\text{m}$  in diameter”<sup>356</sup>. This means that the presence of red blood cells or iron-oxide particles can not account for the smooth completely red fibers from bloodstain areas. Note that McCrone made a blood paint by diluting “3% blood in distilled water”, thereby automatically hemolysing (= breaking open) the red blood cells,<sup>357</sup> and the result on linen cloth was a fairly smooth coating of black-brown material on the linen fibers.<sup>358</sup> The smooth red coating on the Shroud is more in correspondence with a liquid acid hemolysate that mordanted a starch-madder coating pinkish-red.

<sup>351</sup> Rogers, A Chemist’s..., 2008, fig. V-4

<sup>352</sup> Used from R.N. Rogers, A Chemist’s Perspective on the Shroud of Turin, Ed. B.M. Schwartz, 2008, fig. V-4, p. 25

[http://books.google.fr/books?id=p-n84sc4ayYC&printsec=frontcover&hl=fr&source=gbs\\_ge\\_summary\\_r&cad=0#v=onepage&q&f=false](http://books.google.fr/books?id=p-n84sc4ayYC&printsec=frontcover&hl=fr&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false), with permission from Schwartz, STERA, Inc.

<sup>353</sup> [http://en.wikipedia.org/wiki/Red\\_blood\\_cell](http://en.wikipedia.org/wiki/Red_blood_cell)

<sup>354</sup> McCrone, Judgement Day..., 1999, p. 167

<sup>355</sup> Fanti et al., Evidences for..., 2005, A6

<sup>356</sup> Heller and Adler, A Chemical..., 1981, TOM 37

<sup>357</sup> “Fig. 1 –Stroma of a normal water-hemolyzed erythrocyte (osmium fixed). 10,000 X. ... Blood was drawn from the cubital vein and one part hemolyzed in 30 parts of distilled water for 3 minutes.” Braunsteiner et al., 1956, <http://bloodjournal.hematologylibrary.org/content/11/8/753.full.pdf> p. 754; see also 2.4.2.5.

<sup>358</sup> McCrone, Judgement Day..., 1999, p. 95, colorplate 4, fig. 23 and 24, text on p. 97, and color plate 5, fig. 27 on p. 99. See two of these photos on <http://www.mcri.org/home/section/63-64-293/the-latest-shroud-update>; The color of the bloodpaint in all these three photos in the book is brownish black, while the iron oxide in photos on the same page is red., The red color in an online photo of the bloodpaint result on cloth (fig. 18 in McCrone, The Shroud of Turin..., 1990 [http://www.mcri.org/CMSuploads/the\\_microscope\\_%20shroud.pdf?PHPSESSID=80273de2627d22c16744a4492a689496](http://www.mcri.org/CMSuploads/the_microscope_%20shroud.pdf?PHPSESSID=80273de2627d22c16744a4492a689496)) is either not accurate or of a much earlier moment after painting and before oxidation of the blood material. The online figure also shows a different representation of the iron content (micrograms per spot) than the photo in the book Judgement Day... (micrograms per  $\text{cm}^2$ ).

Hemolysate is cytoplasm released from a broken red blood cell and is a red, probably viscous, fluid as it has a very high concentration of the protein hemoglobin. Schwalbe and Rogers reported “At 50X magnification the “blood” looks as if it were applied as a viscous fluid which then flowed around the thread [41, 59] and soaked through to the opposite side of the cloth where it is also visible. The meniscus characteristics of viscous fluids can be seen throughout the “blood” areas. Thread fibers are matted and cemented together.”<sup>359</sup> “The earlier microscopic investigations of Frache et al. [59] revealed the “blood” threads to have slanting or diagonal bands of “granulation” that ranged in color from yellow to red. Filogamo and Zina [60] similarly reported seeing granular particles but apparently nothing resembling red corpuscles.”<sup>360</sup> These observations would be consistent with a viscous hemolysate that flowed on and through the Shroud and dried to granular particles.

The non-birefringent reddish particulates observed on other fibers and ranging to about 3  $\mu\text{m}$ , might be acid heme dimer aggregates from the hemolysate. An unhydrated heme monomer (Fe(III)PPIX) has been approximated by a sphere with a radius of 0.48 nm<sup>361</sup> (1 nm = 0.001  $\mu\text{m}$ ), and its dimers could have formed submicron to 3  $\mu\text{m}$  amorphous particles by aggregation. These aggregates on their own would probably be brown but on the background of a pinkish heme-madder lake on the interface of the aggregate and the Shroud fiber would look reddish (pink plus brown looks red). Also acid methemoglobin crystals – of which one was found on the Shroud (see 2.1.1.1) – could have formed from hemolysate. The possible formation mechanisms of the various kinds of bloodstains are discussed in chapter 5.

#### 2.4.1. Separate serum not red

As the serum found on Shroud fibers from sticky-tapes was golden yellow, there cannot have been much red or brown hemoglobin in this separate serum. “If as little as 0.5% of the red blood cells are hemolyzed, the released hemoglobin will cause the serum or plasma to appear pale red or cherry red in color.[4]”<sup>362</sup> This means that any significant rupture of red blood cells (hemolysis) only could have taken place outside the body and when most plasma/serum had already separated from the red blood cells.

Separation of the plasma/serum from the red blood cells would not only prevent a strong red clot formation in/on the cloth, but also expose the red blood cells to a closer contact with the possibly hemolytic coating of the Shroud. Lack of plasma on the Shroud-RBC interface, plus hemolysis taking place on the surface of the Shroud, may explain why very few red blood cells are now found on the Shroud. Of course red blood cells may have been on the Shroud in larger amounts before they abraded from the layer formed by the hemolysate.

#### 2.4.2. Hemolysis mechanisms

##### 2.4.2.1. Leech digest

In a medicinal leech, the sucked up blood that is in the gut of the leech quickly loses its plasma: it seems to be rapidly excreted by the leech.<sup>363</sup> On the other hand, “Morphological preservation of erythrocytes ingested by leeches has been observed for up to 18 months”<sup>364</sup>, although certainly also hemolysis takes place inside the gut to allow the digestion of the proteins inside the cell, which are the nutrients of the leech. So, a red ‘paint’ made of leech digest, which might have been used for

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<sup>359</sup> Schwalbe and Rogers, *Physics and Chemistry...*, 1982, p. 36

<sup>360</sup> Schwalbe and Rogers, *Physics and Chemistry...*, 1982, p. 36-37

<sup>361</sup> “If we approximate the unhydrated Fe(III)PPIX monomer by a sphere with a radius of 4.8 Å ...” (Villiers et al., *Speciation and structure...*, 2007, p. 110 <http://link.springer.com/article/10.1007%2Fs00775-006-0170-1> ).

<sup>362</sup> <http://en.wikipedia.org/wiki/Hemolysis> , caption of figure on the right hand side

<sup>363</sup> Posting by C.S. Berry <http://shroudofturinwithoutallthehype.wordpress.com/2013/03/02/shroudie-alert-day-12-time-now-to-write-that-long-overdue-letter-to-the-royal-society/>

<sup>364</sup> Kearse, comment <http://shroudstory.com/2013/04/04/blood-clotting-and-the-strange-case-of-brother-hirudo/>

painting the bloodstains, as suggested by Berry,<sup>365</sup> would in a way resemble the substance of plasmaless/serumless blood. Remaining red blood cells in the leech digest could have hemolysed on the Shroud in the same way as authentic serumless blood may have done.

#### 2.4.2.2. Saponins - madder

Saponins are organic detergents (washing molecules), found in many plants, and known for their hemolytic activity; they consist of a lipophilic moiety (the aglycone) that binds to lipids, and a sugar moiety that binds to water.<sup>366</sup> Schwalbe and Rogers reported “microscopic, chemical laser probe, or mass spectrometric examinations” yielded no support for the presence of saponins on image fibers.<sup>367</sup> Note that PMS was not really done on controls (= non-image-non-scorch fibers): the only ‘control’ was a Raes sample<sup>368</sup> that released early furfural, which is best explained by the Raes corner being a light scorch area.<sup>369</sup> On image or scorch fibers the saponins could or would have been broken down by image formation or scorching.

#### Madder root contains saponins

The root of the madder plant that is used for dyeing (*Rubia tinctorum*) contains saponins: “The alcohol-water extract contained, besides free sugars, saponins also ... After evaporation under reduced pressure, the brown syrupy mass gave tests for the presence of saponins. A small portion of the extract was well shaken with water when a fairly permanent lather was observed. Acid hydrolysis of a test sample of the crude extract with 5% H<sub>2</sub>SO<sub>4</sub> aq. gave an ether soluble product which gave Liebermann-Burchard test. A solution of the aqueous alcohol extract in water, on cooling, deposited colourless cubic crystals, m.p. 182-4°; acetate m.p. 72-73°. It was identified as sucrose.”<sup>370</sup> Sucrose is “a nonreducing sugar”.<sup>371</sup> Also the root of Indian madder, *Rubia cordifolia*, can be used for dyeing, and contains saponins.<sup>372</sup> The foam number (or froth number) of the foam produced by saponins in the extracts of the stems of these two madder species is identical (166.0), but the foam number of *Rubia cordifolia* root (200.0) is a bit higher than the foam number of *Rubia tinctorum* root (154.0).<sup>373</sup> Moderate stirring of *Rubia tinctorum* root powder in water at room temperature (or in hot water) caused a foam to appear that remained, while the liquid was standing still, for at least an hour; also a *Rubia tinctorum* root powder ‘tea’, which after cooling down had spontaneously become more acid, probably by lactic acid from fermentation, and which was then acidified further with vinegar to render it yellow, yielded a stable foam after mild shaking (see fig. 2.50). A correlation between foam number and hemolytic activity was found in 1962.<sup>374</sup> In the case of an amaranthus flour, a

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<sup>365</sup> Berry, It’s still looking good for leech digesta as the source of blood on the Shroud of Turin, March 3, 2013, <http://shroudofturinwithoutallthehype.wordpress.com/2012/08/04/its-still-looking-good-for-leech-digesta-as-the-source-of-blood-on-the-shroud-of-turin/> and <http://shroudofturinwithoutallthehype.wordpress.com/2013/03/02/shroudie-alert-day-12-time-now-to-write-that-long-overdue-letter-to-the-royal-society/>

<sup>366</sup> <http://en.wikipedia.org/wiki/Saponin> ; “The technique proposed to determine red blood cell methemoglobin, as a percentage, is based on stabilization at 60 moles/L using a phosphate buffer solution followed by hemolysis with 1% saponin” Rechetzki et al., 2012, *Rev Bras Hematol Hemoter.*, <http://www.scielo.br/pdf/rbhh/v34n1/v34n1a07.pdf> p. 15

<sup>367</sup> Schwalbe and Rogers, *Physics and Chemistry...*, 1982, p. 14

<sup>368</sup> Rogers, *Pyrolysis/Mass Spectrometry...*, 2004, p. 2

<sup>369</sup> Hoeven, *Internal selvedge...*, 2012, 2.5.1.

<sup>370</sup> V.V.S. Murti, T.R. Seshadri, S. Sivakumaran, A Study of Madder, the Roots of *Rubia tinctorum* Linn., *Indian Journal of Chemistry*, Vol. 8, Sept. 1970, pp. 779-782.

<sup>371</sup> <http://en.wikipedia.org/wiki/Sucrose>

<sup>372</sup> “roots of *Rubia cordifolia* contain saponins” (Patil et al., Antihyperglycemic, antistress and nootropic activity of roots of *Rubia cordifolia* Linn, *Indian Journal of Experimental Biology*, Vol. 44, Dec 2006, pp. 987-992, [http://www.researchgate.net/publication/6622865\\_Antihyperglycemic\\_antistress\\_and\\_nootropic\\_activity\\_of\\_roots\\_of\\_Rubia\\_cordifolia\\_Linn/file/3deec51aa189380045.pdf](http://www.researchgate.net/publication/6622865_Antihyperglycemic_antistress_and_nootropic_activity_of_roots_of_Rubia_cordifolia_Linn/file/3deec51aa189380045.pdf))

<sup>373</sup> RG Dengre, KN Patel, MB Chauhan, Comparative studies of *Rubia cordifolia* Linn. and *Rubia tinctorum* Linn (Rubiaceae), *Ancient Science of Life*, Vol No. XIII Nos. 1 & 2, July-October 1993, Pages 165 - 179 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3336544/pdf/ASL-13-165.pdf>, Table 5.

<sup>374</sup> “Vacek et al. (25) showed that correlations exist between foam number, surface tension, hemolytic activity” (Jones and Elliot, 1969, <https://dl.sciencesocieties.org/publications/cs/abstracts/9/6/CS0090060688>) – here, the reference probably is to Vacek, L. and Sedlak, B., Comparison of physical, chemical and biological properties of saponins. *Gunma J. Med. Sci.*, 11, 1-6, 1962), as referred to in Khalil and El-Adawy, 1994: “Correlations between foam number, surface

concentrate containing saponins and with a foam number <100 had a non-zero hemolytic activity, viz. 1-2.<sup>375</sup> Still, it would be useful to know the exact species of the saponin(s) in *Rubia tinctorum* root. As the Lieberman-Burchard test is a test for cholesterol, which is a sterol,<sup>376</sup> madder root saponins probably include a sterol-type saponin, which is the type of saponin that generally gives much higher rates of hemolysis than the triterpene type.<sup>377</sup> On the other hand, the bark (the outermost layer on stems and roots) of plants of the Rosaceae family, to which madder belongs, contains “very polar saponins” which are also present in *Saponaria officinalis* (the traditional soapwort) and which are “among the biggest with nine to ten oses bound to a pentacyclic triterpenoid acid” and are “hemolytic”.<sup>378</sup>



Fig. 2.50. Stable foam arising from moderate stirring of madder root powder in hot tap water (left) and from mild shaking of the same extract that had spontaneously acidified/fermented and then was acidified further by adding vinegar (right). ©AvdH

#### No hydrolysis of madder saponins in a slightly acidic extract

The published photos of the color changes of the Raes fibers with pH suggest that alizarin of madder was the Shroud’s main background colorant. The color of the fibers turned from yellow-brown to bright yellow by adding a strong acid,<sup>379</sup> so the original color on the fibers is not one corresponding

tension, haemolytic activity, and toxicity after subcutaneous administration of saponin have been reported by Vacek and Sedlak (1962).” <http://www.sciencedirect.com/science/article/pii/0308814694901201>

<sup>375</sup> “Hemolytic activity (hemolysis degree) 1-2 Foam index <100”, Escudero et al., 2004, Comparison of the chemical composition and nutritional value of *Amaranthus cruentus* flour and its protein concentrate, <http://link.springer.com/article/10.1007/s11130-004-0033-3>

<sup>376</sup> [http://en.wikipedia.org/wiki/Liebermann%E2%80%93Burchard\\_test](http://en.wikipedia.org/wiki/Liebermann%E2%80%93Burchard_test) ; <http://en.wikipedia.org/wiki/Cholesterol>

<sup>377</sup> “The name ‘saponin’ denotes a large and chemically heterogeneous group of sterol glycosides and triterpene glycosides linked to one or two polar oligosaccharides. The rates of hemolysis induced by sterol glycosides are generally much higher than those of triterpene glycosides (24). ... 24. Takechi M & Tanaka Y (1995). Haemolytic time course differences between steroid and triterpenoid saponins. *Planta Medica*, 61: 76-77.” Yuldasheva al., *Brazilian Journal of Medical and Biological Research*, 2005, [http://www.scielo.br/scielo.php?pid=S0100-879X2005000700009&script=sci\\_arttext](http://www.scielo.br/scielo.php?pid=S0100-879X2005000700009&script=sci_arttext)

<sup>378</sup> “Among the plants producing triterpenoid saponins, some contain great amounts of very polar saponins, essentially in the rhizome and the roots (*Saponaria officinalis* L., *Gypsophila* sp., Caryophyllaceae) or in the bark (*Quillaja saponaria* Mol., *Quillaja smegmadermos* D.C., Rosaceae). These saponins are among the biggest with nine to ten oses bound to a pentacyclic triterpenoid acid. Their amphiphilic structure confers to them some well-known properties such as detergent, emulsive, hemolytic and toxic substances.” Henry, *Biotechnology in Agriculture and Forestry* 1989, [http://link.springer.com/chapter/10.1007/978-3-642-73617-9\\_24#page-1](http://link.springer.com/chapter/10.1007/978-3-642-73617-9_24#page-1); [http://en.wikipedia.org/wiki/Rubia\\_tinctorum](http://en.wikipedia.org/wiki/Rubia_tinctorum)

<sup>379</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, fig. 12 and 13, p. 17-18: “The yellow-brown encrustation shown in figure 12 swelled and became more transparent as it soaked. The color instantly changed to bright yellow in 6N hydrochloric acid (HCl), and the coating was reduced in density as the fibers were soaked in the acid (figure 13).”

to a strong acidity. Alizarin is yellow at pH  $\leq 5$  and pink at pH 8-10. Purpurin is yellow-orange at pH  $\leq 3.5$  and pink at pH  $\sim 6-9$ .<sup>380</sup> The apparent slight acidity of the Shroud's yellow-brown dye, e.g. at pH 5, most probably was not strong enough to break (= hydrolyse) the madder saponins into their aglycone (sapogenin) and sugar moiety, which hydrolysis would have destroyed the hemolytic activity. Steroidal saponins of yucca hydrolyse only partially in a pH as low as 1-2 at about the boiling point, and the resulting degraded saponins then need even harsher methods for complete hydrolysis, to destroy the saponin and release the sapogenin.<sup>381</sup> Triterpene saponins of ginseng need concentrated HCl to hydrolyse at room temperature.<sup>382</sup> Partial hydrolysis of the primeveroside glycosides of alizarin and lucidin of madder root occurred by 2-3 hours boiling in a 1% H<sub>2</sub>SO<sub>4</sub> aqueous solution; their total hydrolysis was achieved by 3 hours boiling in an aqueous 5% H<sub>2</sub>SO<sub>4</sub> solution, and also acid hydrolysis of (some of) the madder saponins was done in 5% H<sub>2</sub>SO<sub>4</sub> aq.<sup>383</sup> The pH of 5% H<sub>2</sub>SO<sub>4</sub> aq. and that of 1% H<sub>2</sub>SO<sub>4</sub> aq is less than 1.0,<sup>384</sup> and thus much less than the apparent pH of the madder dye used on the Shroud.

A dye can be extracted from madder root without adding any acid, for instance by mere stirring of the powdered root in neutral water of 35-65 °C for about 4 hours; in this process "hydrolysis of ruberythric acid can be performed by endogenous madder root enzymes. In this enzymatic process also nordamnacanthal is formed out of lucidin primeveroside ... Preferably the temperature is kept below 65°C. At a higher temperature, the enzymes start to be denatured. ... the transformation of ruberythric acid and lucidin primeveroside into alizarin and lucidin, respectively, can be performed in demi-water, ultra-pure water and even tap water."<sup>385</sup> Temperatures above 65 °C denature the active enzymes, but a dye would be extracted anyway: madder root contains both free colorants (e.g. alizarin and purpurin)<sup>386</sup> and their glycosides. Besides, ruberythric acid, the primeveroside glycoside of alizarin, is a colorant as well: along with alizarin and purpurin, it is one of the "colour components present in plants of the Rubiaceae family"<sup>387</sup>. So, a simple boiling-water extract of madder root would be a dye and contain saponin; after cooling down, the liquid could have fermented<sup>388</sup> (cf. fig. 2.51) – which would have removed the sucrose – and have been slightly acidified and then used as a yellow

<sup>380</sup> Miliani et al., Acidichromic effects..., 2000, p. 143-144

<sup>381</sup> "According to the invention, aqueous extracts of the plant tissue, containing the saponin, are acidified to about pH 1 to 2 and heated, preferably at about the boiling point, until partial hydrolysis and precipitation of saponin occurs. The partially hydrolysed saponin is then recovered, as by decantation, filtration or centrifuging, and may be readily hydrolysed further to sapogenin by conventional methods. This process has two principal advantages over the conventional one-step, strong acid hydrolysis: (a) ... (b) a great saving in reagent cost is achieved because the amount of acid required to acidify the plant extract to pH 1-2 is only 5 to 10% of the amount required to produce the 2-normal solution used in the conventional process." Partial acidic hydrolysis of steroidal saponins, Patent US 2780620 A, <http://www.google.nl/patents/US2780620?printsec=description&hl=nl&dq=hydrolysis+saponins+pH>

<sup>382</sup> "Acid hydrolysis of diol-type Ginseng saponin with conc. HCl at room temperature yielded chlorinated sapogenin. ... The fundamental skeleton of the genuine saponins is dammarane-type tetracyclic triterpen, and that of only ginsenoside Ro is oleanane-type pentacyclic triterpene, which is a minor component in ginseng." S. Shibata, Chemistry and Cancer Preventing Activities of Ginseng Saponins and Some Related Triterpenoid Compounds, *J Korean Med Sci* 2001; 16(Suppl): S28-37, ISSN 1011-8934 <http://atkinginseng.com/Articles/Cancer%20Preventing%20Activities.pdf> p.S30

<sup>383</sup> V.V.S. Murti, T.R. Seshadri, S. Sivakumaran, A Study of Madder, the Roots of *Rubia tinctorum* Linn., *Indian Journal of Chemistry*, Vol. 8, Sept. 1970, pp. 779-782

<sup>384</sup> The pH of 5% H<sub>2</sub>SO<sub>4</sub> aq. would be 0, and that of 1% H<sub>2</sub>SO<sub>4</sub> aq. about 0.7.

<sup>385</sup> "The optimum pH range varies with the extraction time. The optimum pH for 30 minutes reaction time is about 6. At higher and lower pH's the percentage conversion lowers rapidly. At 4 hours the pH optimum lies between 6 and 8." Method for the production of a dye preparation based on madder root - European Patent EP1191070, <http://www.freepatentsonline.com/EP1191070.html>

<sup>386</sup> Singh et al., 9,10-Anthraquinones..., 2004, Table 1, entries 10 and 29, <http://onlinelibrary.wiley.com/doi/10.1002/cbdv.200490088/abstract;jsessionid=E8C42C392CA61DCF5ACA2FC6D648B994.f03t01>

<sup>387</sup> Organic Mass Spectrometry in Art and Archaeology, ed. Maria Perla Colombini, Francesca Modugno, 2009, [http://books.google.nl/books?hl=nl&lr=&id=46klmqOb\\_gAC&oi=fnd&pg=PA3&dq=Rubia+tinctorum+xylose+arabinos&ots=1urMD4C3OX&sig=vn4Uuiyp4hMrNYAAweKgXQGtZKk#v=onepage&q=Rubia%20tinctorum&f=false](http://books.google.nl/books?hl=nl&lr=&id=46klmqOb_gAC&oi=fnd&pg=PA3&dq=Rubia+tinctorum+xylose+arabinos&ots=1urMD4C3OX&sig=vn4Uuiyp4hMrNYAAweKgXQGtZKk#v=onepage&q=Rubia%20tinctorum&f=false) p. 370, fig. 13.2. p. 370

<sup>388</sup> "(f) When you finish dyeing, throw the liquor and madder roots away, if possible on the compost, as the liquid ferments and the roots go mouldy very quickly." [http://wildcolours.co.uk/html/madder\\_dyeing.html](http://wildcolours.co.uk/html/madder_dyeing.html)

dye and fabric brightener on the fabric that would become the Shroud. Madder dye can even be extracted from chopped and crushed madder root in cold water and also applied to fabric in cold water (room temperature) – this is the time-consuming ‘Nest Rubio’ technique for madder.<sup>389</sup>

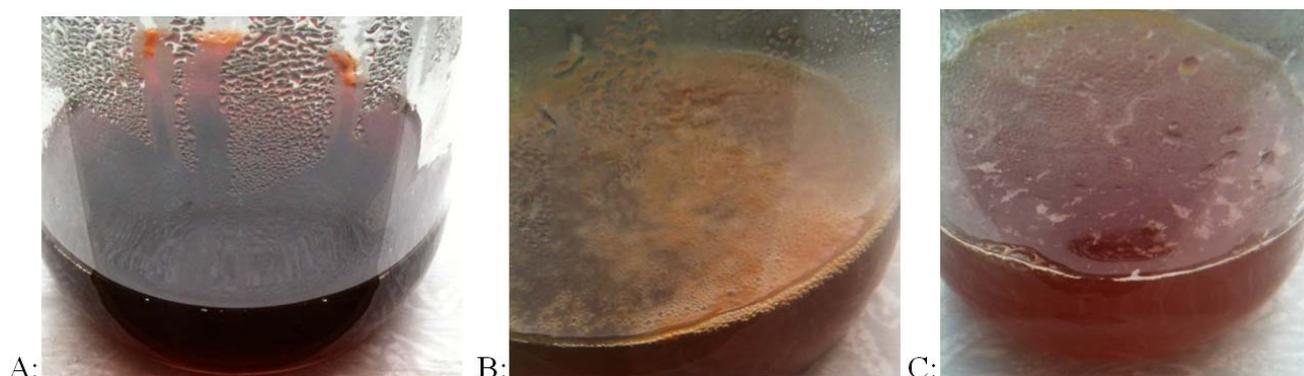


Fig. 2.51. A and B: same extract, made by gently stirring *Rubia tinctorum* root powder in hot water for 45 min, spontaneously fermented in a closed glass jar. Photos two hours and four days after extraction, respectively. The pH dropped from 6.5-7 to 4.5-5, in these four days. C: a madder root ‘tea’ fermenting two days after preparation. ©AvdH

A predominant presence of saponins from a *Saponaria officinalis* (soapweed) residue is precluded by many characteristics of the Shroud, which are consistent with the presence of madder dye (see 4.4).

#### 2.4.2.3. Drying

In drying out, any red blood cell will eventually collapse onto itself and be minimally damaged, and release some hemoglobin and breakdown products, but most of the content remains “within”.<sup>390</sup> In red blood cells lysed by saponins, larger and permanent holes are formed in the cell wall<sup>391</sup> before it dries out, through which more of the content is released. So, on a cloth that is not treated with hemolytic detergents, such as saponins, less (and denser/drier) hemolysate would ultimately be released, and more (almost) intact cells would probably still be visible. Yet, as quoted above in 2.2., a red viscous fluid reached the reverse side of the Shroud in many places and hardly any cells are seen, at least on sticky-tapes. So, hemolysis by a mere drying process is less probable than hemolysis (also) by saponins.

#### 2.4.2.4. Ozon and light

Ozone (O<sub>3</sub>) gas has a hemolytic effect on red blood cells. In *in vitro* tests on a fish red blood cell suspension, “Ozone exposure induced hemolysis, formation of methemoglobin, and RBC membrane lipid peroxidation ... neither ozone nor its derivative directly attacked from the outside of the cell, but ozone that penetrated through the membrane derived the reactive oxygen species from Hb inside of the cell”; also in bovine and swine erythrocytes, ozon induced hemolysis and methemoglobin

<sup>389</sup> chopping and crushing: [http://wildcolours.co.uk/html/madder\\_roots.html](http://wildcolours.co.uk/html/madder_roots.html) and cold water dyeing: [http://wildcolours.co.uk/html/madder\\_dye\\_nest\\_rubio.html](http://wildcolours.co.uk/html/madder_dye_nest_rubio.html) ; “There is an article by Nest Rubio in the Spring 1993 issue of Spin-Off Magazine on cold water dyeing and it is a MOST excellent article, I've been using this technique since '94.” <http://www.backwoodshome.com/forum/vb/archive/index.php/t-399.html>

<sup>390</sup> Point 4. of the comment of cell biologist and immunologist Kelly Kearse, <http://www.shroudstory.com/2013/05/25/an-important-and-highly-informative-guest-posting-by-paul-maloney/#comment-35016>

<sup>391</sup> “saponin-lysed erythrocytes do not reseal, thus indicating an irreversible damage of the lipid bilayer. ... Transmission electron microscopy revealed destruction of the lipid bilayer and emergence of multilamellar buds in saponin-lysed ghosts.” Baumann et al., Hemolysis of human erythrocytes with saponin affects the membrane structure, *Acta Histochemica*, 2000, <http://www.sciencedirect.com/science/article/pii/S0065128104700136>

formation.<sup>392</sup> It was also found that “ferrous hemoglobin potentiates ozone-induced lipid peroxidation while methemoglobin, resulting primarily from nitrogen dioxide, inhibits this process.”<sup>393</sup> An *in vivo* study on guinea pigs found “a higher level of methemoglobin in the ozone exposed guinea pigs than in the air exposed controls, but only when the blood samples were taken at 1/2 an hour after ozone exposure.”<sup>394</sup> For humans, “Within the ozone therapeutic range, we have never detected methemoglobin. Washed erythrocytes or ghosts resuspended in buffered saline, without any natural antioxidants are very sensitive to ozone (53,95) but these data cannot be compared to our experimental condition. ... Extracellular (particularly uric acid and ascorbic acid) and intracellular antioxidants quench most of the ozone dose.”<sup>395</sup> In 2006, it was “shown by a series of systematic studies on model molecules, on the isolated prosthetic groups, on isolated hemoglobin (methemoglobin) and on whole blood that the action of ozone is specifically directed toward the prosthetic haeme groups of this protein causing their fission into oxidized degradation products. Therefore, ozone is selectively bound by haeme groups of hemoglobin”; the study also showed “experimental evidences about the degradation of the methemoglobin macromolecule caused by ozone and about the fact that the main reaction target are the prosthetic groups.”<sup>396</sup> From these results, it seems that in a bloodstain that has lost most of its plasma/serum, ozone could have had an oxidizing and heme degrading and hemolytic effect. If hemolysis already had taken place before exposure to ozone, the hemolysate would only get (more) oxidized and degraded after exposure to ozone. In the case of the Shroud, ozone may have been produced during image formation (e.g. during an image- and light- and ozon-producing corona discharge<sup>397</sup>), but if this ozone caused the hemolysis that produced or supplemented the necessary amount of fluid hemolysate to form bloodstains on the reverse side of the Shroud, the blood can not have been dry at that moment. This renders mere hemolysis by ozone less plausible for the Shroud case: the imprinted scourge clots probably only got wet again after the bloodflows elsewhere on the skin had dried (see 5.5.4). The same applies to cell membrane damage and hemolysis caused by photo-oxidation.<sup>398</sup>

#### 2.4.2.5. Wet cloth – hypotonic hemolysis

Distilled or hypotonic water, which has a lower ion concentration than the red blood cell content, gets sucked into the cell by osmosis and makes it burst. A microscopic view of such a water-hemolyzed erythrocyte shows a cell that has burst in many places.<sup>399</sup> If the Shroud was soaking wet when blood got on it, the red blood cells may have been lysed by the water. The colored heme products in that case most probably would have diffused to the reverse side of the Shroud in all of its bloodstains. Yet, the Shroud has bloodstains that are only visible on the observe side. For instance, many scourge marks, and the tip of the lower, darker, bloodrivulet in the middle of the dorsal image, at the height of the small of the back, are almost not present on the reverse side.<sup>400</sup> This favours the

<sup>392</sup> Fukunaga et al., Mechanism of oxidative damage to fish red blood cells by ozone, 1999, <http://onlinelibrary.wiley.com/doi/10.1080/713803566/full>; Meadows et al, 1986, <http://www.sciencedirect.com/science/article/pii/0006291X8691243X>; Cataldo et al., 2005, <http://www.sciencedirect.com/science/article/pii/S0141391005000947>

<sup>393</sup> Goldstein, 1976, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1475003/pdf/envhper00488-0102.pdf>

<sup>394</sup> Ballew et al., 1983, <http://www.tandfonline.com/doi/abs/10.1080/10934528309375126>

<sup>395</sup> Bocci, 1996, <http://oxidation-therapy.com/pdfs/OzoneBioregulator.pdf>

<sup>396</sup> Cataldo, 2006, <http://www.tandfonline.com/doi/abs/10.1080/01919510600900290#.UoJ7Fr5gWUk>

<sup>397</sup> “CD has applications, for example, in photocopying or ozone and NOx manufacturing. ... CD also forms: - UV luminescence; - heating; - ozone (O3) and other relative substances such as nitric acid (NHO3); - acoustic effect; - electromagnetic interference.” Fanti, Body Image Formation Hypotheses based on Corona Discharge: Discussion, p. 4-5

<sup>398</sup> Phototherapy may cause hemolysis if bilirubin is bound to the red blood cell wall (Ostrea et al., 1985, Red Cell Membrane Lipid Peroxidation and Hemolysis Secondary to Phototherapy, <http://onlinelibrary.wiley.com/doi/10.1111/j.1651-2227.1985.tb10987.x/abstract;jsessionid=1AED4EC6E32C5C8CA7CDE251A64E7C50.f04t01>); Bilirubin is unaffected by ozon (Shinriki et al., 1998, <http://www.ncbi.nlm.nih.gov/pubmed/10069448>).

<sup>399</sup> “Fig. 1 –Stroma of a normal water-hemolyzed erythrocyte (osmium fixed). 10,000 X. ... Blood was drawn from the cubital vein and one part hemolyzed in 30 parts of distilled water for 3 minutes.” <http://bloodjournal.hematologylibrary.org/content/11/8/753.full.pdf> p. 754

<sup>400</sup> See the photographs of the observe and reverse sides of the Shroud on the Wiki of the Shroud Science Group <http://shroud.wikispaces.com/PROPERTIES>

hypothesis that the Shroud was dry and, in these particular bloodstains, also the bloodmaterial (e.g. clot or transposed bloodstain) was relatively dry, or wet in only a very small amount, when it touched the observe side.

#### 2.4.2.6. Toluene

“Toluene can be used to break open red blood cells in order to extract hemoglobin in biochemistry experiments.”<sup>401</sup> Toluene was the solvent used by Heller and Adler to free Shroud fibers from sticky-tapes. Red particles – not meaning ‘red blood cells’ – which they saw on the optically anisotropic sticky-tapes were freed from the tape for further assay, both for polarization studies (e.g. harvesting of birefringent iron-oxide particles<sup>402</sup>) and for microscopical colorimetric tests, fluorimetric tests, and chemical microspotting.<sup>403</sup>

### 2.5. Hydroxyproline in red particles on Zina-thread

Hydroxyproline is a major component of collagen, the main structural protein of the various connective tissues in animals and humans. That its signal was observed in Pyrolysis Mass Spectrometry of a specific Shroud sample with red material on it,<sup>404</sup> may be explained by the presence of collagen on the sample, or the presence of blood, or both. Normal human blood serum contains hydroxyproline at a level that can be detected by immunological techniques<sup>405</sup> that are much less sensitive than PMS; “serum levels of hydroxyproline may be evaluated in patients as a measure of liver and renal function”.<sup>406</sup>

As the particular Shroud sample was the Zina-thread, and this thread was from both a blood area and a heel area,<sup>407</sup> and the heel is also a part of the sole<sup>408</sup>, and as there is no red material on the upper part of the heels but only on the soles<sup>409</sup>, the red material must have been in the area of the sole of the foot. The location called B1D by the Gilberts, at the sole of the foot, is called “heel” by Pellicori;<sup>410</sup> also Heller used the word “heel” for the location on the foot where dirt was detected by reflectance spectrometry and microscopy.<sup>411</sup>

It has been assumed by the physician Gambescia that two nails pierced the right foot: “one nail pierced the metatarsal area of one foot while a second nail passed through the front of the ankle and through the foot”; it is called “completely hypothetical” by Zugibe, but it is also the opinion of Desalvo, while Battaglini confirms that it is “quite difficult to describe, with due technical precision, the position of the feet of the Man of the Shroud”.<sup>412</sup> Also Bevilacqua et al. claimed in 2014 that the

<sup>401</sup> <https://en.wikipedia.org/wiki/Toluene>

<sup>402</sup> Heller, Report on..., 1983, p. 195

<sup>403</sup> Heller and Adler, A Chemical..., TOM 37

<sup>404</sup> Rogers, A Chemist's..., 2008, p. 56

<sup>405</sup> For instance with the ELISA kit for hydroxyproline [http://www.mybiosource.com/datasheet.php?products\\_id=452577](http://www.mybiosource.com/datasheet.php?products_id=452577)

<sup>406</sup> Comment of cell biologist and immunologist K. Kears <http://www.shroudstory.com/2013/04/04/blood-clotting-and-the-strange-case-of-brother-hirudo/>

<sup>407</sup> “One of the analytical methods used during the STURP studies was pyrolysis mass spectrometry. ... one (“the Zina thread”) was a complete yarn segment that had been withdrawn from the heel image area” Rogers, Studies on..., 2005; “At least one of the blood samples (the “Zina thread” from the image heel) showed a strong peak for hydroxyproline at low temperature. This amino-acid is present in animal proteins including blood proteins or collagen.” Heimbürger, A detailed, p. 9; The sample called “Zina heel” was characterized as Image yes, Scorch no, Blood yes, Water no, in the table on p. 2 of Rogers, Pyrolysis/mass spectrometry..., 2004, <http://www.shroud.com/pdfs/rogers4.pdf>

<sup>408</sup> “In human anatomy, the heel is the prominence at the posterior end of the foot. It is based on the projection of one bone, the calcaneus or heel bone, behind the articulation of the bones of the lower leg.” <http://en.wikipedia.org/wiki/Heel>

<sup>409</sup> see ShroudScope <http://www.dshroud.com/shroudScope/shroudScope.shtml?zl=8&image=4&lon=15123&lat=2218>

<sup>410</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, fig. 18, p. 1936; Pellicori, Spectral properties..., 1980, p. 1919

<sup>411</sup> Heller, Report on..., 1983, p. 112

<sup>412</sup> Gambescia is cited on [http://articles.philly.com/1986-07-10/news/26096073\\_1\\_joseph-gambescia-nail-bala-cynwyd](http://articles.philly.com/1986-07-10/news/26096073_1_joseph-gambescia-nail-bala-cynwyd); Zugibe, The Crucifixion of Jesus: A Forensic Inquiry, p. 93-94 [http://books.google.nl/books?id=\\_iU4CPSvDK4C&pg=PA93&lpg=PA93&dq=shroud+two+nails+heel+right+foot&source=bl&ots=AkqM5MyV17&sig=O6xLbIsU9vxZBCepoOCyOKh7t1w&hl=nl&sa=X&ei=sU-](http://books.google.nl/books?id=_iU4CPSvDK4C&pg=PA93&lpg=PA93&dq=shroud+two+nails+heel+right+foot&source=bl&ots=AkqM5MyV17&sig=O6xLbIsU9vxZBCepoOCyOKh7t1w&hl=nl&sa=X&ei=sU-)

right foot had two nail wounds: “The third discovery is to do with the right foot of the Man of the Shroud: it was nailed to the cross twice. An analysis of the imprint of the sole of the right foot shows two nails were driven into it: one between the second and third metatarsal and another at heel level which other academics had not spotted clearly.”<sup>413</sup>

So, it seems that hydroxyproline could have originated from a nail wound in the heel of the right foot or from the centre of the sole, from which it may have flowed down to the heel with the blood while the body lay on the Shroud. It seems hydroxyproline may even result from abraded skin on the sole of the foot.<sup>414</sup>

Traces of hydroxyproline would probably not be visible in the absorbance spectrum of the bloodstain. Hydroxyproline quenches the fluorescence of human serum albumin,<sup>415</sup> and in solution probably is colourless.

## 2.6. High Na and Cl levels on reverse side

Relatively high levels of Na and Cl were found in organic red particles that were found in dust vacuumed from the reverse side of Shroud; natron was also found in this dust.<sup>416</sup> Nothing is said about the levels of Na and Cl and natron on the observe side, probably because no dust was vacuumed from this side. The 1978 X-ray fluorescence study, the equipment of which allowed detection of elements with atomic number greater than 16, might perhaps have detected a relatively high level of Cl (with atomic number 17), but the element is not mentioned in the study. After having discussed calcium, strontium, iron, potassium, rubidium, zirconium, yttrium, it says that “The scattered radiation interference discussed in connection with Fig. 8(b) limit detection sensitivity for several other potentially interesting elements.”<sup>417</sup>

Perhaps, at Jesus’ preliminary burial (cf. John 19:42 20:2 Luke 23:56-24:1), natron and/or salt crystals were sprinkled on the wooden bier or on top of the doubled shroud to retard decomposition of the body at least until the completion of the burial on the day after the Sabbath, when women took spices to the tomb. “‘Natron’ was used in Palestine for the corpse dewatering.”<sup>418</sup> The Jewish Encyclopedia says on the preparation for burial: “As soon as the last breath was drawn, the eyes of the dead were closed by the oldest or the most distinguished son or next relative (Gen. xlvii. 4), the mouth was shut, and kept in position by a band on the cheek-bones, and the body placed upon sand or salt on the floor to retard decomposition, metal or glass being put upon the navel to prevent swelling. Then the body was washed and anointed with aromatic unguents, and wrapped in linen clothes.”<sup>419</sup> Presumably, the dead body was not undressed right after the moment of death but only

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[HU52VJoGg0QXF9YDoCg&ved=0CC4Q6AEwADgK#v=onepage&q=shroud%20two%20nails%20heel%20right%20fo](http://www.gizapyramid.com/LECTURE-SHROUD2.htm)  
[ot&f=false](http://www.gizapyramid.com/LECTURE-SHROUD2.htm); “On this dorsal image the imprint of the right foot can be seen. It appears that two nails were used. One was first placed through the right angle joint and this fixed the right foot to the cross. Then the left foot was placed over the right and a second nail driven through the fleshy section of both feet.” Desalvo, Shroud of Turin picture tour ..., <http://www.gizapyramid.com/LECTURE-SHROUD2.htm>; “Even if one admits that the holes we see are each in the approximate area of the second intermetatarsal space, it would not be physically possible to make the two surfaces come together, (the R instep and the L sole), oriented anatomically and functionally in diametrically opposite directions. And if one admits that the tarso-metatarsal articulations of the two feet had been subjected to a distortion in pronation so exceedingly violent as to lacerate the various and robust ligamentous formations in these areas, one would have to renounce the idea that the feet rotated on the nail.” Battaglini, Considerations on..., 1983, <http://www.shroud.com/pdfs/ssi09part3.pdf>

<sup>413</sup> <http://vaticaninsider.lastampa.it/en/world-news/detail/articolo/sindone-shroud-sudario-33948/>

<sup>414</sup> Comment of K. Kearse, <http://shroudstory.com/2013/04/04/blood-clotting-and-the-strange-case-of-brother-hirudo/>

<sup>415</sup> Zhou et al., 2012, <http://pubs.rsc.org/en/content/articlelanding/2012/ob/c2ob25967b>

<sup>416</sup> Fanti and Gaeta, Il mistero della Sindone, p. 181, also mentioned in Svensson and Heimburger, Forensic aspects..., 2012, p. 2520 (pdf . 8); Evidence C34 referring to Riggi 1982 (Fanti et al., Evidences for..., 2005,

<http://www.shroud.com/fanti3en.pdf> p. 5)

<sup>417</sup> Morris et al., X-ray fluorescence..., 1980

<sup>418</sup> Fanti and Marinelli, Results of..., p. 5 (no. 12)

<sup>419</sup> Jewish Encyclopedia, item “Burial”, referring to (Shab. xxiii. 5; Sem. i. 2, 3; Acts ix. 37; John xi. 44, xii. 7, xix. 39 *et seq.*, xx. 6 *et seq.*; Matt. xxvii. 59; Mark xv. 46 *et seq.*; Luke xxiii. 53 *et seq.*; Testament of Abraham, xx.)

<http://www.jewishencyclopedia.com/view.jsp?artid=1607&letter=B&search=burial>

right before it was washed. In that case, the sand/salt would only have touched the outsides of the ordinary clothes. Likewise, salt may have only touched the outside of the enveloping Shroud.

### 3. SURVIVAL OF CLOTH, BLOOD AND SERUM – PRESERVATIVE COATING

Heller wrote on the experiences of the STURP team in 1978: “The team expected the Shroud to be in a delicate condition. It was not. It was supple, strong, and felt almost like a new, expensive tablecloth. The weave, a relatively tight herringbone twill, had not only right-angle strength, but diagonal strength as well. We were to determine subsequently that, though it was covered with mildew spores, there was no mildew on it. We still have no explanation for this or for its splendid condition.”<sup>420</sup> An explanation for the splendid condition of the Shroud may be the presence of a waterresistant sealing coating of retrograded and polished starch, which, when still gelatinous, had been used as a lubricant during weaving. That the mildew spores on the Shroud are not active, might be explained by the presence antimicrobial preservatives in this coating.

#### 3.1. Myrrh and aloes – antibacterial and antifungal

Baima Bollone wrote that “the blood on the Shroud is perfectly preserved, without any traces of pollution, which is likely to be due to the inhibitory presence of aloes and myrrh.”<sup>421</sup> Baima Bollone observed a positive response for myrrh and aloes “for the dust samples from the Shroud. In particular, the preparations from C12a and C8a showed the presence of fluorescent lumps either free or adherent to the fibres, while on the preparations from D2c only free lumps were observed”, but he observed a “totally negative response for ... Shroud fibers”.<sup>422</sup> Nitowski tells in a video about the finding of a “substance” “congruable to myrrh” by microscopy and about a photomicrograph showing a fiber “enocoated with blood, myrrh and aloe”.<sup>423</sup> Two photomicrographs of aloe and myrrh particles on Shroud samples, with photos of reference particles, are online in a poster session by Fanti and Faccini.<sup>424</sup> In a letter of February 27, 1986, Nitowski wrote “It amazes me that the U.S. scientists missed so much. One can clearly see large chunks of calcium, myrrh, and aloes on the tapes and these things are not difficult to identify!”<sup>425</sup> The same is expressed in a press release of her ESSJ team: “June Press Release ... (2) Through comparative examination of the Mylar tapes from the Shroud of Turin, ESSJ has found the presence of myrrh and aloes on the Shroud, something not documented previously by American scientists.”<sup>426</sup>

In 1983, Heller had indeed reported: “Adler and I had found no traces of aloe, myrrh, or any other spice. We had found no oils, though they may have oxidized away.”<sup>427</sup> This complies with what Rogers wrote in 2004: “Biblical accounts suggested several types of compounds that might have appeared on the cloth (e.g., aloes, myrrh, sebaceous secretions, etc.). We planned and executed chemical analytical methods that could detect them in 1978. Those methods were extremely sensitive, but they did not detect squalene or myrrh.”<sup>428</sup> But a very important detail, which should

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<sup>420</sup> Heller, Report on..., 1983, p. 116-117; on these pages Heller even suggests that the main cloth of the Shroud is the strongest part of the cloth, instead of any of the patches sewn on after the 1532 AD fire.

<sup>421</sup> Baima Bollone, The Forensic Characteristics..., Proceedings Turin 2000, p. 213

<sup>422</sup> Baima Bollone and Gaglio, Demonstration of..., 1984, <http://www.shroud.com/pdfs/ssi13part3.pdf>

<sup>423</sup> Nitowski = Sister Damian OCD, video <http://tp.holyshroudguild.org/media/NewProject.swf>

<sup>424</sup> Fanti and Faccini, Sindone: la scienza spiega la fede – Turin Shroud: science explains faith, <http://www.dii.unipd.it/-giulio.fanti/research/Sindone/Mostra%20Sindone.pdf>, p. 15

<sup>425</sup> Nitowski = Sister Damian OCD, handwritten letter to Father Rinaldi, 1986, page 3

[http://www.scribd.com/fullscreen/45011592?access\\_key=key-y74jz4s46kysncgjwsj&allow\\_share=false&escape=false&show\\_recommendations=false&view\\_mode=scroll](http://www.scribd.com/fullscreen/45011592?access_key=key-y74jz4s46kysncgjwsj&allow_share=false&escape=false&show_recommendations=false&view_mode=scroll) = Scribd document on right hand side of <http://www.holyshroudguild.org/sister-damian-of-the-cross-ocd-dr-eugenia-nitowski.html>

<sup>426</sup> p. 3 of June 1985 press release that starts on p. 8 of the Scribd document

[http://www.scribd.com/fullscreen/45010690?access\\_key=key-48x44lgtvyb9eggdljr&allow\\_share=false&escape=false&show\\_recommendations=false&view\\_mode=scroll](http://www.scribd.com/fullscreen/45010690?access_key=key-48x44lgtvyb9eggdljr&allow_share=false&escape=false&show_recommendations=false&view_mode=scroll) on the left hand side of <http://www.holyshroudguild.org/sister-damian-of-the-cross-ocd-dr-eugenia-nitowski.html> )

<sup>427</sup> Heller, Report on..., 1983, p. 209

[http://books.google.fr/books?ei=dNPnUbiBLoL\\_PKercAH&hl=fr&id=o9glK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&focus=searchwithinvolume&q=aloe](http://books.google.fr/books?ei=dNPnUbiBLoL_PKercAH&hl=fr&id=o9glK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&focus=searchwithinvolume&q=aloe)

<sup>428</sup> Rogers, Frequently Asked Questions..., 2004, FAQ #15

have been mentioned, or at least considered, here by Rogers,<sup>429</sup> is that the fibers were washed with xylene and toluene<sup>430</sup> (see 4.2.1.1), which substances are solvents for oil<sup>431</sup>: “Washing them with solvents ... washing was guaranteed to remove some of the impurities. ... McCrone had probably ruined our chances of finding squalene and triglycerides by pyrolysis/MS”.<sup>432</sup> This is in the 2008 posthumous book of Rogers. “Triglycerides are the main constituents of vegetable oil”<sup>433</sup>, like the oil of the plants aloe and myrrh.

Therefore, the difference in observations might be due to the circumstance that Baima Bollone and Nitowski identified “lumps” and “chunks” as myrrh and aloe, while Heller and Adler and Rogers perhaps only sought for traces of myrrh and aloe “oil” and “compounds” on the surface of linen fibers pulled or cut from the sticky tapes and washed with xylene/toluene.

The “oleogum-resin” and “volatile oil” of myrrh “possesses antibacterial and antifungal activity against *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*”<sup>434</sup> and myrrh’s essential oil also has activity against the fungi “*Aspergillus flavus*, *A. niger* and *Penicillium citrinum*”.<sup>435</sup> The Biblical aloe used as a perfume in burial ceremonies, such as the aloe in the “mixture of myrrh and aloes” brought by Nicodemus for the burial of Jesus (John 19:39), was *Aquilaria agallocha*.<sup>436</sup> Extracts of this plant “showed moderate zones of inhibition (14 - 18 mm) against all the bacteria tested”, viz. “pathogenic bacteria such as *Shigella flexneri*, *Bacillus brevis*, *Pseudomonas aeruginosa* and *Bacillus subtilis*”.<sup>437</sup>

Pellicori of STURP made laboratory stains of myrrh and aloes on linen, scorched some areas of these stains, and observed that “Neither myrrh nor aloes stains fluoresced.”<sup>438</sup> For this reason it is likely that, besides myrrh and aloes lumps, another, possibly preservative, substance is present on the atypically fluorescent Shroud (see 4.2.4).

### 3.2. Saponaria – antibacterial and antioxidant

“*Saponaria* is toxic, and it is a potent preservative.”<sup>439</sup> A study showed that “the aerial parts of *Artemisia absinthum*, *Artemisia santonicum* and *Saponaria officinalis* exhibit antibacterial activity against a number of bacteria”, and that “Methanol extracts of the three species analyzed showed high antioxidant activity” and that “There was a positive correlation (R = 0.819) between the total

<sup>429</sup> Note that Rogers did write in this same paper of 2004 (in FAQ #18 on a hypothesized bioplastic coating) – so after he had written about the madder dye in the Raes sample in his paper Scientific method..., 2002 – that Shroud fibers for Raman and Pyrolysis/Mass spectrometry were washed “free of adhesive with xylene (not a solvent for any “bioplastic polymers”)” (Rogers, Frequently Asked Questions..., 2004, FAQ #18).

<sup>430</sup> Rogers, A Chemist’s..., 2008, p. 37; Heller and Adler, A Chemical..., 1981, TOM 37

<sup>431</sup> Xylene is “insoluble or very slightly soluble in water” but “more soluble in oil”

<http://www.sciencelab.com/msds.php?msdsId=9927647>; cf. “Xylene-based inhibitor solves crude oil wax problems in Niger Delta pipeline” <http://www.ogj.com/articles/print/volume-103/issue-10/transportation/xylene-based-inhibitor-solves-crude-oil-wax-problems-in-niger-delta-pipeline.html>;

“Toluene occurs naturally at low levels in crude oil” <https://en.wikipedia.org/wiki/Toluene>; “The optimum ratio of toluene to palm oil is 1:1 (v/v). ... toluene

is the most promising solvent in promoting transmethylation” Krisnangkura et al., 1992

<http://link.springer.com/article/10.1007/BF02540569>

<sup>432</sup> Rogers, A Chemist’s..., 2008, p. 52 [http://books.google.fr/books?id=p-n84sc4ayYC&printsec=frontcover&hl=fr&source=gbs\\_ge\\_summary\\_r&cad=0#v=onepage&q=ruined&f=false](http://books.google.fr/books?id=p-n84sc4ayYC&printsec=frontcover&hl=fr&source=gbs_ge_summary_r&cad=0#v=onepage&q=ruined&f=false)

<sup>433</sup> <http://en.wikipedia.org/wiki/Triglycerides>

<sup>434</sup> “15. Myrrh Botanical name: *Commiphora molmol* Family: Burseraceae Part used: Gum resin, stem and leaves Chemical constituent as preservative: Oleogum-resin, volatile oil Preservative action: Myrrh possesses antibacterial and antifungal activity against *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* 53, 54. (53. Kumar P. and Clark M. Saunder W.B., Ed. Clinical Medicine, 5th Edn, (2002) 54. Saeed M.A. and Sabir A.W., Antibacterial activities of some constituents from oleo-gumresin of *Commiphora mukul*. Fitoterapia, 75(2): 204-208, (2004))” (Sing, 2010, <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.185.800&rep=rep1&type=pdf>)

<sup>435</sup> Batool, 2007, <http://www.jnus.org/pdf/1/2007/1/256.pdf>

<sup>436</sup> Boi, The Ethnocultural significance ..., 2012, p. 3-4

<sup>437</sup> Dash, 2008, <http://www.ajol.info/index.php/ajb/article/download/59366/47665>

<sup>438</sup> “The laboratory stains discussed in Sec. IV.B, both scorched and unscorched, were examined for visual fluorescence when irradiated with the 366-nm Hb line. The basic linen blue-white fluorescence changed to faint yellow-green with baking. Neither myrrh nor aloes stains fluoresced.” Pellicori, Spectral Properties..., 1980, p. 1919.

<sup>439</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 5-6

phenolic content and the antioxidant activity measured in the plant samples.”<sup>440</sup> It was also found, in a study using *Quillaja saponaria* saponins and *Yucca schidigera* plant extract, that “Saponins have the potential to modulate microbial growth in natural and artificial fermenters”; “All the saponins and the plant extract increased growth of *Escherichia coli* up to a certain concentration and thereafter decreased growth.”<sup>441</sup> An in vivo study on saponin-treated rats showed that “the beneficial effects of saponin on serum lipids were related to a direct saponin antioxidant activity.”<sup>442</sup> However, many characteristics of Saponaria preclude its active and predominant presence on the Shroud (see 4.4).

### 3.3. Madder – antimicrobial, antifungal, insecticidal, antioxidant

Alizarin is an antifungal agent<sup>443</sup>, and so is purpurin.<sup>444</sup> Xanthopurpurin, rubiadin, and nordamnacanthal are potent antimicrobial agents,<sup>445</sup> and they are present in a water extract of *Rubia tinctorum* roots.<sup>446</sup> A madder extract is also insecticidal.<sup>447</sup> Lucidin-3-*O*-primeveroside, which is particularly present in *Rubia tinctorum*, drives off the varied carpet beetle (*Anthrenus verbasci* L.), also called the museum bug, which not only scavenges on other dead insects but also hair, feathers, and natural fibers.<sup>448</sup> A comparative study on ethanol, methanol, ethyl acetate, and water extracts of *Rubia tinctorum* showed that water extracts of the roots were not antimicrobially active against ten out of ten specific Gram (+) and Gram (-) bacteria, but was active against two out of three specific yeasts and three out of three specific fungi and actinomycetes.<sup>449</sup> Water extracts of the aerial parts of the plant acted the same, except that they are only active against one out of three yeasts. Among the tested Gram (-) bacteria was *Escherichia coli*, which is one of the lactic acid bacteria and belongs to “the healthy microflora of human mucosal surfaces”.<sup>450</sup> If the madder root extract was made slightly acidic, for instance by adding some wine vinegar, it would not only make the red-orange root extract<sup>451</sup> an acidic and thus yellow dye but the acidity would also give the extract more resistance to fungi and some resistance to bacteria, except the acetic and lactic acid bacteria of vinegar itself.<sup>452</sup> Its lactic acid bacteria would start the fermentation of madder’s free sucrose to lactate/lactic acid and

<sup>440</sup> Sengul et al., 2011, <http://ijpr.sbmu.ac.ir/index.php/daru/article/view/?action=articleInfo&article=877>

<sup>441</sup> Sen et al., 2002, <http://onlinelibrary.wiley.com/doi/10.1046/j.1472-765X.1998.00379.x/abstract>

<sup>442</sup> Rodrigues et al., 2005, <http://informahealthcare.com/doi/abs/10.1080/09637480500081738> – in the abstract, a soybean flavonoid (2-phenyl-benzopyrane) is called a saponin.

<sup>443</sup> Handbook of acid-base indicators, R.W. Sabnis, 2008,

[http://f3.tiera.ru/3/Chemistry/Analytical%20chemistry/Sabnis%20R.W.%20Handbook%20of%20acid-base%20indicators%20\(CRC.%202007\)\(ISBN%200849382181\)\(416s\).pdf](http://f3.tiera.ru/3/Chemistry/Analytical%20chemistry/Sabnis%20R.W.%20Handbook%20of%20acid-base%20indicators%20(CRC.%202007)(ISBN%200849382181)(416s).pdf) p. 11 (pdf page 28)

<sup>444</sup> “we have demonstrated the potent *in vitro* antifungal activity of purpurin, a natural red anthraquinone pigment in madder roots (*Rubia tinctorum* L.), against a panel of six pathogenic *Candida* species” Wai-Kei Tsang et al., 2013,

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0086032>

<sup>445</sup> Naidu et al., 2009, <http://www.scribd.com/doc/23867259/Antimicrobial-Agents-From-Rubia-Cordifolia>

<sup>446</sup> <http://www.freepatentsonline.com/EP1191070.html>

<sup>447</sup> <http://fwxc.com/article-187231.html>

<sup>448</sup> Art Néss Proaño Gaibor de Vries, 2011, <http://www.collectiewijzer.nl/2011/02/24/rubiaceae-dyestuff-repellents-and-museumbugs/>

<sup>449</sup> Kalyoncu et al., Antimicrobial Activity of Common Madder (*Rubia tinctorum* L.), *Phytotherapy Research* 20/6, 490-492 (2006), <http://onlinelibrary.wiley.com/doi/10.1002/ptr.1884/abstract>

<sup>450</sup> [http://en.wikipedia.org/wiki/Lactic\\_acid\\_bacteria](http://en.wikipedia.org/wiki/Lactic_acid_bacteria)

<sup>451</sup> Madder roots are red-orange [http://wildcolours.co.uk/html/natural-dyes\\_13.html](http://wildcolours.co.uk/html/natural-dyes_13.html)

<sup>452</sup> “As in yogurt, when the acidity rises due to lactic acid-fermenting organisms, many other pathogenic microorganisms are killed.” [http://en.wikipedia.org/wiki/Lactic\\_acid\\_fermentation](http://en.wikipedia.org/wiki/Lactic_acid_fermentation); “Vinegar, throughout history, has been reputed to have strong antibacterial properties. One test by Good Housekeeping’s microbiologist found that 5% vinegar is 90% effective against mold and 99.9% effective against bacteria” <http://en.wikipedia.org/wiki/Vinegar#Cleaning>; “In addition to acetic acid, fruit vinegars often contain citric, malic, lactic, and tartaric acids and may also include phenolics, some of which are produced as a result of fermentation.” Shahidi et al., 2008,

<http://apjcn.nhri.org.tw/server/APJCN/17/s1/380.pdf>; lactic acid is a “major” organic acid in wine vinegars: “The method was successfully validated and enables the reliable separation of major organic acids in wine vinegars (ie tartaric, citric, malic, lactic and acetic acid) in about 25 min.” Morales et al., 1998, <http://www.sciencedirect.com/science/article/pii/S002196739800572X>

possibly some CO<sub>2</sub> and alcohol,<sup>453</sup> which also is a preservative. Madder contains, besides saponins, also rubiadin, which is a potent antioxidant,<sup>454</sup> and many other polyphenolic antioxidant compounds.

### 3.4. Leech saliva antibiotics

In the medicinal leech, *Hirudo medicinalis*, which feeds on sucked up blood, “feeding results in a dramatic rise of the metabolic rate as indicated by O<sub>2</sub> consumption, excretion of NH<sub>3</sub> and elimination of ions and water, each showing a characteristic time-course. ... Respiration, NH<sub>3</sub> excretion and the rise of proteolytic activity are inhibited by the antibiotic kanamycin.”<sup>455</sup> Kanamycin is a bacteriocidal antibiotic,<sup>456</sup> so it seems that bacteria in the leech gut are an important part of the digestive mechanism. This means that there are probably little natural antibacterial preservatives in the leech gut. Saliva of feeding leeches contains anticoagulants, vasodilators and anti-inflammatory agents.<sup>457</sup> It also contains hyaluronidase, which increases interstitial viscosity, and has “antibiotic effects”; yet, these effects probably aren’t large, for bacterial infections belong to the possible complications of treatment with leeches and “The benefit of prophylactic antibiotics during treatment with leeches has been reported”.<sup>458</sup>

### 3.5. Mordant protects madder lake from degradation

Not only would madder constituents have a preserving activity on blood products, a heme mordant may also have a protecting activity on madder. A study using an alum mordant showed that “The mordant has a protecting action on degradation” of *Rubia tinctorum* dyestuff; in ageing, “The blue spectral shifts observed for purpurin and madder samples (and even for alizarin to a lesser extent) were markedly reduced in the presence of mordant showing again the role of alum in improving the dye lightfastness.”<sup>459</sup> The high stability of pinkish-red madder lake is shown for instance by its presence on an ancient Egyptian painting,<sup>460</sup> a 6th century AD Byzantine manuscript (fig. 2.15),<sup>461</sup> and 7-9<sup>th</sup> century AD cloths.<sup>462</sup>

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<sup>453</sup> “Lactic acid fermentation is a biological process by which glucose, fructose, and sucrose are converted into cellular energy and the metabolite lactate. ... In *homolactic fermentation*, one molecule of glucose is ultimately converted to two molecules of lactic acid. *Heterolactic fermentation*, in contrast, yields carbon dioxide and ethanol in addition to lactic acid, in a process called the phosphoketolase pathway.” [http://en.wikipedia.org/wiki/Lactic\\_acid\\_fermentation](http://en.wikipedia.org/wiki/Lactic_acid_fermentation)

<sup>454</sup> Kunwar et al., 2009, <http://www.docstoc.com/docs/19972326/Indigenous-Use-and-Ethnopharmacology-of-Medicinal-Plants-in-Far> p. 18 (p. 14 of Docstoc file) = <http://lib-ojs3.lib.sfu.ca:8114/index.php/era/article/view/223>

<sup>455</sup> Zebe et al., 1986, <http://www.sciencedirect.com/science/article/pii/0300962986900411>

<sup>456</sup> <http://en.wikipedia.org/wiki/Kanamycin>

<sup>457</sup> “1. History of hirudotherapy. In medieval and early modern medicine, *Hirudo medicinalis* were used to remove blood from patients in an attempt to balance the 'biological humours'. ... Bdelins, Anti-inflammatory, inhibits plasmin, trypsin and acrosin.”, Medicinal leech therapy (Hirudotherapy) : A brief overview, Singh, 2010,

<http://www.sciencedirect.com/science/article/pii/S1744388109001339>; “Plasma-kallikrein-inhibitory activity from the European leech, *H. medicinalis*, was demonstrated to be present in the stasin-type serine proteinase inhibitor from the medicinal leech salivary gland and to be involved in anticoagulant, vasoactive or anti-inflammatory actions [24, 25].”

Kim and Kang, 1998, <http://onlinelibrary.wiley.com/doi/10.1046/j.1432-1327.1998.2540692.x/pdf>

<sup>458</sup> Gröbe et al., 2012, Leech therapy in reconstructive maxillofacial surgery,

<http://www.sciencedirect.com/science/article/pii/S0278239111003119> ; [http://en.wikipedia.org/wiki/Hirudo\\_medicinalis](http://en.wikipedia.org/wiki/Hirudo_medicinalis)

referring to Wells et al., <http://www.ncbi.nlm.nih.gov/pubmed/8479316> ; Whitaker et al., 2005,

<http://www.sciencedirect.com/science/article/pii/S0266435604002165>

<sup>459</sup> Clementi et al., A spectrometric..., 2007, <http://www.sciencedirect.com/science/article/pii/S0003267007009464>, p. 54 (Conclusions) and p. 52

<sup>460</sup> “Madder lake Pinkish-red, clear isotropic masses”, Sack, Tahk and Peters, A technical examination of an ancient Egyptian painting on canvas, SP Sack, FC Tahk, T Peters Jr - *Studies in Conservation*, 1981 – *JSTOR*,

<http://www.jstor.org/discover/10.2307/1505817?uid=3738736&uid=2&uid=4&sid=21102611584517>

<sup>461</sup> the manuscript called *Vienna Dioskurides*, see Aceto et al., First analytical..., 2012,

<http://www.sciencedirect.com/science/article/pii/S1386142512004568>;

<sup>462</sup> Orska-Gawrýs et al., 2003, sample #1 and #5 of fig. 5, which at sample #10 says “no mordant”, implying Fe and Al are mordants <http://ars.els-cdn.com/content/image/1-s2.0-S0021967303000839-gr5.gif> of

<http://www.sciencedirect.com/science/article/pii/S0021967303000839>

## 4. MADDER ON STARCH COATING

### First-century temple mantle brightener

All various special features of the Shroud's bloodstains – the red color, the other chemical and physical properties, their probable formation and their preservation – can be explained by the mere presence of a waterresistant starch-madder coating on the Shroud.

Many arguments for the presence of this coating have already been given in the article “Internal selvedge in starched and dyed temple mantle – no invisible repair in Turin Shroud – no Maillard reaction”; this 2012 article and its preceding 2011 article, updated in June 2013, “The seam and missing corners of the Turin Shroud as characteristics of John Mark's temple garment”, have already shown that the Shroud most probably is a first-century Jewish temple mantle. There it was discussed that the Shroud is a fabric

1) in which “faults in the preparation of the shafts point to a specifically ancient twill weave manufacturing method”<sup>463</sup>, and the combination of its extremely fine threads (ca. 0.25 mm diameter, about 38 x 25 threads per cm<sup>2</sup>) and extremely fine technical fibers (10-20 micrometer diameter<sup>464</sup>) and herringbone weave is “incomparable”<sup>465</sup>

2) which has a probably Pharisaic enlarged border, cut and resewn at manufacture before the transverse hems were sewn across by the same professional hand,<sup>466</sup> and sewn in a special stitching type (two lines of parallel overcast stitches (no counter stitches) for the seam, both applied from the reverse side, nearly invisible on the observe)<sup>467</sup> similar to the stitching of seams found in first-century Masada, Judea, and a stitching type that the ancient Egyptians only used to sew a fringeless braid to a garment – for other purposes, even on garments, simpler stitching types were used, e.g. a

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<sup>463</sup> Textile expert G. Vial, reviewed in Sept./Oct. 1990 *BSTS newsletter* 26,9, <http://www.shroud.com/pdfs/n26part9.pdf> p. 2,

<sup>464</sup> Gian Marco Rinaldi, Autogol a Tucson, <http://sindone.weebly.com/autogoltucson.html>; Fanti et al., Evidences for..., 2005, introduction p. 2, and evidence A6 <http://www.shroud.com/pdfs/doclist.pdf>

<sup>465</sup> G. Vial, reviewed in Sept./Oct. 1990 *BSTS newsletter* 26,9, <http://www.shroud.com/pdfs/n26part9.pdf> p. 2,

<sup>466</sup> The raw cut edges inside the seam show no fray and the warp threads are continuous across the seam, as shown by X-ray photography (Adler and A. and Whanger, Concerning the Side Strip..., 1997, <http://www.shroud.com/adler2.htm>), and the seam may be located at a woven-in selvedge, consisting of warp threads that have cotton spun in (Hoeven, Internal selvedge..., 2012, par. 2.1.); drawing of type of seam and stitching of side seam <http://shroudofturin.files.wordpress.com/2013/12/60.jpg>; photo of seam plus hem: Heimbürger, Cotton in Raes..., 2009, fig. 15, <http://www.shroud.com/pdfs/thibault7part3.pdf>. In August 2014, Bracaglia of the Holy Shroud Guild disclosed that Raes had written, in confidential letters to Vial and Kim 13/11/1989, that of his microscopic preparations of threads of part 1 (main Shroud) of the Raes corner, not all had cotton, and that only very few microscopic preparations of part 2 (border strip) of the Raes corner were made:<sup>466</sup> “It is true that in my report of 1973 I mentioned that I found traces of cotton fibers only in the main body of the Shroud. I must however add that even for the main body I did not find traces of cotton in all microscopical preparations. On the other hand I had only a very small part of the strip added to the main body and I could only make a few microscopical preparations of the fibers on the strip”. ; “Raes also states in his letter to Fr. Kim That “I could not agree with him and that in my opinion the cotton fibers were not superficial fibers”.” (Comments by “Giorgio / HSG”, August 9, 2014 at 1:30 pm, and further down at 3:48 pm, <http://shroudstory.com/2014/08/07/comment-promoted-thibault-heimburger-on-rogers-discoveries/>). Vial's presentation in Rome 1993 noted there is little doubt that, speaking about part 2 of the Raes sample compared with part 1, “the fabric is the same” and that this “lack of cotton does not seem to be very determining” (video Rome 1993, Vial <https://www.youtube.com/watch?v=r-JO2pbJQFQ> from 2:03). This means that not only part 1 and part 2 of the Raes sample are probably the same fabric, but also the main Shroud may be of the same fabric, e.g. of linen with some spun-in cotton in some parts of some threads. The reported quantitative difference between the cotton in certain Raes warp threads (10-20% cotton) and a thread from the main Shroud near the radiocarbon sample (2% of thin cotton) is not significant anylonger, since even parts of Raes threads can contain no cotton at all; traces of cotton were found in other, more centrally located samples of the main Shroud (Hoeven, Internal selvedge..., 2.1.2.). Also the Zina thread from the heel area of the main Shroud seems to have contained some cotton, as most of the fibers in the envelope that contained the “picked to pieces” Zina thread were cotton, which Rogers' book interpreted as “obviously detached from the rag-bond paper while the sample was being picked to pieces” (Rogers, A Chemist's..., 2008, p. 26 and 38), but he may have been mistaken. So perhaps there is no internal selvedge, woven of cotton-linen spun-together thread, after all, but the whole Shroud ununiformly has spun-in cotton.

<sup>467</sup> See a digital copy of two pages (with photos and drawing) from the English version of an article by textile expert and Shroud conservator Flury-Lemberg (probably from the Flury-Lemberg, Die Leinwand des Turiner..., Proceedings of the International Shroud Symposium, Turin 2000) at <http://shroudofturin.files.wordpress.com/2013/12/60.jpg>

“run and fell” seam, combining a running stitch line and an overcast stitch line –,<sup>468</sup> 3) which probably has an extraordinary coating of retrograded starch and madder on top of the starch, which both would be washed out in a single hot wash<sup>469</sup> – washing was not allowed for Jewish temple garments.<sup>470</sup>

Wheat starch that was gelatinized in hot water – i.e., got a loose structure with water molecules inside – to form a viscous paste, probably was wiped on the warp threads to protect them during weaving. This starch eventually dried and thus retrograded – i.e., returned to a more organized dense structure that resists the entrance of cold water – and formed a thick (cold-water) insoluble starch layer on the cloth. This thick layer would have been largely washed from the cloth after weaving and then dried and retrograded again to form a thin insoluble starch film on the threads. The cloth that was meant to be a temple garment was then dyed with an acidic and fluorescent madder dye, and probably glazed by firmly rubbing it with a glass ball or slickstone, such as the Viking-type linen smoother or the Dutch smoothing balls from the 8th and 9th centuries<sup>471</sup>, to render the starch film more dense and sealing, and thus dirt repellent and lustrous. Number B14 of the Shroud evidence list says “The TS linen has a lustrous finish (Rogers, 1978-1981).”<sup>472</sup> The fluorescence of the Shroud that is unlike the fluorescence of other aged linen, and that is discussed more extensively in 4.2.3 and 4.2.4, was a reason for Rogers to assume the presence of a fluorescent coating on the Shroud.<sup>473</sup>

Madder fluorescence may be the reason why only a few clothes in the New Testament are called ‘lampran’ = “shining”, viz. king Herod’s robe (Luke 23:11 WH BBE), and the clothes of a notional man that enters the synagogue (James 2:2-3), and the clothes of the angel that appeared to Cornelius (Acts 10:30). Shining linen, even pure and shining linen, is only mentioned for the clothes of the angels that came out of the sanctuary (‘naos’) in the Court of the Priests of the temple (“and come forth did the seven messengers having the seven plagues, out of the sanctuary, clothed in linen, pure and shining, and girded round the breasts with golden girdles” Rev. 15:6) and for the bride of the Lamb – the bride of Christ, the eternal high priest-king – (“and there was given to her that she may be arrayed with fine linen, pure and shining, for the fine linen is the righteous acts of the saints” Rev. 19:8, both Young’s Literal Translation); the Greek text has λινον καθαρον και λαμπρον - ‘linon katharon kai lampron’ - for the angels, and βυσσινον καθαρον και λαμπρον - ‘byssinon katharon kai lampron’ - for the bride (Textus Receptus).<sup>474</sup> Here ‘byssinos’ means ‘fine linen’, which certainly

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<sup>468</sup> In the Proceedings of the 2000 Turin symposium, Flury-Lemberg shows a drawing of the type of seam (visible in Soons’ presentation The Halo around..., 2012, slide 11, <http://www.shroud.com/pdfs/soonspanppt.pdf> and also in <http://shroudofturin.files.wordpress.com/2013/12/60.jpg>), and calls it “seam type Masada”: “Abb. 3 a Zeichnung: Nahttyp Masada” (Flury-Lemberg, Die Leinwand des Turiner..., proceedings 2000, p. 34). It might be described as a kind of rolled seam, secured with two lines of tiny overcast stitches. It is interesting that the ancient Egyptians specifically used two lines of overcast stitches to sew on a fringeless braid along an edge of a garment: “When a braid was added to a garment, one of several techniques was used, depending on the nature of the braid and the place where it was to be attached. If it was a fringed braid placed at the lower edge of a garment, it would normally be secured with one line of overcast stitching (e.g. Carter no. 367i; Cairo JE 62625). On the other hand, two lines of overcast stitching were used to sew on fringeless braids, whether along an edge of a garment or down the middle” (Nicholson and Shaw, 2000, Ancient Egyptian materials and technology, p. 282-283, [http://books.google.co.uk/books?id=Vj7A9jJrZP0C&printsec=frontcover&dq=Ancient+Egyptian+materials+and+technology,+Cambridge+University+Press,+2000&hl=nl&sa=X&ei=pSv0UN7RC\\_Sr0AXYsoHwBQ&ved=0CC0Q6AEwAA&q=fringeless&f=false#v=snippet&q=fringeless&f=false](http://books.google.co.uk/books?id=Vj7A9jJrZP0C&printsec=frontcover&dq=Ancient+Egyptian+materials+and+technology,+Cambridge+University+Press,+2000&hl=nl&sa=X&ei=pSv0UN7RC_Sr0AXYsoHwBQ&ved=0CC0Q6AEwAA&q=fringeless&f=false#v=snippet&q=fringeless&f=false)); the “run and fell seam” is shown in its fig. 11.11.e.

<sup>469</sup> Water of about 60 degrees C or more solves retrograded starch: see fig. 1, 2, 3, and 4, in which R<sub>T</sub> is retrograded starch, of Fernández-Martin et al., 2008, [http://www.researchgate.net/publication/233885487\\_Pressurization\\_of\\_some\\_starches\\_compared\\_to\\_heating\\_Calorimetric\\_thermo-optical\\_and\\_X-ray\\_examination/file/72e7e51b8e22d2a185.pdf](http://www.researchgate.net/publication/233885487_Pressurization_of_some_starches_compared_to_heating_Calorimetric_thermo-optical_and_X-ray_examination/file/72e7e51b8e22d2a185.pdf)

<sup>470</sup> Maimonides, Mishneh Torah, Kli Hamikdash, chapter 8, Halacha 4-5, [http://www.chabad.org/library/article\\_cdo/aid/1008233/jewish/Chapter-8.htm](http://www.chabad.org/library/article_cdo/aid/1008233/jewish/Chapter-8.htm)

<sup>471</sup> <http://www.oldandinteresting.com/antique-irons-smoothers-mangles.aspx>; <http://www.huntsearch.gla.ac.uk/cgi-bin/foxweb/huntsearch/DetailedResults.fwx?collection=archaeology&SearchTerm=B.1914.861&reqMethod=Link>; <http://www.wegwijslezer.nl/php/vreemd.php?selpage=55#reaction951> : “bollen uit de 8e en 9e eeuw”

<sup>472</sup> Fanti et al, Evidences for..., 2005, evidence B14

<sup>473</sup> Rogers, A Chemist’s..., 2008, p. 40

<sup>474</sup> <http://www.biblegateway.com/passage/?search=Revelation%2015%3A6%3B%2019%3A8&version=YLT;TR1550>

applies to the linen of the Shroud and which was the material prescribed for the high priest's white "coat in checker work of fine linen" (Ex 28:39 RSV, Vulg)<sup>475</sup>. 'Katharos', also used for the linen cloth/mantle bought by Joseph of Arimathea (Matthew 27:59) means 'ritually clean'<sup>476</sup>, as was required for all objects and persons in the temple. 'Lampros' (= shining) may refer to the fluorescent property of the pale yellow madder coating that rendered these clothes, including the Shroud, "shining". The sealing starch film on the Shroud probably also retarded the ageing and decomposition of its extremely fine linen. That no other linen cloths like the Shroud have been found, is probably due to the Jewish prescription that all linen temple garments that became soiled, should not be laundered but cut and made into wicks for the temple's oil lamps; in 70 AD the whole Jewish temple was burnt.<sup>477</sup>

## 4.1. Starch

### 4.1.1. Strippable sealing film

Rogers wrote he found starch on threads from the Raes corner (iodine test) – beneath a yellow-brown flaking (starch) gum crust – and on a main-Shroud sample (a reddish color with iodine azide reagent) and on "linen fibers from the TS" by pyrolysis mass spectrometry, Kohlbeck detected starch on the Raes sample (iodine test) and perhaps on a main Shroud sample from the lance wound area, and McCrone microscopically identified starch grains on main Shroud sample(s).<sup>478</sup> Rogers even wrote that "The hypothesis on carbohydrate impurities is supported by observations of traces of some starch fractions on image fibers", while Heller and Adler reported that "body image" fibers gave a negative "iodine – iodide" test for starch.<sup>479</sup> On image fibers, starch may have been transformed by the image formation process.

Nitowski, who was Sister Damian OCD and worked with Kohlbeck and studied many tape samples from all over the Shroud, seems to have been worried about the whole Shroud when she wrote: "September 15, 1985 ... I know what Professor Raes said about the small quantity of cotton, however, Kohlbeck found that not only cotton, but linen fibrils do have a starch coating. I would be worried about a possible reaction between the starch and the red dye from the silk storage cloth."<sup>480</sup> The cloth of the Shroud "does not readily absorb water"<sup>481</sup> – although linen is renowned for its capability to absorb moisture rapidly<sup>482</sup> –, and is supple and strong in both lateral and diagonal

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<sup>475</sup> <http://www.biblegateway.com/passage/?search=Exodus%2028:39&version=RSV;VULGATE>

<sup>476</sup> Cf. John 13,10 Rev 15,6 Ezr 6,20 Lev 24,6 2Ch 13,11 LXX ; Former Professor of Religious Studies D. Fulbright states that "it is indisputable that καθαρος in Matthew 27:59 is a reference to Levitical purity" (Fulbright, "A Clean Cloth"- What Greek Word Usage Tells Us about the Burial Wrappings of Jesus, BSTS Newsletter, No. 62, Dec. 2005, p. 15-17, <http://www.shroud.com/pdfs/n62part7.pdf>); Grossi, Jewish Shrouds..., 2013, p. 26

<sup>477</sup> "Whenever any of the priestly garments become soiled, they are not bleached or laundered. Instead, they are left to be used for wicks and he should wear new ones. 21 (21: For there should be no expressions of poverty in a place of wealth (Zevachim 88b))." (Maimonides, Mishneh Torah, Kli Hamikdash, chapter 8, Halacha 4-5, [http://www.chabad.org/library/article\\_cdo/aid/1008233/jewish/Chapter-8.htm](http://www.chabad.org/library/article_cdo/aid/1008233/jewish/Chapter-8.htm); [http://en.wikipedia.org/wiki/Destruction\\_of\\_the\\_temple](http://en.wikipedia.org/wiki/Destruction_of_the_temple)

<sup>478</sup> Hoeven, Internal selvedge..., 2012, paragraph 2.2. ; then, in 2012, I interpreted the text on Kohlbeck's "observation" as applying to starch in the lance wound area, but now, on second thoughts, I think the "observation" in the lance wound area may be only the observed blackening effect of microscopy oil on red particles, not the observation of starch on Raes threads: Bracaglia of the Holy Shroud Guild wrote "Dr. Kohlbeck explained to me that Sue Benford contacted him and requested if he could send her his microscopic photographs of the lance wound area where Dr. Kohlbeck made his observation. (6-BF). She explained to him that she believes what Dr. Heller thought was blood is actually the gum,dye,mordant coating which Dr. Kohlbeck referred in his findings as Starch." (Bracaglia, Raes Problematic Threads, part 3, [http://holyshroudguild.org/dr-raes-problematic-threads\\_3.html](http://holyshroudguild.org/dr-raes-problematic-threads_3.html)); McCrone, Judgement Day for the Turin Shroud, p. 85

<sup>479</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 30; Heller and Adler, A Chemical..., 1981, text p. 43 and Table 7, p. 54

<sup>480</sup> Nitowski, Handwritten letter, 1986, p. 2 of Scribd document on [http://www.scribd.com/fullscreen/45010690?access\\_key=key-48x44lgtcyb9eggdljr&allow\\_share=false&escape=false&show\\_recommendations=false&view\\_mode=scroll](http://www.scribd.com/fullscreen/45010690?access_key=key-48x44lgtcyb9eggdljr&allow_share=false&escape=false&show_recommendations=false&view_mode=scroll)

<sup>481</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 32

directions.<sup>483</sup> An age-estimation, based on certain mechanical properties of the linen fibers of the Shroud, yielded a date of 400 AD  $\pm$  400 years (95% confidence level); in this estimation, the systematic effect of the 1532 AD fire was estimated as only 10% of the ageing effect that was measured in linen that was decolored by heating in an oven for 2 hours in 200 °C - this artificial ageing effect was comparable to that of a few centuries.<sup>484</sup> If the Shroud's raw 'mechanical' date had been corrected with a larger 'fire-ageing' effect than only this estimated 10% of a few centuries, the Shroud would have seemed even younger than 400 AD. A waterresistant starch coating is a protective layer against wear for the surface fibers and also reduces the amount of water that gets into the Shroud quickly, and would also be a physical barrier for microbes to reach and eat the linen itself.

The strippable thin layer ("Ghost") on the outside of "all superficial linen fibers on Shroud samples", including background fibers, is called "colored"<sup>485</sup> and is continuous across linen fiber nodes<sup>486</sup> – see for instance the photo published by Heimburger of the yellow image layer that seems partly missing on a fiber node,<sup>487</sup> and also the SEM photos in the figures 7, 8, 9, and A5 to A8, published by Fanti, Calliari and Canovaro.<sup>488</sup> This colored layer has been estimated to be thicker than might be expected of a mere primary cell wall<sup>489</sup>.

Garza-Valdez wrote on some Shroud threads: "Riggi kept the bulk of the three pieces of textile, but as he cut some threads for me, they sounded brittle, and it struck me as an indication of the presence of something more than mere fabric. When, with fine scissors, I cut a thread myself, it was as if I were cutting a plasticized fishing line or a series of thin copper wires, very different from the feeling I got when cutting a thread from a contemporary piece of linen."<sup>490</sup>

As already listed in 2012,<sup>491</sup> there are many differences between the microscopic visibility of the coating on the main Shroud sticky-tapes and that of the scorched coating on the Raes threads: it is not just that 1) there is no, or hardly any, old cotton in the main Shroud, and, moreover, the 2% old cotton found in a thread from the edge of the radiocarbon sample at the main Shroud was 2) not on its surface but a spun-in contaminant, and 3) is much thinner than the surface cotton in the Raes threads. Also 4) the coating found on linen fibers of the main Shroud would have been much thinner<sup>492</sup> and 5) thus with much less yellow-brown color than on an eventual thin old cotton surface

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<sup>482</sup> "Linen is highly hygroscopic as it is capable to rapidly absorb and yield moisture. It evaporates water as quickly as the pond surface. Linen can absorb up to 20% of its own weight in moisture while still feeling dry to the touch. That explains why linen cloth always feels fresh and cool" <http://www.purelinen.com.au/purelinen-linen-facts> ; "Hydrophilic nature of flax fibre - The ability of flax fibre to absorb water mainly hinges on the content of the non-cellulosic part (mainly hemicelluloses) and the porous content of the fibre (Bledzki and Gassan 1999) which is related to the fibre structure and composition." Oraj, 2008, <http://ecommons.usask.ca/bitstream/handle/10388/etd-11252008-111438/MSc-Thesis-RahimOraji-November2008-.pdf?sequence=1>, referring to Bledzki, A.K. and J. Gassan. 1999, *Progress in Polymer Science* 24: 221-274, <http://www.sciencedirect.com/science/article/pii/S0079670098000185>

<sup>483</sup> Heller, Report on..., 1983, p. 116-117

<sup>484</sup> Fanti and Gaeta, Il mistero della Sindone, 2013, p. 100, 102

<sup>485</sup> "Phase-contrast photomicrographs show that there is a very thin coating on the outside of all superficial linen fibers on Shroud samples named "Ghost"; "Ghosts" are colored (carbohydrate) impurity layers pulled from a linen fiber by the adhesive of the sampling tape and they were found on background, light-scorch and image sticky tapes (Zugibe and Rogers 1978, Rogers 2002)." Fanti et al., Evidences for..., 2005, A3, <http://www.shroud.com/pdfs/doclist.pdf>

<sup>486</sup> "14) The color of image fibers was often stripped off of their surfaces, leaving molds of the fibers in the adhesive.

Growth nodes can be seen in the molds." (Rogers, Frequently Asked Questions..., 2004, p. 16)

<sup>487</sup> Heimburger, A detailed..., 2008, p. 4, <http://www.shroud.com/pdfs/thibault%20final%2001.pdf>

<sup>488</sup> Fanti, Calliari and Canovaro, Analysis of micro-particles..., 2012, <http://www.dii.unipd.it/-giulio.fanti/research/Sindone/Paper%20Valencia.pdf>

<sup>489</sup> "The colored layers show all of the same chemical properties observed on intact image fibers (see 12 above). All of the color is on the surfaces of the fibers. The colored layer is 200-600 nanometers thick." (Rogers, Frequently Asked Questions..., 2004, p. 16); Fanti and Botella et al., Microscopic and macroscopic..., 2010; "200 nm (1 nm = 10<sup>-9</sup> m), i.e. the thickness of the primary cell wall of the single linen fiber." Lazzaro et al., Sub-micrometer coloration..., 2010, p. 1; see also Hoeven, Internal Selvedge..., 2012, paragraph 2.2.1

<sup>490</sup> Garza-Valdes, The DNA of God?, 2001, end of chapter 3, p. 28

<sup>491</sup> Hoeven, Internal Selvedge..., 2.5.3.

<sup>492</sup> Rogers wrote about the Raes crust: "The encrustation is heaviest on cotton fibers, it is the vehicle for the yellow-brown color". (Rogers and Arnoldi, Scientific Method..., 2002, p. 17) "The thickness of the coating on the Raes yarn varies greatly. Cotton fibers tend to have much thicker coatings than linen fibers; however, I would guess that the coating

fiber. 6) The sticky-tape samples broke fibers from the top of the weave of the main Shroud, which may have a thinner coating than fibers from the down-parts of the weave, between the intersection of warp and weft threads (originally or by later abrasion); only a (Raes) thread contained and showed all parts of its weave. So, for six accumulative reasons, the chance to find the Raes coating on the top of the weave of the main Shroud is much smaller than in a Raes thread. Indeed, only the “Ghosts” on sticky-tapes made some researchers wonder if there is or isn’t a coating on the Shroud fibers, because the Ghost is thinner than the wavelength of the light used in microscopy, and thus invisible when still on the fiber. Moreover, 7) the indexes of refraction of the Raes coating, on one hand, and of the sticky-tape adhesive, of linen lengthwise, of cotton, and of 1.515-index microscopy immersion oil, on the other, are all approximately the same (very close to 1.515)<sup>493</sup>, so also this contributes to the invisibility of a Raes coating when on the fiber, also on samples of the main Shroud. According to Rogers, even a thick Raes coating could be completely invisible: “The index of the coating on the Raes samples varies a little, but it is very close to 1.515: It can be completely invisible on a normally prepared slide.”<sup>494</sup> Only the Ghosts were discernable as coatings, because they were empty.<sup>495</sup> Furthermore, 8) the color of the coating would only be yellow-brown in scorch areas of the Shroud, 9) the coating would only swell and dissolve in water if it had been scorched to starch gum – unscorched non-image fibers would still have a coating of insoluble retrograded starch, and 10) a very thin scorched flaking coating may have been washed away along with the sticky-tape adhesive when the scorched fiber was removed from the sticky-tape by washing with toluene in preparation for further microchemical testing. These flakes may have already loosened from the scorched fibers when McCrone stuck all Mylar sticky-tapes to microscope slides and then pulled them off and stuck them to microscope cover slips, during which twofold operation, “hard compression of the Mylar onto the glass slides had squeezed everything together.”<sup>496</sup>

Rogers, in his posthumous book “A Chemist’s Perspective on the Shroud of Turin”, says and shows in the book’s fig. VII-3 (fig. 4.1), that a yellow stripped off encrustation from a weakly scorched fiber of the main Shroud is present on sticky tape I1B (#4 on the dorsal map): “A search of tape samples from lightly-scorched areas revealed ghosts that appeared to be identical to those from image areas. Thin layers of colored impurities had stripped off from scorched fibers that were completely isolated from image areas (figure VII-3). Scorched fibers from the sample shown in the figure (STURP sample I1B) were very slightly colored”; the caption to this figure reads “Figure VII-3: A line of yellow flakes stripped off of one side of a lightly-scorched fiber (800X). The outline of

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does not average more than about 2  $\mu\text{m}$  thick.” (Ibid. p. 27) On the ‘Ghosts’ of image fibers: “The coating is too thin to measure accurately with a standard microscope; however, it appears to be 200-600 nanometers thick (in the range of a wavelength of visible light).” (Rogers, Frequently asked questions..., 2004, p. 11) “The color of the image is indeed a result of a thin coating. “Thin” is the important word. ... flakes can be seen in the “ghosts” on the sampling tapes ... I have been unable to measure its index. ... The thickness of the image color must be less than a sodium-D wavelength (589 nanometers).” (Rogers, A Chemist’s..., 2008, p. 92); When describing a photomicrograph of a warp thread of the radiocarbon sample in 1.345-index oil, Rogers writes: “Figure IX-3 ... Notice that both cotton fibers are completely covered by a colored layer. Some of the linen fibers are nearly clean.” (Rogers, A Chemist’s..., 2008, p. 67)<sup>493</sup> “The two indices of cotton are close to that of the adhesive. Birefringence is first-order white. The index of linen across the fiber is appreciably lower than that of the adhesive.” Rogers and Arnoldi, Scientific Method..., 2002, p.14 “The index of refraction of a normal linen fiber parallel to its length is nearly identical to that of the adhesive on the sampling tapes (it nearly disappears). That index is very close to 1.515. The index across the fiber is appreciably lower than the adhesive. The indexes of refraction and crystallinity of image fibers are identical to unaffected fibers.” Rogers, Frequently Asked Questions..., 2004, p. 15; “The image-color coating seems to be amorphous, but I have been unable to measure its index. ... The usual immersion oil used by microscopists has an index of 1.515, because a normal microscope slide is made of crown glass with an index of 1.517 at 589 nanometers. The index of the coating on the Raes samples varies a little, but it is very close to 1.515: It can be completely invisible on a normally prepared slide. Water with an index of 1.33 can not be used as an immersion liquid to enhance contrast, because the coating swells and dissolves.” Rogers and Arnoldi, Scientific Method..., 2002, p. 27

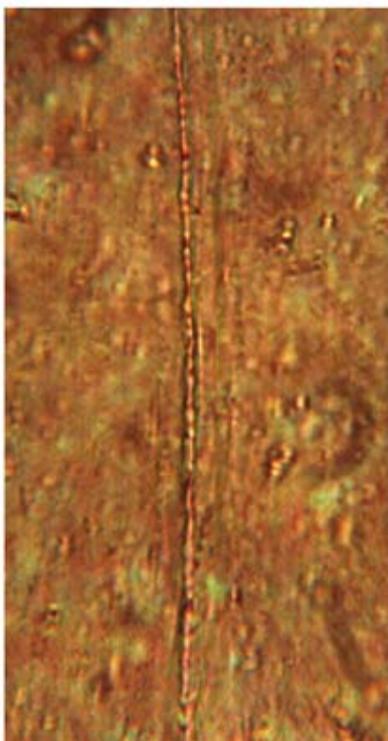
<sup>494</sup> Rogers, Frequently asked questions..., 2002, p. 26

<sup>495</sup> “Figure 5: “Ghost” on sample 1EB. The tape was pulled from the calf of the leg. There is no fiber in the horizontal line, proved by rotating the sample between crossed polarizers. Cellulose is birefringent. The line shows a faint-yellow image color.” Rogers and Arnoldi, Scientific Method..., 2002, p. 7

<sup>496</sup> Rogers, A Chemist’s, p. 24; Heller, Report on..., 1983, p. 163

the other side of the fiber and some dispersed flakes are visible.”<sup>497</sup> This confirms that, in light scorches on the main Shroud, there is a scorched strippable flaked coating that is similar to the one in the lightly scorched Raes corner.

More arguments for the presence of a starch-madder coating on the whole Shroud have already been published in 2012.<sup>498</sup> Now, this chapter will extend this evidence, and the next chapter will differentiate between madder and Saponaria, showing there is much evidence against the presence of Saponaria.



*Fig. 4.1. Photomicrograph (800X) of a sticky tape from a lightly scorched area of the main Shroud (sample IIB) ©2005 Raymond N. Rogers Collection, STERA, Inc.*<sup>499</sup>

#### **4.1.2. Hot water washed out starch – blue fluorescence**

The images of the ventral shoulders, prominently depicted on the 1516 AD Belgian Lier copy and the possibly pre-1532 Xabregas copy,<sup>500</sup> if they were ever that prominently present on the Shroud, seem to have moved and disappeared with the firefighting water that got on the Shroud in 1532 AD. Inside these waterstains one would at least expect to see scorch marks, where the coating of starch had scorched into a yellow-brown soluble starch gum layer: starch gum consists of cold-water-soluble carbohydrate molecules, viz. dextrans; also the image layer may have scorched into a soluble layer. Now it is very meaningful that the inside of some of the 1532 AD water stains<sup>501</sup>, also where the image of a shoulder/arm might be expected, seems lighter than the surrounding background (see e.g. fig. 4.2, of which the photos on the right, from Miller and Pellicori, p. 81, are from the ventral shoulder areas).

The UV-fluorescence of the inside of the ‘washing’ small waterstains looks darker and more blue than the outside: the UV photos in the article of Miller and Pellicori show this on p. 76 (dorsal calves

<sup>497</sup> Rogers, A Chemist’s..., 2008, p. 45-46, Fig. VII-3

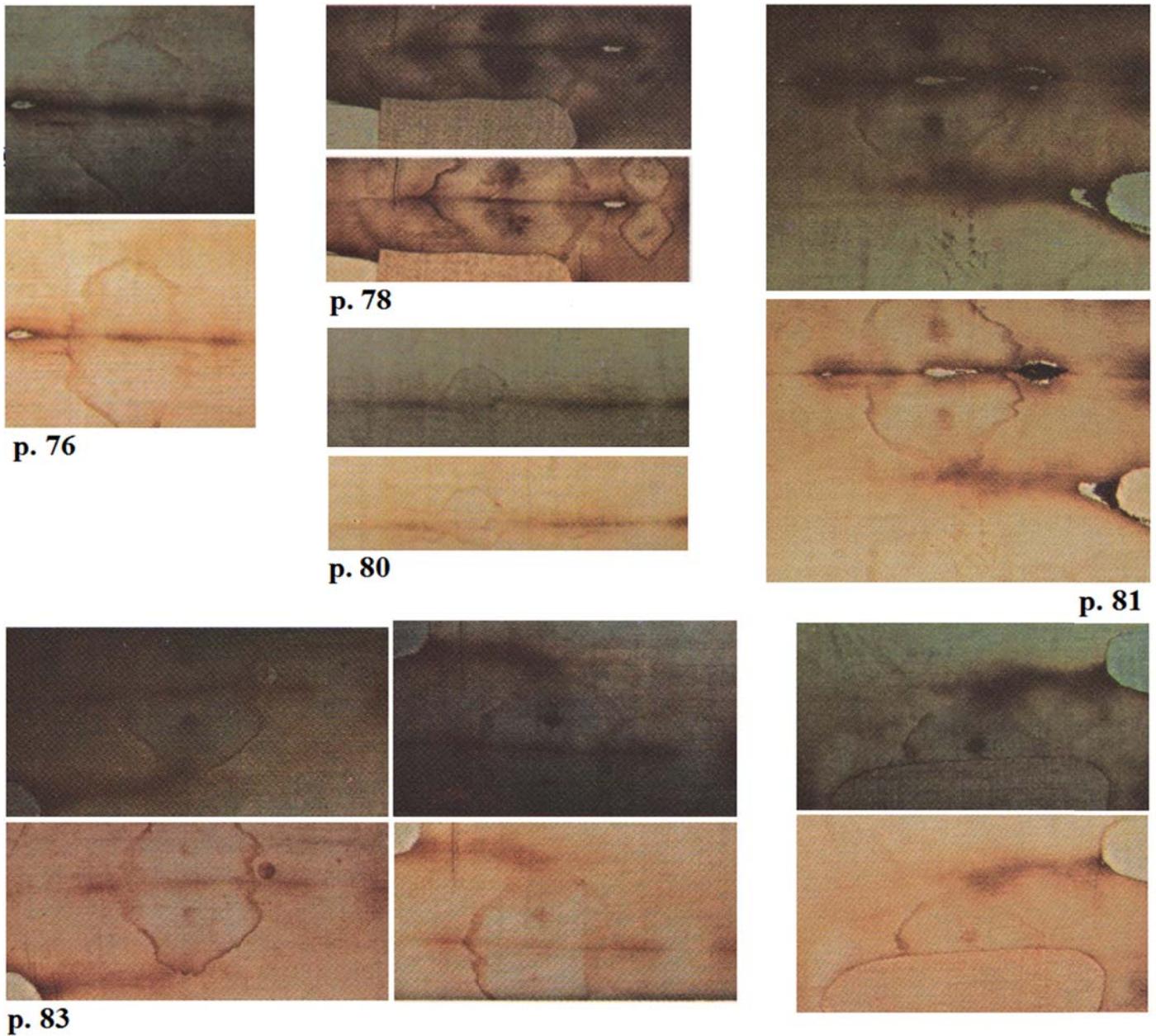
<sup>498</sup> Hoeven, Internal selvedge..., 2012

<sup>499</sup> Used from R.N. Rogers, A Chemist’s Perspective on the Shroud of Turin, Ed. B.M. Schwartz, 2008, fig. VII-3, p. 46, with permission from STERA, Inc.

<sup>500</sup> <http://www.imagochristiorganizationinc.com/about-the-shroud.php> and Van Haelst, The Lier Shroud..., <http://www.west.net/~shroud/pdfs/ssi20part5.pdf>, p. 10-11 and Fig. 1c on its p. 16 and 17

<sup>501</sup> <http://www.sindonology.org/shroudScope/shroudScope.shtml?zl=9&image=4&lon=11827&lat=3834> and perhaps <http://www.sindonology.org/shroudScope/shroudScope.shtml?zl=9&image=4&lon=16955&lat=3945>

area) at the water stain at the lower right corner of the photo; at p. 78 (dorsal shoulders area), the small rhombic ‘owls eyes’ in the upper and lower right of the photo are darker and blue in UV, but lighter than their surroundings in visible light; also at p. 80 (dorsal top of head area), the inside of the small stain, at the center of the lower margin of the photo, is more blue and darker than the stain’s surroundings in UV, but lighter in visible light; at p. 81 (ventral shoulders area), parts of the ‘pears’, at the centre of the photo, on top and below, are more blue and darker; at p. 83 (ventral below-knees area), both in the upper and lower part of the photo, the pear halves are more blue and darker in UV, but lighter in visible light (see e.g. fig. 4.2).



*Fig. 4.2. UV-fluorescence photos of the Shroud at 1532 AD waterstains (cropped from figures of Miller and Pellicori, 1981) ©Biocommunications Association, Inc.<sup>502</sup>*

- p. 76: at the height of the dorsal calves*
- p. 78: " " dorsal shoulders*
- p. 80: " " dorsal top of head*
- p. 81: " " ventral shoulders*
- p. 83: " " ventral below the knees*

<sup>502</sup> Images reprinted from Miller & Pellicori, *Journal of Biological Photography*, Vol 49, No 3, 1981, p. 71-85, with permission from the publisher, the Biocommunications Association, Inc.

This means that the firefighting water probably moved the scorched starch-madder coating and perhaps scorched image layer to the watermark borders. If this water had become hot because the Shroud itself was still hot when the water moved through it, or because it had run across and fallen from a hot silver reliquary, it may also have removed an unscorched background or image layer that was still cold-water-insoluble, as seems to have occurred, for instance in the small waterstain shown above (fig. 4.2, p. 80). Water of about 60 °C or more can remove a retrograded starch layer<sup>503</sup> including a topping fluorescent madder dye. It has been reported that “the “color” of the yellow body image fibrils is neither altered nor extracted by ... water”, but it was not specified whether also hot water was used.<sup>504</sup> The water of the waterstains of the other symmetry pattern – the large diamond shaped waterstains that are in a different pattern than the burns and small waterstains – probably was cold and got into a cold Shroud before the 1532 AD fire, for this water did not wash away the body image or the starch in the now lightly scorched Raes corner: this corner’s surface fibers now still have both starch and starch gum.<sup>505</sup>

#### **4.1.3. FTIR spectra of Raes samples are similar to FTIR spectra of main Shroud non-image fibers and of linen**

##### **4.1.3.1. Raes FTIR spectra are similar to main Shroud non-image FTIR spectra**

The FTIR spectrum of a large piece of crust from the Raes corner, in which Rogers found starch, a non-proteinaceous gum (viz. starch gum), and madder dye, is very similar to the FTIR spectrum of the yellow end of the thread from the Raes area that has been called “Raes sample #1” and a “spliced fiber”.<sup>506</sup> The text of Villarreal’s slide showing these FTIR spectra reads: “Data taken from a large piece of crust are quite similar to data from the Shroud thread. Spectrum may be dominated by embedded fibers” (see fig. 4.3, in which the upper, green curve is called “crust” and the lower, blue curve “shroud - region 2”).

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<sup>503</sup> See fig. 1, 2, 3, and 4, in which R<sub>T</sub> is retrograded starch, of Fernández-Martin et al., 2008, [http://www.researchgate.net/publication/233885487\\_Pressurization\\_of\\_some\\_starches\\_compared\\_to\\_heating\\_Calorimetric\\_thermo-optical\\_and\\_X-ray\\_examination/file/72e7e51b8e22d2a185.pdf](http://www.researchgate.net/publication/233885487_Pressurization_of_some_starches_compared_to_heating_Calorimetric_thermo-optical_and_X-ray_examination/file/72e7e51b8e22d2a185.pdf)

<sup>504</sup> Heller and Adler, *A Chemical...*, 1981, TOM p. 43

<sup>505</sup> Described in Hoeven, *Internal selvedge...*, 2012, par. 2.2.2., 2.5.1. and 4.3.

<sup>506</sup> Roberto and Roberta Villarreal, *A New Look...*, 2012, p. 3.

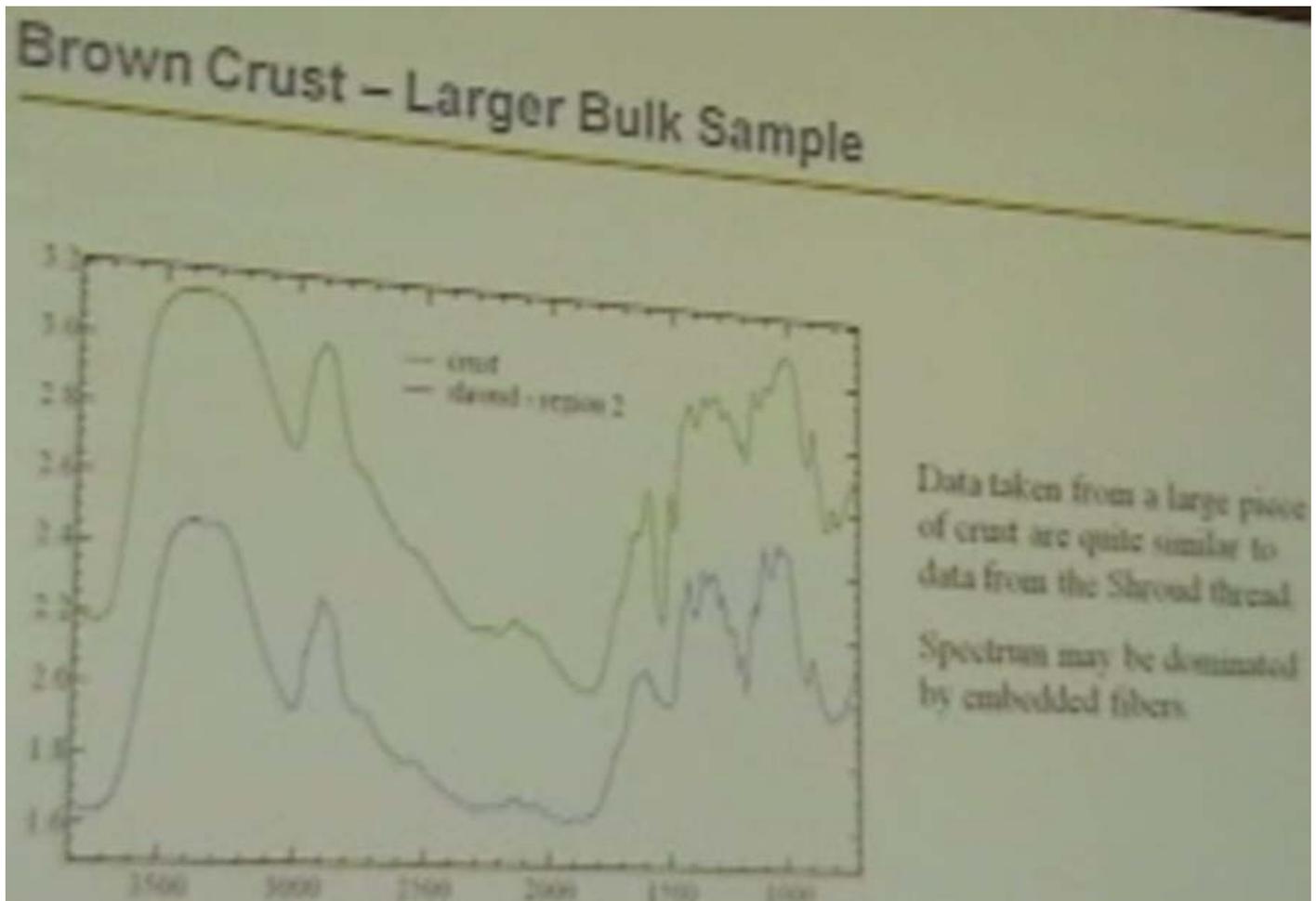


Fig. 4.3. FTIR spectra of brown crust and thread region 2, Villarreal's 2008 presentation ©R. Breault, Shroud of Turin Education Project Inc.<sup>507</sup>

(green curve = crust; blue curve = shroud – region 2; The slide of Villarreal was projected and/or filmed in perspective, which made the horizontal scale slightly non-linear: toward lower wavenumbers (toward the right part of plot) the FTIR plot is slightly compressed. The vertical axes on the left and right are not parallel. For this figure, the screen shot of the video has been slightly rotated to render the right part of the plot strictly vertical; the left part of the plot is still skew.)

These two FTIR spectra of Villarreal, most interestingly, are also very similar to the FTIR spectra of non-image fibers from the surface of the main Shroud, as published by Adler, Selzer, and DeBlase<sup>508</sup> (four FTIR spectra of non-image fibers, see fig. 4.4), and by Adler, in another article, in *The Orphaned Manuscript* on page 83 (one spectrum of a non-image fiber sample, in one plot with one spectrum of a “radiocarbon warp” fiber, and four other spectra, all represented in his figure 1, with a single and linear horizontal scale, see fig. 4.5).

<sup>507</sup> Used from R. Breault's video of R. Villarreal's 2008 presentation, published by Shroud University, [www.shrouduniversity.com/videos/villareal.wmv](http://www.shrouduniversity.com/videos/villareal.wmv), screenshot at 22:30, with permission of R. Breault, Shroud of Turin Education Project Inc., [www.ShroudEncouter.com](http://www.ShroudEncouter.com); also visible at 6:15 of <https://www.youtube.com/watch?v=9kBpplTK044>

<sup>508</sup> in Adler, *The Orphaned Manuscript* on page 99, and in Minor M. et al. (eds.), *The Shroud of Turin - Unraveling the Mystery* (Proceedings Dallas 1998) on p. 171

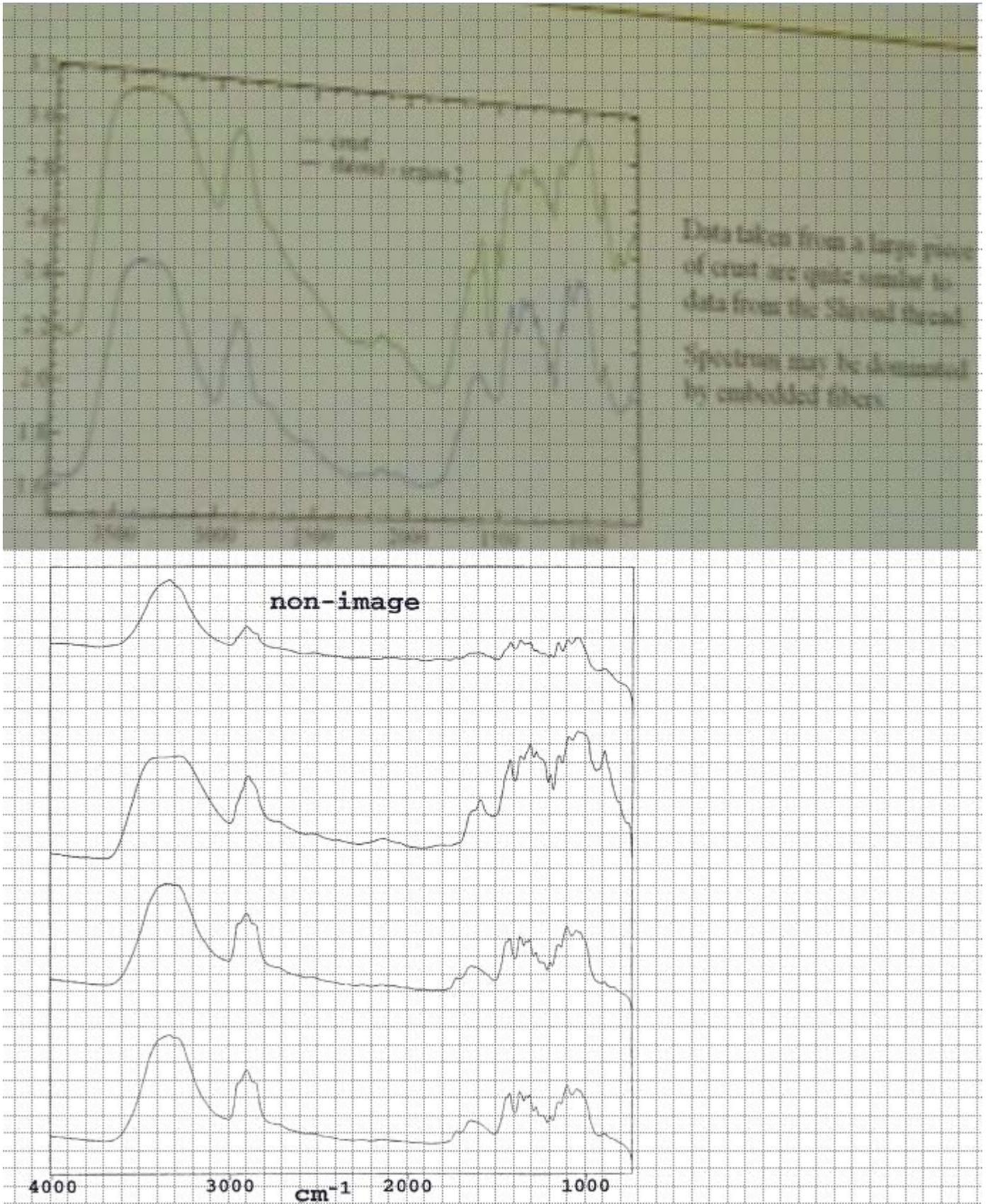


Fig. 4.4. Rough comparison (by added lath) of Villarreal's FTIR spectra of Raes crust (large piece) and Raes thread (©R. Breault, Shroud of Turin Education Project Inc.<sup>509</sup>) to the FTIR spectra of

<sup>509</sup> Adapted from R. Breault's video of R. Villarreal's 2008 presentation, published by Shroud University, [www.shrouduniversity.com/videos/villarreal.wmv](http://www.shrouduniversity.com/videos/villarreal.wmv), screenshot at 22:30, with permission of R. Breault, Shroud of Turin

main Shroud non-image fibers from the 2002 book of the proceedings of Dallas 1998 (©A.D. Adler, R. Selzer, F. DeBlase<sup>510</sup>)

This fig. 1 of Adler's 1996 article (TOM p. 83) shows that its "non-image" FTIR spectrum is not much different from its "radiocarbon warp" FTIR spectrum. This last FTIR spectrum is the upper curve in fig. 2, "inner warp", of Adler, Selzer and DeBlase's article, published in 2002 in the book of the proceedings of the 1998 Dallas Symposium<sup>511</sup>, in which also three other radiocarbon "inner warp" FTIR spectra are shown (see fig. 4.6), and four "outer warp" and four "weft" radiocarbon FTIR spectra and four "non-image" FTIR spectra. Unfortunately, on pages 99-101 of The Orphaned Manuscript of 2002 (reproducing the curves of Adler, Selzer and DeBlase's 2002 article), the scale marks on the horizontal axes of the FTIR plots are not spaced linearly, and the non-image curves have been stretched horizontally with respect to the radiocarbon curves.<sup>512</sup> Fortunately, the curves are represented with a linear scale and without stretching of the non-image curves in their original article in the book of the Dallas proceedings.<sup>513</sup> Alignment of these plots shows even more clearly that the "non-image" FTIR spectra are quite similar to the radiocarbon "inner warp" FTIR spectra (see fig. 4.6), and also to at least one "outer warp" and one "weft" FTIR spectrum. This is confirmed by the wavenumbers of peaks in the 1680-1540  $\text{cm}^{-1}$  region, given in the text of Adler, Selzer and DeBlase's article: "The position (given in  $\text{cm}^{-1}$ ) ... non-image (1593, 1643) ... radiocarbon pattern (1590, 1643, both strong)."<sup>514</sup>

The linear horizontal scale of Adler's fig. 1 of 1996 (TOM p. 83), and of the plots in the book of the proceedings, can be aligned with a fine decimal lath, which allows estimating peak wavenumbers in an enhanced view of the curves (uncertainty estimated  $\pm 10 \text{ cm}^{-1}$ , cf. fig. 4.5 and 4.6 below, here covered with a coarse lath, and not enhanced). The article says "Transmission spectra were collected from 4011.6 to 739.8  $\text{cm}^{-1}$  at 8  $\text{cm}^{-1}$  resolution, 64 scans" and "the objective was adjusted to produce the best optical image of the specimen. .... control experiments showed that only this focus could provide reproducible spectra of a sample without distorting the relative intensities of the high vs. low frequency patterns of the spectrum."<sup>515</sup>

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Education Project Inc., [www.ShroudEncouter.com](http://www.ShroudEncouter.com); also visible at 6:15 of <https://www.youtube.com/watch?v=9kBpplTK044>

<sup>510</sup> Adapted from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, in Minor M. et al. (eds.), The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, Compiled by Michael Minor, Alexander Books, Alexander - North Carolina, 2002, pp. 166-181, © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer

<sup>511</sup> Adler, Selzer and DeBlase, Further Spectroscopic..., 2002, TOM p. 99 = fig. 2 on p. 170 of the book Minor M. et al. (eds.), The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, published by Alexander Books; this book reads "Copyright ©2002 Michael Minor" on p.2

<sup>512</sup> Scroll back from

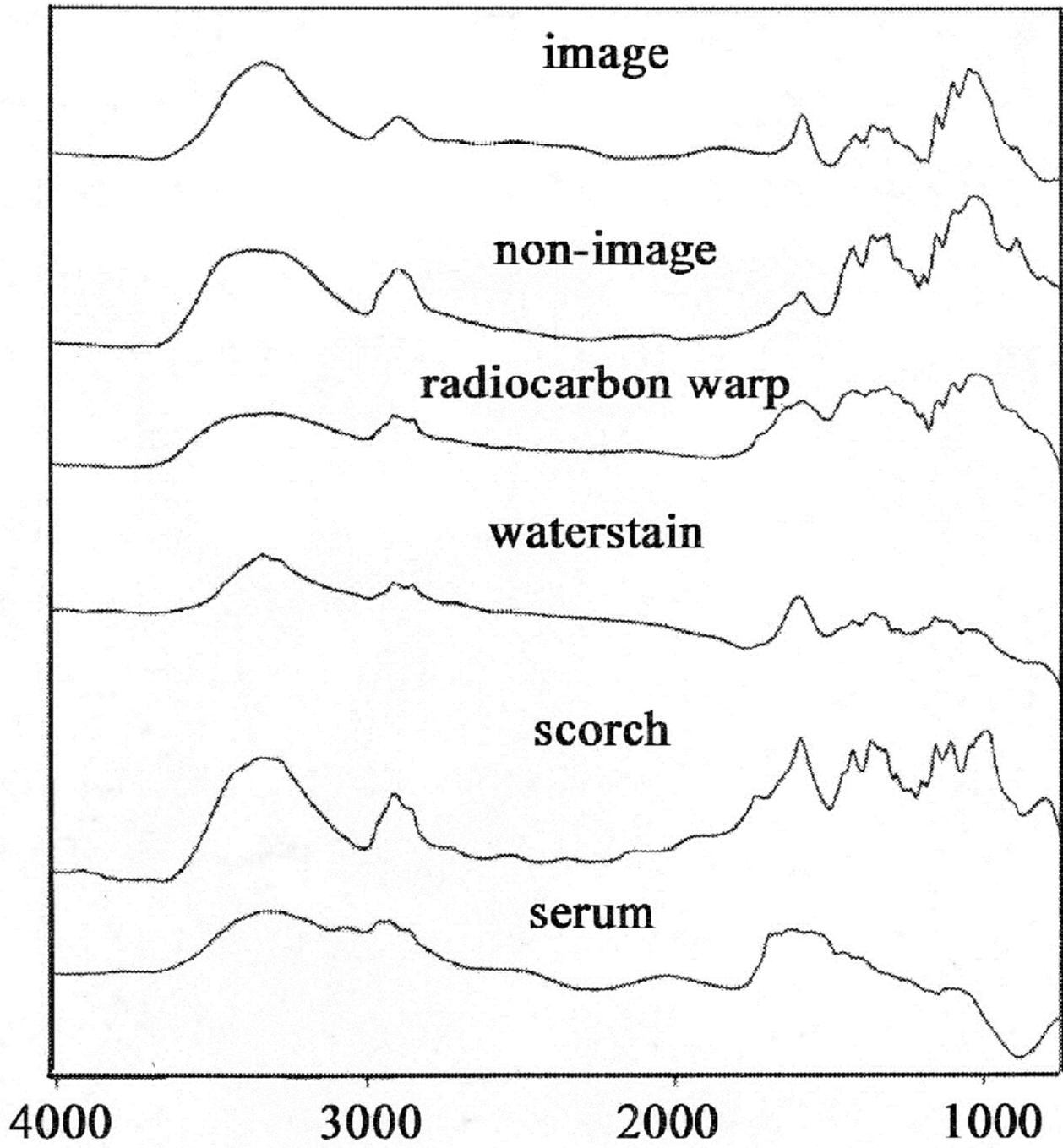
<http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMlcq90QWto4GAAw&ved=0CDEQ6AEwAA#v=snippet&q=clot%20controls&f=false>

<sup>513</sup> Minor M. et al. (eds.), The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, Alexander Books, Alexander NC (USA), 2002 (contains Adler, Selzer and DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin) <http://www.alibris.co.uk/search/books/isbn/9781570901683>

<sup>514</sup> Adler, Selzer and DeBlase, Further Spectroscopic..., 2002, TOM 96

<sup>515</sup> Adler, Selzer and DeBlase, Further Spectroscopic..., 2002, TOM 95

A:



B:

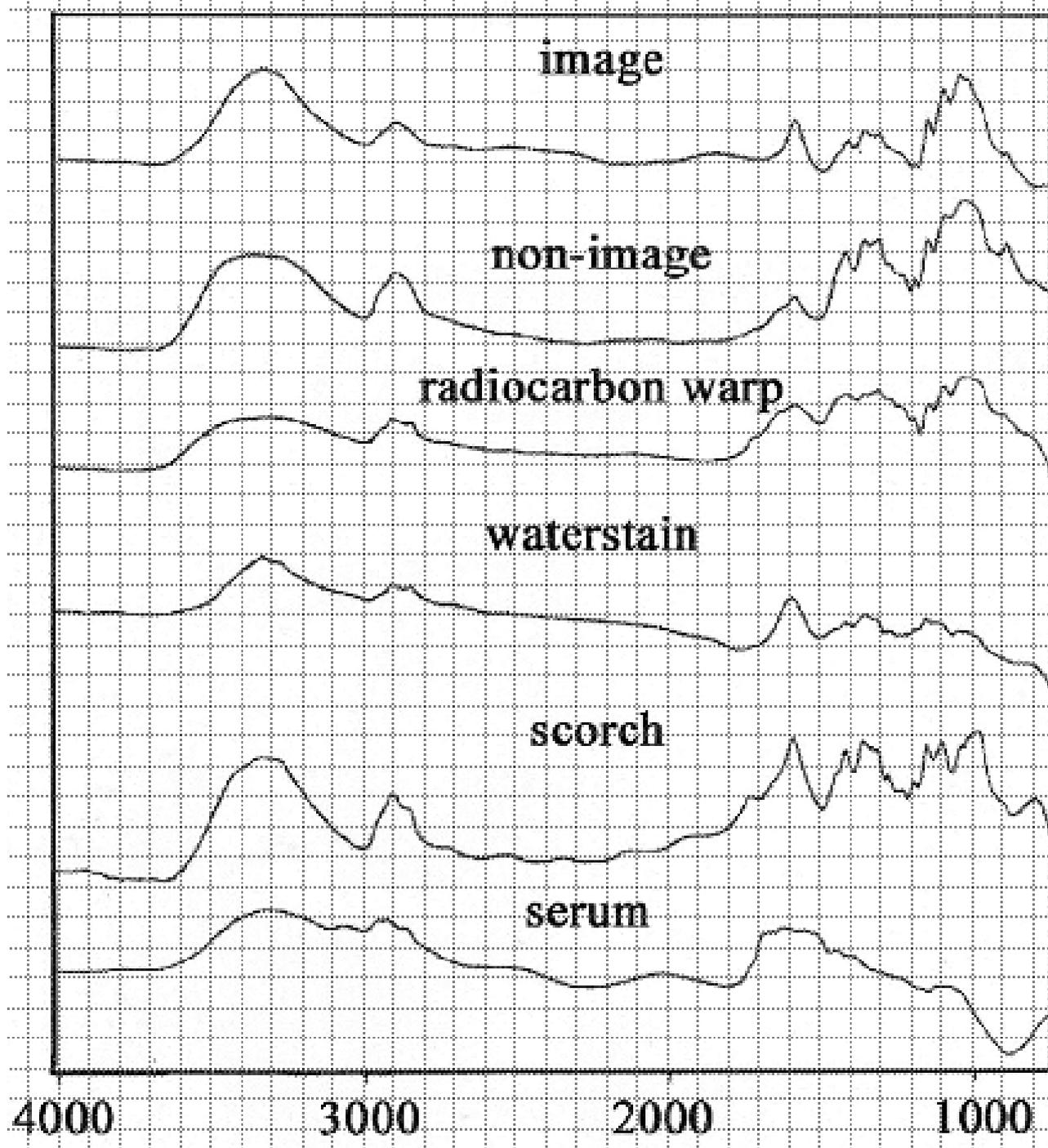
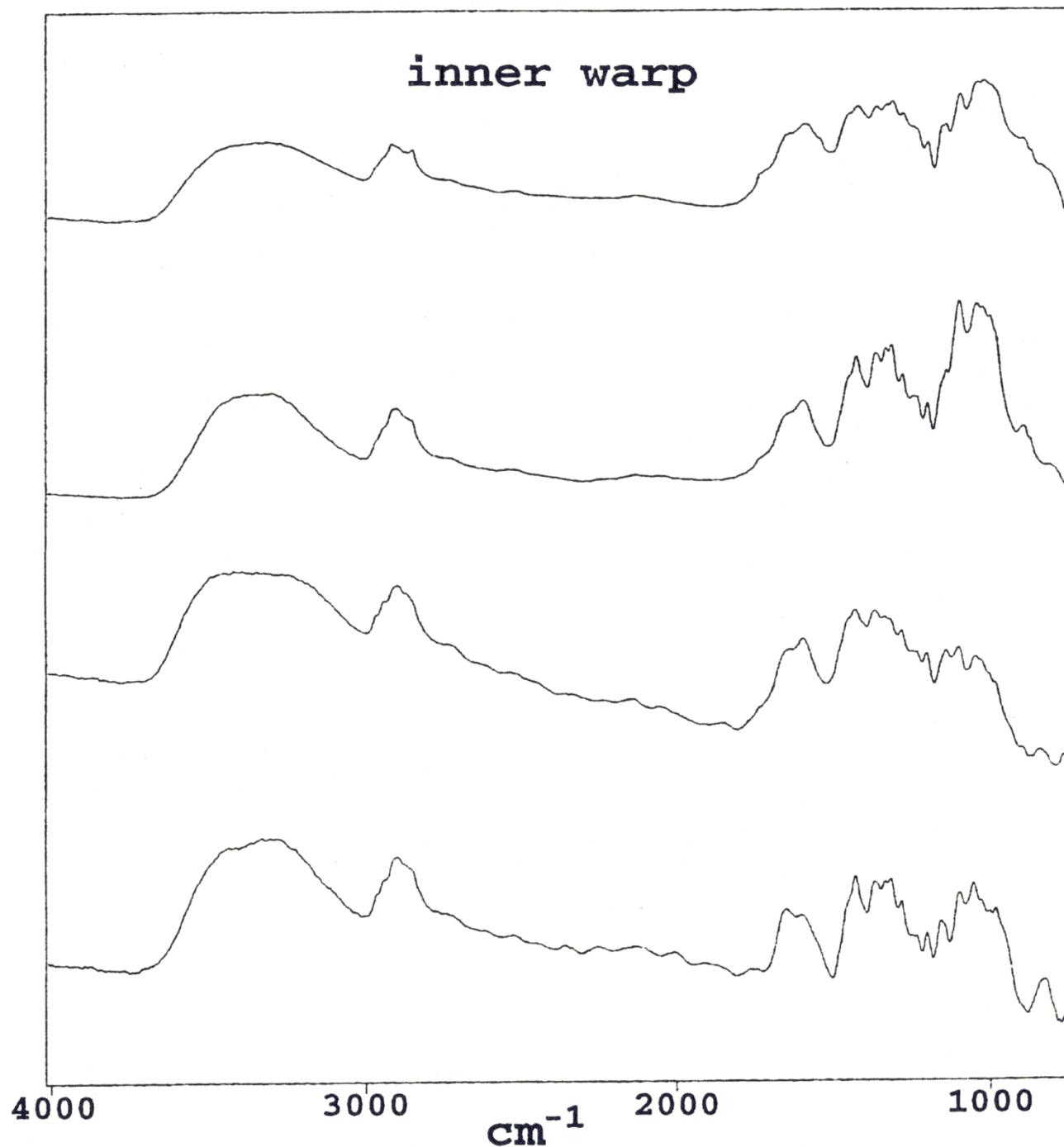


Fig. 4.5. A: FTIR spectra of Adler's figure 1 of "Updating Recent Studies on the Shroud of Turin" 1996 (*The Orphaned Manuscript* p. 83), ©ACS<sup>516</sup> B: *Idem*, covered with lath ©ACS<sup>517</sup>

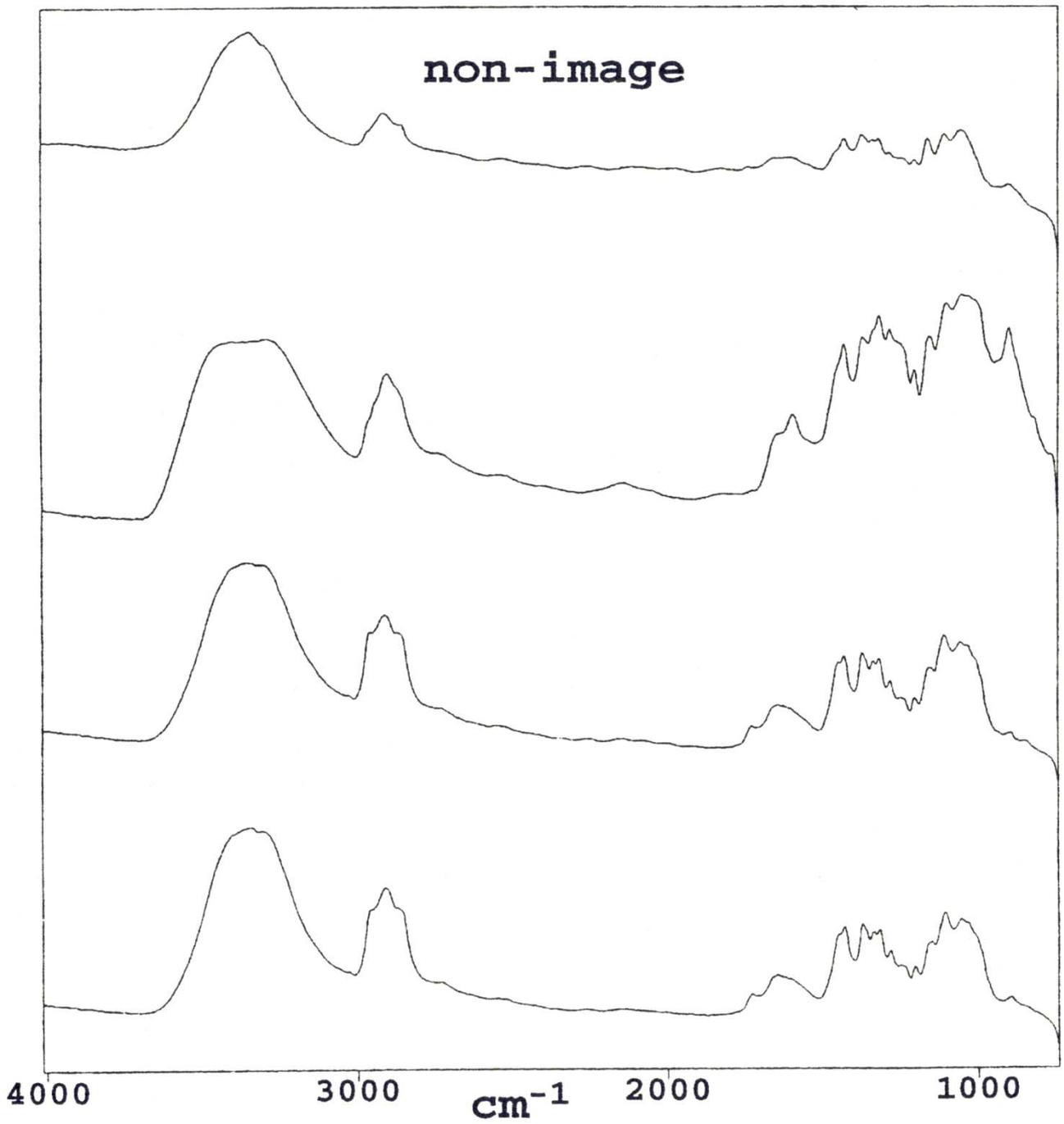
<sup>516</sup> Used with permission from A.D. Adler, *Updating Recent Studies on the Shroud of Turin*, *Archeological Chemistry*, chapter 17, pp. 223-228, *ACS Symposium Series*, Vol. 625, May 1996, Chapter DOI: 10.1021/bk-1996-0625.ch017, ISBN13: 9780841233959 eISBN: 9780841215740, <http://pubs.acs.org/doi/abs/10.1021/bk-1996-0625.ch017?prevSearch=Updating%2Brecent%2Bstudies%2Bon%2Bthe%2Bshroud%2Bof%2Bturin&searchHistoryKey=> Copyright © 1996 American Chemical Society. (fig. 1, also in *The Orphaned Manuscript* (TOM) p. 83 and online at <http://www.sindone.info/ADLER.PDF>).

<sup>517</sup> Adapted with permission from A.D. Adler, *Updating Recent Studies on the Shroud of Turin*, *Archeological Chemistry*, chapter 17, pp. 223-228, *ACS Symposium Series*, Vol. 625, May 1996, Chapter DOI: 10.1021/bk-1996-0625.ch017, ISBN13: 9780841233959 eISBN: 9780841215740, <http://pubs.acs.org/doi/abs/10.1021/bk-1996-0625.ch017?prevSearch=Updating%2Brecent%2Bstudies%2Bon%2Bthe%2Bshroud%2Bof%2Bturin&searchHistoryKey=>

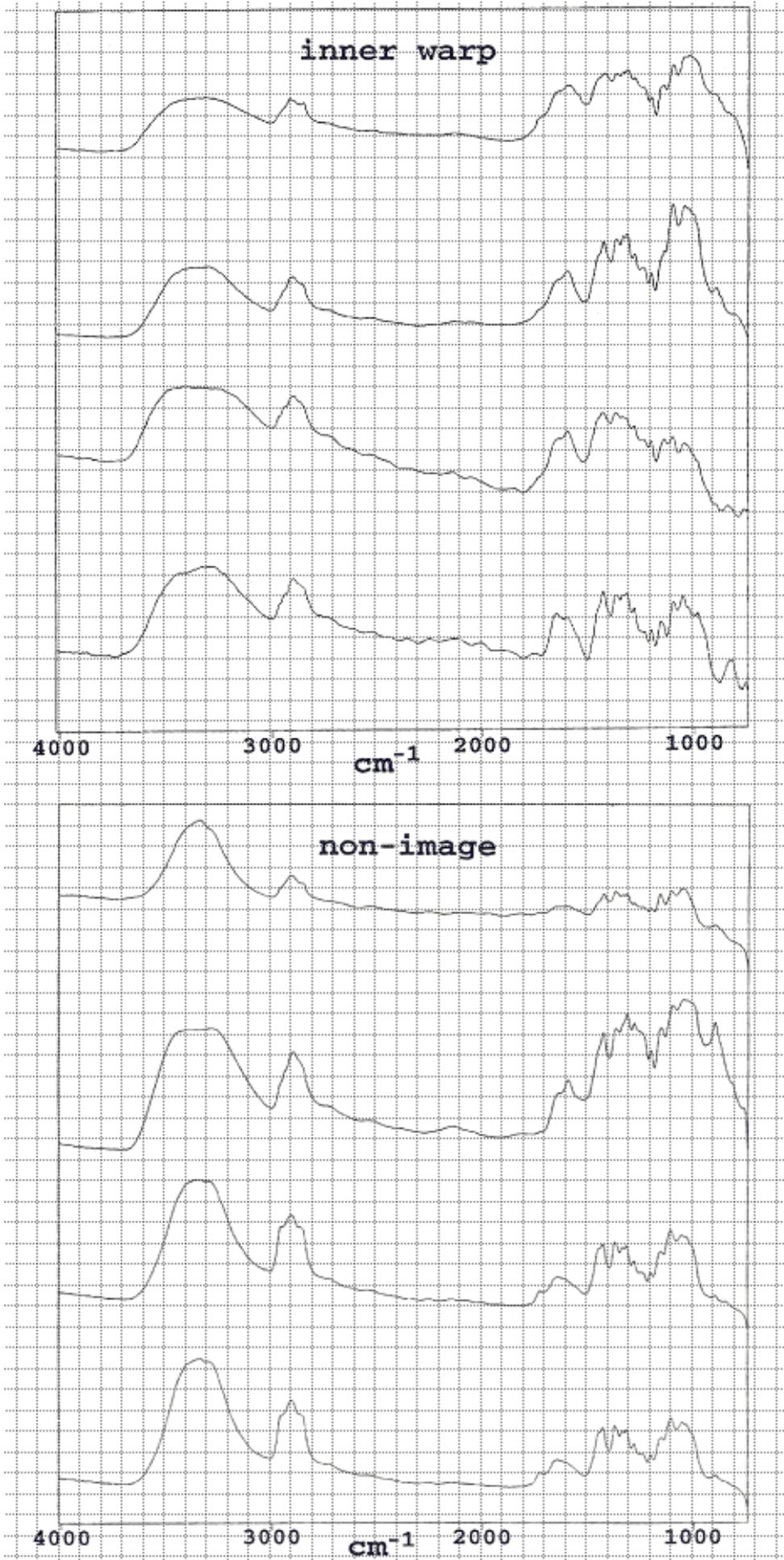
A:



*Fig. 2 Typical FTIR absorbance patterns of radiocarbon sample inner warp fibers*



*Fig. 4 Typical FTIR absorbance patterns of non-image fibers*



B:

*Fig. 4.6. A: Two original plots of FTIR spectra of radiocarbon inner warp and main Shroud non-image fibers from the 2002 book of the proceedings of Dallas 1998 ©A.D. Ader, R. Selzer, F. DeBlase<sup>518</sup> B: Idem, aligned and covered with lath ©A.D. Ader, R. Selzer, F. DeBlase<sup>519</sup>*

#### 4.1.3.2. No adequate linen or Shroud standard used for comparison in 2008

Robert Villarreal, who had received the Raes samples for analysis from Schwartz, said in his 2008 presentation in Ohio that “we did not have an actual Shroud linen standard”, meaning a standard for the linen of the main Shroud.<sup>520</sup> Apparently, the Shroud non-image FTIR spectra that had been published by Adler, Selzer and Deblase in 2002, were unknown to Villarreal. However, in the C14 forum discussion in Ohio 2008, after Villarreal’s presentation, Fanti told the audience, including Schwartz, Benford and Villarreal, that he had Shroud linen from beside the carbonating sample (received from Fondazione 3M, taken from beside the so-called ‘Reserva’ that was left after four pieces of the cut sample were sent to the C14 labs) and that he had analysed it with FTIR and Raman spectroscopy etc., and that he had presented the preliminary results at Shroud Science Group level – “so you also have them” Fanti said –, and that these fingerprint results of Fanti’s 3M sample are the same as those of the main Shroud.<sup>521</sup>

Furthermore, the “linen standard” Villarreal used in his comparisons was not representative of new or aged linen either (see fig. 4.16): their top black curve for “linen standard”, with its absent 3400  $\text{cm}^{-1}$  OH-band and its very high ca. 1700  $\text{cm}^{-1}$  C=O peak, is not typical of linen at all.<sup>522</sup> For comparison, see a modern FTIR spectrum of linen in fig. 4.7 below, and FTIR spectra of “New linen” and of the aged linen of the 1803 AD “Victory Sail” in fig. 4.8 below. During the C14 forum in Ohio 2008, archeologist Maloney<sup>523</sup> offered to give Villarreal some old linen, not from the Shroud, and Villarreal accepted to analyze them, and the next day it was agreed that the results would be published by Schwartz on his website.<sup>524</sup>

Thirdly, for some reason, also the cotton standard presented by Villarreal in 2008<sup>525</sup> looks different from other cotton FTIR spectra, available online.<sup>526</sup>

<sup>518</sup> Used from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, in Minor M. et al. (eds.), The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-181, © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer

<sup>519</sup> Adapted from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, in Minor M. et al. (eds.), The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-181, © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer

<sup>520</sup> Villarreal, 2008 presentation, [www.shrouduniversity.com/videos/villareal.wmv](http://www.shrouduniversity.com/videos/villareal.wmv) at 36:55, also in part 5 <https://www.youtube.com/watch?v=tMord0YLILE> at ca 4:10

<sup>521</sup> Breault, 2008 C 14 Forum, video at <https://www.youtube.com/watch?v=o0A44IthPSs>, hear and see Fanti from 11:17, speaking about the FTIR results from 17:06-18:00 and saying that the 3M piece is linen at 19:25-27.

<sup>522</sup> Villarreal, 2008 presentation, 20:55 and 23:00 of video <http://www.shrouduniversity.com/videos/villareal.wmv> of ShroudUniversity.com, also at 4:25 and 6:30 of <https://www.youtube.com/watch?v=9kBpplTK044> (part 3on YouTube)

<sup>523</sup> <http://www.stlouishshroudconference.com/app-presenters/Maloney>

<sup>524</sup> Breault, 2008 C 14 Forum, video at <https://www.youtube.com/watch?v=o0A44IthPSs>: try to hear the plenary discussion from 24:27: “Bob, I can get you some aged linen. ... about fifteen – thirteen hundred ...” ... “a mummy foot” ... “linen fibers of different ages.” Villarreal answered: “okay, anything I can get...” 26:13-17. In a session the next morning (see “2008 C143 Recap”, <https://www.youtube.com/watch?v=DFrRtqFwC-g>), Accetta recapitulated the plan of the forum (0:00-5:22) and Villarreal and Schwartz arranged that the results of Villarreal’s analysis would be published on Schwartz’ website (15:02-16:25).

<sup>525</sup> Villarreal, 2008 presentation, slide at 20:55 of video at <http://www.shrouduniversity.com/videos/villareal.wmv> of ShroudUniversity.com, also at 4:25 of <https://www.youtube.com/watch?v=9kBpplTK044> (part 3of YouTube version)

<sup>526</sup> Cotton: [http://tera.chem.ut.ee/IR\\_spectra/images/stories/cotton.png](http://tera.chem.ut.ee/IR_spectra/images/stories/cotton.png) on [http://tera.chem.ut.ee/IR\\_spectra/index.php?option=com\\_content&view=article&id=132&Itemid=101](http://tera.chem.ut.ee/IR_spectra/index.php?option=com_content&view=article&id=132&Itemid=101) and Cotton wool: [http://tera.chem.ut.ee/IR\\_spectra/images/stories/cotton\\_wool.png](http://tera.chem.ut.ee/IR_spectra/images/stories/cotton_wool.png) on [http://tera.chem.ut.ee/IR\\_spectra/index.php?option=com\\_content&view=article&id=136&Itemid=105](http://tera.chem.ut.ee/IR_spectra/index.php?option=com_content&view=article&id=136&Itemid=105)

Therefore, also Villarreal's conclusions of 2008 – based on these anomalous standards – that linen and cotton have “Very different” FTIR spectra and that the Raes samples are “definitely not linen”, are meaningless. What is more, they are incorrect, as will be shown below. In Valencia 2012, Robert and Roberta Villarreal presented a slide showing adequate linen and cotton FTIR standards,<sup>527</sup> and corrected the earlier conclusion about the Shroud fibers by writing: “The three samples that we looked at have cotton. ... The FTIR analysis gave very definite peaks showing the functional groups of cotton and linen.”<sup>528</sup>

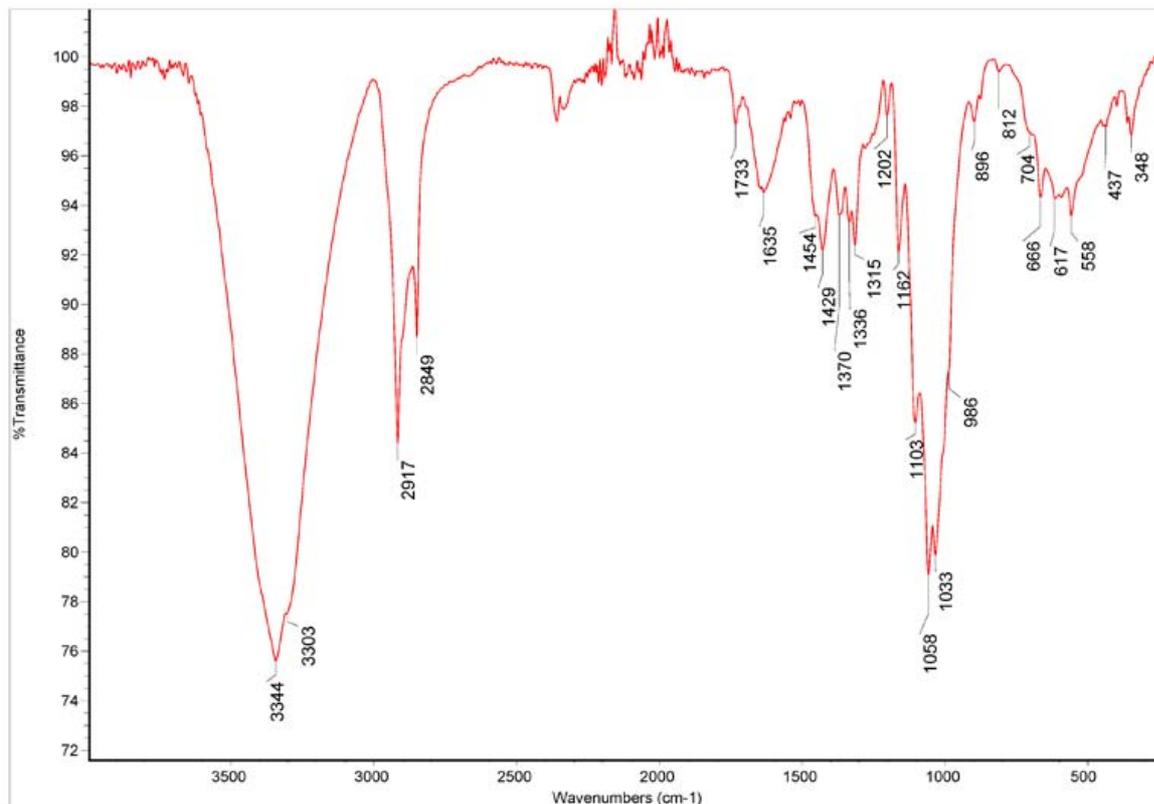


Fig. 4.7. FTIR spectrum of linen. Used from the Database of ATR-IR spectra of materials related to paints and coatings ©University of Tartu, Estonia, Institute of Chemistry<sup>529</sup>

<sup>527</sup>Villarreal and Villarreal, A New Look..., 2012 presentation slides, <http://www.shroud.com/pdfs/villarrealvppt.pdf> #23

<sup>528</sup> Villarreal and Villarreal, A New Look..., 2012 presentation text, <http://www.shroud.com/pdfs/villarrealvtxt.pdf>, p. 3

<sup>529</sup> Used from “Database of ATR-IR spectra of materials related to paints and coatings”, University of Tartu (Estonia), Institute of Chemistry, Chair of Analytical Chemistry, online at [http://tera.chem.ut.ee/IR\\_spectrum/index.php?option=com\\_content&view=article&id=94&Itemid=60](http://tera.chem.ut.ee/IR_spectrum/index.php?option=com_content&view=article&id=94&Itemid=60) and [http://tera.chem.ut.ee/IR\\_spectrum/index.php?option=com\\_content&view=article&id=131&Itemid=100](http://tera.chem.ut.ee/IR_spectrum/index.php?option=com_content&view=article&id=131&Itemid=100) with permission from the publisher

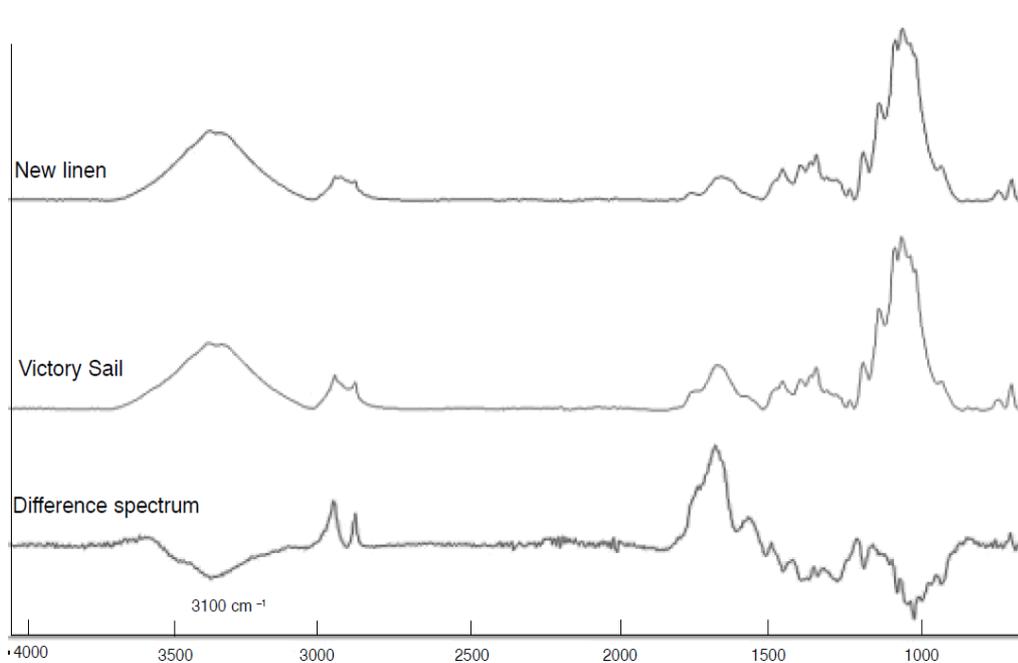


Fig. 4.8. FTIR spectra of “New linen” and the “Victory Sail” (1803 AD) and their “Difference spectrum” ©P. Wyeth, Textiles Conservation Centre, Southampton University<sup>530</sup>

#### 4.1.3.3. Linen, cotton and starch FTIRs are similar to each other and to Shroud FTIRs

In fact, the FTIR spectra of linen, cotton and starch are very similar to each other (see fig. 4.9 below), which is only natural, as these materials all mainly consist of glucose units, either linked to each other by beta linkage in cellulose (in linen and cotton) or by alpha linkage in starch. In 2011, it was said and shown: “In FTIR analysis (Fig. 4), since the chemical nature of starch and cellulose is mostly similar, many of the characteristic peaks overlap and hence distinction is difficult.”<sup>531</sup> This means that the cotton fiber percentages, found in Raes and radiocarbon threads by Rogers (10-20% long cotton fibers in thread R7, also in the core of this cotton-linen blend thread) and by Fanti (2% thin cotton contamination, in a linen thread from just next to the radiocarbon sample),<sup>532</sup> are not contradicted by FTIR results, and that these results also do not readily preclude or show the presence of the starch that was detected on Raes threads and fibers by wet chemistry.<sup>533</sup>

<sup>530</sup> Used from [http://wwwchem.uwimona.edu.jm/courses/CHEM2402/Textiles/Veg\\_Fibres.html](http://wwwchem.uwimona.edu.jm/courses/CHEM2402/Textiles/Veg_Fibres.html) (also at <http://www.rsc.org/Education/Teachers/Resources/Inspirational/resources/3.3.3.pdf>) with permission from P. Wyeth, Textiles Conservation Centre, Winchester Campus, Southampton University

<sup>531</sup> Vigneshwaran et al., 2011, <http://link.springer.com/article/10.1007/s13204-011-0020-5/fulltext.html>

<sup>532</sup> “Cotton is found in R7 as a very long bundle (or bundles) of several fibers running all along the thread with some of them penetrating in the core, ... taking in account ... fibers forming long bundles or sometimes “nodes” or individual relatively long fibers) there is about 15% of cotton in the outer part ... and about 10% of cotton in the core of the thread. ... R7 is definitely some kind of blended thread: cotton (10%-20%)/ linen (80-90%). ... Both kinds of fibers have been spun together to obtain the thread.” Heimburger, Cotton in Raes..., 2009, part 3, p.1; Rogers declared to the Shroud Science Group: “I have found copious amounts of cotton at the core of all of the yarn segments I have dissected.” Communique to the Shroud Science Group, March 5, 2004, 2:30 AM, cited in Marino and Prior, Chronological History..., 2008, p. 18; cf. “Cotton is not a simple surface contaminant: It occurs throughout the Raes threads.” Rogers and Arnoldi, Scientific Method..., 2002, p. 14; “Figure IX-3: Cotton and linen fibers from a warp thread of the radiocarbon sample.” Rogers, A Chemist’s..., 2008, p. 66-67; Heimburger, Cotton in Raes..., 2009, part 3, p. 4, and appendix by Fanti, p. 3

<sup>533</sup> Rogers, Comments On..., 2001, p. 13-14; Rogers and Arnoldi, Scientific Method..., 2002, p. 7, 30, Rogers, A Chemist’s..., 2008, p. 44; Fanti and Schwartz et al., Evidences for..., 2005, A15; Rogers, Frequently Asked Questions..., 2004, p. 11; Bracaglia of the Holy Shroud Guild wrote “Dr. Kohlbeck explained to me that Sue Benford contacted him and requested if he could send her his microscopic photographs of the lance wound area where Dr. Kohlbeck made his observation. (6-BF). She explained to him that she believes what Dr. Heller thought was blood is actually the gum,dye,mordant coating which Dr. Kohlbeck referred in his findings as Starch.” (Bracaglia, Raes Problematic Threads, part 3); Kohlbeck wrote: “There were additional observations such as finding solubilized starch on

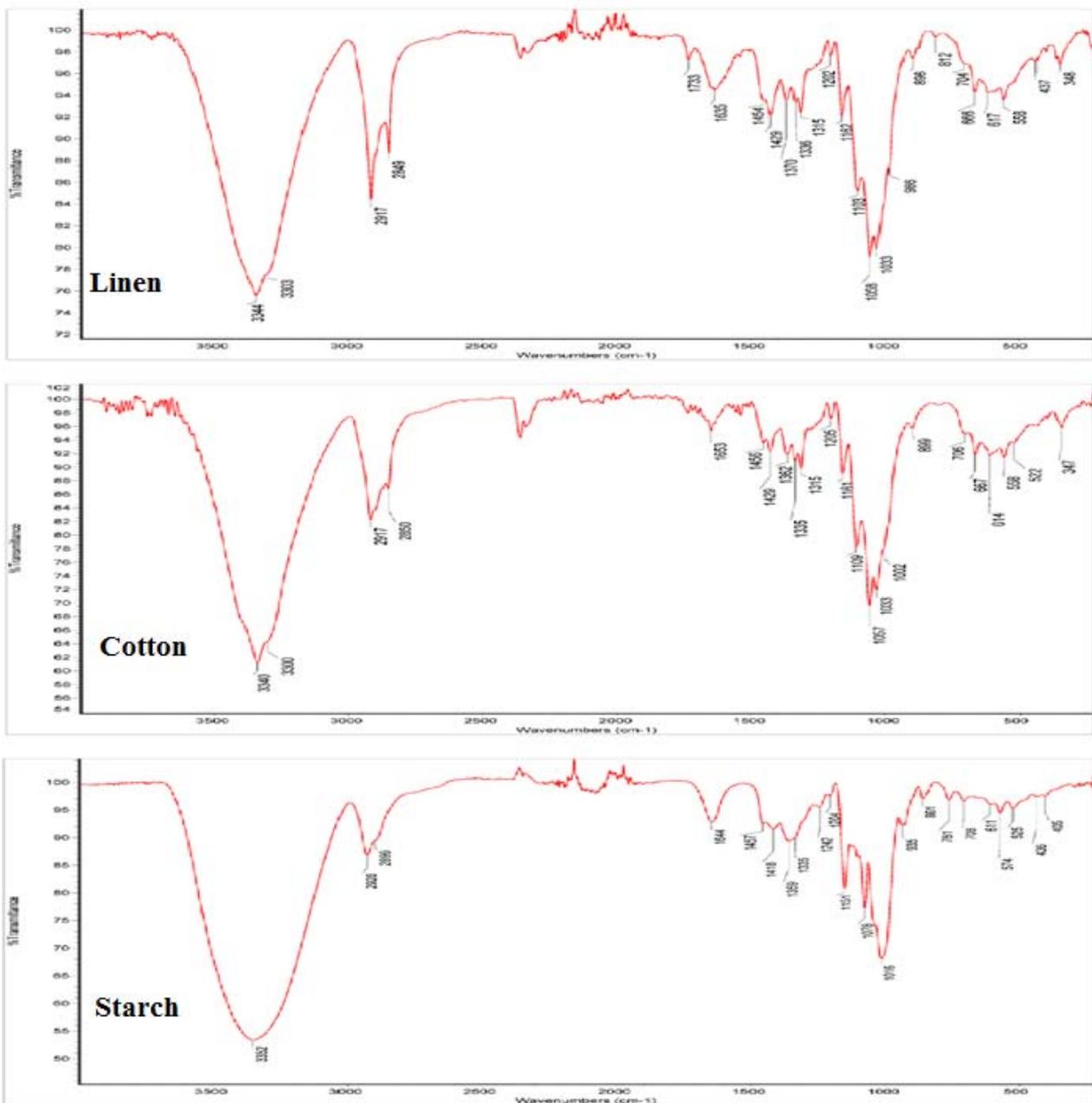


Fig. 4.9. FTIR spectra of linen, cotton, and starch. Used from the Database of ATR-IR spectra of materials related to paints and coatings ©University of Tartu, Estonia, Institute of Chemistry<sup>534</sup>

For comparison, see the FTIR spectrum of Gum Arabic (fig. 4.10). Here the relative intensities of the three low-wavenumber bands are unlike those of the Raes thread or even Raes crust. Because no protein has been found in the Raes corner, the presence of Gum Arabic – which does contain proteins – has already been precluded anyway.<sup>535</sup> Also, the cotton fibers found in the Raes corner were spun-

Professor Rae’s samples and cotton fibers with the linen fibers which Prof. Rae identified as a mid-east cotton.”

Bracaglia, Dr Nitowski’s..., <http://holyshroudguild.org/dr-nitowski-new.html>

<sup>534</sup> Adapted (stretched and aligned) from Database of ATR-IR spectra of materials related to paints and coatings, University of Tartu (Estonia), Institute of Chemistry, Chair of Analytical Chemistry, with permission from the publisher, online at

[http://tera.chem.ut.ee/IR\\_spectra/](http://tera.chem.ut.ee/IR_spectra/):

[http://tera.chem.ut.ee/IR\\_spectra/index.php?option=com\\_content&view=article&id=131&Itemid=100](http://tera.chem.ut.ee/IR_spectra/index.php?option=com_content&view=article&id=131&Itemid=100)

[http://tera.chem.ut.ee/IR\\_spectra/index.php?option=com\\_content&view=article&id=132&Itemid=101](http://tera.chem.ut.ee/IR_spectra/index.php?option=com_content&view=article&id=132&Itemid=101)

[http://tera.chem.ut.ee/IR\\_spectra/index.php?option=com\\_content&view=article&id=113&Itemid=81](http://tera.chem.ut.ee/IR_spectra/index.php?option=com_content&view=article&id=113&Itemid=81)

<sup>535</sup> Hoeven, Internal selvedge..., 2012, 2.5.1.

in with the linen fibers,<sup>536</sup> so could not be just a local addition glued to the cloth as a coating applied in a cleaning procedure.<sup>537</sup>

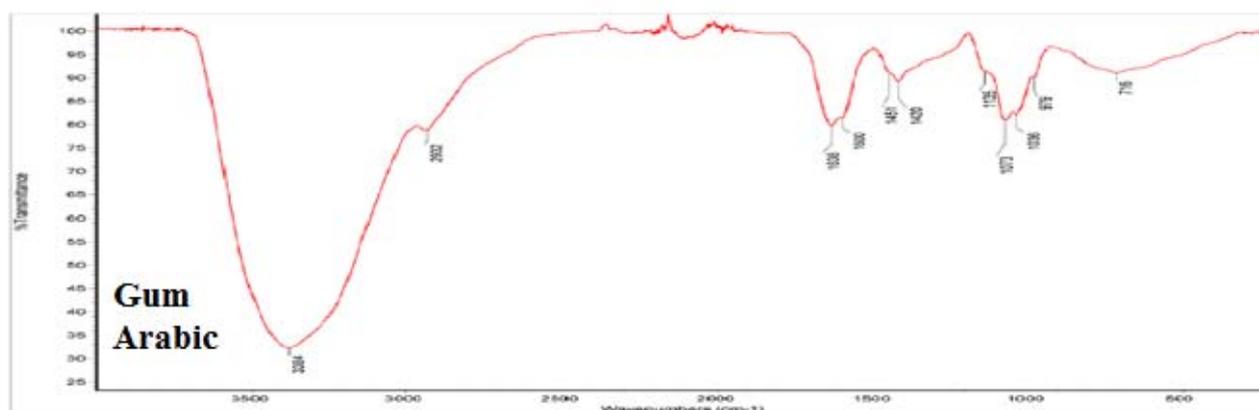


Fig. 4.10. FTIR spectrum of Gum Arabic. Used from the Database of ATR-IR spectra of materials related to paints and coatings ©University of Tartu, Estonia, Institute of Chemistry<sup>538</sup>

The overall similarity between linen and Shroud FTIR spectra (especially those of non-image, image, Raes and radiocarbon) is obvious (see fig. 4.11 below).

#### 4.1.3.4. “Backing cloth” FTIRs ‘older’ than main Shroud

The FTIR spectra of new linen and of linen of 1803 AD (the “Victory Sail”) are shown in fig. 4.8 above. The online text that accompanies the linen FTIR spectra of fig. 4.8 says “The deterioration over the years has come in part from the aerial oxidation of the alcohol groups to carboxylic acids. This can be seen in the difference spectrum calculated from the IR spectrum of fresh linen compared to that of the sail. The band at 1720 cm<sup>-1</sup> being due to acid formation (C=O) and the negative band at ~3100 cm<sup>-1</sup> showing the loss of the OH groups. The two bands at 2930 cm<sup>-1</sup> and 2850 cm<sup>-1</sup> show that the sail has gained some oils or waxes that were not originally present.” When the above linen spectra (unfortunately, with a non-linear scale) are aligned to the non-image spectra with a linear scale (by aligning the small peak in the trough between the 1350 and 1000 cm<sup>-1</sup> bands, and the trough between the ~1700 and 1350 band of the linen spectra, see fig. 4.11 below), the strong difference band at “1720 cm<sup>-1</sup>” appears to consist of a peak at 1650 with a shoulder at 1720 cm<sup>-1</sup>. The negative bands around 1350 and 1000 cm<sup>-1</sup> show that these bands have decreased in ageing. Fanti and Gaeta wrote, in 2013, that the ratio of the OH band and the C=O band is a parameter that is adequate for estimating the age of linen cloths.<sup>539</sup>

Moroni, at the Rome 1993 Shroud conference, showed FTIR spectra of a linen cloth before and after heating in a closed container: in the FTIR spectrum of the heated and yellowed fibers, there also is a marked increase in the band at 1640 cm<sup>-1</sup> relative to the adjacent bands at 1605 and 1720 cm<sup>-1</sup> (fig. 4.12).<sup>540</sup>

<sup>536</sup> Heimburger, Cotton in Raes..., 2009, part 3, p.1, cited above in a note (also Raes, Rogers and others, on this subject, are cited in Hoeven, Internal Selvedge..., 2012, 2.1.)

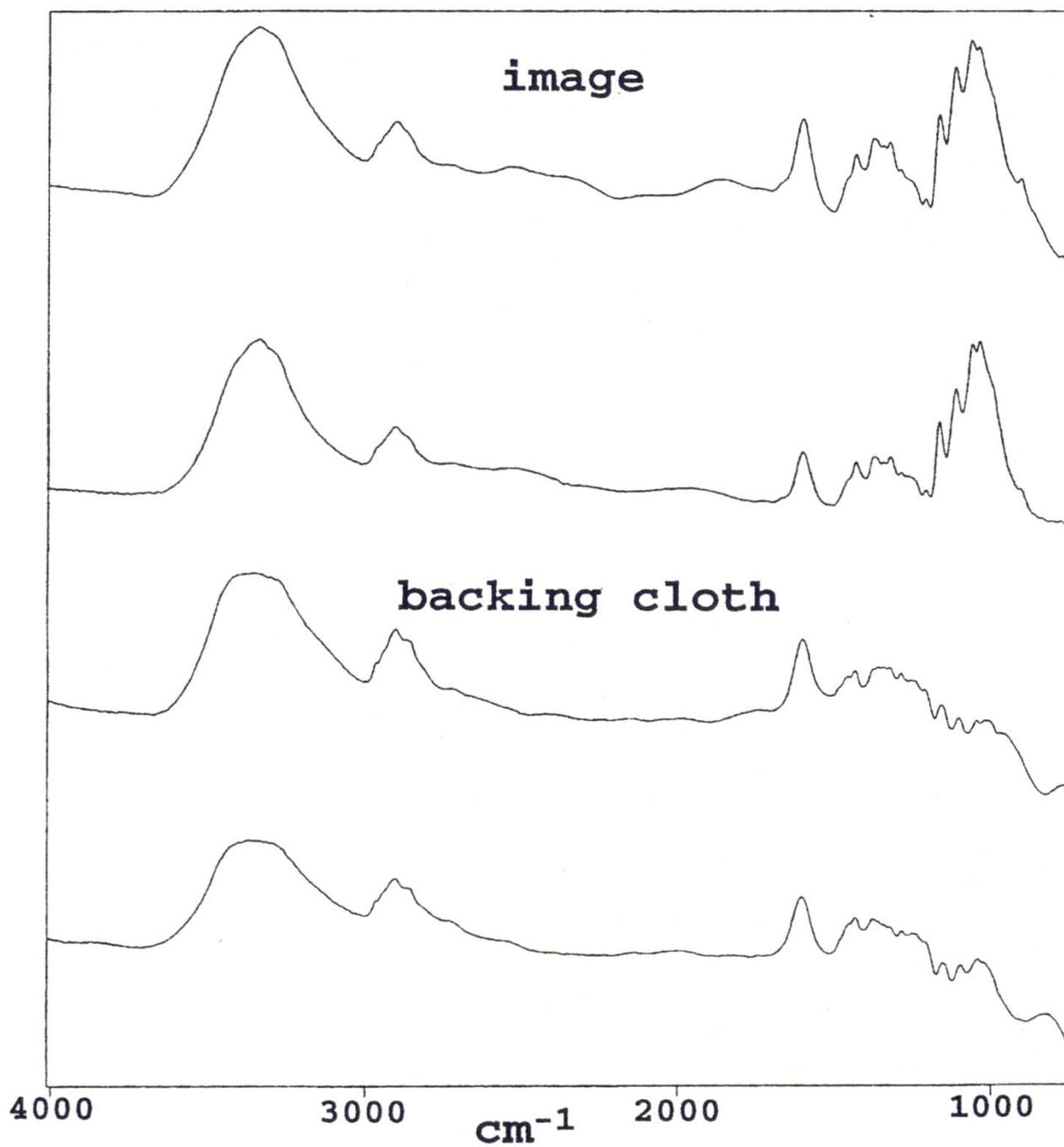
<sup>537</sup> Such a coating of glued-on cotton was suggested by Moon, Coloured Dissolved Organic Matter..., 2013, p. 20

<sup>538</sup> Adapted (stretched) from Database of ATR-IR spectra of materials related to paints and coatings, University of Tartu (Estonia), Institute of Chemistry, Chair of Analytical Chemistry, with permission from the publisher, online at [http://tera.chem.ut.ee/IR\\_spectrum/index.php?option=com\\_content&view=article&id=94&Itemid=60](http://tera.chem.ut.ee/IR_spectrum/index.php?option=com_content&view=article&id=94&Itemid=60) [http://tera.chem.ut.ee/IR\\_spectrum/index.php?option=com\\_content&view=article&id=107&Itemid=77](http://tera.chem.ut.ee/IR_spectrum/index.php?option=com_content&view=article&id=107&Itemid=77)

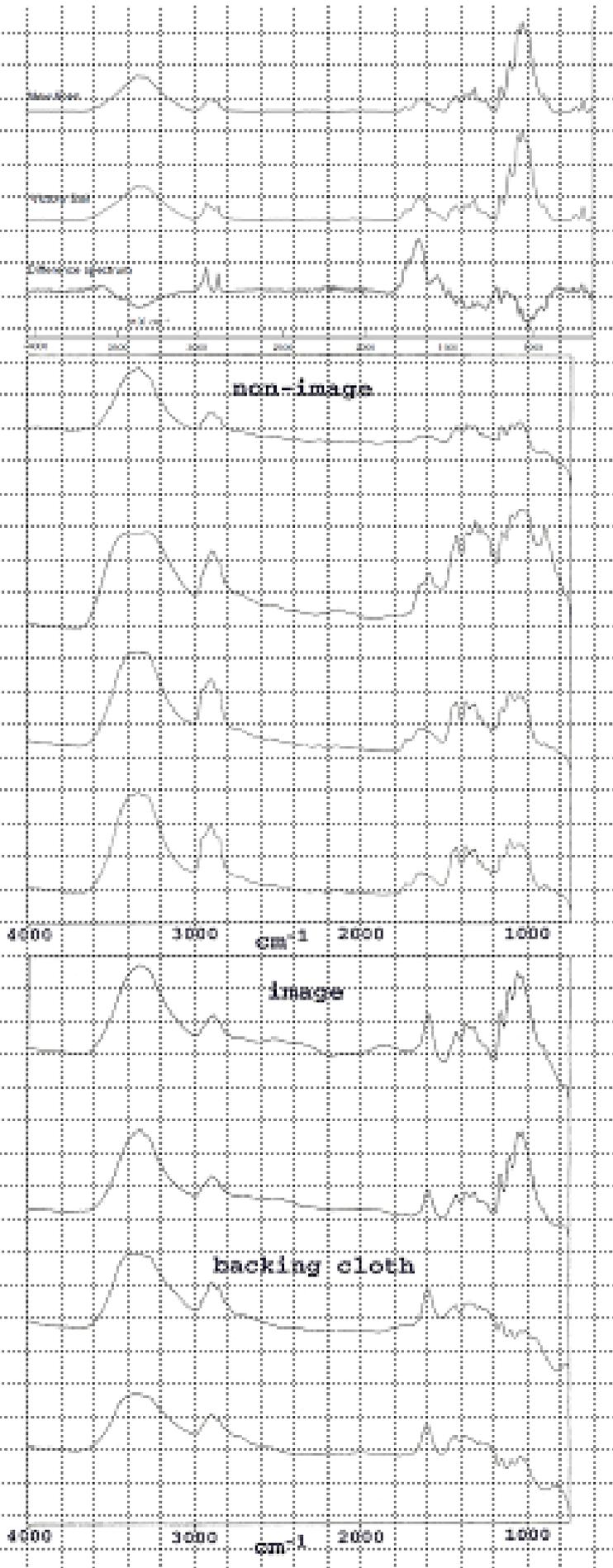
<sup>539</sup> Fanti and Gaeta, Il Mistero della Sindone, 2013, p. 88

<sup>540</sup> Mario Moroni, The Age and Proposal for photo-colormetric Control of the Shroud. Presentation Rome 1993, <https://www.youtube.com/watch?v=79mf-lmWM6o>, at 2:10 (“linen cloth”) and at ca. 15:25 -16:35 (FTIR spectra of A (red = “image” = yellowed cloth) and B (blue = “background” = white cloth)).

A:



*Fig. 7 Typical FTIR absorbance patterns of image and backing cloth*



B:

Fig. 4.11. (above) A: FTIR spectra of image and “backing cloth” fibers of the Shroud ©A.D. Adler, R. Selzer and F. DeBlase<sup>541</sup> B: Comparison of FTIR spectra with added lath. Top: New linen/Victory Sail ©P. Wyeth, Textiles Conservation Centre, Southampton University<sup>542</sup>. Centre and bottom: non-image and image and backing cloth ©A.D. Adler, R. Selzer and F. DeBlase<sup>543</sup>

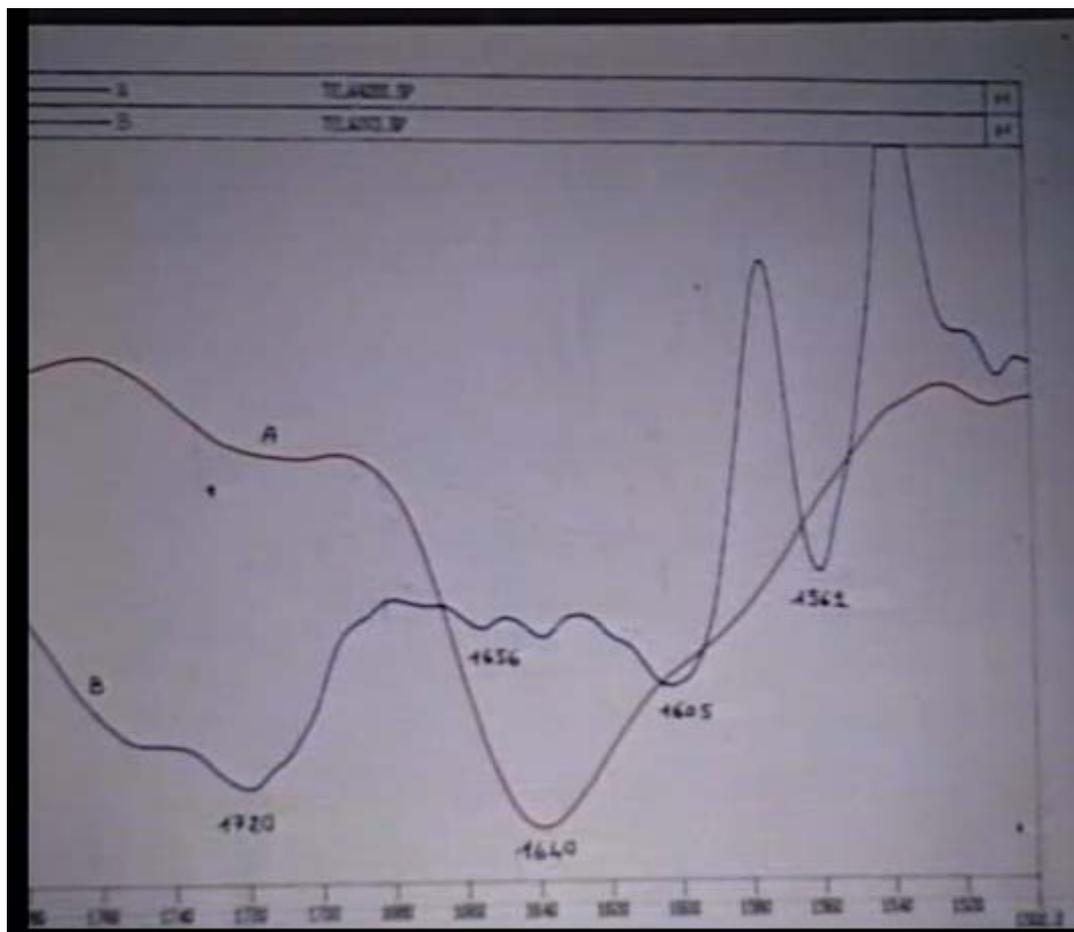


Fig. 4.12. FTIR spectra (detail 1500-1800  $\text{cm}^{-1}$ , in transmission mode) of a folded cloth of white linen, heated in a closed container, as presented by Moroni, in Rome 1993.<sup>544</sup> A (red): yellowed fibers (“image”) yield a clear 1640  $\text{cm}^{-1}$  peak; B (blue curve): white fibers (“background”) yield 1720, 1656, 1505 and 1562  $\text{cm}^{-1}$  peaks. ©R. Breault<sup>545</sup>

#### Backing cloth FTIR spectra probably waterstain FTIR spectra

These Victory Sail and new linen FTIRs, of relatively new material, look quite similar to the FTIRs of non-image fibers from the main Shroud and also to the FTIRs of the Raes samples (crust and encrusted thread end), also because the relative intensities of the  $\sim 1650$ ,  $1350$  and  $1000 \text{ cm}^{-1}$  bands

<sup>541</sup> Used from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, in Minor M. et al. (eds.), The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-181, © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer

<sup>542</sup> Adapted from [http://wwwchem.uwimona.edu.jm/courses/CHEM2402/Textiles/Veg\\_Fibres.html](http://wwwchem.uwimona.edu.jm/courses/CHEM2402/Textiles/Veg_Fibres.html) (also at <http://www.rsc.org/Education/Teachers/Resources/Inspirational/resources/3.3.3.pdf>) with permission from P. Wyeth, Textiles Conservation Centre, Winchester Campus, Southampton University

<sup>543</sup> Adapted from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, in Minor M. et al. (eds.), The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-181, © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer

<sup>544</sup> Moroni, M., The Age and Proposal for photo-colometric Control of the Shroud. Presentation Rome 1993, video by R. Breault at <https://www.youtube.com/watch?v=79mf-ImWM6o>, slide with these FTIR spectra from 15:45

<sup>545</sup> Used from R. Breault’s video <https://www.youtube.com/watch?v=79mf-ImWM6o> with his permission.

increase in this order in all their curves. In the two FTIRs of “backing cloth” fibers of the Shroud<sup>546</sup>, on the other hand, these bands have decreasing heights: the ~1650 band is higher than the 1350 band, which in turn is higher than the 1000 cm<sup>-1</sup> band (see fig. 4.11). Also the ratio of the OH band to the C=O band in the Shroud non-image FTIRs is higher than in the backing cloth FTIRs. In these aspects of the FTIRs, the 1534 AD backing cloth’s linen thus, oddly, looks older than the Shroud linen to which it was attached only much later. An interesting observation in this regard, made by Flury-Lemberg, is that “It was necessary to remove the Holland cloth because it was creating tension on the linen of the Shroud. Over the centuries the Holland cloth seems to have shrunk. ... A large piece of an approximately hundred year old pure unbleached linen was in fact used to replace the Holland cloth.”<sup>547</sup> The backing cloth is called ‘the Holland cloth’ and “an authentic, documented sample of Medieval linen”.<sup>548</sup> Its tension-creating shrinkage – cf. “No one sews a piece of unshrunk cloth on an old garment; if he does, the patch tears away from it, the new from the old, and a worse tear is made” (Mark 2:21) – probably means that the linen of the Shroud – dating from 1353 AD or earlier – had already fully shrunk in 1534 AD, and that the Holland cloth is younger than the Shroud, and indeed is from 1534 AD and thus about 450 years old.

Another odd feature of the “backing cloth” FTIRs is that they only have a single peak at 1600 cm<sup>-1</sup> instead of 1650 or 1720 cm<sup>-1</sup>, the expected values for medieval pure linen. The 1600 cm<sup>-1</sup> peak is not present in the Victory Sail FTIR, but is present in nearly all Shroud FTIRs, and it is especially clear in its “waterstain” FTIRs, that also have decreasing intensities of the 1600, 1350 and 1000 cm<sup>-1</sup> bands (see fig. 4.5 (TOM 83) above and fig. 4.13 below).

The backing cloth probably was not waterstained, for it was applied after the 1532 fire, and most probably also after the big waterstains got into the Shroud. The (probably) two fibers that were used for the two “backing cloth” FTIRs<sup>549</sup> were from a sticky tape that was applied to the not-covered front surface of the backing cloth next to the Raes corner, and that was called 1FH and provided to Heller and Adler by Rogers.<sup>550</sup> It seems probable that the two FTIR fibers were in fact Shroud waterstain fibers that had been transposed from elsewhere on the Shroud by folding, for instance from the big waterstain at the opposite corner of the Shroud, at the other side of the ventral feet. In 1532 the Shroud was damaged in a folded state, and the burn marks show that it had been folded along the longitudinal axis first, with the image inside, making the observe side of the Raes corner touch the observe side of the waterstain at the opposite corner of the same short edge.<sup>551</sup> In 1534, after the backing cloth had been applied, one may have used the same way of folding, thus making the not covered corner of the backing cloth touch the waterstain at the opposite corner. Just as it was “found” that “The blood had abraded off in many places and was transposed everywhere”<sup>552</sup>, also Shroud fibers may have abraded off and been transposed. This fiber abrasion may especially have taken place in the corners, where the Shroud had been held and handled a lot – all corners now show dirt deposits – and where the surface probably was more damaged and had more damaged and

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<sup>546</sup> two curves in fig. 7., Adler, Selzer and DeBlase, *Further Spectroscopic...*, 2002, TOM 100

<sup>547</sup> Transcription of 2005 interview of Flury-Lemberg in “The Third International Dallas Shroud Conference”, *BSTS Newsletter* 62, Dec. 2005, <http://www.shroud.com/pdfs/n62part2.pdf>

<sup>548</sup> “Nuns patched burn holes and stitched the Shroud to a reinforcing cloth that is now known as the Holland cloth. I also sampled it in 1978. The Holland cloth provides an authentic, documented sample of Medieval linen.” (Rogers and Arnoldi, *Scientific Method...*, 2002, p. 14)

<sup>549</sup> In Adler’s presentation in the Dallas in 1998 conference, <http://shrouduniversity.com/podcasts/aladler.mp3> at 9:50, he said on four non-image spectra: “These were four fibres. They were transferred to the salt plates we used for the FTIR microscope and you’ll see the patterns are in fact quite similar. These fibres, this one came from the head area, the chest area, the knee area, and the foot area. So here’s four fibres taken over two metres of length of the cloth in fact giving one pattern.” This means that also an FTIR spectrum labelled “backing cloth” may have been made on only one fiber.

<sup>550</sup> Sticky tape #1 on the original STURP map (Figure 2 on p. 15 of 2nd scribd document by Sr. Nitowski, *Criteria for authentication of...*, 1986, on <http://holyshroudguild.org/dr-nitowski-new.html>); “Nuns patched burn holes and stitched the Shroud to a reinforcing cloth that is now known as the Holland cloth. I also sampled it in 1978. The Holland cloth provides an authentic, documented sample of Medieval linen.” (Rogers and Arnoldi, *Scientific Method...*, 2002, p. 14); Table 1: “1FH Patch cloth”, and text Acknowledgments, Heller and Adler, *A Chemical...*, 1981, TOM 48-49

<sup>551</sup> Gueresschi and Salcito, *Further Studies...*, 2005

<sup>552</sup> Heller, *Report on...*, 1983 p. 196; Heller checked the transposal of red particles by folding and unfolding an old Spanish linen sample with bloodstains: “tiny blood particles could be seen all over.” (Ibid.)

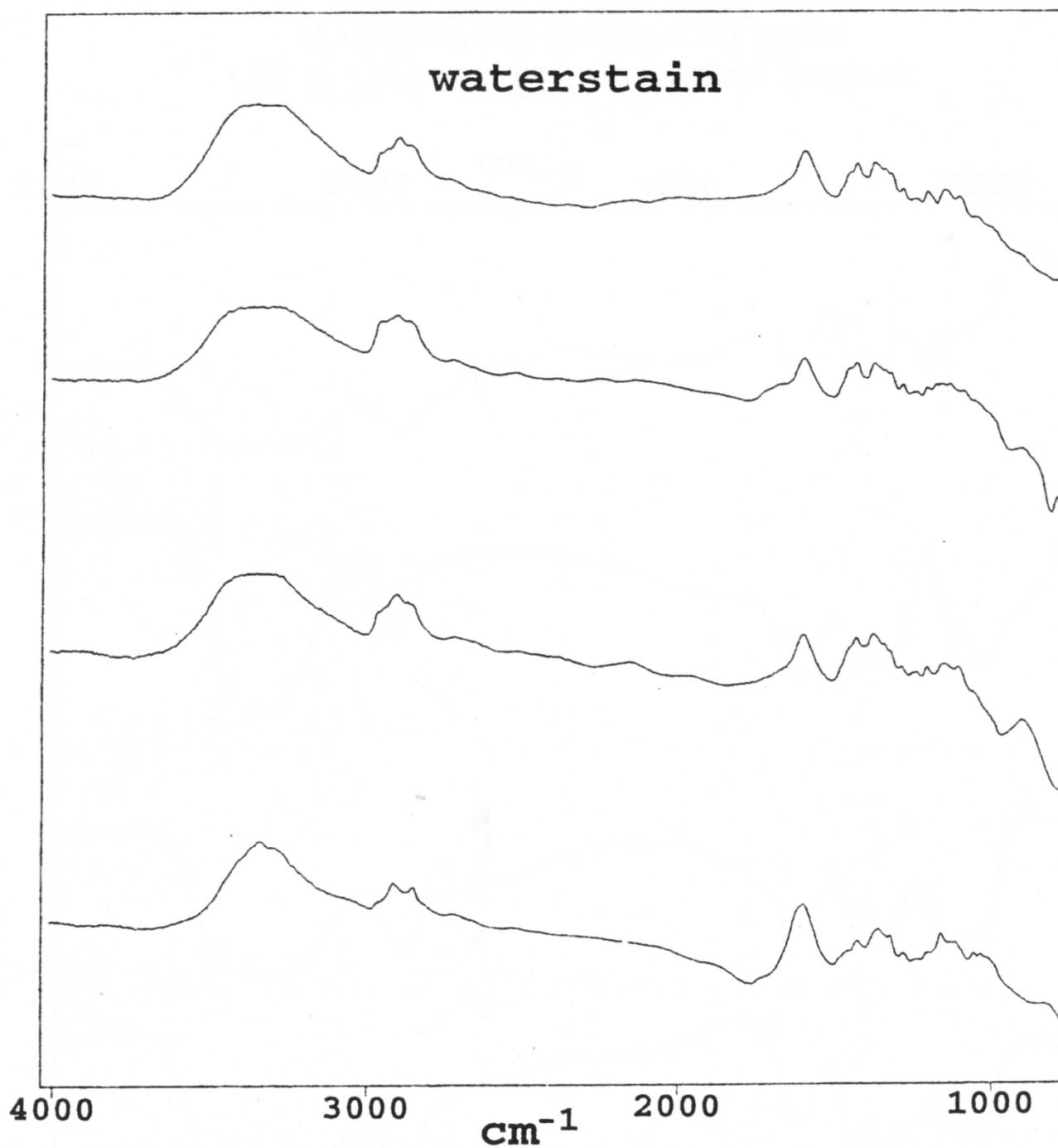
broken fibers than surfaces that were not touched and handled. Abraded waterstain fibers may have been transposed to the not-covered backing cloth by direct contact and friction during and after folding. Note that occasional artists' pigments were found on the sticky-tape samples of the Shroud, and that these are assumed to have been transposed from Shroud replica's to the Shroud by direct contact, when a replica was laid on the Shroud only once for the so-called 'sanctification' of the replica.<sup>553</sup> The abraded waterstain fibers, transposed to the surface of the backing cloth, would have been picked up by the sticky-tape first, before real backing cloth fibers. Unfortunately, McCrone had firmly stuck all Mylar sticky-tape samples to microscope slides, then he had pulled them off and stuck them down to microscope cover slips, and Heller said that "hard compression of the Mylar onto the glass slides had squeezed everything together", which rendered any distinction of upper or deeper surface material by Heller and Adler impossible.<sup>554</sup>

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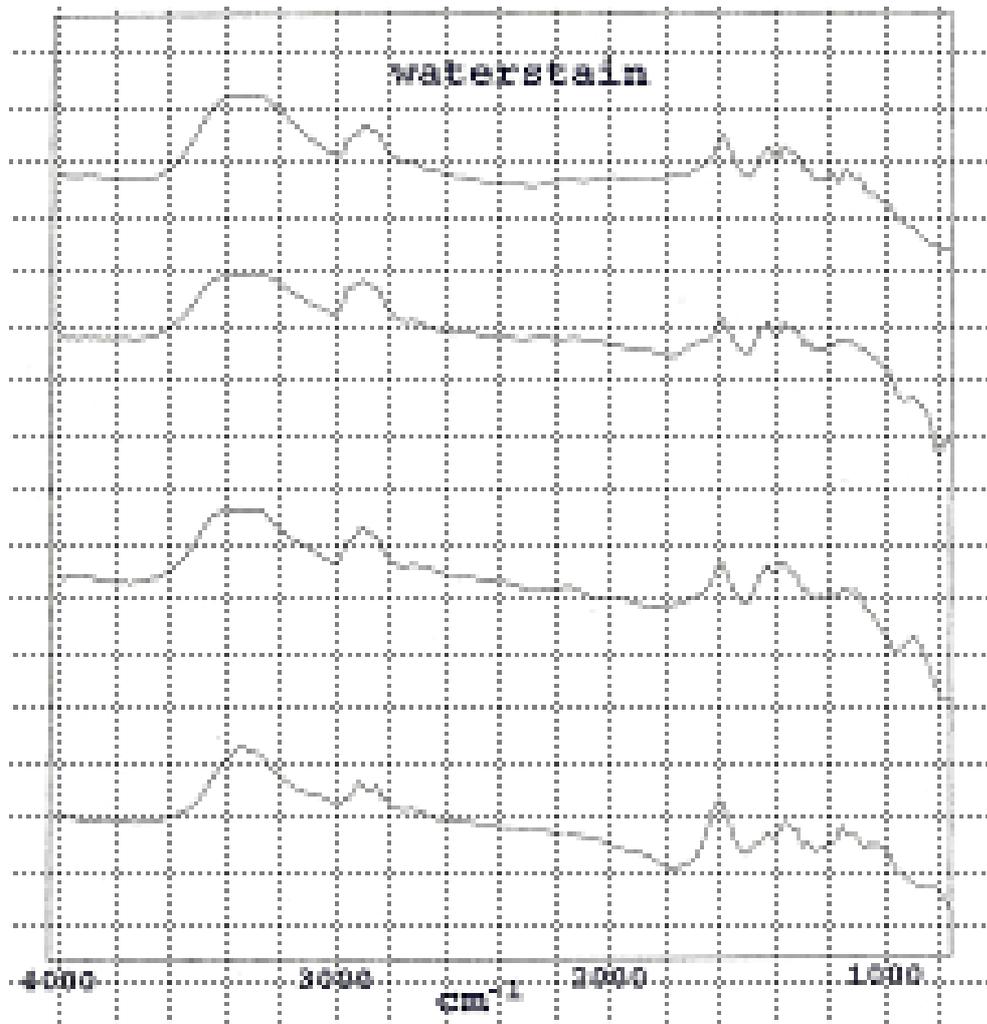
<sup>553</sup> Adler, *The Shroud fabric...*, 2002, TOM 119; Ford, *The Shroud of Turin's...*, 2000, p. 17

<sup>554</sup> "Walter McCrone took it upon himself to stick all of the samples down to microscope slides. He did not reserve *any* samples in a pristine state. ... he pulled the tapes off of the slides and stuck all of them down to microscope cover slips. This destroyed much of the physical evidence we had sought."; "Walter McCrone ... contaminated all of our tape samples by sticking them to microscope slides. All of the fibers were immersed in the tape's adhesive." Rogers, *A Chemist's*, p. 24, 52; Heller, *Report on...*, 1983, p. 163

A:



*Fig. 5 Typical FTIR absorbance patterns of waterstain fibers*



B:

Fig. 4.13. A: FTIR spectra of waterstain fibers of the Shroud and B: Idem, covered with lath ©A.D. Adler, R. Selzer and F. DeBlase<sup>555</sup>

<sup>555</sup> Used and adapted from A.D. Adler, R. Selzer, and F. DeBlase, Further Spectroscopic Investigations of Samples of the Shroud of Turin, in Minor M. et al. (eds.), The Shroud of Turin - Unraveling the Mystery - Proceedings of the 1998 Dallas Symposium, Compiled by Michael Minor, Alexander Books, Alexander – North Carolina, 2002, pp. 166-181, © A.D. Adler, R. Selzer, F. DeBlase, with permission from R. Selzer

#### 4.1.3.5. Wavenumber comparison – pectin, no protein, possible madder component

A survey of the estimated and published wavenumbers is given in table 7. They are those of Villarreal's FTIRs of the Raes crust (thin fragment and large piece from region 1 and 2 of Raes thread #1) and a few Raes threads (Raes thread #1 – region 1 and 2 –, and Raes threads #7 and #14 – which had spun-in cotton (Heimbürger found up to 20% cotton in R7, also in the core)<sup>556</sup> and on which starch and madder was detected<sup>557</sup>). Also the wavenumbers of the FTIRs of Adler et al., pertaining to main Shroud non-image fibers, image fibers, and backing cloth fibers, are listed. The table also shows the wavenumbers of cotton, linen, (wheat) starch, amylopectin, alizarin, purpurin and squalene (for alizarin and purpurin FTIRs, see fig. 4.14 and 4.15). In the table, the color of the cell refers to the substance that may be the source (wavenumber within  $\pm 10\text{ cm}^{-1}$  from the substance's reference wavenumber), with priority for linen, then for starch, then madder (except for the thin crust fragment, where starch and madder were given priority over linen). Amylopectin is the main component of retrograded starch, as starch's amylose leaches out in the gelatinisation that takes place before retrogradation. The starch on the Shroud, if applied at weaving, would have retrograded twice, and probably was polished as well. In the Raes corner it would also have turned into starch gum by light scorching, in which many alpha links would have been broken, yielding large dextrans. Squalene is a component of human sebum, which is probably present in the handling dirt that was deposited in the corners by human hands.

Some small non-image peaks/shoulders that are also present in the Raes/radiocarbon FTIRs but that are not seen in image FTIRs might be due to starch and madder. This will be discussed in the following. The resolution/representation of at least the Shroud FTIRs is too poor ( $8\text{ cm}^{-1}$  resolution of the 1998 equipment<sup>558</sup> and the uncertainty of the readings is estimated to be  $\pm 10\text{ cm}^{-1}$  for Adler's plots and more for those of the screenshots of Villarreal's presentation) and the number of possible substances (with their overlaps) is perhaps too large to use the wavenumbers as evidence for the presence of starch and madder on the non-image fibers of the main Shroud (also because wavenumbers of other madder constituents of madder dye than alizarin and purpurin and possible degradation products have not been listed). But the apparent match of wavenumbers does not preclude it.

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<sup>556</sup> “Rogers found old cotton, with a yellow-brown coating and spun with the linen, in the Raes threads R5 (warp), R7 (weft) and R14 (warp or weft). [Rogers and Arnoldi, *Scientific Method*, p. 14 and 17; “Figure 6 shows fibrils from Raes thread #5. ... You can see one cotton twist (lower right), but the field of view at 400X is too narrow to see any other twists. Twists are about 1.25mm apart. According to Raes, this would identify the cotton as *herbaceum*. Each major division of the reticule is 0.026 mm.” Rogers, Supportive comments, p. 2 and fig. 6 on p. 5] “R7 is definitely some kind of blended thread: cotton (10%-20%)/ linen (80-90%). There is more cotton in the outer part than in the core. Both kinds of fibers have been spun together to obtain the thread.” [Heimbürger, *Cotton in Raes*, part 3, p.1; Rogers declared to the Shroud Science Group: “I have found copious amounts of cotton at the core of all of the yarn segments I have dissected.” *Communique to the Shroud Science Group*, March 5, 2004, 2:30 AM, cited in Marino and Prior, *Chronological History*, p. 18; cf. “Cotton is not a simple surface contaminant: It occurs throughout the Raes threads.” Rogers and Arnoldi, *Scientific Method*, p. 14] “Raes # 7 is about 10 mm in length”. [Heimbürger, *Cotton in Raes*, part 3, p. 2] Rogers said of Raes thread R14: “When the cotton fiber was drawn out of the thread, it showed reversals about 1.2-mm apart.” [Rogers and Arnoldi, *Scientific Method*, p. 14] So, this would match the roughly 8 reversals per cm of the ancient type *Gossypium herbaceum*, observed by Raes.” (Hoeven, *Internal Selvage*..., 2012, 2.1.1.)

<sup>557</sup> Rogers and Arnoldi, *Scientific Method*..., 2002, p. 17-19

<sup>558</sup> For the FTIRs presented by Adler et al. at the 1998 Dallas Symposium (Adler, Selzer and DeBlase, *Further Spectroscopic*..., 2002, TOM 95)



Table 7. FTIR wavenumbers of various relevant substances. Dark green: cotton<sup>559</sup>; light green: linen<sup>560</sup>; dark blue: starches<sup>561</sup> (light blue marking the  $\alpha$ -link wavenumbers); light blue: amylopectin<sup>562</sup>; light orange: alizarin<sup>563</sup>; dark orange: purpurin<sup>564</sup>; brown: squalene<sup>565</sup>; white: Shroud samples (1<sup>st</sup> boxed-in column Raes crust thin; then one column Raes crust bulk, three columns Raes thread #1, one column Raes thread #7, one column Raes thread #14<sup>566</sup>, four columns radiocarbon area weft, four columns radiocarbon area inner warp, five boxed-in columns non-image, three columns image, two columns backing cloth (light blue and yellow marking starch and madder wavenumbers, respectively<sup>567</sup>).

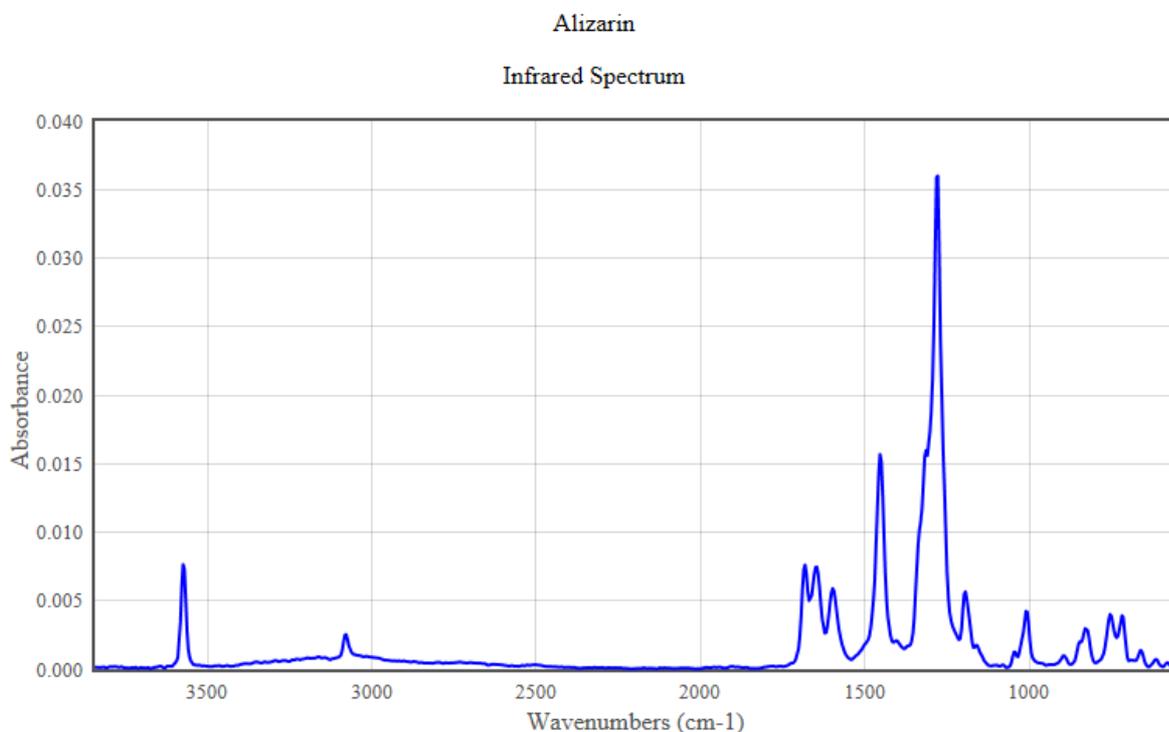


Fig. 4.14. FTIR spectrum of alizarin ©NIST<sup>568</sup>

<sup>559</sup> [http://tera.chem.ut.ee/IR\\_spectra/index.php?option=com\\_content&view=article&id=132&Itemid=101](http://tera.chem.ut.ee/IR_spectra/index.php?option=com_content&view=article&id=132&Itemid=101)

<sup>560</sup> [http://tera.chem.ut.ee/IR\\_spectra/index.php?option=com\\_content&view=article&id=131&Itemid=100](http://tera.chem.ut.ee/IR_spectra/index.php?option=com_content&view=article&id=131&Itemid=100)

<sup>561</sup> [http://tera.chem.ut.ee/IR\\_spectra/index.php?option=com\\_content&view=article&id=113&Itemid=81](http://tera.chem.ut.ee/IR_spectra/index.php?option=com_content&view=article&id=113&Itemid=81); Nobrega et al., 2012, text at "Infrared spectroscopy" in [http://www.scielo.br/scielo.php?pid=S0104-14282012000500013&script=sci\\_arttext](http://www.scielo.br/scielo.php?pid=S0104-14282012000500013&script=sci_arttext) (cf. Fig 1a and 1b); wheat starch

[http://img.springerimages.com/Images/Springer/JOU=10973/VOL=2010.99/ISU=2/ART=2009\\_194/MediaObjects/WATER\\_10973\\_2009\\_194\\_Fig1\\_HTML.jpg](http://img.springerimages.com/Images/Springer/JOU=10973/VOL=2010.99/ISU=2/ART=2009_194/MediaObjects/WATER_10973_2009_194_Fig1_HTML.jpg); wheat starch MFA [http://cameo.mfa.org/images/thumb/8/89/MFA-Wheat\\_starch.jpg/300px-MFA-Wheat\\_starch.jpg](http://cameo.mfa.org/images/thumb/8/89/MFA-Wheat_starch.jpg/300px-MFA-Wheat_starch.jpg); amylopectin-rich starch

<http://www.scielo.br/img/fbpe/mr/v4n2/n2a05f01.gif>

<sup>562</sup> <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0142961212002864-gr1.jpg> in Zhou et al, 2012,

<http://www.sciencedirect.com/science/article/pii/S0142961212002864>; <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0144861710007940-gr1.jpg> in Lu et al, 2011,

<http://www.sciencedirect.com/science/article/pii/S0144861710007940>

<sup>563</sup> NIST Chemistry WebBook <http://webbook.nist.gov/cgi/cbook.cgi?Spec=C72480&Index=0&Type=IR&Large=on>; synthetic alizarin CAMEO [http://cameo.mfa.org/images/3/32/PR083\\_Alizarin%2C\\_synthetic.jpg](http://cameo.mfa.org/images/3/32/PR083_Alizarin%2C_synthetic.jpg)

<sup>564</sup> MFA <http://cameo.mfa.org/wiki/File:MFA-Purpurin.jpg>

<sup>565</sup> NIST Chemistry WebBook <http://webbook.nist.gov/cgi/cbook.cgi?ID=C7683649&Mask=80>

<sup>566</sup> all Raes samples: Villarreal, presentation Ohio 2008, [www.shrouduniversity.com/videos/villarreal.wmv](http://www.shrouduniversity.com/videos/villarreal.wmv)

<sup>567</sup> all radiocarbon, non-image, image, backing cloth samples: Adler, Selzer and DeBlase, Further spectroscopic..., 2002 (Minor M. et al. (eds.), Unraveling..., Proceedings, 2002), except first non-image and first image column: Alder, Updating Recent..., 1996 (TOM 83)

<sup>568</sup> Used from the NIST Chemistry WebBook

<http://webbook.nist.gov/cgi/cbook.cgi?ID=C72480&Units=SI&Mask=80#IR-Spec> © NIST, with permission from NIST Chemical Sciences Division

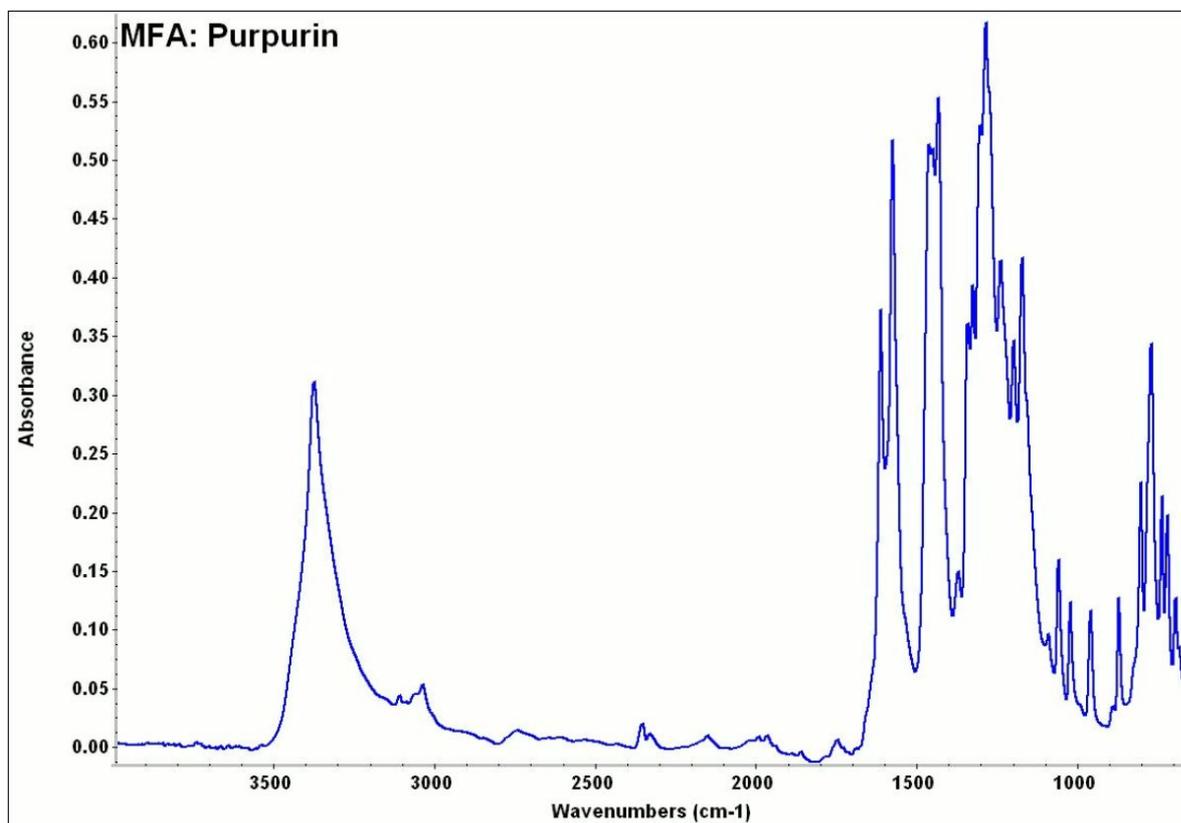


Fig. 4.15. FTIR spectrum of purpurin, Photograph ©2013 Museum of Fine Arts, Boston<sup>569</sup>

### Missing peaks

Villarreal's slide introducing the FTIR analysis read: "Analysis was performed using an FTIR microscope in reflectance mode, sampling at most a few fibers per spectrum. Several (4-6) spectra were obtained at each chosen position along the length of the thread. Issue: Some spectral characteristics depend on the vertical position of the fibers relative to the focal plane (fibers must lie flat for reliable data)."<sup>570</sup> So, if some linen, starch or madder peaks are missing in some Raes FTIRs, this may be one of the reasons. The FTIRs by Adler, Selzer and DeBlase were measured in transmission mode. Other reasons for the missing of some madder peaks may be: 1) madder would have been largely washed off by the xylene/toluene that washed off the sticky-tape adhesive (see 4.2.1.1.), 2) madder peaks were dominated by the much larger presence of linen, and probably even of starch, and 3) madder consists of more than just alizarin and purpurin, and therefore madder peaks may be a bit shifted from the locations of peaks of pure undegraded alizarin and purpurin.

### Thin crust fragment without linen peaks

The peaks in the FTIR with the written text "What appeared to be a thin fragment of crust"<sup>571</sup> from a Raes thread can be completely assigned to starch/amylopectin and alizarin and purpurin – all containing –OH –, and the "olefinic" features, reported on the slide (fig. 4.16), may be due to sebum components of handling dirt deposits. The peak assignment of the "thin fragment" FTIR does not need the presence of linen or cotton. The FTIR of the large piece of crust does seem to need the presence of some linen and even cotton, which is not impossible and would comply with Villarreal's remark that its FTIR spectrum is that similar to the FTIR of region 2 of the thread, that the FTIR of

<sup>569</sup> Used from the Conservation & Arts Materials Encyclopedia Online (<http://cameo.mfa.org/wiki/File:MFA-Purpurin.jpg>) with permission from the Museum of Fine Arts, Boston (<http://www.mfa.org/>)

<sup>570</sup> Villarreal, presentation at Ohio 2008, [www.shrouduniversity.com/videos/villarreal.wmv](http://www.shrouduniversity.com/videos/villarreal.wmv) at 19:53; also in its part 3, at <https://www.youtube.com/watch?v=9kBpplTK044> at 3:25.

<sup>571</sup> Perhaps the spectrum is a calculated difference spectrum, e.g. between region 1 and region 2 after the large piece of crust had come off of the thread.

the large piece of crust “may be dominated by embedded fibers”.<sup>572</sup> Also, all large peaks of the Raes/radiocarbon and main Shroud non-image FTIRs can be assigned to linen, starch and alizarin/purpurin. The presence of cotton and sebum components on the Raes samples is not precluded.

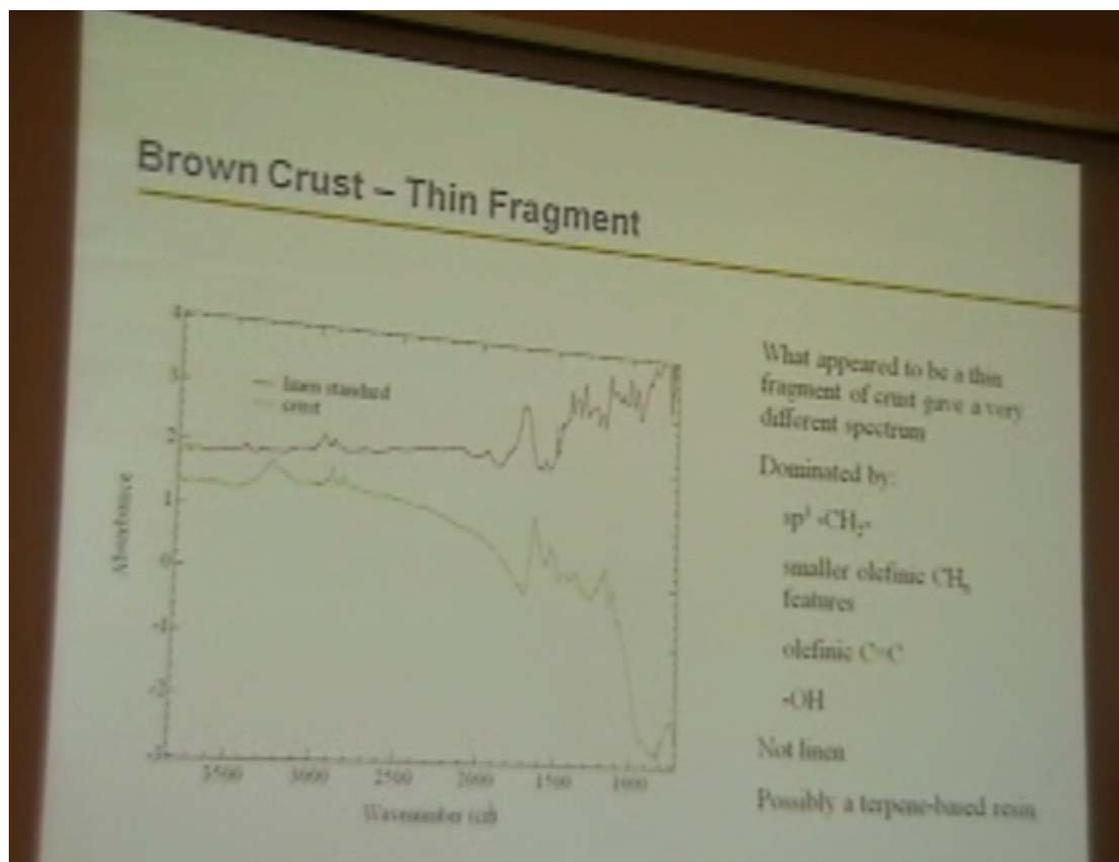


Fig. 4.16. FTIR of “What appeared to be a thin fragment of crust”, green curve = “crust”, black curve = “linen standard”, 2008 presentation of Villarreal ©R. Breault, Shroud of Turin Education Project Inc.<sup>573</sup>

#### Alpha linking of starch in Shroud FTIR spectra

All three bands that are typical for the glycosidic alpha-linking in starch are discernable in some of the Shroud’s FTIRs. These bands are small bands at or near 764, 860, and 929  $\text{cm}^{-1}$ , in native starch and other starch reference FTIRs.<sup>574</sup> The first band, at ca. 765  $\text{cm}^{-1}$ , seems present in both the Raes/radiocarbon and non-image and even image and backing cloth FTIRs, so this is not conclusive evidence for the presence of starch, unless there is also starch on the “backing cloth” fibers. The second band, at ca. 855-880  $\text{cm}^{-1}$ , seems indeed missing on the backing cloth, but its presence on image fibers makes its assignment to starch doubtful, as no (intact) starch was detected on image fibers by Heller and Adler. The third band, at ca. 915-935  $\text{cm}^{-1}$ , seems only present in the

<sup>572</sup> “Data taken from a large piece of crust are quite similar to data from the Shroud thread. Spectrum may be dominated by embedded fibers” text on slide at 22:43, Villarreal presentation 2008 Ohio

[www.shrouduniversity.com/videos/villarreal.wmv](http://www.shrouduniversity.com/videos/villarreal.wmv)

<sup>573</sup> Used from R. Breault’s video of R. Villarreal’s 2008 presentation, published by Shroud University, [www.shrouduniversity.com/videos/villarreal.wmv](http://www.shrouduniversity.com/videos/villarreal.wmv), screenshot at 23:00, with permission of R. Breault, Shroud of Turin Education Project Inc., [www.ShroudEncouter.com](http://www.ShroudEncouter.com); also visible at 6:30 of <https://www.youtube.com/watch?v=9kBpplTK044> (part 3 of YouTube version)

<sup>574</sup> “bands at 764, 860 and 929  $\text{cm}^{-1}$  confirmed the  $\alpha$ -configuration of glycosidic linkage typical for starch [22].” Capek et al., 2010, <http://link.springer.com/article/10.1007/s10973-009-0194-1> on their fig. 1 [http://img.springerimages.com/Images/Springer/JOU=10973/VOL=2010.99/ISU=2/ART=2009\\_194/MediaObjects/WATER\\_10973\\_2009\\_194\\_Fig1\\_HTML.jpg](http://img.springerimages.com/Images/Springer/JOU=10973/VOL=2010.99/ISU=2/ART=2009_194/MediaObjects/WATER_10973_2009_194_Fig1_HTML.jpg); cf. native starch FTIR (NS) in <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0969806X12004550-gr1.jpg> in El-Rehim et al., 2013, <http://www.sciencedirect.com/science/article/pii/S0969806X12004550>

Raes/radiocarbon and non-image FTIRs, and this could reflect the presence of starch on only the Raes, radiocarbon and non-image fibers.

#### Pectin but no protein in non-image and radiocarbon FTIRs

In his presentation in Dallas in 1998, Adler made an intriguing remark on the FTIRs of the non-image fibers, which is not in the article in the proceedings: “There is one feature to point out, however, that there is an enhanced metal carboxyl, eh metal ester absorption here as compared with ordinary linen. That does tend to confirm the identification made a year ago at Nice by Stephen Mottin that the fluorescence of the background of the cloth may be due to the presence of pectic substances. We’re gonna follow that up.”<sup>575</sup> In his article in the proceedings of the 1999 Richmond conference, Adler indeed wrote about positive indicative test results for pectic substances with ruthenium red on non-image fibers, but, oddly, still said spectral analysis was needed: “It would appear that Mottin’s hypothesis is correct, pectic substances are present, but the matter should still be confirmed by spectral analysis.”<sup>576</sup> Pectin is not fluorescent.<sup>577</sup> Esters have FTIR absorption in the 1765-1715  $\text{cm}^{-1}$  region,<sup>578</sup> and pectin of dried apple pomace has FTIR absorption at 1747  $\text{cm}^{-1}$  (“esterified carboxyl groups”) and 1647  $\text{cm}^{-1}$  (probably “free carboxyl groups”).<sup>579</sup> The non-image fibers, Raes thread #7, and region 1 of Raes thread #1 have similar bands at 1725-1735  $\text{cm}^{-1}$ , six of the eight radiocarbon fibers have such bands at 1735-1740  $\text{cm}^{-1}$  (the other two have a band at 1765 and 1770  $\text{cm}^{-1}$ , respectively). Also a 1640-1650  $\text{cm}^{-1}$  band is present in most of these FTIRs. This indicates that the pectin contents of the main Shroud’s non-image fibers and the Raes/radiocarbon fibers are not very different.

Another interesting remark in Adler’s presentation, after having showed the non-image FTIRs, is “Here’s one of the serum coated fibers. And the thing that is distinctive about this is one in this region can clearly detect the amide I, II, III groups typical of proteins. Those absorptions were lacking in the previous fibers. They were not also found, incidentally, in the radiocarbon fibers, so any suggestions there is secundar..., secondary amides responsible for some of the problems for the radiocarbon dating data are in fact not validated by the IR.”<sup>580</sup> This means that the suggestion that gum Arabic, which contains proteins, is present in the Raes/radiocarbon corner, as was suggested by Rogers,<sup>581</sup> is not validated by FTIR spectroscopy.

#### Unique 830 and 1710 and 2140 and 2740 $\text{cm}^{-1}$ bands due to madder?

In the <1000 and 1700-2800  $\text{cm}^{-1}$  ranges, there are less linen and starch peaks, so any madder peaks have a chance to be more discernable here, than in the ranges that are dominated by linen and starch. Bands at 830, 1710, 2140 and 2740  $\text{cm}^{-1}$ , which are present only in Raes, radiocarbon, and non-image FTIRs, might be madder peaks. They can not be assigned to standard linen or starch and are not present in image and “backing cloth” FTIRs.

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<sup>575</sup> Adler, Presentation in Dallas in 1998, Further Spectroscopic..., <http://shrouduniversity.com/podcasts/aladler.mp3> at 11:00

<sup>576</sup> Adler, The Nature of..., 1999, TOM 106-107

<sup>577</sup> “Histology of Plant Extracellular Matrix” ascribes no fluorescence to pectin, but to lignin only (a light blue fluorescence) [http://www.cas.muohio.edu/~meicenrd/ANATOMY/Ch4\\_Histology/lab4.html](http://www.cas.muohio.edu/~meicenrd/ANATOMY/Ch4_Histology/lab4.html); and for a study of “Interaction of various pectin formulations with porcine colonic tissues” pectins had to be made fluorescent artificially (“Fluorescence-labeled pectins were prepared by the conjugation of fluoresceinamine to the molecules of P-25, P-94, and P-N by Belder’s method [17].” LinShu Liu et al., p. 5908), in order to be able to observe the pectins’ behaviour in the colonic tissues <http://ddr.nal.usda.gov/bitstream/10113/37497/1/IND44306122.pdf> / <http://www.sciencedirect.com/science/article/pii/S0142961205002243>

<sup>578</sup> Table of Foothill College, <http://www.foothill.edu/psme/armstrong/ir.shtml>

<sup>579</sup> Fig. 1 and text of de Fátima Sato et al., 2011, [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S1807-86212011000300001](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1807-86212011000300001)

<sup>580</sup> Adler, Presentation Dallas 1998, Further Spectroscopic..., <http://shrouduniversity.com/podcasts/aladler.mp3> at 13:28

<sup>581</sup> “Gum arabic, a complex mixture of polysaccharides and glycoproteins” [http://en.wikipedia.org/wiki/Gum\\_arabic](http://en.wikipedia.org/wiki/Gum_arabic); Rogers and Arnoldi, Scientific Method..., 2002, p. 21

### 1225-1235 cm<sup>-1</sup> band due to madder and/or linen?

Where the band/broad shoulder at 1225-1235 cm<sup>-1</sup> that is present in almost all Raes and non-image FTIRs cannot be assigned to starch because another, neighbouring, peak is more similar to the nearest standard starch peak, it can be assigned to madder (purpurin), and also to a similar form in standard linen or cotton FTIRs. It is not present in two of the three published FTIRs of image fibers, perhaps because it is due to, or partly due to, a madder component. As the form at 1225-1235 is also present in the “backing cloth” FTIRs, and the identity of the fibers that produced these FTIRs is not certain, no definitive conclusions can be drawn.

### 1280 cm<sup>-1</sup> peaks due to madder? Not Saponaria

The peak at ca. 1280 cm<sup>-1</sup>, which is present in nearly all Shroud related FTIRs, is not present in the standards for linen or starch, but is present as the strongest peak in those of both alizarin and purpurin (see fig. 4.14 and 4.15). As also the image and “backing cloth” FTIRs have this peak its assignment is not certain. Perhaps the peak is due to the vibration of a structure of alizarin or purpurin that is left intact when these dyestuffs are degraded to substances without acidichromic (dye) properties. The peak seems not attributable to Saponaria (soapweed) for its quillaia saponin (a quillaic acid glycoside) has bands at ca. 1215-1260 and 1600-1640 cm<sup>-1</sup> and a ca. 1730 cm<sup>-1</sup> peak,<sup>582</sup> and Saponaria’s other characteristics are in contrast with most Shroud properties anyway (see 4.4.).

### 1475 cm<sup>-1</sup> shoulder due to purpurin?

The shoulder at 1475-1480 cm<sup>-1</sup>, which is present in some of the radiocarbon, non-image, waterstain and scorch FTIRs, cannot be assigned to linen, cotton, starch or aged linen (linen’s 1454 cm<sup>-1</sup> shoulder has only become broader in ageing), and may represent the high 1470 cm<sup>-1</sup> peak of insoluble and heat resistant purpurin. It is not present in the image FTIRs, which would comply with the absence of dye on image fibers. As the form at 1475 is also present in the “backing cloth” FTIRs, and the identity of the fibers that produced these FTIRs is not certain, no definitive conclusions can be drawn.

### The image’s increasing 1600 band at expense of the 1650 band is not typical of linen ageing but rather of madder and madder degradation and starch yellowing

Adler, Selzer and DeBlase wrote that “Comparison of the tabulated data on carbonyl frequencies<sup>21</sup> is most revealing. The position (given in cm<sup>-1</sup>) and relative intensity of the peaks in the carboxylic acid salt region (1650-1540) and conjugated ketone region (1680-1640) show an apparent progressive oxidation-type pattern with the non-image (1593, 1643) the weakest, then water stained (broad 1697, weak 1640), then image (strong 1694, 1645 shoulder), then scorch (1591, broad 1645), and finally the radiocarbon pattern (1590, 1643, both strong).”<sup>583</sup> Here, their 1697 and 1694 values in the waterstain and image pattern are erroneous, and apparently 100 cm<sup>-1</sup> too high, for the waterstain and image curves have no peak at ca. 1700 but at ca. 1600 cm<sup>-1</sup>, just like the other Shroud curves (see TOM 83 in fig. 4.5). The “apparent progressive oxidation-type pattern” takes the increasing peaks at ca. 1600 and 1650 into account. Oxidation of organic substances is likely to produce carbonyls (C=O functional groups), and the 1600 and 1650 peaks are indeed in the carbonyl (C=O) spectral region. A table showing the characteristic wavenumbers for a number of relevant organic structures is in table 8. It shows that all C=O groups in saturated structures have wavenumbers well above 1600 cm<sup>-1</sup> (1715-1680 for aldehydes, 1700-1665 for ketones, 1730-1717 for esters, and 1715-1680 for

<sup>582</sup> Dixit et al., 2010, Fungal Growth Inhibition of Regenerated Cellulose Nanofibrous Membranes Containing Quillaia Saponin, <http://link.springer.com/article/10.1007/s00244-010-9493-6#page-1>, its figure 2 a, [http://img.springerimages.com/Images/Springer/JOU=00244/VOL=2010.59/ISU=3/ART=9493/MediaObjects/WATER\\_244\\_2010\\_9493\\_Fig2\\_HTML.jpg](http://img.springerimages.com/Images/Springer/JOU=00244/VOL=2010.59/ISU=3/ART=9493/MediaObjects/WATER_244_2010_9493_Fig2_HTML.jpg)

<sup>583</sup> Adler, Selzer and DeBlase, Further Spectroscopic..., 2002, TOM 96 (21 = D. Lin-Vien, N. Colthup, W. Fately, and J. Grasselli, The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules, 1991, pp. 117-146 [http://books.google.nl/books?id=bYWNSi6abvwC&printsec=frontcover&dq=infrared+and+raman+organic&hl=nl&sa=X&ei=VL-1Uur\\_G8jC0QWys4C4DO&redir\\_esc=y#v=onepage&q=carboxylic%20acid%20&f=false](http://books.google.nl/books?id=bYWNSi6abvwC&printsec=frontcover&dq=infrared+and+raman+organic&hl=nl&sa=X&ei=VL-1Uur_G8jC0QWys4C4DO&redir_esc=y#v=onepage&q=carboxylic%20acid%20&f=false); Lin-Vien’s table “9.1. C=O Spectral Regions” is online at p. 119.)

carboxylic acids), and that of the C=O groups in conjugated structures only carboxylic acid salts (mentioned by Adler) and aromatic compounds have C=O wavenumbers at 1600 cm<sup>-1</sup>.

		linen	image chromophore		not demon- strated on Shroud	aromatic compounds (e.g. madder and its degradation products)
OH stretch	<b>3650- 3550</b> alcohols, free *	<b>3200-3550</b> alcohols, hydrogen bonded* (cellulose)	<b>3200-2500</b> carboxylic acids *			
C=O stretch				<b>1730-1665</b> saturated*		
				<b>1750-1590</b> conjugated <sup>o</sup>		
			<b>1715-1680</b> carboxylic acids, conjugated *		<b>1650-1540 and 1450-1360</b> carboxylic acid salts <sup>#</sup>	<b>1640-1580</b> 1,3-diketones (enol form) <sup>#</sup> <b>1670-1600</b> substituted benzophenones and substituted quinones <sup>#</sup> <b>1602-1586</b> diphenquinones (mixed with C=C ring vibrations) <sup>#</sup> <b>1695/1698</b> vanillin C=O stretch <sup>@</sup>
C=C stretch				<b>1650-1600</b> alkene, conjugated* <b>1625-1585</b> conjugated <sup>o</sup>		<b>1625-1440</b> aromatic* ~ <b>1600</b> aryl/substituted benzene ring* <sup>#</sup> <b>1500</b> benzene <sup>o</sup> <b>1495/1506</b> toluene (methyl- benzene) <sup>^</sup>
?				<b>1656-1641</b> undegraded starch <b>1650</b> linen ageing Victory Sail <sup>0</sup> <b>1635</b> linen (absorbed water?)		
?						<b>1599</b> and <b>1685</b> acetophenone* <b>1604</b> isopropyl benzene*
?						~ <b>1500</b> and ~ <b>1600</b> o-xylene (1,2- dimethyl-benzene) <sup>□</sup> ~ <b>1500</b> and ~ <b>1600</b> m-xylene (1,3- dimethyl-benzene) <sup>Δ</sup> ~ <b>1500</b> p-xylene (1,4-dimethyl- benzene) <sup>□</sup> <b>1605/1614</b> toluene (methyl- benzene) <sup>^</sup>
?						~ <b>1705</b> and ~ <b>1610</b> lignin <sup>+</sup> (this probably is the FT-IR the equivalent of the <b>1605</b> Raman ring-stretching mode of phenolic lignin in linen <sup>584</sup> ) <b>1698</b> and <b>1600</b> and <b>1509</b> vanillin <sup>@</sup>

Table 8. FTIR wavenumbers for relevant structures

\* = Foothill College, <http://www.foothill.edu/psme/armstrong/ir.shtml>

# = Lin-Vien, Handbook,

<http://books.google.nl/books?id=bYWNSi6abvwC&printsec=frontcover&dq=The+Handbook+of+Infrared+and+Raman>

<sup>584</sup> Edwards et al., 2006 <http://onlinelibrary.wiley.com/doi/10.1002/jrs.1609/pdf>; cf. the closeness of the FT-IR and Raman 1600 cm<sup>-1</sup> peaks in the aromatics mesitylene and indene, on p. 3 of [http://in.materials.drexel.edu/blogs/280\\_advanced\\_materials\\_lab/attachment/3738.ashx](http://in.materials.drexel.edu/blogs/280_advanced_materials_lab/attachment/3738.ashx)

[+Characteristic+Frequencies+of+Organic+...&hl=nl&sa=X&ei=V-DOUqj3Bq2a0QXG-oHwDw&ved=0CDUO6AEwAA#v=onepage&q=C%3DO&f=false](#) scroll back from p. 120 to p. 119

° = Chemanalytical <http://www.chemanalytical.com/ft-ir-spectra>

□ = NIST <http://webbook.nist.gov/cgi/cbook.cgi?ID=C95476&Mask=80#IR-Spec> and <http://webbook.nist.gov/cgi/cbook.cgi?ID=C106423&Type=IR-SPEC&Index=4>

△ = Hanhong Group [http://www.hanhonggroup.com/ir/img/108-38-3\\_IR1.gif](http://www.hanhonggroup.com/ir/img/108-38-3_IR1.gif)

+ = USGS <http://pubs.usgs.gov/sir/2004/5292/images/FigB1.gif>

0 = Victory Sail [http://wwwchem.uwimona.edu/jm/courses/CHEM2402/Textiles/Veg\\_Fibres.html](http://wwwchem.uwimona.edu/jm/courses/CHEM2402/Textiles/Veg_Fibres.html)

^ = Michigan State University, <https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/Images/toluene.gif/> <http://orgchem.colorado.edu/Spectroscopy/irtutor/aromaticsir.html>

@ = Michigan State University <https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/Images/vanilin2.gif> on <https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/infrared/irspec1.htm> / Hanhong Group [http://www.hanhonggroup.com/ir/img/121-33-5\\_IR1.gif](http://www.hanhonggroup.com/ir/img/121-33-5_IR1.gif)

### *Not aged linen or carboxylic acid salts*

Now it is important to note that, besides that not an increase at  $1600\text{ cm}^{-1}$  but an increase at  $1650\text{ cm}^{-1}$  and  $1720\text{ cm}^{-1}$  is typical of age-oxidized linen (see 4.1.3.4 above)<sup>585</sup>, also the presence of carboxylic acid salts – a combination of a metal and deprotonated carboxylic acid<sup>586</sup> – has not been demonstrated on the Shroud image. The chromophore of the image, which is characterized by a sharp  $1600\text{ cm}^{-1}$  peak with hardly any  $1650$  shoulder (or  $1720$  peak), has been described as “aldehyde and carboxyl groups”, also called “aldehyde and cellulosic carboxyl”, and “aldehydes and cellulosic carboxyl functional groups ... Thus ... a mixture of conjugated carbonyl structures”, and “a conjugated carbonyl”, and “carbonyl [sic] groups as a chromophore”, and “a conjugated carbonyl (or alpha dicarbonyl groups)”<sup>587</sup>. It was assumed that these groups were produced from cellulose by “acid oxidizing conditions”<sup>588</sup> in an “acid catalyzed oxidation”, which is the effect that most simply describes the image formation effect, according to Heller and Adler.<sup>589</sup> Heller specified that “The conjugated carbonyl is the end product of dehydrating acid oxidation. (By contrast, alkaline oxidation produces no color.)”<sup>590</sup>

But if an oxidation is catalysed by acid, the resulting products would also be acid/protonated, and not deprotonated as in carboxylic acid salts. Adler explained this in an interview, saying that “When you eventually oxidize cellulose you produce an acidic structure. So, it is very logical that after the reaction goes for a bit, it’s going to be acid catalysed” and that, at the start of the image formation, threads with “different degrees of degradation” would have “started out at different acidities. [...] There’s already acid there, in the structure of these batches of fibers.”<sup>591</sup> So, right after acidic image formation (in cellulose or otherwise), there would have been no carboxylic acid salts, but rather carboxylic acids, which do not produce a FTIR peak at  $1600\text{ cm}^{-1}$ . Ageing of pure cellulose yields “the appearance of a shoulder centered at around  $1730\text{ cm}^{-1}$  due to the carboxyl and/or carbonyl groups”<sup>592</sup>.

<sup>585</sup> See paragraph 4.1.3.4 and [http://wwwchem.uwimona.edu/jm/courses/CHEM2402/Textiles/Victory\\_sail\\_FTIR.gif](http://wwwchem.uwimona.edu/jm/courses/CHEM2402/Textiles/Victory_sail_FTIR.gif) on [http://wwwchem.uwimona.edu/jm/courses/CHEM2402/Textiles/Veg\\_Fibres.html](http://wwwchem.uwimona.edu/jm/courses/CHEM2402/Textiles/Veg_Fibres.html) and Edwards et al., 2006, <http://onlinelibrary.wiley.com/doi/10.1002/jrs.1609/pdf>

<sup>586</sup> <http://en.wikipedia.org/wiki/Carboxylate>; Acetic acid is a carboxylic acid, and, in solution, “Above a pH of 5.5, acetic acid converts to acetate” (<http://en.wikipedia.org/wiki/Acetate>), but on the Shroud carboxylic acids would not be in a (aqueous) solution and their protons could not escape on their own.

<sup>587</sup> Heller and Adler, A Chemical..., 1981, TOM 46, 43; Adler, Chemical and Physical..., 2002, TOM 22, 23; Heller and Adler, A Chemical..., 1981, 44; Heller, Report on..., 1983, p. 199

<sup>588</sup> Heller and Adler, A Chemical..., 1981, TOM 46

<sup>589</sup> Case, The Shroud of Tuim..., 1996, p. 63, 83

<sup>590</sup> Heller, Report on..., 1983, p. 200; cf. Berry’s experiments: alkali treated cotton cloth, then washed and dried, yielded less scorch color <http://shroudofturinwithoutallthehype.wordpress.com/2013/10/27/why-is-the-shroud-image-so-superficial-half-tone-and-striated-is-it-on-raised-ribs-of-primary-cell-wall-hemicellulose/> and acid (lemon juice +sugar) treated linen cloth yielded more scorch color than untreated linen

<http://shroudofturinwithoutallthehype.wordpress.com/2012/10/25/refining-a-model-childrens-invisible-writing-trick-with-lemon-juice-allows-thermal-imprinting-scorching-at-a-much-reduced-temperature/>

<sup>591</sup> Case, The Shroud of Turin..., 1996, p. 62 and 82-83

<sup>592</sup> Cocca et al., 2011, <http://www.hindawi.com/journals/isrn.materials.science/2011/863083/>

### *Not xylene/toluene or lignin or hemicellulose or water*

The Shroud fibers that were from the sticky-tapes had been freed from the adhesive by xylene or toluene; xylene was detectable as a contaminant in PMS.<sup>593</sup> Xylene is a benzene ring with two methyl functional groups. Xylene (o-, m-, and p-xylene) has a strong FTIR peak at 1500 cm<sup>-1</sup>, where none of the Shroud samples shows a peak or shoulder. Ortho-xylene, meta-xylene and toluene have a peak at 1600 cm<sup>-1</sup>, but as no xylene or toluene was used for preparing the Raes and radiocarbon samples, taken from whole threads, and these samples' FTIRs also show the characteristic 1600 peak, it is improbable that xylene or toluene is causing it.

Also lignin and hemicellulose both have a peak at ca. 1610 cm<sup>-1</sup>,<sup>594</sup> but lignin and hemicellulose, as small constituents of linen, would already be accounted for in the new linen FTIR, which only shows a minimal 1600 shoulder. In the oxidatively aged linen of the Victory Sail,<sup>595</sup> and also in Moroni's linen that had yellowed from heating in a closed container<sup>596</sup> (fig. 4.12), the 1600 FTIR intensity has decreased relative to the ~1650 intensity. So, the increased 1600 peak and disappeared 1650 peak in Shroud image fibers can not be assigned to an increased presence of lignin and hemicellulose. Vanillin, an oxidation product of lignin, has strong peaks at 1698, 1600 and 1509 cm<sup>-1</sup>,<sup>597</sup> but the image FTIRs have no band at ca. 1700 cm<sup>-1</sup>.

The FTIR spectrum of water that is in the NIST Chemistry webbook, shows a peak at 1640 cm<sup>-1</sup>, and a study on hydrated aluminium sulfate assigned a peak at 1655 cm<sup>-1</sup> to molecular water.<sup>598</sup> In FTIR analysis of aged cellulose, "the absorbed and bonded water is in the region of carbonyl group (between 1635 and 1670 cm<sup>-1</sup>)".<sup>599</sup> Therefore, if "the image fibrils are simply more dehydratively oxidized than the non-image fibrils",<sup>600</sup> it is unlikely that the increased 1600 cm<sup>-1</sup> peak and disappeared 1650 cm<sup>-1</sup> peak in image FTIRs is due to an increased water content, which would rather yield an increased ca. 1640 cm<sup>-1</sup> peak.

### *Possibly madder and its degradation products*

Now it is very interesting to investigate whether aromatic madder dyestuffs, such as alizarin and purpurin, during image formation and ageing may have oxidized/degraded to the aromatic substances, which do not behave as a dye anymore, but which do have C=O frequencies at 1600 cm<sup>-1</sup>. The C=O groups of 1,3 diketones in enol form, listed in the table to which Adler referred, have wavenumbers in the 1640-1580 range and are present in alizarin, which has such an enol form, viz. a 1,10 keto tautomer<sup>601</sup>. Alizarin has peaks at 1596, 1646/1625 and 1680/1710 cm<sup>-1</sup>, and purpurin at 1580, 1620 (see fig. 4.14 and 4.15 above<sup>602</sup>). Of these peaks, at least one peak is probably completely or largely due to any or all of the three benzene/aryl rings in the dyestuffs, for C=C stretch vibrations in benzene compounds are at 1625-1440 cm<sup>-1</sup>. When the central ring of alizarin and purpurin is broken

<sup>593</sup> toluene: Heller and Adler, *A Chemical...*, 1981, TOM 37; "The map shows some contamination with the xylene that was used to wash adhesive off of the fibers." Rogers, *Pyrolysis/Mass Spectrometry...*, 2004, p. 5

<sup>594</sup> hemicellulose: purple curve in fig. 6 on page 4 of J.L. Mass,

[http://tighar.org/Projects/Earhart/Archives/Research/Bulletins/62\\_LotionBottle/Scientific%20Analysis%20of%20Fine%20Art%20Report%201180.pdf](http://tighar.org/Projects/Earhart/Archives/Research/Bulletins/62_LotionBottle/Scientific%20Analysis%20of%20Fine%20Art%20Report%201180.pdf); lignin in <http://pubs.usgs.gov/sir/2004/5292/images/figB1.gif>

<sup>595</sup> "There is marked diminution in the spectral band intensity of the C=C stretching mode at 1605 cm<sup>-1</sup> for the historic sailcloth specimens, which may be attributed to oxidation in use and storage and on display." Edwards et al., 2006, Raman spectroscopic analysis of a unique linen (etc)., <http://onlinelibrary.wiley.com/doi/10.1002/jrs.1609/pdf>; cf.

Garside and Wyeth 2005, [http://eprints.soton.ac.uk/18716/1/ARC-AnHis-Garside-118-125.pdf?origin=publication\\_detail](http://eprints.soton.ac.uk/18716/1/ARC-AnHis-Garside-118-125.pdf?origin=publication_detail)

<sup>596</sup> Moroni, *The Age and Proposal for photo-colormetric Control of the Shroud*. Presentation Rome 1993, videoed by Breault, <https://www.youtube.com/watch?v=79mf-ImWM6o>

<sup>597</sup> vanillin, 4-hydroxy-3-methoxybenzaldehyde <http://en.wikipedia.org/wiki/Vanillin#mediaviewer/File:Vanillin2.svg> has the following FTIR in CCl<sub>4</sub>: [http://www.hanhonggroup.com/ir/img/121-33-5\\_IR1.gif](http://www.hanhonggroup.com/ir/img/121-33-5_IR1.gif) (cf. )

<sup>598</sup> <http://webbook.nist.gov/cgi/cbook.cgi?ID=C7732185&Units=SI&Type=IR-SPEC&Index=1#IR-SPEC> and Contreras et al., 2006, <http://www.azom.com/article.aspx?ArticleID=3517>, respectively

<sup>599</sup> Cocca et al., 2011, <http://www.hindawi.com/journals/isrn.materials.science/2011/863083/>

<sup>600</sup> Heller and Adler, *A Chemical...*, 1981, TOM 46

<sup>601</sup> Claro et al., *The use of...*, 2008, fig. 1; and Miliani et al., *Acidichromic effects...*, 2000, Scheme 2

<sup>602</sup> Alizarin: <http://webbook.nist.gov/cgi/cbook.cgi?Spec=C72480&Index=0&Type=IR&Large=on>  
Purpurin: <http://cameo.mfa.org/images/thumb/1/11/MFA-Purpurin.jpg/300px-MFA-Purpurin.jpg>

– which is the first step in the oxidation of alizarin<sup>603</sup> –, these molecules open up and turn into structures similar to benzophenones, of which the C=O peaks are in the 1670-1600 region and thus possibly superimposed on their aryl peak, dependent on the substituted groups. For instance, “Benzophenone with two *para*-dimethylamino groups on it has a C=O frequency shifted to 1600 cm<sup>-1</sup>, which is superimposed on the ring band at 1600 cm<sup>-1</sup>.”<sup>604</sup> Further oxidation/degradation/polymerization of the madder derivatives may result in single ring compounds – such as quinones –, and perhaps diphenoquinones, which have C=O peaks in the small 1602-1586 cm<sup>-1</sup> region: “In the double ring compounds, 3, 3', 5, 5'- tetraalkyl-4,4'-diphenoquinones, a strong IR band is seen involving out-of-phase C=O stretch mixed with ring vibrations in the region 1602-1586 cm<sup>-1</sup>”<sup>605</sup>

So, it seems possible that, on Shroud image fibers, some peaks of the originally present madder C=O were shifted and, along with peaks of any newly produced C=O, were superimposed on the degraded madder C=C peak during image formation.

This would be consistent with what Adler, Selzer and DeBlase called the “apparent progressive oxidation-type pattern” in non-image, image and scorch fibers. On image fibers, the 1600 peak seems enhanced at expense of the 1650 band, but in scorch fibers, both the the 1650 band (as in age-oxidized linen) and the 1600 band are enhanced (fig. 4.17), which corresponds to the microscopic observations: on image fibers only the outmost layer (starch-madder coating) is colored (acid oxidized),<sup>606</sup> but on scorch fibers the whole fiber (also the linen) is colored<sup>607</sup> (presumably anaerobically pyrolysed in the red fluorescing scorches, and possibly oxidized in the green fluorescing scorches<sup>608</sup>). As hydroxymethylfurfural and furfural are anaerobic pyrolysis products of cellulose of linen,<sup>609</sup> and a Seliwanoff's test detected furfurals in main Shroud scorch fibers,<sup>610</sup> the “broad 1645” band<sup>611</sup> in scorched Shroud fibers may also contain bands of hydroxymethylfurfural aggregates/derivatives (having their strongest band at 1637cm<sup>-1</sup><sup>612</sup>) and, to a lesser extent, furfural

<sup>603</sup> “the first step in the oxidation of alizarin is the cleavage of the aromatic ring in the C-C bond near the C=O group to form small carbonyl species and colorless intermediates, mainly phthalic acid.” Pirillo et al., 2010, Horseradish Peroxidase and Hematin as Biocatalists for Alizarin Degradation Using Hydrogen Peroxide, <http://pubs.acs.org/doi/abs/10.1021/ie901528y> p. 6750

<sup>604</sup> D. Lin-Vien, N. Colthup, W. Fatel, and J. Grasselli, The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules, 1991, pp. 117-146 (p. 133, this page was completely online in 2013 [http://books.google.nl/books?id=bYWNSi6abvwC&printsec=frontcover&dq=infrared+and+raman+organic&hl=nl&sa=X&ei=VL-1Uur\\_G8jC0QWys4C4DQ&redir\\_esc=y#v=snippet&q=superimposed&f=false](http://books.google.nl/books?id=bYWNSi6abvwC&printsec=frontcover&dq=infrared+and+raman+organic&hl=nl&sa=X&ei=VL-1Uur_G8jC0QWys4C4DQ&redir_esc=y#v=snippet&q=superimposed&f=false)) [http://books.google.nl/books?id=bYWNSi6abvwC&printsec=frontcover&dq=infrared+and+raman+organic&hl=nl&sa=X&ei=VL-1Uur\\_G8jC0QWys4C4DQ&redir\\_esc=y#v=onepage&q=carboxylic%20acid%20&f=false](http://books.google.nl/books?id=bYWNSi6abvwC&printsec=frontcover&dq=infrared+and+raman+organic&hl=nl&sa=X&ei=VL-1Uur_G8jC0QWys4C4DQ&redir_esc=y#v=onepage&q=carboxylic%20acid%20&f=false))

<sup>605</sup> D. Lin-Vien, N. Colthup, W. Fatel, and J. Grasselli, The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules, 1991, pp. 117-146 (p. 133, this page was completely online in 2013 [http://books.google.nl/books?id=bYWNSi6abvwC&printsec=frontcover&dq=infrared+and+raman+organic&hl=nl&sa=X&ei=VL-1Uur\\_G8jC0QWys4C4DQ&redir\\_esc=y#v=onepage&q=carboxylic%20acid%20&f=false](http://books.google.nl/books?id=bYWNSi6abvwC&printsec=frontcover&dq=infrared+and+raman+organic&hl=nl&sa=X&ei=VL-1Uur_G8jC0QWys4C4DQ&redir_esc=y#v=onepage&q=carboxylic%20acid%20&f=false))

<sup>606</sup> Cross sections of image fibers did not show color in the cellulose, according to Adler, as reported by Rogers, cited by Porter (<http://shroudofturin.wordpress.com/2012/05/07/the-body-image-is-created-by-molecular-change-of-linen-fibres-really/>).

<sup>607</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 9

<sup>608</sup> The Shroud's red fluorescing scorches are assumed to have been produced by anaerobic pyrolysis = heat without oxygen (cf. Pellicori, Spectral properties..., 1980, p. 1919); “Laboratory-produced scorches emit a bright greenish-yellow fluorescence if they were produced in air and reddish if produced under conditions of limited available oxygen.” Miller and Pellicori, Ultraviolet photography..., 1981, p. 84). Rogers' book says that Pellicori reported that “the margins of the scorches fluoresced in the green, entirely different than the background of the Shroud”, but doesn't give a reference (Rogers, A Chemist's..., 2008, p. 20). These green fluorescing scorches may have been oxidized, e.g. after opening of the reliquary that kept the Shroud during the 1532 fire.

<sup>609</sup> “Cellulose pyrolyzes to produce hydroxymethylfurfural (mass 126), which begins to deformylate in a series reaction to produce furfural (mass 96).” Rogers, Studies on..., 2005, p. 192; hemicellulose, a minor constituent of linen, also produces furfural in pyrolysis (cf. <http://en.wikipedia.org/wiki/Furfural#Production>).

<sup>610</sup> Rogers, A Chemist's..., 2008, p. 40

<sup>611</sup> Adler, Selzer and DeBlase, Further spectroscopic..., 2002, TOM 96

<sup>612</sup> 1637 cm<sup>-1</sup> of hydroxymethyl derivatives: Zhang et al., First identification of primary nanoparticles in the aggregation of HMF, 2005, fig. 3, <http://full-text.com/article/First+identification+of+primary+nanoparticles+in+the+aggregation+of+HMF.d90.html>; 1675 cm<sup>-1</sup>: pure hydroxymethylfurfural FTIR, <http://watsonnoke.com/wp-content/uploads/2012/12/5-Hydroxymethylfurfural-CAS67-47->

(its strongest band is at 1670-1690,<sup>613</sup> probably due to the aldehyde group, but this group would be lost in polymerization) and/or there is simply more water between the scorched (broken and degraded) cellulose chains. Also the high and broad ca. 1600 band of the scorched fibers may contain the strong 1604  $\text{cm}^{-1}$  band of hydroxymethylfurfural derivatives<sup>614</sup>.

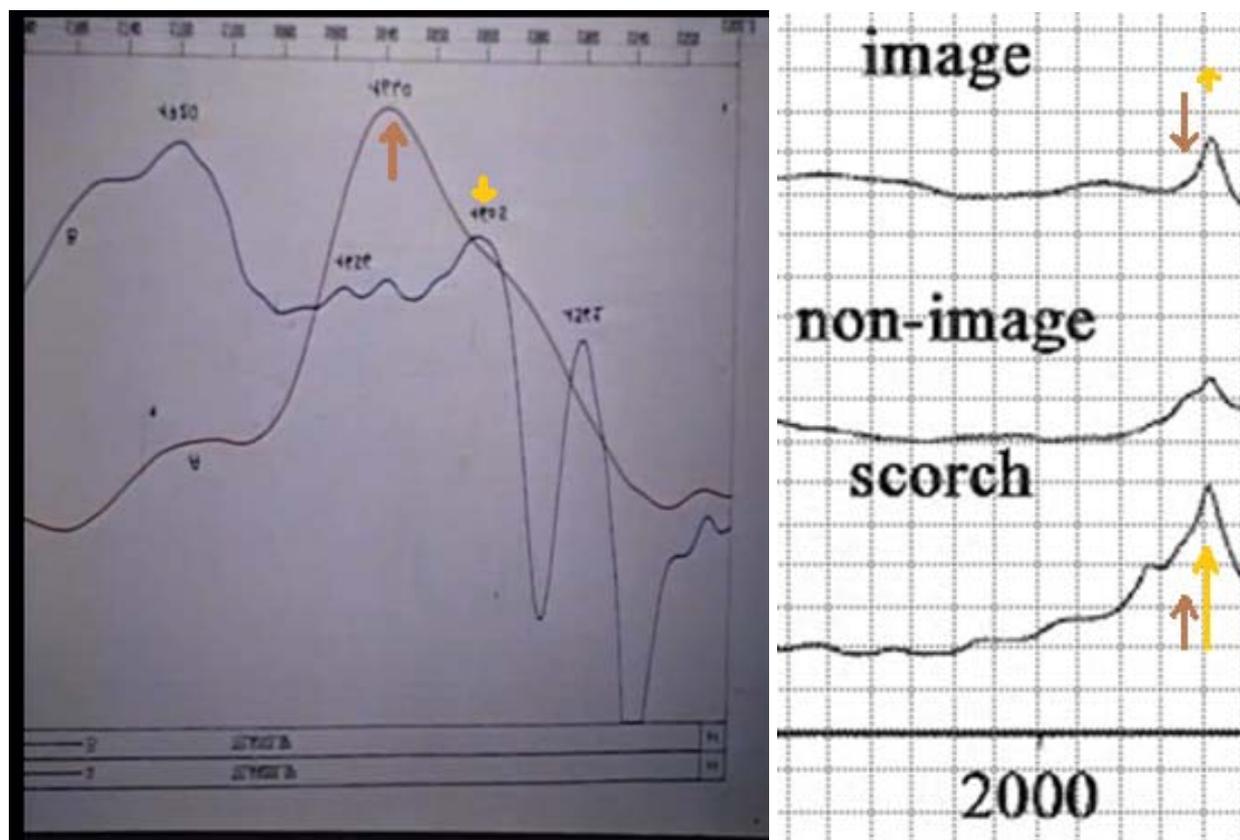


Fig. 4.17. Comparison of  $\sim 1600$  (yellow arrows) and  $\sim 1650 \text{ cm}^{-1}$  (brown arrows) bands in white (blue curve B) and heat-yellowed (red curve A) linen fibers and Shroud non-image, image and scorch fibers. Linen details from fig. 4.12, FTIR 1500-1780  $\text{cm}^{-1}$ , mirrored upside-down (Moroni's 1993 presentation ©R. Breault<sup>615</sup>); Shroud details from fig. 4.5, FTIR 1500-2600  $\text{cm}^{-1}$  (Adler, Updating recent studies on the Shroud of Turin, 1996, ©ACS<sup>616</sup>)

#### Possibly starch yellowing products

Starch, when still undegraded, has a peak at 1656-1641  $\text{cm}^{-1}$ . After a yellowing by dehydration, oxidation, and conjugation, as has been assumed to have occurred on image fibers, no furfural or hydroxymethylfurfural (HMF) was found on them, for Rogers reported a negative Seliwanoff's test,

0-FTIR-and-HPLC.jpg on <http://watsonnoke.com/5-hydroxymethylfurfural-cas-67-47-0/5-hydroxymethylfurfural-cas67-47-0-ftir-and-hplc/>

<sup>613</sup> <http://webbook.nist.gov/cgi/cbook.cgi?ID=C98011&Type=IR-SPEC&Index=1#IR-SPEC>; cf. Hanhong group, [http://www.hanhonggroup.com/ir/ir\\_en/RB04010017.html](http://www.hanhonggroup.com/ir/ir_en/RB04010017.html)

<sup>614</sup> Zhang et al., First identification of primary nanoparticles in the aggregation of HMF, 2005, fig. 3, <http://full-text.com/article/First+identification+of+primary+nanoparticles+in+the+aggregation+of+HMF,d90.html>

<sup>615</sup> Moroni, M., The Age and Proposal for photo-colometric Control of the Shroud. Presentation Rome 1993, video by R. Breault at <https://www.youtube.com/watch?v=79mf-ImWM6o>, these FTIR details from 15:45

<sup>616</sup> Used (and adapted) with permission from A.D. Adler, Updating Recent Studies on the Shroud of Turin, Archeological Chemistry, chapter 17, pp. 223-228, *ACS Symposium Series*, Vol. 625, May 1996, Chapter DOI: 10.1021/bk-1996-0625.ch017, ISBN13: 9780841233959 eISBN: 9780841215740, <http://pubs.acs.org/doi/abs/10.1021/bk-1996-0625.ch017?prevSearch=Updating%2Brecent%2Bstudies%2Bon%2Bthe%2BShroud%2Bof%2BTurin&searchHistoryKey=> Copyright © 1996 American Chemical Society. (fig. 1, also in The Orphaned Manuscript (TOM) p. 83 and online at <http://www.sindone.info/ADLER.PDF>).

but this might be due to polymerisation/condensation of furfural.<sup>617</sup> Polymerisation of HMF is perhaps also possible. Yet, the Seliwanoff's test did detect furfurals in main Shroud scorch fibers.<sup>618</sup> Hydroxymethylfurfural is an aromatic pyrolysis product of cellulose and starch, while furfural is an aromatic pyrolysis product of hemicellulose of linen's primary cell wall and of hydroxymethylfurfural. Although no absolute temperatures were reported, pyrolysis mass spectrometry of image fibers seems not to have shown early release of these furfurals, but this may also have been due to polymerisation.<sup>619</sup> The strongest FTIR band of hydroxymethylfurfural aggregates/derivatives, at  $1637\text{ cm}^{-1}$ , is not clearly present in image fibers (there is just perhaps a tiny band at  $1640$  in one of the three image FTIRs). Furfurals are fluorescent, also when polymerized, and probably fluoresce red when produced by anaerobic scorching of unbleached linen (probably forming furfural-lignin degradation polymers), and perhaps fluoresce green if they can be produced by aerobic scorching.<sup>620</sup>

In image areas, where only the topmost layer of the fibers is colored, no red fluorescence is observed, while the scorch marks have completely colored fibers and detectable furfural (Seliwanoff's test) and fluoresce red with green borders.<sup>621</sup> If only a starch-madder coating became straw yellow on image fibers, any furfurals in it could not polymerize with lignin or lignin degradation products to form a red fluorescing polymer. The fluorescence of the image, which is weaker than that of the background, is further discussed in 4.2.5. below.

If furfurals are absent in the image layer, as suggested by the negative Seliwanoff's test and by the weaker fluorescence, the straw yellow color on image fibers might be due to alkene (i.e., not aromatic) conjugated C=C bonds<sup>622</sup>, which produce a peak at  $1650\text{-}1600\text{ cm}^{-1}$ , even at  $1625\text{-}1585\text{ cm}^{-1}$ , according to the Chemanalytical data.<sup>623</sup> However, alkenes also have a strong =C-H bend peak at  $995\text{-}685\text{ cm}^{-1}$ ,<sup>624</sup> where image fibers do not have a more pronounced peak than non-image fibers, which makes alkenes less probable as the image's main chromophores. If alkenes belong to the image's chromophores, they might be degradation/oxidation products of either linen's primary cell wall, or starch, or madder, or a combination of them.

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<sup>617</sup> Rogers, A Chemist's..., 2008, p. 39-40; the Seliwanoff's test detects both kinds of furfural, for the text on Wikipedia mentions furfural and the figure shows hydroxymethylfurfural <http://en.wikipedia.org/wiki/Seliwanoff>

<sup>618</sup> Rogers, A Chemist's..., 2008, p. 40

<sup>619</sup> Rogers, Studies on..., 2005, p. 192; Rogers, A Chemist's..., 2008, p. 39-40

<sup>620</sup> "Miller's experiment ... with burning linen in a limited-oxygen atmosphere had produced a furfural-type material, which fluoresced in the ultraviolet. This jibed with the ultraviolet reflectance spectra of the Shroud itself." (Heller, Report on, p.175). "Also, [furfural's chemical structure](#) shows that furfural after polymerisation by bonding at its aldehyde group would still fluoresce. The aldehyde group of furfural is its only difference with [furan](#), which has just a single hydrogen atom instead of the aldehyde group. The ring of furfural is equal to the ring of furan, and this ring has only two double bonds. So, as [furan fluoresces](#), a furfural polymer, having changed or lost its complete aldehyde group but still having this same furan ring, would also fluoresce. At least some furfural polymers, bonded by furfural's aldehyde group, fluoresce measurably, such as the furfural-biphtalate resin: its chemical structure shows a resin of furfural bound by its aldehyde group ([Scheme 2](#)). Its fluorescence is a bit weaker than that of pure furfural, but still much stronger than the hardly fluorescent biphtalate ([Fig. 12](#)). Also in the [furfural-naphthol resin](#) the aldehyde group of furfural has bonded to naphthol (Scheme 3), and then there was condensation of the naphthol-furfural molecules at the oxygen atom of the (former) aldehyde group, so, also here the furan ring has been preserved (Scheme 5). This "condensed furfurylol Naphthol pigment" fluoresces and, as its relative intensity is stronger than that of mere naphthol (Fig. 4), the furan ring still contributes to the fluorescence of the polymer." Hoeven, comment

<http://shroudofturinwithoutallthehype.wordpress.com/2012/10/23/thibaults-principle-true-for-a-stepped-template-maybe-but-not-an-artistic-bas-relief-with-rounded-contours-and-gentler-relief/#comment-1224>; "Laboratory-produced scorches emit a bright greenish-yellow fluorescence if they were produced in air and reddish if produced under conditions of limited available oxygen." (Miller and Pellicori, Ultraviolet Fluorescence..., 1981, p. 84)

<sup>621</sup> "Pellicori reported that ... the margins of the scorches fluoresced in the green, entirely different than the background of the Shroud." Rogers, A Chemist's..., 2008, p. 20 - no reference to Pellicori's report is given here ; Ibid. p. 40

<sup>622</sup> Berry, Raymond N Rogers: STURP supremo chemist (RIP) who sadly lost the plot (due to an apparent blind spot, it would seem, for those ultra-thin and highly superficial primary cell walls of flax and linen fibres)., FAQ 12, <http://shroudofturinwithoutallthehype.wordpress.com/2013/01/05/raymond-n-rogers-sturp-supremo-chemist-rip-who-sadly-lost-the-plot-due-to-an-apparent-blind-spot-it-would-seem-for-those-ultra-thin-and-highly-superficial-primary-cell-walls-of-flax-and-linen/>

<sup>623</sup> <http://www.chemanalytical.com/ft-ir-spectra>

<sup>624</sup> Foothill spectral table <http://www.foothill.edu/psme/armstrong/ir.shtml>

### *Correction for systematic error in age estimation by FTIR parameters*

If it is correct that madder compounds contribute to the 1650-1600 band in non-image FTIRs, especially in non-image fibers that were not necessarily washed with xylene/toluene, such as those vacuumed from the Shroud, this would be part of the explanation why the uncorrected age found for such Shroud non-image fibers by means of several measured FTIR parameters, among which the C=O to OH ratio, was 752 BC  $\pm$  400 years, i.e., much older than the age of 400 AD  $\pm$  400 years, estimated by the mechanical properties of Shroud fibers.<sup>625</sup> what was assumed to be only (age-enhanced) C=O of linen probably was also C=C of original madder plus C=O of madder that is much less balanced by OH than C=O of aged linen is<sup>626</sup>. The systematic error in the FTIR age estimation related to the effects of the 1532 AD fire may have been much smaller than as estimated by Fanti and Gaeta.<sup>627</sup> Their age estimation by Raman spectroscopy, on the other hand, yielding a date of 200 BC  $\pm$  500 years, focused on the COC group at 1097 cm<sup>-1</sup> and the C-OH group at 3251 cm<sup>-1</sup>,<sup>628</sup> which are glycosidic groups common to linen and starch – the OH- stretch of alcohols with intermolecular H-bonds is at 3200-3550 cm<sup>-1</sup> –, and therefore probably was not skewed (much) by madder groups or extra starch.

## **4.2. Madder dye**

### **4.2.1. Visible color and wet acid-base chemistry**

Heller and Adler reported on “the fibrils and particles found on the Shroud samples” that “There are (a) the clear to pale yellow background fibrils (non-image) of the cloth which bear no image or blood stains”; elsewhere they call background fibers “pale yellow fibrils (non-image)” and “pale yellow non-image fibrils of the Shroud”.<sup>629</sup>

Bracaglia wrote on unpublished material in the Holy Shroud Guild archives: “Dr. McCrone writes on June 13th 1979, “Sample 3AF (finger image) shows the largest percentage of colored fibre examined it closely the fibers are un-uniform colored over lengths exceeding weave units hence the color is not a surface effect-possible mechanism includes heat or liquid treatment. There are in this sample and others a number of yellow amorphous tubular flaked like a material resin. ( I thought in the part they could be aloes or etc).”<sup>630</sup> Here, McCrone is probably speaking about both the strippable straw yellow image layer and the pale yellow strippable layer of non-image fibers,<sup>631</sup> for he says the un-uniform color exceeds weave units. Note that, in image fibers, “with few exceptions, we find that the yellow coloration of the fibrils is interrupted as the thread goes beneath a crossing thread in the weave pattern. Those few exceptions where yellowing appears to pass under the crossing thread seem due to mechanical stretching of the cloth, because these are usually accompanied by a region of uncoloured fibrils at the opposite end of the exposed thread where it comes up from beneath a crossing thread.”<sup>632</sup>

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<sup>625</sup> Linen fibers vacuumed from the Shroud: Fanti and Gaeta, *Il Mistero...*, 2013, p. 80; Certain specific cleaning operations were performed on samples before the FTIR analysis, for example on fabrics that were contaminated with sand: *Ibid.*, p. 84; *Ibid.*, p. 90, 102

<sup>626</sup> Undegraded alizarin has two OH groups and two C=O groups and every 14 C atoms, while undegraded cellulose has three OH groups and no C=O groups on every 6 C atoms (cf. <http://en.wikipedia.org/wiki/Alizarin> and <http://en.wikipedia.org/wiki/Cellulose> ).

<sup>627</sup> The systematic effect due to the fire was estimated as 452 years ageing, resulting in a corrected FTIR-age of 300 AD  $\pm$  400 years ( $\alpha = 0.05$ ) for the Shroud (Fanti and Gaeta, *Il Mistero...*, 2013, p. 91)

<sup>628</sup> Fanti and Gaeta, *Il mistero...*, 2013, p. 93-94

<sup>629</sup> “There are (a) the clear to pale yellow background fibrils (non-image) of the cloth which bear no image or blood stains.” (Heller and Adler, *A Chemical...*, 1981, TOM 36); “the fibrils can be seen to form a progression with pale yellow fibrils (non-image) staining the weakest” (*Ibid.*, TOM 43); “Proteases had absolutely no effect on the yellow (body) image or pale yellow non-image fibrils of the Shroud.” (*Ibid.*, TOM 41).

<sup>630</sup> Bracaglia, *Raes Problematic...*, [http://holyshroudguild.org/dr-raes-problematic-threads\\_2.html](http://holyshroudguild.org/dr-raes-problematic-threads_2.html)

<sup>631</sup> Both called ‘Ghost’ in Fanti et al., *Evidences for...*, 2005, Evidence A3 <http://www.shroud.com/pdfs/doclist.pdf>

<sup>632</sup> Jumper et al., *A Comprehensive Examination...*, 1984, p. 450

A large part of the original assumed madder on main Shroud non-image fibers on the cloth may have degraded in ageing or have sublimated (= evaporated from the solid phase), for instance in the heat of the 1532 AD fire. The sublimation temperature of alizarin is only 368-498 K = 95-225 °C.<sup>633</sup>

The madder in the Raes corner turned yellow, red and purple according to alizarin's acidichromism: according to Rogers, "Alizarin is used as an acid-base (pH) indicator in chemical analysis. It is yellow below a pH of 5.6 red above a pH of 7.2 (figure 14), changing to purple above 11.0 (figure 15). This agrees with observations on the coating."<sup>634</sup> The 2010 Handbook of Biological Dyes and Stains says that alizarin is yellow up to pH 5.5, red at pH 6.8 to 10.1, and purple from pH 12.1.<sup>635</sup> Purpurin, another component of madder dye, when in water-dioxane solution with increasing pH "changes from yellow-orange (pH ≤ 3.5) to pink (pH ≈ 6-9) to violet (pH ≥ 12)" and "In the ground state, purpurin (pK<sub>1</sub> = 4.7; pK<sub>2</sub> = 9.5) is a stronger acid than alizarin (pK<sub>1</sub> = 6.6; pK<sub>2</sub> = 12.4) in both the first and second dissociation steps".<sup>636</sup>

Rogers showed fibers from a Raes thread and wrote "The yellow-brown encrustation shown in figure 12 swelled and became more transparent as it soaked. The color instantly changed to bright yellow in 6N hydrochloric acid (HCl) ... (figure 13)."<sup>637</sup> This observed color change means that the original color probably was not bright yellow and thus not of a dye with pH lower than 3.5-4.5 where both purpurin and alizarin are fully protonated and yellow. Perhaps the dye used on the Shroud had a pH 4.5 – 5.5, where alizarin is yellow and purpurin (yellow-) orange.

#### 4.2.1.1. Xylene and toluene are solvents of madder

Rogers reported that "Walter McCrone had ignored agreements on how the STURP samples were to be observed, and he contaminated all of our tape samples by sticking them to microscope slides. All of the fibers were immersed in the tape's adhesive. ... Washing them with solvents was certain to contaminate the samples with traces of the solvents ... More irritating was the fact that washing was guaranteed to remove some of the impurities. ... McCrone had probably ruined our chances of finding squalene and triglycerides by pyrolysis/MS".<sup>638</sup> The fibers pulled from the sticky tapes and fibers on small pieces of excised tape were washed with xylene by Joan Janney and also by Heller and Adler, in Colorado Springs: "We then washed the fibers and excised tape samples with xylene to remove adhering adhesive. It was a laborious process, and nobody involved had much good to say about Walter."<sup>639</sup> In Connecticut, Heller and Adler used toluene to free fibers and particles from sticky-tape adhesive.<sup>640</sup>

Starch is not soluble in xylene or toluene<sup>641</sup>, but alizarin – virtually insoluble in water and moderately soluble in ethanol – is soluble in xylene and toluene,<sup>642</sup> and so is purpurin.<sup>643</sup> Therefore, the

<sup>633</sup> NIST alizarin <http://webbook.nist.gov/cgi/cbook.cgi?ID=C72480&Units=SI&Mask=4#Thermo-Phase>

<sup>634</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 18-19, Fig. 14 <http://www.shroud.com/pdfs/rogers2.pdf> at pH 8 the color "reddened"; "The colored coating shows all of these changes as a function of pH." Rogers, Studies on..., 2005, p. 191-192

<sup>635</sup> R.W. Sabnis, Handbook of Biological Dyes and Stains – Synthesis and Industrial Applications, 2010, John Wiley & Sons, [http://samples.sainsburysebooks.co.uk/9780470586235\\_sample\\_382294.pdf#page=30](http://samples.sainsburysebooks.co.uk/9780470586235_sample_382294.pdf#page=30) (book page 10, pdf page 32)

<sup>636</sup> Miliani et al., Acidichromic effects..., 2000, (the dissociation constants were "measured in mixed solvents, where the dielectric constant is reduced compared with pure water. However, the dielectric constant decrease for the mixture used [water-dioxane (2:1 v/v)], calculated by the empirical equation reported by Anderson, is relatively modest, hence the effect on the pKs does not exceed one pK unit." (p. 144, 148 )

<sup>637</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 18

<sup>638</sup> Rogers, A Chemist's..., 2008, p. 52

<sup>639</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 37; cf. Heller, Report on..., 1983, p. 159 , on the sessions in Colorado Springs

<sup>640</sup> "To prepare a specimen for testing, the portion of tape containing it was excised by scalpel from the sample. It was then washed free of the tweezer-held tape with toluene into a spot plate well. The adhesive was then removed by repeated washings with toluene." Heller and Adler, A Chemical..., 1981, TOM 37; cf. Heller, Report on..., 1983, p. 181, on Heller and Adler's work in Connecticut

<sup>641</sup> Starch is soluble in hot water, which is a polar solvent. Xylene and toluene are very non-polar solvents (cf. <http://www.sciencedirect.com/science/article/pii/0021961475901597> and "non-polar organic solvents such as the aromatics benzene and toluene." <https://en.wikipedia.org/wiki/Toluene>) and therefore should not dissolve starch.

<sup>642</sup> R.W. Sabnis, Handbook of Biological Dyes and Stains – Synthesis and Industrial Applications, 2010, John Wiley & Sons, [http://samples.sainsburysebooks.co.uk/9780470586235\\_sample\\_382294.pdf#page=30](http://samples.sainsburysebooks.co.uk/9780470586235_sample_382294.pdf#page=30) (book page 10, pdf page

xylene/toluene would have washed off (most of) the yellow madder dye from the fibers. This is a very important fact and a serious complication of the interpretation of the wet chemistry done on fibers and particles from sticky tape samples. It probably explains why there is no report of detection by wet chemistry of any dye on non-image fibers from the tape samples of the main Shroud, while madder dye was easily detected on unwashed Raes threads by wet chemistry.

#### 4.2.1.2. No deliberate dye test on non-image fibers by Heller and Adler

Alizarin and purpurin are phenols. Heller recounted: “Adler ... proceeded to test image fibrils for phenols, riboflavin, steroids, ... They were all negative. ... We then set up a more formal protocol for the serious work. ... I told him that, first and foremost, I wanted to do a large series of controls. We could use the three-hundred-year-old Spanish linen for that purpose.”<sup>644</sup> So, this testing for phenols and other structures was done outside of the protocol and was not done on “controls” (for which they would use old Spanish linen), let alone on non-image fibers. Heller and Adler’s article “A Chemical Investigation of the Shroud of Turin” lists the structures mentioned by Heller, and also porphyrins, primary amines, aldehydes, cellulosic carboxyls, and Saponaria extract, in its Table 7, and says: “The methods employed in testing for specific organic structures and functional groups of possible interest are given in Table 7. These test were performed on the uncoated fibrils: body image, non-image and scorch fibrils. With the exception of positive aldehyde and cellulosic carboxyl tests, all other species tested for tested negatively. Thus we see no evidence for the stains or dyes on the body image fibrils at levels that would be evident to the eye.”<sup>645</sup> The second sentence does not necessarily mean that all of these test were performed on all three kinds of uncoated fibrils. The text also explicitly says on these negative tests that “this does not preclude the possibility that some of these substances may have resided on the cloth in the past and been “lost” over time through oxidation, degradation, etc. ... positive tests in some cases would have been more meaningful than the negative tests.”<sup>646</sup> And, when discussing further tests on image fibers, it is stated on the image chromophore: “Thus the solvent, redox, and acid-base tests are also consistent with the lack of any applied stains or dyes, but are consistent with the chemistry of carbony groups [sic] as a chromophore.”<sup>647</sup> The abstract of the article states “There is no chemical evidence for the application of any pigments, stains, or dyes on the cloth to produce the image found thereon.”<sup>648</sup> The same seems to be said further on in the article: “In view of the range of our chemical testing for metal pigments and organic stains and dyes, we found no evidence for the application of any such known materials on this cloth. Whatever the image is due to, it would appear most unlikely that it has been painted as we normally speak of such a process.”<sup>649</sup> Here, Heller and Adler speak of application of materials, to which the image would be due – materials that are applied on a cloth as in a painting; so, this lack of evidence for applied image materials would not preclude the presence of a dye as part of the imageless cloth itself (“this cloth”), present everywhere before the image got onto it.

Rogers made it very clear, for, when trying to show that the Raes and radiocarbon area is a repair in the main Shroud and having showed there is dye on the Raes thraeds, he only says “No dye could be

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32); cf. datasheet alizarin: “Soluble in alcohol, benzene, toluene, xylene, pyridine, carbon disulfide, glacial acetic acid.” <http://datasheets.scbt.com/sc-214519.pdf>

<sup>643</sup> datasheet purpurin: “Soluble in alcohol (forming red solutions), in ether (intensely yellow with fluorescence), xylene (dark yellow), boiling alum solution (red), benzene, toluene” <http://datasheets.scbt.com/sc-205822.pdf>; A US patent states “An ink jet head was produced in the same manner as in Example 2 except that a xylene solution of 1,2-dihydroxyanthraquinone was applied as a material absorbing the i-line to the ink flow path pattern”, and specifies that “Examples of the compound absorbing the i-line and having one or more phenolic hydroxyl groups include ... anthraquinones such as 1-hydroxyanthraquinone, 1,2-dihydroxyanthraquinone (alizarin), 1,4-dihydroxyanthraquinone (quinizarin), 1,5-dihydroxy-anthraquinone (anthrarufin), 2,6-dihydroxyanthraquinone (anthraflavic acid) and 1,2,4-trihydroxyanthraquinone (purpurin) ... These may be used either singly or in any combination thereof.” <http://www.google.com/patents/US8304177>

<sup>644</sup> Heller, Report on..., 1983, p. 171-172

<sup>645</sup> Heller and Adler, A Chemical..., 1981, TOM 43

<sup>646</sup> Heller and Adler, A Chemical..., 1981, TOM 43

<sup>647</sup> Heller and Adler, A Chemical..., 1981, TOM 44

<sup>648</sup> Heller and Adler, A Chemical..., 1981, TOM 35

<sup>649</sup> Heller and Adler, A Chemical..., 1981, TOM 46

detected on any image fibers.”<sup>650</sup> If it had been determined that no dye is present on any non-image fibers, he should and would have said it here; instead, he explicitly says that no dye could be detected on any image fibers. Note, that, in this same text of 2002, he did say that “The encrustation on Raes samples is almost certainly a plant gum. The gum does not appear on any of the other linen samples that are associated with the Shroud of Turin.”<sup>651</sup> Nevertheless, his posthumous 2008 book says and shows, in its fig. VII-3 (fig. 4.1), that a yellow strippable encrustation is present on weakly scorched fibers of the main Shroud.

Rogers and Schwalbe also only wrote about the absence of dye on image fibers: “Microchemical studies of yellow fibrils taken from tape samples of the pure-image area have shown no indication for the presence of dyes, stains, inorganic pigments, or protein-, starch-, or wax-based painting media” and “the yellow fibril discoloration does not result from any likely (non-ferrous) inorganic or lake pigment (see note 5). Additional tests for organic dyes and stains gave similar results. Heller and Adler could not extract the yellow color with strong acids, strong bases, or a variety of organic solvents”.<sup>652</sup> It seems they all only knew of (deliberate) dye tests on image fibers.

#### 4.2.1.3. Indirect dye tests: yellow in acid – pinkish in weak base

Non-image fibers probably were not deliberately tested for dyes using wet chemistry. Most madder dye probably was washed from the fibers that were removed from sticky-tapes anyway. But indirect test results were not conflicting with presence of a residue of acid madder dye on non-image fibers.

“We began by using nonimage background fibrils. After thirty minutes in sulfuric acid, they had the rich color and chemistry of an image fibril. ... the image fibrils were much more corroded than the nonimage fibrils. The sulfuric acid-treated fibrils looked identical with the image fibrils under phase contrast microscopy. We also made pseudo-image fibrils from the Spanish linen and other control linens.”<sup>653</sup> Here, the acid-induced straw yellow color of the non-image fibers is consistent with the “bright yellow” color of madder in acid, as was seen by Rogers on Raes fibers in 6N hydrochloric acid.<sup>654</sup> Also the straw-yellow fibers near the so-called ‘poker-holes’, photo-macro- and -micrographed by Maloney,<sup>655</sup> may be due to some of the acid that probably formed the ‘poker-holes’ themselves and colored the pale yellow madder dye on the cloth more yellow. Flury-Lemberg stated that the holes and their borders and their accompanying black-brown stains were all stains that must have been made by a liquid with an acidic character.<sup>656</sup>

The hydrazine which, according to Heller’s book, was applied to solve red particles on a set of collected (i.e. xylene-washed) Shroud fibers and which produced a pink hemochromagen color<sup>657</sup>, is a weak base with a  $pK_b$  5.90.<sup>658</sup> Therefore, the observed pink color may have been about the same as the color of any remaining yellow madder on the fibers that would have become more pinkish because of the increased alkalinity. Alizarin is “red” in a pH of about 7,2-11, or, more precisely,

<sup>650</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, p. 18

<sup>651</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, p. 21

<sup>652</sup> Rogers and Schwalbe, *Physics and Chemistry...*, 1982, p. 31 and 13

<sup>653</sup> Heller, *Report on...*, 1983, p. 199-200

<sup>654</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, fig. 13, p. 18.

<sup>655</sup> “Some of the tapes, are samples taken in the pre-dating 1192 area. Paul Maloney photo macro- and -micrographed this burn area sample. These photographs do show clearly the presence of straw-yellow fibers in the scorched areas.” Van Haelst, *The Red Stains...*, 1997 (what Van Haelst called the “pre-dating 1192 area” is the ‘poker-holes’ area, which is the area of the small holes in the pattern of the letter L).

<sup>656</sup> “Die Flecken müssen durch eine Flüssigkeit sauren Charakters entstanden sein. ... Dass es sich um eine Flüssigkeit handeln muss und nicht etwa um flüssiges Metall, wie später in Chambéry, zeigt ein erhalten gebliebener Flecken auf dem Tuch. Abb. 6b: ... An seiner Rändern beobachten wir einen breiten Hof mit zarten schwarzen Linien, die anzeigen, dass sich hier einst eine flüssige Substanz in das Gewebe eingesogen haben muss. Diese Flüssigkeit ist im Zentrum des Fleckens aufgetroffen, hat sich ausgebreitet bis sie an die Rändern des Fleckens soweit aufgebraucht war, dass sie das Gewebe nur noch leicht zu netzen vermochte. Die Tatsache, dass nur die tief liegenden Rillen des Körpergrates betroffen wurden, zeigt, dass die Flüssigkeit von der Rückseite eingedrungen ist.” Flury-Lemberg, *Die Leinwand ...*, *Proceedings Turin 2000*, p.26-27; cf. Guerreschi and Salcito, *Further studies...*, 2005, p. 9

<sup>657</sup> “we began to collect individual fibrils with red dots. One by one they were placed on the slide. .... Adler added the hydrazine, and the red particles began dissolving. ... they’re producing the typical hemochromagen color.” Heller, *Report on...*, 1983, p. 164-165; “pink hemochromagen-like color” Heller and Adler, *A Chemical...*, 2002, TOM 39

<sup>658</sup> <http://en.wikipedia.org/wiki/Hydrazine>

“pale pink” in the pH 8-10 range.<sup>659</sup> In this respect, it is significant that Heller and Adler’s article specifically called the effect of hydrazine on mostly red colored shards the formation of “a characteristic pink hemochromagen-like color”.<sup>660</sup>

Also the two calcium tests were tests that are done in alkaline media. One was the “o-cresolphthalein complexone” test and the other was the glyoxal bis (2-hydroxyanil) (GBHA) test, and either of the tests or both yielded a “strong positive” test result on non-image fibers.<sup>661</sup> The o-cresolphthalein complexone test is done in a pH 12 buffer and uses the absorbance at 575 nm as a measure for the calcium present.<sup>662</sup> Maximum absorbance at 575 nm corresponds to a pink-to-purple visible color, which in its turn corresponds to the pink-to-purple color of madder at pH 12 (alizarin is pink at pH 8-10 and blue at pH  $\geq$  13).<sup>663</sup> Madder thus may have contributed to the strong positivity of this calcium test. Note, that the alkaline pH is useful for the testing of calcium ions already in solution, as in blood serum, in which the test is reported to give accurate results.<sup>664</sup> But the test’s buffered alkaline pH 12 by itself would not have detached chelated calcium from organic tissue, so, if there was actual calcium contributing to the Shroud’s positive calcium test, the cresolphthalein must have detached the calcium from the linen and bound it to cresolphthalein.<sup>665</sup>

Also the test that uses glyoxal bis (2-hydroxyanil) (GBHA), which is a Schiff base, uses an ethanol solution, made alkaline with aqueous NaOH, and gives a “red-violet” test color by complexation with “calcium ions”.<sup>666</sup> Also here, a pinkish madder color would probably not have differed much from the red-violet test color and, if present, would have contributed to the visual positivity of the test. The test is effective in detecting calcium of soluble and even insoluble calcium salts,<sup>667</sup> in which calcium is bound by ion binding, which is less strong than the “chelated covalent form” of binding

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<sup>659</sup> “red above a pH of 7.2 (figure 14), changing to purple above 11.0 (figure 15)” (Rogers and Arnoldi, *Scientific Method...*, 2002, p. 18), and “With decreasing acidity ... the solution turned from yellow to pale pink. ... In the pH 8-10 range, the spectrum did not show appreciable variations” (Miliani et al., *Acidichromic effects...*, 2000, p. 143).

<sup>660</sup> “If the shards are barely covered, i.e., microspotted with a film of hydrazine, they slowly dissolve and give a characteristic pink hemochromagen-like color.”<sup>33</sup> (33 = Hemberg and Legge, 1949)” (Heller and Adler, *A Chemical...*, 2002, TOM 39); “mostly red colored” (Heller and Adler, *A Chemical...*, 1981, TOM 50).

<sup>661</sup> Heller and Adler, *A Chemical...*, 1981, Table 6, TOM 53, and references on p. 56: ““60 Second Calcium Test Kit”, American Monitor Corp.” ... “Feigl, *Spot Tests In Inorganic Analysis*, 5<sup>th</sup> ed., 1953”; “Clear Off-Image Areas ... Chemistry. The fibrils all give a strong positive calcium reaction and a positive reaction for iron (even at test reagent conditions, pH 4.5, and even where Fe<sub>2</sub>O<sub>3</sub> particles are not visible), indicating that ion exchanged (chelated) bound iron is present.” (Jumper et al., *A Comprehensive...*, 1984, p. 463-465

<sup>662</sup> West, *Clinical Chemistry* vol. 29/6, 1983, <http://www.clinchem.org/content/29/6/1315.1.full.pdf+html>

<sup>663</sup> Miliani et al., *Acidichromic effects...*, 2000, p. 143

<sup>664</sup> West, *Clinical Chemistry* vol. 29/6, 1983, <http://www.clinchem.org/content/29/6/1315.1.full.pdf+html>

<sup>665</sup> “It is noted that calcium is strongly chelated above pH 6” (Nikiforuc et al., 1953, <http://jdr.sagepub.com/content/32/6/859.short> <http://jdr.sagepub.com/content/32/6/859.extract> ) and “Theoretically EDTA can bind the equivalent amount of calcium ion above pH 7 if there is no competing chelating agent” (Yoon et al, 1998, <http://www.sciencedirect.com/science/article/pii/S0043135497004168>); EDTA is Ethylenediaminetetraacetic acid, but the test only uses cresolphthalein complexone, diethylamine, potassium cyanide, dimethyl sulfoxide and 8-hydroxyquinolone (West, *Clinical Chemistry* vol. 29/6, 1983, <http://www.clinchem.org/content/29/6/1315.1.full.pdf+html>

<sup>666</sup> Feigl and Anger, *Spot Tests In Inorganic Analysis*, 6<sup>th</sup> edition, 1972, [http://books.google.nl/books?id=H06Q8GGqNUC&pg=PA28&dq=Feigl,+Spot+Tests+In+Inorganic+Analysis,+1953&hl=nl&sa=X&ei=G9D7UrzYJcqF4ASwvYCOBw&redir\\_esc=y#v=onepage&q=glyoxal%20bis&f=false](http://books.google.nl/books?id=H06Q8GGqNUC&pg=PA28&dq=Feigl,+Spot+Tests+In+Inorganic+Analysis,+1953&hl=nl&sa=X&ei=G9D7UrzYJcqF4ASwvYCOBw&redir_esc=y#v=onepage&q=glyoxal%20bis&f=false) p. 401 (“With the exception of aluminum and gallium, trivalent ions of Group III of the periodic system react with glyoxal bis-2-hydroxyanil to give red-violet, chloroform soluble chelates. In the absence of Ca, Sr and Ba which also react (see page 161), this behaviour forms the basis of a test for rare earth salts.”) and p. 161-162 (“The above Schiff base (I) forms with metal ions colored inner complex salts. ... Procedure: ... one drop of a 10% sodium hydroxide solution and one drop of 10% sodium carbonate solution”) and p. 102 (“the calcium ions are then detected by their reaction with glyoxal bis (2-hydroxy-anil)”; see also: “the original solution containing 2 ml of 0.4% GBHA in absolute ethanol, and 0.3 ml of aqueous 5% NaOH, and limited to staining only soluble calcium salts, was modified as follows: 1, 2 ml of 0.4% GBHA in absolute ethanol in 0.6 ml of 10% aqueous NaOH; 11, 0.1 gm GBHA in 2 ml of 3.4% NaOH in 75% ethanol. ... Although the modified methods tested on models failed to stain reagent grade CaCO<sub>3</sub> and Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> crystals completely, apatite in developing vertebrae and calcified plaques in soft tissues were stained intensely red.” of Kashiwa et al., 1964, *Biotechnic & Histochemistry*, 1964, 39 (6) pp 359-367

<http://informahealthcare.com/doi/abs/10.3109/10520296409063307>

<sup>667</sup> Kashiwa et al., 1964, <http://informahealthcare.com/doi/abs/10.3109/10520296409063307>

between calcium and linen, assumed to exist on the Shroud by Heller and Adler.<sup>668</sup> So, also in the GBHA test, it is uncertain to which extent calcium actually contributed to the positive test result. The water that formed the big diamond shaped waterstains, did not irreversibly decolour the insides of the stains visibly. So, the original water of these stains probably was not (strongly) alkaline or acidic. Rogers showed that fibers of a madder-dyed thread from the Raes corner still looked yellow-brown through the microscope, when mounted in water.<sup>669</sup> An experiment showed that when demineralized water at room temperature is sucked into a pale yellow, starched, hot-water-washed, madder-dyed piece of linen, the waterstain is only a bit deeper yellow while wet, and, after drying, does not yield a different color than the background, but only perhaps a little lighter hue (fig. 4.18).



Cold water spreading in the starched-washed-and-madder-dyed linen within a minute



Then drying within ten minutes



Same pale yellow dried linen beside white linen, 2.5 hours after wetting, by two different cameras

*Fig. 4.18. Neutral cold demineralized water does not change the color of madder-dyed linen. The color of the dyed linen is pale yellow to the eye, more like the color of the last photo by a second camera. All of these photos are unadjusted. ©AvdH*

#### 4.2.1.4. Pinkish hue of Shroud in some photographs

In some online photographs of the Shroud, it has a pinkish hue,<sup>670</sup> also in the online color-calibrated Haltadefinizione photo<sup>671</sup> (cf. fig. 5.6). A phenomenon that can be seen on madder-dyed linen, is

<sup>668</sup> [http://en.wikipedia.org/wiki/Noncovalent\\_bonding#Ionic](http://en.wikipedia.org/wiki/Noncovalent_bonding#Ionic) ; Heller and Adler, A Chemical..., 1981, TOM 41; During the retting of flax in order to free its linen fibers, Ca is even “more strongly bound” to the linen than Fe, and “the element calcium ... is confirmed chemically and spectroscopically to be in the linen fibers. This explains why the cloth weave is seen clearly” in X-ray radiographs of the Shroud. (Heller and Adler, A Chemical..., 1981, TOM 45, and Adler, Chemical and Physical..., 2002, TOM 17)

<sup>669</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 18, Fig. 12

<sup>670</sup> E.g., in <https://www.facebook.com/photo.php?fbid=304874859605239&set=o.61488680305&type=3&theater> , but also in the Durante photos on Shroud Scope (cf. fig. 2.1)

<sup>671</sup> See the color of Haltadefinizione’s online photo

[http://www.haltadefinizione.com/images/widgetkit/sindone/sindone\\_positivo.jpg](http://www.haltadefinizione.com/images/widgetkit/sindone/sindone_positivo.jpg) . Haltadefinizione gave special

that, even when it looks yellow to the eye, in some light and in some unadjusted photographs, it looks pinkish (cf. fig. 4.18 and 4.19).



in sunlight behind window pane



in shadow of a gray object at sunlit window pane



indoors, in raking daylight

*Fig. 4.19. Same two pieces of linen, pure and starched-washed-and-madder-dyed, respectively, photographed with the same camera at the same automatic setting but in different light; photos are unadjusted. ©AvdH*

#### **4.2.1.5. Acidity from protonated madder and retrograded starch layer made cellulose-bound iron detach from cellulose and precipitate as iron hydroxide**

Alizarin is not easily soluble in cold water,<sup>672</sup> but, as already stated above, the yellow protonated forms of alizarin and purpurin are acids<sup>673</sup> and would have made the water that moved through the cloth inside the waterstains slightly acidic (assuming it was neutral water originally). This acidity probably allowed the release of the “cellulose bound” chelated iron (most probably from the retting of the flax, just as the observed calcium and strontium<sup>674</sup>) and its movement to the waterstain margins: the acidic testcondition for iron, i.e. pH 4.5, used by Heller and Adler, was enough to free the iron and then detect it.<sup>675</sup> In the waterstain margins it probably formed iron hydroxide ( $\text{Fe}(\text{OH})_3$ ) and eventually, by dehydration, iron oxide crystals ( $\text{Fe}_2\text{O}_3$ ), as these crystals were found there, even

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attention to the calibration of the spectrum “which helped faithfully reproduce the actual color”:

<http://www.haltadefinizione.com/en/the-shroud.html>

<sup>672</sup> Alizarin: “slightly to sparingly soluble” <http://en.wikipedia.org/wiki/Alizarin>

<sup>673</sup> alizarin  $\text{pK}_{a1}$  (first deprotonation) = 6.6/7.4;  $\text{pK}_{a2}$  (second deprotonation) = 12.4/11.8; purpurin:  $\text{pK}_{a1}$  = 4.7 and  $\text{pK}_{a2}$  = 9.5, respectively, (pKs determined in 33%/1% dioxane in water) (Miliani et al., *Acidichromic effects...*, 2000, Table 5 and Scheme 2, p. 148)

<sup>674</sup> “The data indicate generally uniform distribution of calcium and strontium over the investigated areas of the cloth. ... Substantially non-uniform concentrations of iron were observed, particularly at the dorsal-foot and side-wound ‘blood’ stain regions.” Morris et al., *X-ray fluorescence...*, 1980 ; “The uncoated fibrils (non-image, image, and water-stain) all give relatively strong positive tests only for Ca and Fe, except for those from the inside of the water stains which give somewhat weaker, though still definite positive reactions.” (Heller and Adler, *A Chemical...*, 1981, TOM 41). “during the retting, the linen fibers act as an ion exchanger, and do you know what ions they take up selectively from water? .... Calcium, strontium, and iron!” (Heller, *Report on...*, 1983, p. 174)

<sup>675</sup> “Clear Off-Image Areas ... Chemistry. The fibrils all give a strong positive calcium reaction and a positive reaction for iron (even at test reagent conditions, pH 4.5, and even where  $\text{Fe}_2\text{O}_3$  particles are not visible), indicating that ion exchanged (chelated) bound iron is present.” (Jumper et al., *A Comprehensive...*, 1984, p. 464-465)

inside intact linen fibers.<sup>676</sup> Adler, who suggested this crystal generation hypothesis, was evidently not sure about the mechanism which made the iron detach from the “cellulose” when the waterstains were formed, for he gave four alternatives<sup>677</sup>: 1) somehow there was “free” iron – but he doesn’t specify how it got to be “free”; 2) it was still bound to “low molecular weight water soluble cellulose degradation products” – but how it would have detached from these products at the margins to form  $\text{Fe}(\text{OH})_3$  is not specified; 3) iron salts that were already present in the water that would make the waterstains – but the insides of the waterstains have a lower iron content than the outside of the waterstains, and the iron is very pure (no contamination of other elements), which precludes that it was an earth pigment that was already present in the water<sup>678</sup>; 4) the water that freed the iron “had reached very high temperatures”, presuming it had run across the very hot silver casket – but later it was determined by Guerreschi and Salcito that the big waterstains (where all but one of Heller and Adler’s waterstain sticky tapes came from<sup>679</sup>) were not formed by the dousing water of the 1532 AD fire<sup>680</sup> – besides, it was not hot water, for otherwise the starch and madder coating would have been washed away from the hottest parts of the stains, just as in the 1532 AD waterstains; furthermore, in 1995, Adler said that it was the heat of the fire that made the  $\text{Fe}(\text{OH})_3$  dry to iron oxide crystals ( $\text{Fe}_2\text{O}_3$ ).<sup>681</sup> The most probable explanation of the pure iron oxide crystals seen in the waterstain margins, even inside intact linen fibers, is therefore that the acid property of the insoluble yellow madder dye made the water acidic, which made the iron, that got bound to the linen during retting, detach from the linen. This iron (atom weight 56), which apparently could not cross the retrograded starch layer to reach the madder on top of it, migrated (‘chromatographed’) to the waterstain margins and precipitated there as  $\text{Fe}(\text{OH})_3$ , probably with  $\text{OH}^-$  from water that had donated its  $\text{H}^+$  to reprotonate the madder. Later, the heat of the 1532 fire may indeed have driven out any remaining water of  $\text{Fe}(\text{OH})_3$ , transforming it into  $\text{Fe}_2\text{O}_3$ .

Apparently, the acid dye, when it dyed the cloth at manufacture, did not detach the chelated iron from the linen to bind it to the dye in red iron madder lakes. The retrograded starch film, then still new and intact and sealing, probably was an effective barrier between the linen inside and the dye solution on the outside, for instance when the cloth was dipped, rapidly and only once, in a relatively cold dye bath (less than 60 °C) and then left to dry horizontally and fast (e.g. in the sun). The following experiments confirm that a retrograded starch coating seems to effectively separate textile fibers from an acid yellow dye.

#### *Experiment: dyeing cloth with acidic yellow madder dye*

A viscous starch paste was made by cooking fine wheat flour in water with a little vinegar for 10 minutes – ca. 200 ml tap water plus 4 ml white wine vinegar yielded a pH of 4-4.5. After the paste had cooled down, it was smeared unto and into both sides of a piece of unbleached linen, which was then left to dry and retrograde. A water extract of *Rubia tinctorum* root powder<sup>682</sup> was made like a cup of tea: boiling water was poored into a cup containing a brown filter bag with madder root powder, and was allowed to cool down and extract further for 7 hours, after which the filter bag was removed and the extract was put in a closed glass jar. The next day some of the red extract was made straw yellow by acidification with vinegar. Pieces were cut from the not-starched linen and the linen

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<sup>676</sup> Heller, Report on..., 1983, p. 178

<sup>677</sup> 1, 2, and 3: Heller and Adler, A Chemical..., 1981, TOM 46; 4: Heller citing Adler in Report on..., 1983, p. 180.

<sup>678</sup> “Tests for metallic species ... The uncoated fibrils (non-image, image, and water stain) all give relatively strong positive tests only for Ca and Fe, except for those from the inside of the water stains which give somewhat weaker, though still definite positive reactions. ... Digestion of about 50 birefringent red coated fibrils concomitantly, with aqua regia and subsequent testing specifically for trace Mn, Co, Ni, and Al established that such impurities could only be present at a level of less than 1%. Chemically and microscopically, therefore, these birefringent red particles appear to be  $\text{Fe}_2\text{O}_3$  and are quite pure by the chemical tests employed.” Heller and Adler, A Chemical..., 1981, TOM 41-42; cf. Heller, Report on..., 1983, pp. 190, 194-196

<sup>679</sup> Heller and Adler, A Chemical..., 1981, Table 1, TOM 49

<sup>680</sup> Guerreschi and Salcito, Futher Studies..., 2005

<sup>681</sup> Case, The Shroud of Turin..., 1996, p. 81

<sup>682</sup> Purchased from Verfmolen “De Kat”, [http://www.verfmolendekat.com/meeckrapwortel-200-gram.html?store=engels\\_english&from\\_store=nederlands\\_dutch](http://www.verfmolendekat.com/meeckrapwortel-200-gram.html?store=engels_english&from_store=nederlands_dutch)

that had been starched three days ago, and each piece was immersed in an unheated bath of the yellow dye for about 30 s, and then left to dry horizontally on a plate, indoors in the shade.

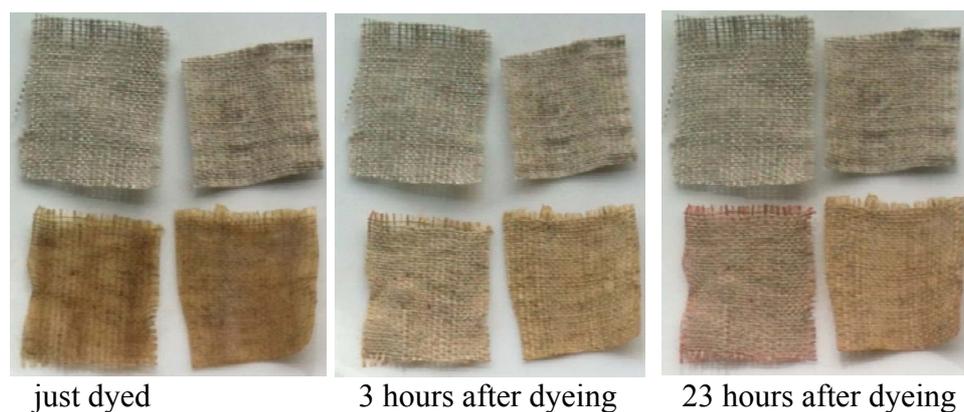


Fig. 4.20. Unbleached linen (control, top left), either starched (top right) or dyed yellow (bottom left) or both (bottom right). ©AvdH

Although both the starched and the not-starched piece had turned yellow by dyeing, only the not-starched piece of cloth slowly turned pink (fig. 4.20), perhaps because the dyestuffs were deprotonated while binding to the linen fibers. Apparently, the slightly acidic starch coating prevented the reddening of the dye on the starched piece of cloth. A similar difference between starched and not-starched cotton, on dyeing with yellow madder dye, was observed (fig. 4.21). In another experiment, a piece of bleached and unbleached linen and a piece of cotton were immersed in vinegar first, and then, while still wet, immersed in yellow madder dye. All pieces remained yellow, except at the tips of a few corner threads.



Fig. 4.21. Cotton cheesecloth dyed with yellow acidic madder root extract. A: Both just dyed. Left piece: not starched, right piece: starched. B: Same pieces of cloth, 9 hours after dyeing. C: Same pieces of cloth plus two controls (top left: not starched, top right: starched), 12 hours after dyeing ©AvdH

That a kind of sealing by retrograded starch occurred on the Shroud is confirmed by what was found in the Raes corner: there, the yellow madder dye was found only on the outer layer of the Raes threads, the cores of the threads being nearly colorless.<sup>683</sup> And today, according to Rogers, the Shroud still “does not readily absorb water”.<sup>684</sup> The water that, after manufacture of the Shroud, made the big waterstains, on the other hand, apparently was sucked into the threads, as in liquid chromatography, for it moved to the waterstain margins carrying the iron forward. And the water apparently was in contact with the cloth long enough to reach even the insides of linen fibers in some locations. The waterstaining configuration suggested by Guerreschi and Salcito, viz. that the Shroud

<sup>683</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, p. 17

<sup>684</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, p. 32

was folded and in a vertically position inside an Essene type jar when water from the bottom of the jar got into it,<sup>685</sup> would comply with such a slow and progressing wetting mechanism.

## 4.2.2. Reflectance curves of clear areas - raw and absolute

### 4.2.2.1. Darker than artificially aged linen

The reflectance spectrum of the Shroud's clear areas is unlike that of artificially aged linen. Pellicori published a plot comparing color and reflectance intensity, and showed that the Shroud reflectance intensity is lower than that of artificially aged linen of the same color: "As explained below, the bake simulates aging of the linen and produces a color close to that of the Shroud clear areas. This is demonstrated in Fig. 7. ... However, the clear background (substrate) reflectance R(550 nm) is reduced to ~0.48 for the laboratory linen compared with 0.36 for the Shroud. Factors such as weave density, thread diameter, and composition and cleanliness influence the reflectance value."<sup>686</sup> This discrepancy in reflectance intensity may be the result of yellow madder dye on the Shroud.

### 4.2.2.2. Possible madder dip in typical raw reflectance scan

The absorbance band of yellowish unmordanted madder dye would be expected at ca. 430 nm.<sup>687</sup> In this region, the shown "Typical raw reflectance scan" of a clear area of the Shroud (fig. 4.22) shows an irregular dip, which is lost in the smoothed "mean" curve, plotted through the mean 410 and 450 nm datapoints of five clear areas. Note, that no mean data point is given between 410 and 450 nm in the original fig. 6 of Gilbert and Gilbert. The absolute reflectance spectrum through 14 mean data points is a more or less straight ascending line from ca. 300 to 750 nm (Gilbert and Gilbert, fig. 6), but "The variation in spectral reflectance from a particular clear area to the mean clear referred to above was generally between  $\pm 3$  and  $\pm 7\%$  across the entire spectrum."<sup>688</sup>

Also note, that the background of the Shroud has a "mottled look throughout" in visible reflectance,<sup>689</sup> and in fluorescence shows blue areas,<sup>690</sup> which probably do not contain as much madder dye as the greenish yellow fluorescing background areas. So, if reflectance of such blue fluorescing regions was included for the calculation of the mean reflectance curve, their lack of madder would have diminished any averaged madder absorbance/reflectance band.

The absorbance of red madder dye on unmordanted wool shows a positive band at ca. 500 nm, which decreases about a third in 12 months' ageing.<sup>691</sup> So, an absorbance band of a yellowish madder dye on an unmordanted Shroud would certainly have decreased a lot in 700-2000 years anyway.

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<sup>685</sup> Guerreschi and Salcito, Further studies..., 2005

<sup>686</sup> Pellicori, Spectral properties..., 1980, p. 1917 (his fig. 7 is online at <http://imagebank.osa.org/getImage.xqy?img=M3cubGFyZ2UsYW8tMTktMTItMTkxMylnMDA3>)

<sup>687</sup> Clementi et al., A Spectrometric..., 2007, Fig. 2 (alizarin on unmordanted wool), <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr2.jpg> in <http://www.sciencedirect.com/science/article/pii/S0003267007009464> and Miliani et al., Acidichromic effects..., 2000, fig. 1 and table 1 (alizarin)

<sup>688</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, p. 1933; the curve reproduced in Pellicori, Spectral properties..., 1980, fig. 6, shows 16 evenly spaced data points from 350 to 750 nm, i.e. 5 more data points than the 11 unevenly spaced datapoints in the 350-750 nm region of the original of Gilbert and Gilbert.

<sup>689</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, p. 1935.

<sup>690</sup> "Background (Non-Image Areas) – Generally yellow-green, with blue flecks, thought to be modern lint contamination." Miller and Pellicori, Ultraviolet Fluorescence..., 1981, Table I, p. 75

<sup>691</sup> Clementi et al., A Spectrometric..., 2007, Fig. 7, <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr7.jpg> in <http://www.sciencedirect.com/science/article/pii/S0003267007009464>

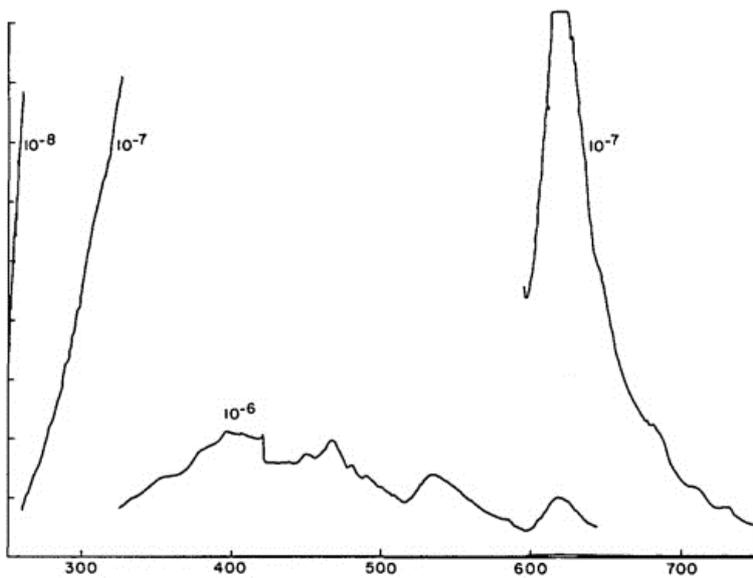
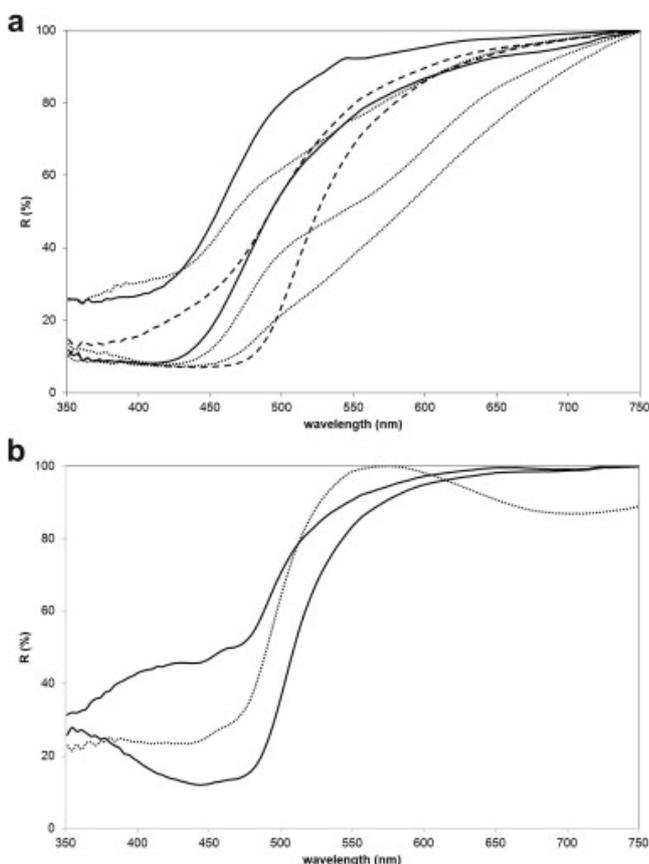


Fig. 4.22. Raw reflectance scan of a clear area of the Shroud ©OSA<sup>692</sup>

#### 4.2.2.3. Limited possibility of discerning yellow dyestuffs

For comparison, see the online fiber optic reflectance spectra of new and old dyed fabrics, which show that the curve is almost straight both for new fustic (yellow) dyed cloth and for yellow areas in a 17<sup>th</sup> century embroidered cloth (see fig. 4.23 and 4.24).<sup>693</sup>



<sup>692</sup> Used from R. Gilbert Jr. and M.M. Gilbert, Ultraviolet-visible reflectance and fluorescence spectra of the Shroud of Turin, *Applied Optics*, 19 (12) 1980, pp. 1930-1936, <http://dx.doi.org/10.1364/AO.19.001930>, fig. 4, with permission from the Optical Society of America

<sup>693</sup> Fig. 5 <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0143720813000594-gr5.jpg>, and fig. 7 <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0143720813000594-gr7.jpg> of Gulmini et al., Identification of dyestuffs in historical textiles: Strong and weak points of a non-invasive approach, *Dyes and Pigments*, Volume 98, Issue 1, July 2013, Pages 136-145, <http://dx.doi.org/10.1016/j.dyepig.2013.02.010>

Fig. 4.23. “(a) Vis-FOR spectra exemplifying those obtained for yellow reference samples (wool dyed with weld (solid lines), fustic (dotted lines) or turmeric (dashed lines) with alum mordant. (b) Vis-FORS spectra exemplifying those obtained for yellow reference samples (wool) dyed with saffron applied directly (solid lines) and post-mordanted with Cu(II) ions (dotted line).” ©Elsevier<sup>694</sup>

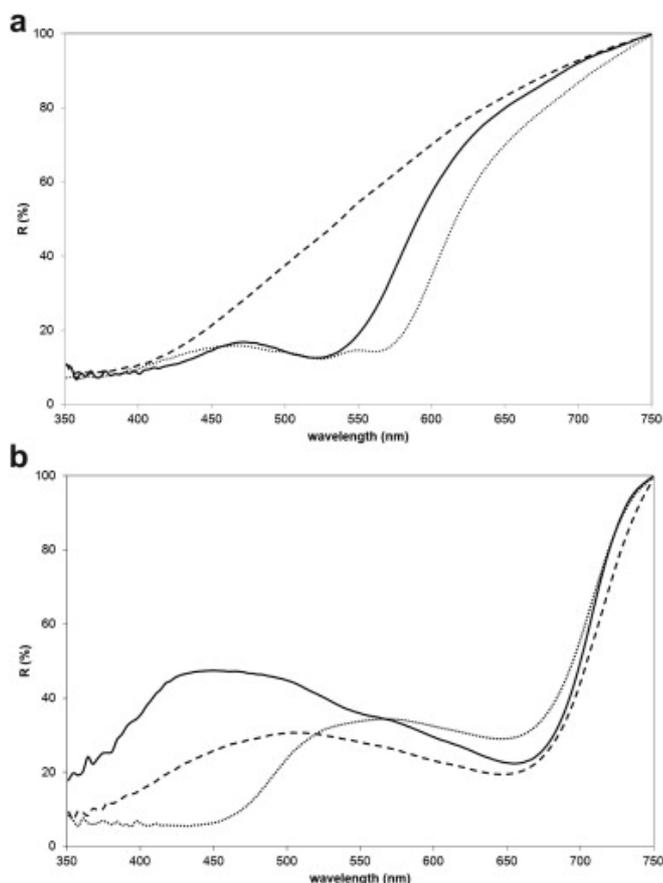


Fig. 4.24. “(a) Vis-FOR spectra obtained for some red or pink (dotted line), yellow (dashed line) and orange–red (solid line) areas on the historical embroidered cloth. (b) Vis-FOR spectra obtained for some blue (solid line), blue green (dashed line) and green (dotted line) areas on the historical embroidered cloth.” ©Elsevier<sup>695</sup>

The curve for the yellow area shows the same shape (~straight line) as the mean background of the Shroud. The article says that “It emerged that visible fibre optics reflectance spectroscopy is poorly selective for yellow dyes based on curcumins and flavonoids, while it generally succeeds in detecting the other dyestuffs considered herein”<sup>696</sup> and “The technique is very efficient in determining indigo and Saxon blue, and readily discriminates animal and vegetable red dyestuffs. Much more limited is

<sup>694</sup> Used from M. Gulmini, A. Idone, E. Diana, D. Gastaldi, D. Vaudan, M. Aceto, Identification of dyestuffs in historical textiles: Strong and weak points of a non-invasive approach, *Dyes and Pigments* Vol 98 Issue 1, July 2013, pp. 136-145, DOI: 10.1016/j.dyepig.2013.02.010, <http://www.sciencedirect.com/science/article/pii/S0143720813000594> (fig. 5 <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0143720813000594-gr5.jpg>), © 2013 Elsevier, Ltd., with permission from Elsevier

<sup>695</sup> Used from M. Gulmini, A. Idone, E. Diana, D. Gastaldi, D. Vaudan, M. Aceto, Identification of dyestuffs in historical textiles: Strong and weak points of a non-invasive approach, *Dyes and Pigments* Vol 98 Issue 1, July 2013, pp. 136-145, DOI: 10.1016/j.dyepig.2013.02.010, <http://www.sciencedirect.com/science/article/pii/S0143720813000594> (fig. 7 <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0143720813000594-gr7.jpg>), © 2013 Elsevier, Ltd., with permission from Elsevier; a photo of the historical embroidered cloth is at <http://www.sciencedirect.com/science/article/pii/S0143720813000594> and <http://ars.els-cdn.com/content/image/1-s2.0-S0143720813000594-fx1.jpg>

<sup>696</sup> Gulmini et al., Identification of ..., 2013, Abstract <http://www.sciencedirect.com/science/article/pii/S0143720813000594>

the possibility of discerning yellow dyestuffs, although a peculiar shape of the reflectance spectra, with sub-bands in the 400-500 nm range, would suggest the presence of carotenoids.”<sup>697</sup>

The dyestuffs of madder are not curcumins or flavonoids or carotenoids, and are not mentioned explicitly in the article. It does say, concerning a further HPLC-DAD-MS analysis: “The analysis detected genistein and luteolin (and some of their glycosylated forms and derivatives), that reveals the use of dyer’s broom (*Genista tinctoria* L.) to obtain the various yellow shades”.<sup>698</sup> Genistein and luteolin are (iso-)flavonoids, occurring in dyer’s broom and weld, respectively, and in neutral pH have an absorbance peak at 325 and 380 nm, respectively, steeply descending to zero at 425 and 440 nm, respectively.<sup>699</sup> So, the straight, slowly ascending line from ca. 400 to 700 nm in the reflectance spectrum of the 17<sup>th</sup> century yellow areas dyed with genistein and luteolin is unlike the steep increase from 325 to 425 nm or from 380 to 440 nm that would be expected for a cloth newly dyed with genistein and luteolin. Similarly, the steep increase from ca. 430 to 525 nm, that would be expected from a cloth newly dyed with yellow madder, would probably not be observable anymore in the aged Turin Shroud reflectance.

#### 4.2.2.4. No lignin-oxidation band

Darkening through oxidation of lignin components results in a change in the 410 nm absorption.<sup>700</sup> That such a band is not seen in the Shroud background reflectance – whether the raw scan or the mean plot – although a 280 nm band, attributable to lignin, is present in the mean plot,<sup>701</sup> may be the result of a sealing starch film on the Shroud, which to some extent protected the lignin of the linen from oxidation during ageing.

#### 4.2.3. Raw fluorescence scan background

The “Typical raw fluorescence scan (point B3A – clear)” made under 365 nm excitation (fig. 4.25)<sup>702</sup> shows, beside the broad band with a peak at 435 nm (blue range), also a shoulder at ca. 540 nm (green range) and a smaller shoulder at 600-620 nm (orange-red), which have become invisible in the smoothed curves (fig. 4.26). The broad band around 435 nm (in the blue range) is probably due to lignin in the linen, as an online fluorescence spectrum of lignin rich pulp has the same shape and peak in the 435 nm region (fig. 4.27),<sup>703</sup> and as unbleached linen and untreated lignin both fluoresce blue<sup>704</sup> (fig. 4.28).

<sup>697</sup> Gulmini et al., Identification of..., 2013, p. 144

<sup>698</sup> Gulmini 2013, Identification of..., 2013, p. 143

<sup>699</sup> Genistein: Zielonka et al., 2003,

[http://www.researchgate.net/publication/9052785\\_Radical\\_scavenging\\_properties\\_of\\_genistein/file/9fcfd51311de79c2a9.pdf](http://www.researchgate.net/publication/9052785_Radical_scavenging_properties_of_genistein/file/9fcfd51311de79c2a9.pdf) and <http://ars.els-cdn.com/content/image/1-s2.0-S0891584903004726-gr1.gif> (in neutral pH) and <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0162013410001480-gr2.jpg> (mordanted). Luteolin and apigenin: Favaro et al.,

Acidochromism and Ionochromism of Luteolin and Apigenin, the Main Components of the Naturally Occurring Yellow Weld: A Spectrophotometric and Fluorimetric Study, 2007, Fig. 4 (neutral pH),

<http://link.springer.com/article/10.1007/s10895-007-0222-0#>

<sup>700</sup> “One of the principal absorption regions that many authors have observed during the darkening and bleaching of pulps is peaked at about 410 nm as can be clearly seen in [Figure 6](#) and in the previously cited curves of Polcin and Rapson, Claesson, Olson and Wennerblom and of Silvy and LeNest. If one may judge from the similarity of the curves for changes in vanillin (in [Figure 6](#)), it is the components of lignin that are responsible for the changes that occur in this region of the spectrum.” Feller, Comments on the Measurement of “Yellowness” in Pulp and Paper, 2011,

<http://cool.conservation-us.org/coolaic/sg/bpg/annual/v06/bp06-04.html>

<sup>701</sup> Lignin absorbance: Fig. 4 <http://ars.els-cdn.com/content/image/1-s2.0-S0032959299000163-gr4.gif> of Fang et al., 1999, <http://www.sciencedirect.com/science/article/pii/S0032959299000163>, and fig. 3 of Feller, 2011,

<http://cool.conservation-us.org/coolaic/sg/bpg/annual/v06/bp06-04.html>; “When the absorption of lignin is involved, Lin and Kringstad considered the absorption at 280 nm to be due to guaiacyl structures; see [Figure 4](#).” (Ibid.)

<sup>702</sup> Gilbert and Gilbert, Ultra-violet visible reflectance..., 1980, Fig. 5 and text

<sup>703</sup> See the figure on p. 3 and the laser wavelength of 337 nm, mentioned at line 61 of page 11 of the docstoc file:

<http://www.docstoc.com/docs/47843952/On-line-Measurement-Of-Lignin-In-Wood-Pulp-By-Color-Shift-Of-Fluorescence---Patent-5486915>

<sup>704</sup> Fluorescence of unbleached linen: Fig. 7 on p. 9 of Fanti, Body Image Formation Hypotheses based on Corona Discharge: Discussion, 2008, <http://www.ohioshroudconference.com/papers/p15.pdf>; fluorescence of untreated lignin in

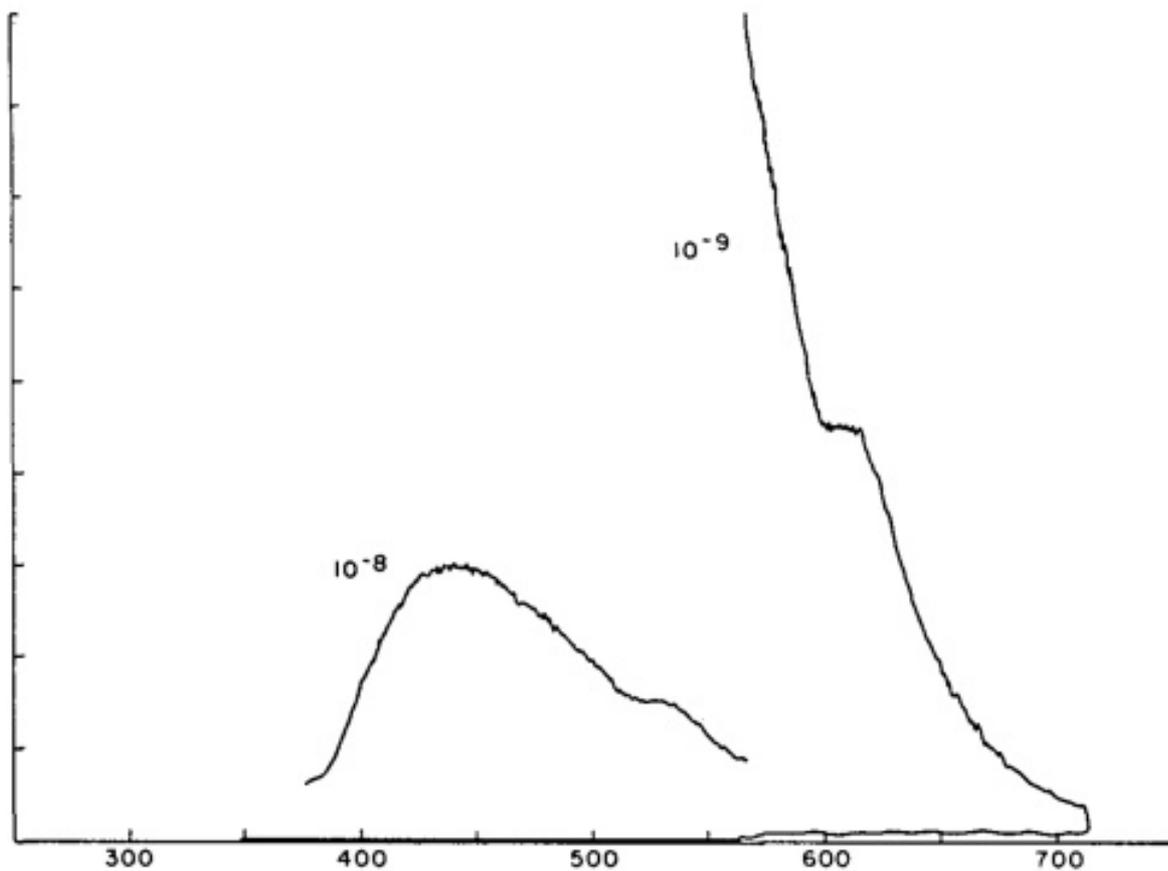


Fig. 4.25. "Typical raw fluorescence scan (point B3A – clear)" of the Shroud ©OSA<sup>705</sup>

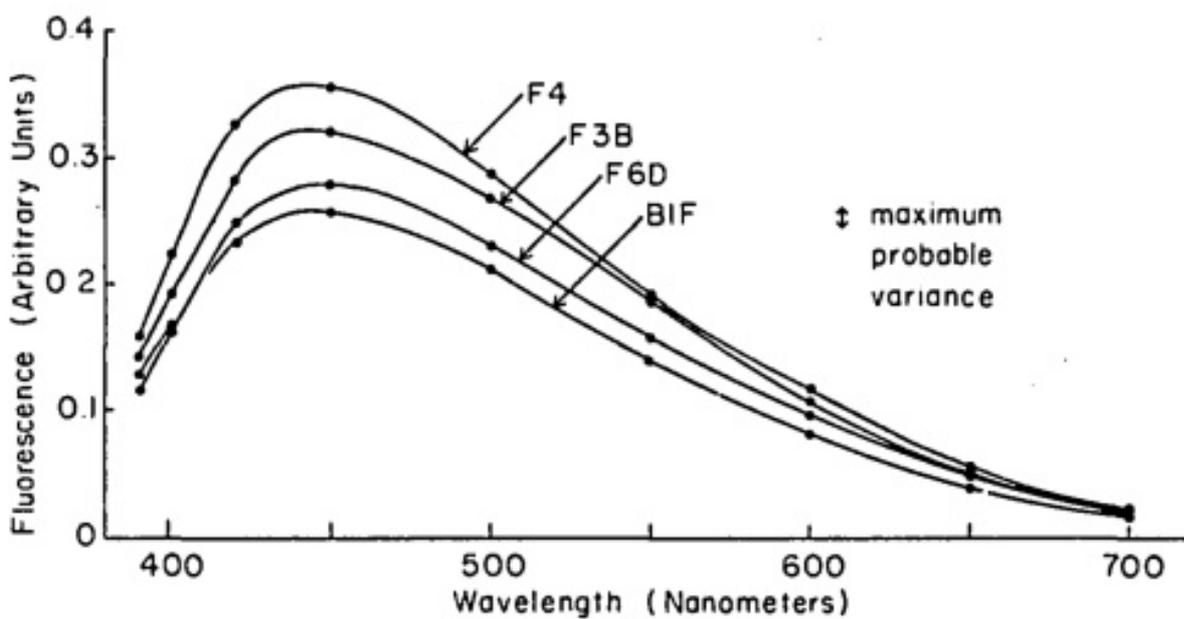


Fig. 4.26. Smoothed fluorescence curves of four clear areas of the Shroud ©OSA<sup>706</sup>

bagasse fiber: Fig. 6 of Coletta et al., 2013, <http://www.biotechnologyforbiofuels.com/content/6/1/43/figure/F6> (see fig. 4.28)

<sup>705</sup> Used from R. Gilbert Jr. and M.M. Gilbert, Ultraviolet-visible reflectance and fluorescence spectra of the Shroud of Turin, *Applied Optics*, 19 (12) 1980, pp. 1930-1936, <http://dx.doi.org/10.1364/AO.19.001930>, fig. 5, with permission from the Optical Society of America

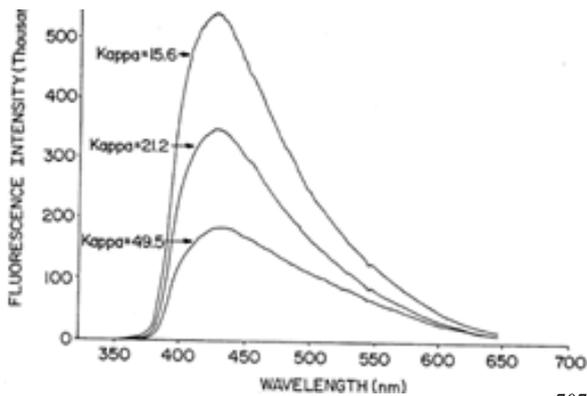
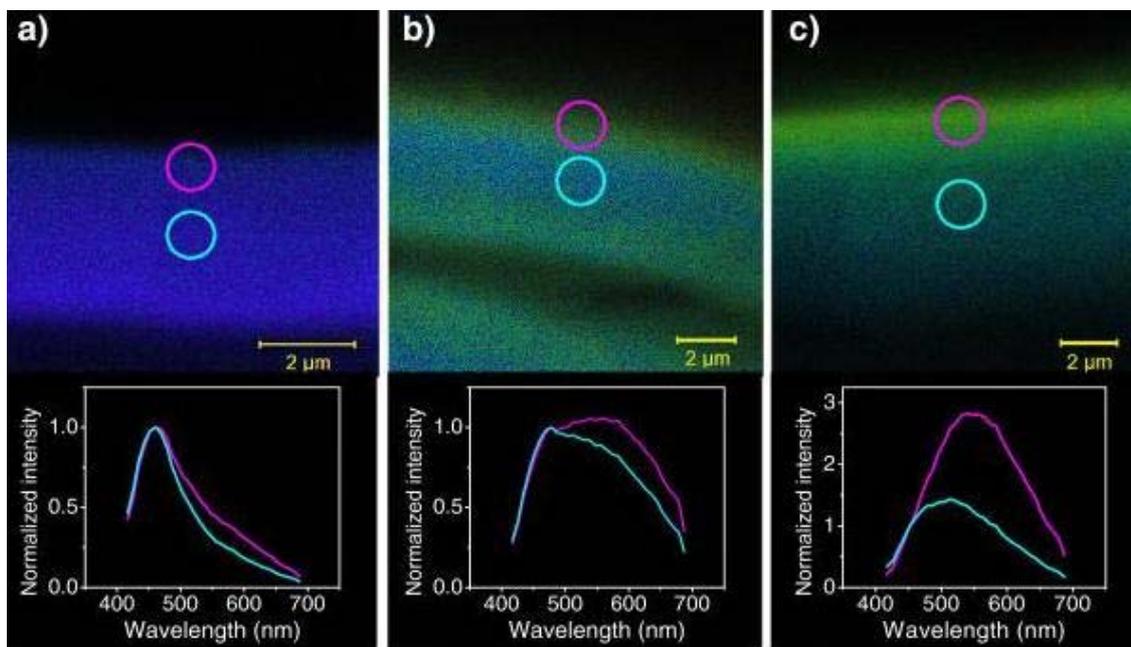


Fig. 4.27. Fluorescence of lignin-rich pulp<sup>707</sup> (public domain)



fluorescence under two 770 nm photons of lignin in cell wall of bagasse fibers, normalized at 478 nm  
 a) untreated                      b) acidic treatment (H<sub>2</sub>SO<sub>4</sub> 1%)                      c) alkali treatment (NaOH 2%)

Fig. 4.28. Fluorescence of lignin-containing microscopic sugarcane bagasse fibers – adapted (= added caption) from Coletta et al., 2013<sup>708</sup> (Creative Commons Attribution Licence<sup>709</sup>)

The raw scan's shoulder at 510-540 nm is probably due to yellow acidic madder dye. An Indian study by Dengre et al., 1993, reported that the fluorescence of a water extract of *Rubia tinctorum* roots is “Yellow Green”, and that “Fluorescence analysis of various solvent extracts of stem and

<sup>706</sup> Used from R. Gilbert Jr. and M.M. Gilbert, Ultraviolet-visible reflectance and fluorescence spectra of the Shroud of Turin, *Applied Optics*, 19 (12) 1980, pp. 1930-1936, <http://dx.doi.org/10.1364/AO.19.001930>, fig. 7, with permission from the Optical Society of America

<sup>707</sup> US Patent <http://www.docstoc.com/docs/47843952/On-line-Measurement-Of-Lignin-In-Wood-Pulp-By-Color-Shift-Of-Fluorescence---Patent-5486915>

<sup>708</sup> Adapted from V.C. Coletta, C.A. Rezende, F.R da Conceição, I. Polikarpov, F.E.G. Guimarães, Mapping the lignin distribution in pretreated sugarcane bagasse by confocal and fluorescence lifetime imaging microscopy, *Biotechnology for Biofuels* 2013 6:43, doi:10.1186/1754-6834-6-43, <http://www.biotechnologyforbiofuels.com/content/6/1/43>, fig. 6 <http://www.biotechnologyforbiofuels.com/content/6/1/43/figure/F6>

<sup>709</sup> The Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>) permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

roots powders, were carried out by the method of Chase and Pratt (1949) (Table 3 and 4)", probably using 365 nm UV light as excitation.<sup>710</sup>

The unmordanted neutral forms of anthraquinones of aged yellow madder at an acidic pH have a combined fluorescence that may have its peak at ca. 540 nm. The neutral form of purpurin, which is yellow-orange and fluoresces about a factor six stronger than alizarin, has a broad flat fluorescence band from ca. 540 to 575 nm, with its peak value at 575 nm (greenish yellow) under 377 nm in water-dioxane at pH 2.5 – in its pink mono-anion form (pH 7), purpurin's fluorescence peak would still be in the greenish-yellow and near 575 nm, viz., at 585 nm; the fluorescence peak of the neutral form of yellow alizarin in water-dioxane at pH 2.5 is at 485 nm (in the blue) – no value is given for a neutral pH, but at pH 9.0 (pale pink mono-anion form) alizarin's fluorescence peak is at 625 nm (in the red).<sup>711</sup> The ratio of alizarin to purpurin in a fresh madder extract depends on the species and perhaps age of the madder plant and on the method of extraction; some recent European studies on madder reported an alizarin fraction of only ca. 15%.<sup>712</sup> But the presence of a larger fraction of alizarin than purpurin in a madder extract, as also has been found, e.g. in 1968,<sup>713</sup> would pull the peak fluorescence of acidic madder towards shorter wavelengths than the 575-585 nm of acidic to neutral purpurin alone.<sup>714</sup> Furthermore, ageing shifts both the purpurin and alizarin fluorescence peak toward shorter wavelengths, and it affects purpurin more than alizarin; 12 months of natural ageing of a red madder-dyed wool sample pulled its fluorescence peak about 50 nm to shorter wavelengths and the amounts of alizarin and purpurin on wool samples were reduced to 67% and 40% of their initial values, respectively.<sup>715</sup> Also, degradation affects madder dye more when it is on cotton than on wool, and the more finely dispersed the dye, the more rapidly it will fade.<sup>716</sup> These are all factors that may have contributed to a more 'green-yellow' fluorescence peak of madder dye on the Shroud. The

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<sup>710</sup> Dengre et al., Comparative studies of *Rubia cordifolia* Linn. and *Rubia tinctorum* Linn (Rubiaceae), *Ancient Science of Life*, 1993, Table 4, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3336544/pdf/ASL-13-165.pdf>; Chase and Pratt, 1949, <http://onlinelibrary.wiley.com/doi/10.1002/jps.3030380612/abstract>; the article of Chitra et al., *Ancient Science of Life*, 2008, also refers to Chase and Pratt 1949, and uses daylight and 365 nm excitation to assess fluorescence color (Table 4 in <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3330866/pdf/ASL-27-9.pdf>).

<sup>711</sup> Fluorescence quantum yields of alizarin, neutral form, is  $5.1 \times 10^{-4}$ ; of purpurin, neutral form  $3.3 \times 10^{-3}$ , mono-anion form  $2.6 \times 10^{-3}$  (Miliani et al., Acidichromic effects..., 2000, Tables 1 to 4, and Figure 2 and 4, p. 143-146, [http://onlinelibrary.wiley.com/doi/10.1002/\(SICI\)1099-1395\(200003\)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract](http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1099-1395(200003)13:3%3C141::AID-POC220%3E3.0.CO;2-J/abstract))

<sup>712</sup> Claro et al., The use of..., 2008, p. 924; Miliani et al., A spectrophotometric..., 1998, 504. Clementi et al., A spectrometric..., 2007, used a madder extract that consisted of 8-9 times more purpurin than alizarin (see their fig. 5c <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr5.jpg> MUU = Madder Unmordanted Unaged), and also Miliani et al., 1998, used a madder extract that consisted of only 15% alizarin (1,2 HAQ) (see their p. 584: "Based on these spectra, the amount of 1,2-HAQ in the sample of natural product was calculated to be around 15%, in contrast with previously reported indication of greater amount of alizarin in madder"); A mature plant yielded 19 anthraquinones, while a one-year old plant yielded only four (Burnett and Thomson, Naturally occurring quinones. Part XV. Biogenesis of the anthraquinones in *Rubia tinctorum* L. (madder), 1968, <http://pubs.rsc.org/en/content/articlelanding/1968/j3/j39680002437#!divAbstract>)

<sup>713</sup> "(ii) Fraction 2 gave alizarin (37 mg.), mp 288-289". mgd. (iii) Fraction 3 consisted of purpurin (18 mg.), red needles, mp 262-263" (from ethanol). (iu) Fraction 4 afforded pseudopurpurin (7 mg.), mp 221-223" (decomp.)" Burnett and Thomson, Naturally occurring quinones. Part XV. Biogenesis of the anthraquinones in *Rubia tinctorum* L. (madder), 1968, <http://pubs.rsc.org/en/content/articlelanding/1968/j3/j39680002437#!divAbstract>; "Natural madder is reported [3] and [7] to be a mixture of alizarin (1,2-di-hydroxyanthraquinone, 1,2-HAQ), as the main colouring matter, and purpurin (1,2,4-tri-hydroxyanthraquinone, 1,2,4-HAQ)." Miliani et al., A Spectrophotometric..., 1998

<sup>714</sup> In red unmordanted "genuine madder" in linseed oil on canvas (probably mono-anions at pH~7) the peak fluorescence (436 nm excitation) is 25 nm to the red of the purpurin peak, while the alizarin peak is about 100 nm to the red of the purpurin peak (Fig. 4 of Miliani et al., A Spectrophotometric..., 1998). So, in acidic madder, the fluorescence peak could also be about 25 nm to the blue of the neutral purpurin peak, as the alizarin peak is about 90 nm to the blue of the purpurin peak (Miliani et al., Acidichromic effects..., 2000). Thus, the flat peak of 540-575 nm of the neutral form of purpurin would correspond to a madder peak at ca. 515- 550 nm.

<sup>715</sup> Clementi et al., A spectrometric..., 2007, Fig. 8, <http://ars.els-cdn.com/content/image/1-s2.0-S0003267007009464-gr8.jpg>. "The blue spectral shifts observed for purpurin and madder samples (and even for alizarin to a lesser extent) were markedly reduced in the presence of mordant ... In the samples dyed with purpurin, the extent of degradation was greater than in those with alizarin, the unaged percentage fraction being about 40% of its initial value on unmordanted wool sample" (Ibid. p. 52).

<sup>716</sup> Clementi et al., A spectrometric..., 2007, p. 47, 52

molar absorption coefficients of alizarin and purpurin are of the same order of magnitude, so, if alizarin is the largest fraction in madder, alizarin's visible color (yellow at  $\text{pH} \leq 5$  in water-dioxane, and at  $\text{pH} < 5.6$  in water) would be the dominant visible color.<sup>717</sup>

#### 4.2.4. Fluorescence photography

Adler wrote: "The background cloth shows a light greenish yellow emission not typical of other known old linen cloths and perhaps suggesting the presence of some type of thin coating of a fluorophore on the original linen".<sup>718</sup> The eight square fluorescence photographs made by STURP in 1978, were made under 335-375 nm UV excitation, and with an effective separation of UV excitation and fluorescence emission by a Hoya L-42 sharp cut filter glass that passes visible light from ~410 nm (= blue) in front of the camera, and a filter cell with salt solution and a C7-54 filter glass which pass UV and violet light upto ~400 nm (= violet) in front of the source ( $10^{-2}$  and  $10^{-3}$  attenuation at 404 nm for source and camera, respectively), resulting in the white teflon-coated magnets<sup>719</sup> dark appearance in the photos; the fluorescence photographs indeed show a weak greenish yellow/yellowish green in the background of the Shroud, and Miller and Pellicori wrote that it showed blue flecks: "Background (Non-Image Areas) - Generally yellow-green, with blue flecks thought to be modern lint contamination".<sup>720</sup> Garza-Valdes reported on Shroud threads from the piece of fabric that was cut in 1988 for the radiocarbon dating – he had received them from Riggi di Numana: "Fibers from the threads taken from the edge of the samples, where there was no image, were pale yellow, and the coating was thinner than in other areas. ... Under ultraviolet light, the fibers had a strong yellowish-green fluorescence".<sup>721</sup>

As already shown, in some waterstains created by the fire fighting water of 1532 AD, the Shroud's fluorescence is (dark) blue but the visible color is lighter than outside the stain, as if a starch-madder coating had been washed from the linen by hot water (fig. 4.2). Here must be noted that even a virtually colorless, or even white looking, faded dye layer can still fluoresce noticeably, as can be seen by comparison of online photos of probably madder-painted statues in visible light and UV light on the "Madder" pages of the site of the Museum of Fine Art Boston.<sup>722</sup>

##### 4.2.4.1. Linen, starched and dyed with yellow madder, can fluoresce green yellow

An experiment with IVN-certified oxygen-bleached linen<sup>723</sup> that was starched with a slightly acidic cooked wheat starch paste and then dyed with different kinds of extracts of *Rubia tinctorum* root showed that acidic yellow madder dye on starched linen can yield different fluorescence colors, among which a greenish yellow color. An extract made as a cup of tea, by pouring boiling water on madder root powder in a filter bag in a cup, after cooling down and storage in a closed glass jar, spontaneously started fermenting and produced small gas bubbles on the surface of the liquid. Within three days, the pH spontaneously dropped from to 6.5-7.0 to 5.0 –5.5 (PeHanon pH strips) and the color simultaneously changed from red to yellow-orange. This spontaneously decolored extract was then further acidified and yellowed with vinegar to  $\text{pH} \leq 4$  and a bright yellow color. The results in daylight and in UV light are shown in fig. 4.29, in which the UV photos show less yellow and orange color of the dyed pieces than when seen directly by the dark-adapted eye. The camera and the eyes

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<sup>717</sup> Miliani et al, Acidichromic effects. ..., 2000, Table 1 and 3, and the individual colors of alizarin and purpurin are mentioned in the text on p. 143-144. Rogers wrote that alizarin "is yellow below a pH of 5.6 and red above a pH of 7.2 (figure 14)" referring to a photomicrograph of reddish Raes fibers in  $\text{NaHCO}_3$  at pH 8.0 (Rogers and Arnoldi, Scientific Method..., 2002, p. 18-19).

<sup>718</sup> Adler, Chemical and Physical..., 2002, p. 13

<sup>719</sup> See Benford and Marino, Discrepancies in..., 2008, <http://www.ohioshroudconference.com/papers/p09.pdf>, white light photo on p. 5, and STURP's tape sample map <http://www.shroud.com/maptap2v.htm>

<sup>720</sup> Miller and Pellicori, Ultraviolet fluorescence..., 1981, p. 71, fig.1 at p. 72, and p. 75, 76

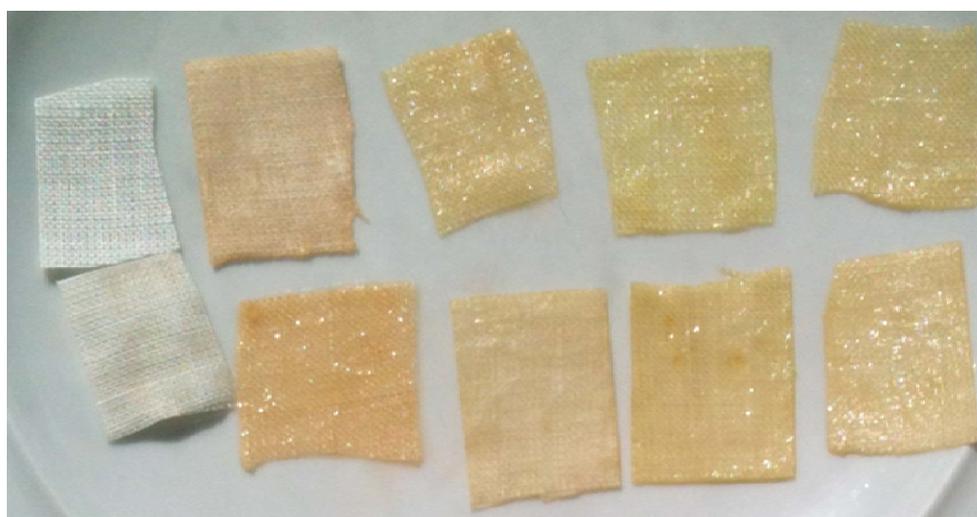
<sup>721</sup> Garza-Valdes, The DNA of God?, 2001, p. 28, 34

<sup>722</sup> [http://www.cameo.mfa.org/wiki/Rose\\_madder](http://www.cameo.mfa.org/wiki/Rose_madder) and <http://www.cameo.mfa.org/wiki/Madder>

<sup>723</sup> It is fine linen with IVN certificate 2013: <http://www.neidig.nu/stoffen/linnen/geweven/1544/6001832-fijnlinnen-wit.html>, which means it was bleached with oxygen (<http://www.naturtextil.com/consumers/quality-seals/best.html>).

were shielded with a UV filter (Hoya L-42<sup>724</sup>, as in Miller and Pellicori's fluorescence photography), but the Wood lamp (peak intensity at ca. 365 nm) was used without an extra visible-light shield and apparently emits a visible blue component, as shown by its specular reflectance from the white plate (fig. 4.30).<sup>725</sup> Yellow fluorescence is hardly picked up by the camera, especially from a distance, for a bright green yellow fluorescence of a yellow madder dye in a glass jar is hardly seen by the camera (fig. 4.31), but is clearly seen by the eye. Because of this blue-leak from the source and the variation in color through variation in distance and the use of a digital camera instead of an analogue film camera, the colors in the UV photos of this paper can not be directly compared to the colors of the official, doubly shielded, UV-fluorescence photos of Miller and Pellicori's article. So, it is difficult to estimate which of the different kinds of madder root extract best fits the Shroud, also because the Shroud has naturally aged and the shown experimentally dyed pieces have not. Yet, because of the many possible fluorescence variations shown in the experiment, a madder extract that would be consistent with the Shroud's colors probably does exist.

Besides, for some reason, various online UV or UV-fluorescence photos of the Shroud show many different colors of the Shroud background areas: rather blue (chest – Weaver and Adler), green (chest and wrist a.o., Miller and Pellicori, cf. fig. 4.2 and 5.3), yellow (chest and wrist and forehead and between head images – Jackson videoed by Breault, wrist – Rogers, fig. 2.30), orange (Raes corner – Rogers and Schwartz), and pinkish orange (wrist – Shroud Science Group Wiki).<sup>726</sup>

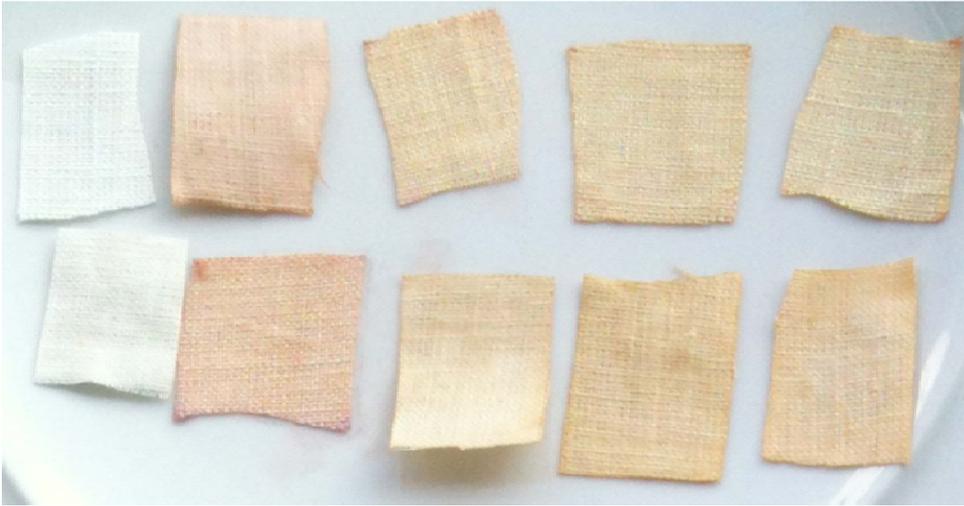


Just dyed. top: not-starched linen, bottom: starched linen, leftmost: controls

<sup>724</sup> <http://www.hoyaoptics.com/pdf/L42.pdf>, 22-9-2014 for sale at <http://www.amazon.com/Hoya-L-42-Sharp-Cut-Filter/dp/B00EAA8GBG>

<sup>725</sup> Wood glass transmits light up to 430 nm (<http://www.komar.org/faq/colorado-cataract-surgery-crystalens/ultra-violet-color-glow/Woods-Glass-Spectrum-Transmission.jpg> cf. [http://en.wikipedia.org/wiki/Wood%27s\\_glass](http://en.wikipedia.org/wiki/Wood%27s_glass)) and the emission spectrum of a black light fluorescent tube, centred at 370 nm, shows a “tiny secondary peak (2)” from the mercury vapor line at 404 nm [http://en.wikipedia.org/wiki/Black\\_light#mediaviewer/File:Fluorescent\\_Black-Light\\_spectrum\\_with\\_peaks\\_labelled.gif](http://en.wikipedia.org/wiki/Black_light#mediaviewer/File:Fluorescent_Black-Light_spectrum_with_peaks_labelled.gif)

<sup>726</sup> Blue “Ultraviolet photograph” of the chest, republished by Adler (Chemical and Physical..., 2002, TOM 14, online at Berry, <http://shroudofturinwithoutallthehype.files.wordpress.com/2012/08/fanti-screen-grab-spear-cropped.png>); green: Miller and Pellicori, printed Journal of Biocommunication, cf. e.g. fig. 4.2 and 5.3; yellow: presentation slides of Jackson, Rome 93' John J. Jackson – New evidence that the Turin Shroud was the Mandylion, <https://www.youtube.com/watch?v=WGCi-FALoDg>, 27:50 forehead, 32:40 chest, 37:45 between head images, and presentation of Jackson, St. Louis Dr. John Jackson – What is missing?, <https://www.youtube.com/watch?v=jyqDaXU4lzE>, 10:35 wrist, and Rogers, Scientific Method..., p. 5, wrist, see <http://shroudstory.files.wordpress.com/2012/02/i8.jpg>; orange – Raes corner: <http://shroudstory.files.wordpress.com/2012/02/i4.jpg> = Fig. 18 in Rogers' 2002 paper “Scientific method applied to the Shroud of Turin” and also on page 5 of his 2004 paper “Frequently Asked Questions (FAQs)”; pinkish orange: “UV photograph” of the wrist, published online by the Shroud Science group, <http://shroud.wikispaces.com/CHARACTERISTICS+OF+BLOODSTAINS>



Same pieces, 4 days after dyeing



Same pieces in UV light, photo taken through 2.5 mm thick Hoya L-42 UV filter, no blue-light filter in front of Wood lamp, 35 days after dyeing



pieces 1, 2, and 3



pieces 3, 4 and 5

Same pieces, same UV lamp, filter and camera and day, just photographed from smaller distances

Fig. 4.29. (above) Dyeing pieces of linen with different madder root extracts. Dyes from left to right: 1. no dye (control), 2. root powder stirred in water at room temperature for ca. 2 h, then filtered through brown filter paper, then acidified, 3. root powder stirred in hot water for ca. 45 min, then filtered through brown filter paper, then acidified, 4. root powder extracted as tea with boiling water, then spontaneously fermented, then acidified, 5. root powder extracted as tea with boiling water then acidified and stored. All dyes had a  $pH \leq 4$ . ©AvdH



Fig. 4.30. Specular reflectance of blue light from a white plate; photo lit with  $\sim 365$  nm Wood lamp unshielded with an extra blue-light filter and photographed through a 2.5 mm thick Hoya L-42 UV filter ©AvdH



Fig. 4.31. Bright green yellow fluorescence of pale yellow madder dye in a glass jar is clearly seen by the eye but hardly seen by the camera from a distance (both through a UV filter). The photos are 'as-is' and brightness-enhanced, respectively. The dye was extracted by heating plus stirring an aqueous suspension of madder root powder for 45 min, and after it had cooled down and spontaneously fermented, it was acidified further by adding vinegar. ©AvdH

#### 4.2.4.2. Blue fluorescence = less print (no acid madder = no catalyst or easy substrate for image formation)

Miller and Pellicori also wrote that in some 'no-print' weave areas (where expected body image is lacking or much less intense) the Shroud is blue fluorescent: "The leg outline and scourge markings are limited by a weave line appearing blue in fluorescent emission where the weave direction changes. This is an area of "no-print"; "Areas in the weave where the image density abruptly decreases (e.g., sides of face) might actually contain very faint images which possibility could be

retrieved by using stimulating radiation of shorter wavelengths. The property of the linen thread that didn't develop image density should also be discovered."<sup>727</sup>

This property may be a lack of starch and yellow-green fluorescing madder dye in these areas, either because the starch and madder was not applied well or because the starch-madder coating had abraded before image formation. Yellow protonated alizarin and purpurin are not easily soluble in cold water, but they are acids (alizarin  $pK_{a1}$  (first deprotonation) = 6.6/7.4;  $pK_{a2}$  (second deprotonation) = 12.4/11.8; purpurin: 4.7 and 9.5, respectively<sup>728</sup>). A fine starch paste, mentioned by the first-century author Pliny as used for papyrus sizing, was prepared by boiling fine flour in water with a small amount of acetic acid (probably vinegar): "minimo aceti aspersu".<sup>729</sup>

The lack of acid madder and acid starch in the blue fluorescent weave areas means that, on the surface, there was a lack of acids that would have been the initial catalysts for the "acid catalyzed oxidation" which is the effect that most simply describes the image formation effect.<sup>730</sup> The blue fluorescent areas are indeed areas of "no print" or actually less print: Castex showed by digital dynamic equalisation that there is a very faint image in these areas at the sides of the face,<sup>731</sup> which means that the madder layer probably was already missing before image formation, which apparently just barely affected the bare linen. If the starch-madder layer had been abraded after image formation, it would have left a colorless fiber with no image at all, for the image layer elsewhere could be stripped from image fibers by the sticky tapes of STURP, leaving colorless fibers, and the cross sections of image fibers didn't show any color "in the cellulose".<sup>732</sup>

An experiment by Berry already showed that application of lemon juice (an acid/sugar combination) made linen more susceptible to scorching.<sup>733</sup> It is well known that paper that contains acid turns yellow by ageing (= oxidation) much faster than acid-free paper.<sup>734</sup> Of the "yellow patina on one surface of many Roman-period papyri from Tebtunis" it is said that it is "possible that the basic overall yellow shade is due to the natural ageing of a layer of paste on the surface", viz. "the light paste wash mentioned by Pliny",<sup>735</sup> which was slightly acidic. An experiment with linen that had been starched with a paste of wheat flour cooked in water with a little vinegar, showed that the thus starched linen scorched much easier than the not-treated pure linen and that also baking in an oven

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<sup>727</sup> Miller and Pellicori, Ultraviolet fluorescence..., 1981, p. 83; 85

<sup>728</sup> Miliani et al., Acidichromic effects..., 2000, Table 5 and Scheme2, p. 148 (pKs determined in 33%/1% dioxane in water)

<sup>729</sup> "glutinum vulgare e pollinis flore temperatur fervente aqua, minimo aceti aspersu, nam fabrile cummisque fragilia sunt." Pliny the Elder, Naturalis Historia 13,23

<http://www.perseus.tufts.edu/hopper/text?doc=Perseus:text:1999.02.0138:book=13:chapter=23&highlight=minimo+aceti+aspersu%2C>, (English translation on

<http://www.perseus.tufts.edu/hopper/text?doc=Perseus%3Atext%3A1999.02.0137%3Abook%3D13%3Achapter%3D26> )

<sup>730</sup> Case, The Shroud of Turin..., 1996, ( interview of Heller and Adler), p. 63, 83

<sup>731</sup> Thierry Castex, Egalisation Dynamique (norme quadratique L2) / Dynamic Equalization

<http://thierrycastex.blogspot.ca/2012/05/egalisation-dynamique-norme-quadratique.html> or

<http://thierrycastex.blogspot.fr/>

<sup>732</sup> "A6) The cellulose of the medullas of the 10-20-micrometer-diameter fibers in image areas is colorless because the colored layer on image fibers can be stripped off, leaving colorless linen fibers (Heller 1981; Rogers 2002)." Fanti et al., Evidences for..., 2005, <http://www.shroud.com/pdfs/doclist.pdf> ; Rogers wrote in an email to the Shroud Science Group: "It is still possible to see places on the sampling tapes from 1978 where image color was stripped off of image fibers. The thin, colored layer is still stuck to the adhesive of the tape. These colored "ghosts" still show all of the chemical properties of the complete image fibers. The image color is not a result of any changes in the cellulose of the linen fibers. The cellulose of the image fibers is still colorless." Carreira, The Shroud of Turin..., 2010,

<http://www.shroud.com/pdfs/carreira.pdf>, p. 30; "Al Adler mounted image fibers in plastic and cut sections normal to the length of the fibers. He did not see any color in the cellulose." Rogers, quoted by Porter, in

<http://shroudofturin.wordpress.com/2012/05/07/the-body-image-is-created-by-molecular-change-of-linen-fibres-really/>

<sup>733</sup> Berry, "Refining a model: children's 'invisible ink' trick with lemon juice allows thermal imprinting ("scorching") at a much reduced temperature", <http://shroudofturinwithoutallthehype.wordpress.com/2012/10/25/refining-a-model-childrens-invisible-writing-trick-with-lemon-juice-allows-thermal-imprinting-scorching-at-a-much-reduced-temperature/>

<sup>734</sup> [http://en.wikipedia.org/wiki/Acid\\_free\\_paper](http://en.wikipedia.org/wiki/Acid_free_paper)

<sup>735</sup> Nicholson and Shaw eds., Ancient Egyptian Materials and Technology, p. 238,

[http://books.google.co.uk/books?id=Vj7A9jRZP0C&printsec=frontcover&dq=Ancient+Egyptian+materials+and+technology,+Cambridge+University+Press,+2000&hl=nl&sa=X&ei=pSv0UN7RC\\_Sr0AXYsoHwBQ&ved=0CC0Q6AEwAA](http://books.google.co.uk/books?id=Vj7A9jRZP0C&printsec=frontcover&dq=Ancient+Egyptian+materials+and+technology,+Cambridge+University+Press,+2000&hl=nl&sa=X&ei=pSv0UN7RC_Sr0AXYsoHwBQ&ved=0CC0Q6AEwAA)

had much more decoloring effect on starched linen than on not-treated pure linen (see fig. 4.32 and 4.33).



Fig. 4.32. A: Three pieces of linen scorched by simultaneously pressing the tip of a hot flatiron on them for 30 s. Top left: starched not-bleached linen, bottom left: (possibly chemically) bleached linen, right: unbleached linen. ©AvdH



Fig. 4.33. Baking starched and not starched linen in an oven at 160 °C. Top left: starched unbleached linen, baked for 15 min, bottom left: bleached linen, baked for 15 min, centre: unbleached linen, baked for 2 hours, right: unbleached linen baked for 7 hours. ©AvdH

If an acidic madder extract on a retrograded acidic starch film is applied to a linen cloth, it would probably also be more susceptible to oxidation and decoloration by the ozone, heat and light that accompany a corona discharge, which would allow for more gradation in areal image density before a cloth is burnt. Alizarin and purpurin are antioxidants,<sup>736</sup> and “reducing agents”, which means that they easily get oxidized.<sup>737</sup> Alizarin dusts even “may form an explosive mixture with air, and any source of ignition, i.e. flame or spark, will cause fire or explosion. ... Avoid contamination with oxidizing agents i.e. nitrates, oxidizing acids, chlorine bleaches, pool chlorine etc. as ignition may result.”<sup>738</sup>

<sup>736</sup> “The structural and electronic properties of hydroxyanthraquinone derivatives, alizarin, purpurin, pseudopurpurin, and their radicals were investigated using density functional theory. It turned out that these three molecules appear to be good candidates for high antioxidant activity species, particularly for pseudopurpurin. ... Phenols are widely used as antioxidants in living organisms.” Jin et al., 2011, Theoretical Study on the Antioxidant Activity of Alizarin, Purpurin, and Pseudopurpurin, DOI: 10.4018/978-1-60960-064-8.ch012, <http://www.igi-global.com/chapter/theoretical-study-antioxidant-activity-alizarin/48371> and <http://www.irma-international.org/viewtitle/48371/>; “The antioxidant activity of purpurin was elucidated by its bond dissociation enthalpy and ionization potential.” Jeremić et al., 2012, Antioxidant and free radical scavenging activity of purpurin, DOI: 10.1007/s00706-011-0695-z, <http://link.springer.com/article/10.1007%2Fs00706-011-0695-z>

<sup>737</sup> “Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.” <https://en.wikipedia.org/wiki/Antioxidant>.

<sup>738</sup> Alizarin datasheet <http://datasheets.scbt.com/sc-214519.pdf>

In the Raes corner, which is a very light scorch area, the madder on Raes thread R14 still behaved as a pH indicator in 2005.<sup>739</sup> The heat that lightly scorched the Raes corner apparently did not burn the madder there, which corresponds to the assumed anaerobic scorching conditions in 1532 AD, creating red-fluorescing pyrolysis products (probably polymerized/condensed furfural), at least in other scorches on the Shroud,<sup>740</sup> and to a probably higher pyrolysis temperature for alizarin.<sup>741</sup> Heller and Adler said that their radiocarbon samples (probably fibers) were “non-fluorescent”, without specifying the excitation wavelength<sup>742</sup>, but Garza-Valdes said his radiocarbon fibers were strongly yellowish-green fluorescent, also without specifying the excitation wavelength. Brown published a photomicrograph, in which Raes thread R14, under 254 nm UV light, shows blue.<sup>743</sup> This blue may be part of the excitation reflecting from a non-fluorescent thread or perhaps blue fluorescence from the neutral form of alizarin dye, which fluorescence is favoured above that of yellow-green fluorescing purpurin dye, when the excitation wavelength decreases.<sup>744</sup>

It seems improbable that the orange-brownish “UV fluorescence photograph” of the Raes corner, published by Rogers, is a pure UV fluorescence photo – it rather seems a UV+white light photo or a UV photo taken without UV filter in front of the camera –, unless there is so much cotton in both the backing cloth and the Shroud here that they fluoresce brown, like some additional small patches on the burns in the official UV-fluorescence photos published by Miller and Pellicori.<sup>745</sup> But whatever

<sup>739</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, p. 17-19

<sup>740</sup> Miller and Pellicori, *Ultraviolet fluorescence...*, 1981, p. 75

<sup>741</sup> the melting point and boiling point of alizarin: 279–83 °C and 430 °C respectively

(<http://en.wikipedia.org/wiki/Alizarin> )

<sup>742</sup> Adler, Selzer and DeBlase, *Further spectroscopic...*, 2002, TOM 94

<sup>743</sup> Brown, *Microscopical Investigation...*, 2005, fig. 3, <http://www.shroud.com/pdfs/brown1.pdf>

<sup>744</sup> “when the exciting wavelength decreases, ... the excitation is in favour of alizarin.” (excitation starting at 436 nm) (Miliani et al, *A spectrophotometric...*, 1998, p. 585); the neutral form of alizarin in water-dioxane has its fluorescence peak at 450-500 nm (blue), but the neutral form of purpurin has its fluorescence peak at 550-600 nm (green yellow), both under 377 nm excitation (Miliani et al, *Acidichromic effect...*, 2000, fig. 2 and 5, see fig. 2.36 and 2.39 above). Purpurin is a stronger fluorophor (Ibid., table 2 and 4), probably under 377 nm excitation, but under excitation at shorter wavelengths the fluorescence of alizarin is apparently favoured. Excitation at 254 nm seems a very strong excitation for alizarin (Ibid., fig. 1 = fig. 2.35 above, absorbance of alizarin); for purpurin 254 nm is beyond the absorbance plot’s scope (Ibid. fig. 3 = fig. 2.37 above).

<sup>745</sup> STURP member Rogers first presented a particular photo (<http://shroudstory.files.wordpress.com/2012/02/i4.jpg>) as the “© 1978 Vernon D. Miller” “UV fluorescence photo of the 14C sampling area” on page 22 (Fig. 18) of his 2002 paper “Scientific method applied to the Shroud of Turin” ([www.shroud.com/pdfs/rogers2.pdf](http://www.shroud.com/pdfs/rogers2.pdf)), and again on page 5 of his 2004 paper “Frequently Asked Questions (FAQs)” (<http://www.shroud.com/pdfs/rogers5faqs.pdf>). Much later, in 2012, it was analysed as such by Morgan ([http://www.academicjournals.org/article/article1380798975\\_Morgan.pdf](http://www.academicjournals.org/article/article1380798975_Morgan.pdf)). It is improbable that the photo is such a pure UV fluorescence photo because of

1) *the blue color of the blue margin*. It is also visible in white light in Benford and Marino, 2008,

<http://www.ohioshroudconference.com/papers/p09.pdf> p. 5 and Schwartz, 2012,

<http://www.shroud.com/pdfs/schwartz1p.pdf> slides 22 and 23.

2) *the white triangle of lighter backing cloth where the Raes sample used to cover it*. Compare this to the ordinary photos, published by Benford and Marino in 2008 (<http://www.ohioshroudconference.com/papers/p09.pdf> p.1, 5, 14, and 15), and especially the Holy Shroud Guild photos 1 and 2 (<http://holyshroudguild.org/discrepancies-in-the-radiocarbon-dating-area-of-the-turin-shroud-m-sue-benford-and-joseph-g-marino.html>), which show this white triangle in ordinary light, while the C14 sample is cut. What substance in the white triangle of the medieval backing cloth (but not in the rest of this cloth) could have fluoresced such a bright light? Miller and Pellicori wrote about their UV-fluorescence photography of 1978 that “The teflon coated magnets, quite visible in white light, are nonfluorescent. This signifies the rejection level of reflected light” (Miller and Pellicori, *Ultraviolet fluorescence...*, 1981, p. 76).

3) *the orange brown color of the Shroud*. This color is very different from the blueish green of the background in copies of the printed official UV fluorescence photos (e.g. Miller and Pellicori, *Journal of Biocommunication*, p. 78), but more like the pinkish orange color of a “UV photograph” of the wrist, published online by the Shroud Science group (<http://shroud.wikispaces.com/CHARACTERISTICS+OF+BLOODSTAINS>), which in turn contrasts with the greenish yellow color of another online UV photo of the wrist published by Rogers (*Scientific Method...*, p. 5, see <http://shroudstory.files.wordpress.com/2012/02/i8.jpg>). Also the greenish blue “Ultraviolet photograph” of the chest, published by Adler (*Chemical and Physical...*, 2002, TOM 14, online at Berry, <http://shroudofturinwithoutallthehype.files.wordpress.com/2012/08/fanti-screen-grab-spear-cropped.png>) showing a bright red fluorescence of scorch marks, is different from the blueish green of the printed official photograph of this area published by Miller and Pellicori (p. 81). So there is a range of different colors of the background in UV photos of

the ‘UV photo’ of the Raes corner is, the spectral anomalies found by digital processing of this photo by Morgan,<sup>746</sup> may simply represent the greasy handling dirt that Shroud conservator and textile expert Flury-Lemberg reported to be present in the corners.<sup>747</sup> Also in the Quad mosaic images, published in the STERA Image Library (which doesn’t show the alleged UV photo of the Raes corner) of STURP photographer Schwartz,<sup>748</sup> the ‘anomalous’ green color is not restricted to the Raes corner. Farey observed that the green color is even present in the bottom left hand corner of each Quad mosaic photo, even where the cloth has no corner, and attributed it, just as the blue color present in the upper half of the photos, to photographic lighting effects.<sup>749</sup>

As the fluorescence spectra of image areas show, besides a reduction of intensity, also a slight red-shift relative to a fluorescence spectrum of the background (fig. 2.34),<sup>750</sup> it might be that in the ‘blue fluorescence = no print’ observation, the cause-effect relation is the other way around: less image formation resulting in a bluer fluorescence. However, blue-vs-green mottling was also observed in the background of the Shroud (“Generally yellow-green, with blue flecks”<sup>751</sup>).

Besides, in Miller and Pellicori’s UV fluorescence photos, where “creases, thread, and shadowing” generally are less apparent, there is a clear dark/light banding, both in the bluer and greener areas, in the whole Shroud, also in the background, outside image areas, and in the fluorescence photos this is even clearer than in white light photos.<sup>752</sup> This supports the assumption that this dark/light banding reflects a property of the non-image thread (or fiber) and is not primarily caused by image formation or shadowing.

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different sources: greenish blue (Adler, chest), blueish green (Miller and Pellicori, chest and wrist), greenish yellow (Rogers, wrist), orange (Rogers, Raes corner), and pinkish orange (Shroud Science Group, wrist).

4) *the shape and viewed area of the photo*. Miller and Pellicori wrote about the 1978 fluorescence photography: “The camera and light source assembly was moved along a rail parallel to the long dimension of the Shroud, which was mounted with its short dimension vertical (Figure 2). The long dimension was divided into eight 53.3-cm square areas for photography and later full-size reconstruction. The areas were intended to be coincident with the black-and-white color separation series taken at another time. The sections were numbered from left to right, beginning at the dorsal feet end of the Shroud. The number-letter coordinate set corresponded with a master reference mosaic and is shown in Figure 3” (Miller and Pellicori, *Ultraviolet fluorescence...*, 1981, p. 73-74). Their published article shows exactly eight consecutive UV photos, all square ones, showing the body image in the middle and the two longitudinal scorch lines just along the edges, and none of them shows the Raes corner or any other corner or selvedge, or even overlaps with the UV photo of the Raes corner. Furthermore, all eight photos have darker margins at the left side of the paper (the side towards the dorsal feet), suggesting the UV lighting was insufficient at this margin of the photo. The ‘UV photo’ of the Raes corner does not show this darker margin, but rather shows signs of digital zooming-in. Perhaps the photo is a cropped part of a preliminary UV+visible light photo, taken without the visible-light filter in front of the source used in the official photographs, or perhaps a UV photo taken without the UV filter in front of the camera. It may have been taken of the ventral half of the Shroud, e.g. when the Shroud was positioned for the transmitted-light photo of the ventral half of the Shroud (see this photo at <http://www.shroud.com/vanhels2.htm> near the bottom of the page), for the orientation and the digital enlargement signs of the ‘UV photo’ of the Raes corner – see also <http://www.shroud.com/pdfs/schwartz1p.pdf> slide 23 – are the same as in the cropped transmitted-light photo of the Raes corner, published by Rogers just below the ‘UV photo’, on his p. 22, in his figures 18 and 19 of *Scientific Method*, <http://www.shroud.com/pdfs/rogers2.pdf>. The UV lighting – shielded by filters – that apparently was not sufficient for lighting the whole square area of the eight official fluorescence photos would probably not have been sufficient for lighting the whole ventral half either.

<sup>746</sup> Morgan, *Digital image processing...*, 2012, [http://www.academicjournals.org/article/article1380798975\\_Morgan.pdf](http://www.academicjournals.org/article/article1380798975_Morgan.pdf)

<sup>747</sup> Flury-Lemberg, *The invisible mending...*, 2007, p. 4 and 5, <http://www.shroud.com/pdfs/n65part5.pdf>

<sup>748</sup> STERA Image Library <http://shroud.com/gallery/pages/Quad%20Mosaic%20Dorsal%20Legs.htm> and <http://shroud.com/gallery/pages/Quad%20Mosaic%20Ventral%20Legs.htm> (both accessed 19 December 2013); cf.

Benford and Marino, *Discrepancies...*, 2008, <http://www.ohioshroudconference.com/papers/p09.pdf> p. 4-6

<sup>749</sup> Farey, *The mystery of...*, 2013, and Farey commenting on Porter’s Shroud of Turin blog at <http://shroudstory.com/2014/01/21/cat-among-the-pigeons/#comment-74160> and <http://shroudstory.com/2014/01/21/cat-among-the-pigeons/#comment-74169>, and linking to his corresponding composite figure <http://i.imgur.com/Mxfe7kS.png>

<sup>750</sup> Gilbert and Gilbert wrote on the image fluorescence spectra that “as the fluorescence level is reduced, the peak shifts slightly to longer wavelengths.” Gilbert and Gilbert, p. 1934, see also their fig. 11: <http://imagebank.osa.org/getImage.xqy?img=M3cubGFyZ2UsYW8tMTktMTItMTkzMC1nMDEx>

<sup>751</sup> Miller and Pellicori, *Ultraviolet fluorescence...*, 1981, p. 75

<sup>752</sup> “Some cloth fluorescent characteristics stand out as different from the white light appearance, namely creases, thread, and shadowing are less apparent in UV. The striation in the weave pattern (warp versus weft) are enhanced in fluorescence.” Miller and Pellicori, *Ultraviolet fluorescent...*, 1981, p. 76

#### 4.2.5. Image fluorescence

The fluorescence of the Shroud's background is greenish-yellow, and the fluorescence of the Shroud image is substantially weaker than that of the background and the peak is very subtly shifted towards the red.<sup>753</sup> Lignin of linen has a broad fluorescence in the blue-green region, with its peak in the blue at ca. 435 nm (fig. 4.27), starch or cellulose is not fluorescent, but a waterextract of madder root (*Rubia tinctorum*) fluoresces "Yellow green",<sup>754</sup> and the fluorescence peak of acid (= protonated) alizarin-plus-purpurin probably is located between 500 and 575 nm, in the green (see 4.2.3. and 4.2.4 above).

If there is a starch-madder coating on the Shroud, during image formation, this starch would probably have turned (more) yellow – for the image layer is straw yellow and more yellow than the background – and perhaps weakly blue fluorescent (ca. 475-500 nm = blue to green) if carbon nano particles were formed.<sup>755</sup> On a not-washed non-image fiber, a pale yellow madder dye on top of the starch has a relatively strong fluorescence because each of the dye's colorants alizarin and purpurin is a hydroxy-antraquinone: a fusion of three benzene rings with two and three hydroxyl groups, respectively. This number of three fused rings is the number that gives the highest fluorescence intensity in benzene derivatives, and the higher the number of hydroxyl groups on the ring the higher the fluorescence intensity.<sup>756</sup> In image formation, these optimally fluorescent colorants would probably have changed into degradation/condensation products with more or less fused benzene rings than the optimal number of three, and less hydroxyl groups – for no phenols were found on image fibers<sup>757</sup> – which degradation products therefore fluoresce much less than the original colorants.<sup>758</sup>

The effect on the total fluorescence of the image fibers would be a substantial reduction of the fluorescence intensity because of the loss of the madder's relatively strong green-yellow fluorescence and the quenching of the blue part of the lignin fluorescence by the straw yellow-reflecting and thus blue-absorbing oxidized starch-madder coating. The relative absorbance spectrum of the image areas has its maximum at ca. 320 nm and decreases almost linearly with increasing wavelengths, so the image layer absorbance is indeed much stronger in the blue than in the green or

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<sup>753</sup> "Figure 11 shows the spectral fluorescence of four body image areas compared with one clear area. Comparing this with Fig. 10 the lower the reflectance of the area, the lower is the fluorescence. Also as the fluorescence level is reduced, the peak shifts slightly to longer wavelengths." – "Comparing Figs. 11 and 13 the body image areas and scorched areas have essentially similar fluorescence characteristics. The main effect of these stains seems to be the quenching of the fluorescence of the underlying cloth. In addition these stains seem to exhibit a low level fluorescence of their own in the 600-700 nm-nm region. This lower level fluorescence is somewhat more pronounced with the scorched areas than the body image areas." (Gilbert and Gilbert, Ultraviolet-visible reflectance, p. 1934-1935)

<sup>754</sup> Dengre et al., Comparative studies of *Rubia cordifolia* Linn. and *Rubia tinctorum* Linn (Rubiaceae), *Ancient Science of Life*, 1993, Table 4, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3336544/pdf/ASL-13-165.pdf>;

<sup>755</sup> "the caramels obtained from commercially purchased sugar (following caramelization) and *jaggery* were analysed by UV-vis and fluorescence spectroscopy. ... under UV light ( $\lambda_{ex} = 365$  nm) it showed blue luminescence. ... Sugar is known to be a nonfluorescent material, but the caramel prepared upon heating sugar showed the emergence of excitation tuneable luminescence, further confirming the formation of CNPs." (CNP = carbon nano particle), see fig. 2 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3337553/figure/f2/> in Palashuddin et al., 2012, Presence of Amorphous Carbon Nanoparticles in Food Caramels, <http://dx.doi.org/10.1038%2Fsrep00383>

<sup>756</sup> "Benzene itself is only weakly fluorescent in the ultraviolet. However, when benzene is substituted by electrophilic groups, such as amino or hydroxyl, the fluorescence is increased. [...] The absorption and fluorescence bands of the hydrocarbons in which a series of benzene rings are fused in a straight chain exhibit increasing wavelengths and increased fluorescence with an increase in the number of rings and conjugated double bonds. The fluorescence yield increases from benzene to naphthalene to anthracene and then drops rapidly for the aromatic hydrocarbons containing more than three phenyl rings in a straight chain." Guibault ed., Practical Fluorescence, 2nd edition, 1990 p. 232; Table 1 on the next page, p. 233, gives the fluorescence excitation and emission maxima of benzene derivatives:

[http://books.google.nl/books?id=7eI3AV14dDsC&pg=PA232&dq=benzene+fluorescence&hl=nl&sa=X&ei=YFLqUoLQOYuY1AX72oGwDg&redir\\_esc=y#v=onepage&q=benzene%20fluorescence&f=false](http://books.google.nl/books?id=7eI3AV14dDsC&pg=PA232&dq=benzene+fluorescence&hl=nl&sa=X&ei=YFLqUoLQOYuY1AX72oGwDg&redir_esc=y#v=onepage&q=benzene%20fluorescence&f=false)

<sup>757</sup> Heller and Adler, A Chemical..., 1981, TOM 43 and 54, table 7; Heller, Report on, p. 198

<sup>758</sup> Loss of the OH-group from benzene having a OCH<sub>3</sub>-group and a OH-group, makes the fluorescence about half a strong and slightly blue-shifted (25 nm shorter peak wavelength) (Guibault, Practical Fluorescence, 1990, [http://books.google.nl/books?id=7eI3AV14dDsC&pg=PA232&dq=benzene+fluorescence&hl=nl&sa=X&ei=YFLqUoLQOYuY1AX72oGwDg&redir\\_esc=y#v=onepage&q=benzene%20fluorescence&f=false](http://books.google.nl/books?id=7eI3AV14dDsC&pg=PA232&dq=benzene+fluorescence&hl=nl&sa=X&ei=YFLqUoLQOYuY1AX72oGwDg&redir_esc=y#v=onepage&q=benzene%20fluorescence&f=false) page 233 Table 1)

red.<sup>759</sup> The color of the remaining fluorescence of the image could be slightly shifted toward the red, for substantially absorbing the blue part of the broad lignin fluorescence makes the peak of the transmitted lignin fluorescence shift towards the red and also any new blue-green fluorescence of oxidized starch could add a small peak red shift. The loss of the green-yellow madder fluorescence would have a blue shifting effect, but apparently this effect is just a bit smaller than the red shifting effects. The effects of subsequent ageing and of the 1532 AD fire on the unknown chemical composition of the image layer is hard to compare to its effect on the sealing coating of retrograded and probably polished starch and madder dye on non-image fibers.

If there is and was no starch-madder coating in the first place and the image layer thus would consist of only the linen's primary cell wall (containing hemicellulose and lignin), this yellow image layer would not only quench any blue lignin fluorescence of the remaining linen, but would probably also still fluoresce itself and in a greener color than the original blue, for it was reported that even (modern bleached) linen that apparently is not fluorescent starts to fluoresce green after having been baked in an oven and thus rendered visibly yellow.<sup>760</sup>

In already lignin-blue fluorescing linen, acid oxidation would probably have made the lignin in the primary cell wall more dense and its fluorescence greener and perhaps also a bit stronger. In a confocal and fluorescence lifetime imaging microscopy study on bagasse fibers, blue-fluorescing lignin in the cell wall of these fibers that were treated with "diluted H<sub>2</sub>SO<sub>4</sub> (1% v/v in water) for 40 minutes at 120°C"<sup>761</sup> (note that treatment with "concentrated H<sub>2</sub>SO<sub>4</sub> for half an hour" made non-image fibers look as image fibers<sup>762</sup>) turned greener fluorescent on the outermost surface of the fiber (fig. 4.28, showing fluorescence by excitation with two 770 nm photons), and the lignin content of the fiber in dry weight percentage increased from 28.2 ± 0.1 to 33.6 ± 0.7 – these were "the lignin concentrations obtained from HPLC data"; after this acid treatment, the green fluorescence of the outermost fiber surface does not look much weaker than the blue fluorescence of the core and perhaps even is a little stronger; unfortunately, the intensities visible on the photos before and after acid treatment can not be compared, because the photos may not all show the same bagasse fiber and

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<sup>759</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, Fig. 9.

<sup>760</sup> "Firstly, I cannot, at any temperature, scorch the stuff without making it fluoresce. This was quite exciting for me as most cloth these days fluoresces anyway (probably being treated with something) but the linen wasn't/didn't until I heated it. Placing a hot spatula on it, in the dark, illuminated only by the light of a Bunsen burner and the UV lamp, produces a dark scorch, of varying depth, surrounded by a clear halo of pale green fluorescence, which also fills up the scorch area itself, except where it is charred to carbon. Carrying out the experiment you suggested, of repeatedly placing the hot object against the cloth until nothing seems to occur, shows that even when the scorch is barely visible, or even not visible at all, the fluorescence is quite clear." (Hugh Farey, October 20, 2012, comment <http://shroudofturinwithoutallthehype.wordpress.com/2012/10/12/stephen-jones-bsc-grad-dip-ed-persists-in-his-mistaken-belief-that-the-shroud-image-is-a-photograph-where-the-scientific-evidence/#comment-959>); "Being a teacher, and anxious to get children to do proper research, I set them to find the lowest temperature we could make linen fluoresce at. Actually it seems a very gradual thing, and some swore they could see it after an hour at 100°C, but it was definitely obvious and quite bright at 150°C. As we were experimenting in 50°C jumps, we can't be more precise – yet..." (Farey, November 7, 2012, comment <http://shroudofturinwithoutallthehype.wordpress.com/2012/10/30/a-leisurely-thinking-aloud-appraisal-of-those-recently-released-shroud-photomicrographs/#comment-1142>); "After cooking a piece of linen for 20 minutes or so at 150C, or after smearing it with myrhh oil (or both, I guess), it glows quite cheerfully under UV light. In the first case it appears a faint yellow-brown under visible light ('yellowed with age'), and in the second it has an oily translucency." (Farey, December 31, 2012, comment <http://shroudstory.com/2012/10/21/thibault-heimburger-now-i-know-for-sure-that-the-ts-body-image-is-not-a-scorch/#comment-21381>); "Having nothing better to do, I shoved a bit of linen into a glass pipe today, and passed methane out of a gas tap through it (and lit it the the other end so as not to die). Then scorched the linen with a Bunsen. There could not be circumstances of less oxygen surrounding the linen. After letting it all cool down, but with the methane still passing over it, I pulled it out and looked at under UV. The same greeny-yellow as ever." (Farey, December 15, 2012, <http://shroudofturinwithoutallthehype.wordpress.com/2012/12/07/lets-poke-em-with-hokum/#comment-1472>).

<sup>761</sup> Coletta et al., 2013 <http://www.biotechnologyforbiofuels.com/content/6/1/43>: "Samples of sugarcane bagasse were treated as described in [1]." 1 =Rezende 2011: "diluted H<sub>2</sub>SO<sub>4</sub> (1% v/v in water) for 40 minutes at 120°C"

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3377919/?tool=pubmed>

<sup>762</sup> There was similarity in "color", "chemistry" and in their corroded appearance under phase contrast microscopy (Heller, Report on..., 1983, p. 200).

the gain in the detectors was adjusted for each photo.<sup>763</sup> So, contrary to alkali treatment,<sup>764</sup> the acid treatment did not remove lignin (a “complex polymer of aromatic alcohols”, viz. of ‘single ring’ phenols<sup>765</sup>) but seems to increase its density, and thereby its fluorescence wavelength, and possibly also its intensity a little, at least under two photon excitation.<sup>766</sup> The same fluorescence green-shift due to concentration increase takes place under ordinary one photon excitation, for instance in pyrene, consisting of four fused benzene rings: “benzene derivatives ... Changes in concentration have also been reported to affect the fluorescence spectrum. As the concentration of pyrene in xylene

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<sup>763</sup> Coletta et al., Mapping the lignin distribution in pretreated sugarcane bagasse by confocal and fluorescence lifetime imaging microscopy, *Biotechnology for Biofuels* 2013, 6:43, full version online at <http://www.biotechnologyforbiofuels.com/content/6/1/43/>; see Fig. 6, <http://www.biotechnologyforbiofuels.com/content/6/1/43/figure/F6>, in which “The fluorescence spectra were normalized at 478 nm.” (article text), and see table 1 <http://www.biotechnologyforbiofuels.com/content/6/1/43/table/T1>. In a personal communication in July 2013, one of the authors of the study wrote “It is a difficult task to rank intensities for samples in figure 6. We were concerned about changes in the spectral shape instead of in intensity. We had to adjust the confocal conditions (mainly the gain in the detectors) for each bagasse fiber to avoid intensity saturation of the 2 photons detectors and to get the ideal count rate for the FLIM measurements in order to suppress after-pulse effects. So, unfortunately, the intensities are not comparable between fibers in Fig.6, but only spectral shape and decay of intensity.” He also wrote “It seems that the acid treatment affects the outermost regions of the fibers. The intensity is not so conclusive because of the broadening of the emission spectrum. In this case the intensity is a little higher in outermost regions as a result of line broadening (the total intensity should be higher in this case).” Here must be taken into account that the individual spectra were all normalized at 478 nm, so, a broader spectrum does not necessarily have a stronger total intensity. Yet, the author, probably on account of the raw spectra, states that the intensity is a little higher in outermost regions.

<sup>764</sup> “NaOH treatments are known for removing lignin from biomass [8,15,16] and result in a considerable decrease of the lignin concentration in the studied samples [1].” Coletta et al., 2013, <http://www.biotechnologyforbiofuels.com/content/6/1/43/>

<sup>765</sup> <http://en.wikipedia.org/wiki/Lignin>

<sup>766</sup> Although no direct mention is made of the fluorescence intensity after acidic treatment, the FLIM article does say for one-photon excitation: “The lower lignin contents in the fibers are thus associated to slower decays.” ... “The displacement of the decay times (Figure 4a) and the variations of their amplitudes (Figure 4b) can be associated to changes in the excited state (exciton) dynamics. This is assigned mostly to the rearrangement and the removal of lignin rather than to the chemical modification of this molecule, caused by the NaOH pretreatment. This statement is consistent with the fact that a considerable fraction of the lignin has been removed by the alkali pretreatment under the conditions applied in this study. Moreover, closely packed molecular arrangements, as those found in the lignin film, favor long range dipole-dipole interactions that, consequently, introduces additional energy transfer channels that compete with its internal relaxation and emission. These concurrent processes are also responsible for exciton migration among lignin molecules, which also increases the probability of quenching at non-radiative sites. All these competing mechanisms raise substantially the exciton radiative decay rate. On the other hand, NaOH pretreatments remove lignin, reducing competing non-radiative channels and migration, which may extend the radiative decay rate.”

And for two-photon excitation: “However, the emission broadens considerably in the range of high wavelengths comprising the visible and the near infrared region. This is a clear indication that the natural lignin arrangement in the untreated bagasse has been strongly changed by the acid treatment, in such a way that new 2P excited states are formed in the very-low-energy tail of lignin density of states.” ... “Great disorder may occur during acid pretreatment because solubilized lignin molecules react with monomers and oligomers to form larger molecules [29], thus affecting the molecular structure of this polymer and its stable or metastable conformations at nanoscale [7]. ... These results show therefore that lignin substructures definitely contribute to its emission and decay properties.” ... “Raw bagasse results presented on Figure 6 show only a slight spectral broadening and a net decrease of 10% in the mean decay time when the region close to the outermost interface of the fiber is compared to its middle region. This is consistent with previous reports on the lignin enriched region in the cell wall boundaries of plants [7]. This scenario changes significantly for the bagasse treated with 1% H<sub>2</sub>SO<sub>4</sub> (Figures 6b and 6b’). The large spectral broadening due to lignin self-interaction, as observed in molecular aggregates, transforms the color image to blue-greenish in the middle region and to green-yellowish close to both cell wall boundaries. When we compare with the native bagasse fiber, the acid treatment produces a reduction of 11% and 7% in the mean decay time evaluated near the outermost interface and in the middle of the fiber, respectively. According to Figure 4a, this corresponds to a variation of about 5% in the lignin fraction.” ... “Our experiments indicate that in untreated bagasse, lignin is arranged in a loose manner with relatively weak interaction between lignin macromolecules. The acid treatment aggregates the non-solubilized lignin thus increasing a number of different nanoenvironments around each molecule whereas the alkali treatment solubilizes and removes lignin.” Coletta et al., *Biotechnology for Biofuels*, 2013, <http://www.biotechnologyforbiofuels.com/content/6/1/43/>

increases, the fluorescence changes from blue to green, but the absorption spectrum remains unchanged”.<sup>767</sup>

#### 4.2.6. SEM-EDS analysis – smooth organic coating embedding particles

In a 2012 article, Fanti, Calliari, and Canovaro published a SEM image of a roughly coated/encrusted linen Shroud fiber, and its EDS, which shows C, O and Ca peaks; another linen Shroud fiber was shown, with a very smooth coating, rendering the linen’s “transverse striations” invisible, and with a single particle adhering to it. The EDS spectrum of the fiber shows O, C, Ca and S.<sup>768</sup> The linen fibers examined by both SEM and EDS were taken from the dust that was vacuumed from between the reverse side of the Shroud and the backing cloth. The article text says that “The detailed analysis shows that the deposits present on the fibers are composed in most cases of Calcium (Ca), therefore we can suggest the presence of Calcium Carbonate (CaCO<sub>3</sub>) encrustations, see Fig. 8 and 9”, and the conclusion even states “Some fibers show the presence of inorganic impurities on their surface; these encrustations seem of calcium carbonate”, without having specified why the impurities are called “inorganic”. Morris, Schwalbe and London said that the calcium and strontium they detected on the Shroud by X-ray fluorescence, “possibly represent airborne dust deposits”, referring to “the rich marble and limestone regions of northern Italy”; they said that, “Although other explanations are possible, the uniform calcium and strontium distributions might be explained simply as dust accumulations.”<sup>769</sup> But this airborne dust would not be “encrusted” to an otherwise uncoated and dry fiber, but loosely embedded between the threads and fibers of the cloth. CaCO<sub>3</sub> in the configuration of a coating would be limescale, which only precipitates from hard water. But calcium is uniformly present all over the Shroud in a relatively large concentration: “X-ray radiographs of the Shroud were taken employing a medical type diagnostic instrument. ... Under the conditions employed, one expects strong absorption characteristics for the element calcium, the evidence for which is confirmed chemically and spectroscopically to be in the linen fibers. This explains why the cloth weave is seen clearly”.<sup>770</sup> A limescale contamination of this scale and uniformity is improbable on a cloth that only shows waterstains in a well restricted diamond shapes in a symmetric pattern. Therefore, the third explanation for the uniform presence of calcium is most probable. It was offered by Heller and Adler, and it says that calcium and strontium and iron got chelated (= covalently bound) to the linen during the retting of the flax.<sup>771</sup> This means that also on the single fibers under SEM and EDS investigation, the observed calcium may simply be present between the linen and the starch-plus-madder coating.

A starch-plus-madder coating is just as “invisible” to EDS as the linen itself, as both are organic (containing C, O, and H). Of course, at manufacture of the cloth, an occasional CaCO<sub>3</sub> (or CaSO<sub>4</sub>) dust particle may have settled on the outside of the drying starch-plus-madder coating as well.

Rogers said that, except on fibers from bloodstain areas, no sulfur compounds were found on the fibers from the sticky tape samples of the observe surface of the Shroud. X-ray fluorescence spectroscopy on the observe side of the cloth – in which hydrocarbons strongly suppress the lower energy peaks of material such as Ca and S on the reverse side, could not detect sulfur anyway, as its

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<sup>767</sup> <http://en.wikipedia.org/wiki/Pyrene>; Guibault, Practical Fluorescence, 1990, 2nd edition, p. 232

[http://books.google.nl/books?id=7eI3AVI4dDsC&pg=PA232&dq=benzene+fluorescence&hl=nl&sa=X&ei=YFLqUoLQOYuY1AX72oGwDg&redir\\_esc=y#v=onepage&q=benzene%20fluorescence&f=false](http://books.google.nl/books?id=7eI3AVI4dDsC&pg=PA232&dq=benzene+fluorescence&hl=nl&sa=X&ei=YFLqUoLQOYuY1AX72oGwDg&redir_esc=y#v=onepage&q=benzene%20fluorescence&f=false)

<sup>768</sup> Fanti et al., Analysis of micro-particles..., 2012, p. 8-9 and Fig. 7-9

<sup>769</sup> Morris, Schwalbe and London, X-ray Fluorescence..., 1980, p. 45, 46

<sup>770</sup> Adler, Chemical and Physical..., 2002, TOM 17

<sup>771</sup> “The data indicate generally uniform distribution of calcium and strontium over the investigated areas of the cloth. ... Substantially non-uniform concentrations of iron were observed, particularly at the dorsal-foot and side-wound ‘blood’stain regions.” Morris et al., X-ray fluorescence..., 1980 ; “The uncoated fibrils (non-image, image, and water-stain) all give relatively strong positive tests only for Ca and Fe, except for those from the inside of the water stains which give somewhat weaker, though still definite positive reactions.” (Heller and Adler, A Chemical..., 1981, TOM 41). Binding of Ca and Fe and Sr during retting: Heller and Adler, A Chemical..., 1981, TOM 45-46; cf. Heller, Report on..., 1983, p. 174)

atomic number 16 was below the detection limit of the equipment.<sup>772</sup> Yet, Fanti found a sulfur peak next to a calcium and oxygen and carbon peak in an EDS spectrum of a non-image fiber that was vacuumed from the reverse side of the Shroud.<sup>773</sup> This sulfur peak might be due to calcium sulfate dihydrate (gypsum), which is a common mineral and can be present, besides calcium carbonate, in clay. Also free mineral particles found in the dust vacuumed from between the reverse side of the Shroud and the backing cloth were analysed: “those mineral particles are very similar to the typical local clay of Jerusalem (and of other Mediterranean areas influenced by the winds of the Sahara desert). They seem to belong of the Illite-Smectite family also containing gypsum.”<sup>774</sup>

#### 4.2.7. Microscopy - Red aluminum lake particles

Red madder lake particles were found in the Raes corner. Rogers photomicrographed them on fibers from the Raes corner, and analysed them and wrote that “The red lakes are diagnostic for Madder root dyes and alum. The solubility characteristics of the red lakes indicate  $\text{AlO}(\text{OH})$ ”, i.e. “hydrous aluminium oxide [ $\text{AlO}(\text{OH})$ ]”, and that “With the gum invisible or swelled lightly in water, it is easy to see the lakes suspended in the gum and stuck to the fibers. Fig. 3 shows (upper left) colloidal red dye lakes suspended in the gum”; on a few “blue lakes” which he analysed, he wrote that “The color suggests alizarin on crystals of calcite or aragonite in the threads”.<sup>775</sup> Fanti and Basso made and published photomicrographs of similar-looking red particles on yellow fibers vacuumed from a relatively large area around the Raes corner, labelled “i”.<sup>776</sup>

##### 4.2.7.1. Similar look of Raes and 3CB (small of back blood area) red particles

It is interesting that also Maloney reported that madder was observed. Unfortunately, he used the terms “rose madder” and “madder rose dye” for the yellow dye that was seen on the Raes samples by Rogers<sup>777</sup>, and also used the term “madder rose” for the red particles seen elsewhere by McCrone, so it is hard to know what he means in the first sentence of this quote: “In photomicrographs the madder rose appears to have been deposited on the cloth while wet. To make the matter more suspicious yet, the madder rose was found on sticky tapes 3-CB and 3-AB both of which were taken from the blood flow area across the back.”<sup>778</sup> A photomicrograph of red-brown particles (on a fiber, though this is not visible) on tape 3CB is online in McCrone’s article of 1990, and another photomicrograph of the same tape 3CB is online on the Facebook page of the Holy Shroud Guild,<sup>779</sup> and a larger one of 3CB

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<sup>772</sup> “The relatively large proportion of calcium (~ 1 w %) is most likely underestimated in these results because no account was taken of its distribution within the cloth. X-ray attenuation by hydrocarbons is greatest at lower energies and in these measurements would strongly suppress the calcium peak.” (Morris et al., X-Ray Fluorescence..., 1980, p. 45); the EDS peak of Ca is at ca. 3.60 and that of S is at ca. 2.30 (Fanti et al., Analysis of micro-particles..., 2012, Fig. 19), so S is at an even lower energy than Ca. “The available equipment allowed detection of elements with atomic numbers greater than 16.” (Morris et al., X-ray Fluorescence..., 1980, p. 40).

<sup>773</sup> Fanti et al., Analysis of micro-particles..., 2012, Fig. 9,

<sup>774</sup> Fanti et al., Analysis of micro-particles..., 2012, p. 13

<sup>775</sup> Rogers, Studies on..., 2005, <http://www.shroud.it/ROGERS-3.PDF> p. 191-192

<sup>776</sup> Fanti and Basso, Statistical analysis..., 2008, <http://www.ohioshroudconference.com/papers/p16.pdf> fig. 2, p.3 and fig. 16, p. 8

<sup>777</sup> Maloney, Presentation Ohio 2008, <http://www.shrouduniversity.com/osucon08/audio/paulmaloney.mp3> at 19:08 and 22:27: “This is what Rogers’ paper in *Thermochimica Acta* does. It confirms the presence of the mordant, aluminum, and reveals the presence of two other items: rose madder and gum Arabic.” (18:57-19:10); “The nature of the repaired area, if it exists, seems to be characterized by a combination of the following five components, based on Ray Rogers’ research: #1. linen-cotton spun yarns, spliced into the Shroud cloth and showing a coating of 2. starch, 3. aluminum mordant and other metallic salts, 4. gum Arabic binder, and 5. madder rose dye.” (21:57-22:30). Compare “a mixture of gum Arabic, mordant and madder root dye” (24:54-59).

<sup>778</sup> Maloney, Science, Archaeology..., 1998, <http://www.freeinquiry.com/skeptic/shroud/as/maloney.html>

<sup>779</sup> McCrone, The Shroud of Turin..., 1990, Fig. 6, [http://www.mcrci.org/CMSuploads/the\\_microscope\\_%20shroud.pdf?PHPSESSID=80273de2627d22c16744a4492a689496](http://www.mcrci.org/CMSuploads/the_microscope_%20shroud.pdf?PHPSESSID=80273de2627d22c16744a4492a689496) (in his book *Judgement day*..., 1999, p. 90-91, McCrone calls the photo of this figure 6 “Figure 22. Another blood-image area fiber with red ochre particles magnified 1500 times”, while “Figure 21. Typical red particles on a 3-CB blood-image linen fiber magnified 600 times.” This figure 21 of the book is not one of the figures in the 1990 article); “3CB FeO<sub>3</sub> Red Hematite ©McCrone” on

of McCrone is online on the site of the Holy Shroud Guild.<sup>780</sup> These last two photomicrographs show bright red particles which are not on a fiber, but stuck in the adhesive of the tape, while also air bubbles between tape and microscope slide are seen. Also various photomicrographs of some red dots on a fiber of sample 6BF as seen by Kohlbeck – one before and the other after having been mounted for a few months in Cargille oil – are on the Facebook page and on the site of the Holy Shroud Guild.<sup>781</sup>

At the Ohio conference of 2008, Maloney, to whom Walter McCrone in 1981 had sent several Kodak transparencies of photos he took of Shroud linen fibers,<sup>782</sup> told about McCrone's Kodak slides: "On those slides, McCrone had written the following note: ["madder rose, linen fiber, medium (blue) sample 3 CB" and sample 3-AB, next slide please, which came from the blood flow across the back nearest the side-strip side of the Shroud and directly adjacent to that flow on linen, itself."<sup>783</sup> The original STURP map, published by the Holy Shroud Guild, indeed locates sample 3AB in the small of back area, but it is unclear whether or not there is a red color at the 5 cm<sup>2</sup> location of sticky tape 3AB.<sup>784</sup> Heller and Adler, who received this sample from Rogers who took the sticky tapes samples in 1978, designate sample 3AB as "Body image area, back, adjacent "lance" wound".<sup>785</sup> In the sample map published by STURP photographer Schwartz, sticky tape sample 3AB is called "Control" and even located in a clear area beside the hip image, not in or near a red flow at all.<sup>786</sup> McCrone, who first got the sample from Rogers, calls sample 3AB "control near right side wound".<sup>787</sup>

So, it seems sample 3AB was not from an actual blood flow itself, but from a clear area adjacent to it. In this case, its red particles might indeed be aluminium-madder lakes – just as in the Raes corner – that perhaps formed when hydrous aluminium oxide particles got stuck to a wet Shroud, for instance right after most of the starch had been washed off, or after the Shroud had had a madder dye bath. Fanti reported that aluminum is one of the elements that are present in mineral particles coming from Mt. Zion and in particles vacuumed from the back of the Shroud; also Kohlbeck detected aluminium in both Jerusalem limestone and in limestone from the Shroud.<sup>788</sup> If many red particles, stuck to the main Shroud, also in non-blood areas, are aluminum-madder lakes, this may mean that madder dye on the entire main Shroud was minimally pre- or post-mordanted by airborne dust particles. Another source of aluminum-madder lake might be the 'sanctification' of painted Shroud

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<https://www.facebook.com/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater#!/photo.php?fbid=10201282441565738&set=oa.10151506022204822&type=1&theater>

<sup>780</sup> [http://www.holyshroudguild.org/uploads/2/7/1/7/2717873/3605614\\_orig.jpg](http://www.holyshroudguild.org/uploads/2/7/1/7/2717873/3605614_orig.jpg) on

<http://www.holyshroudguild.org/drmccrone6.html>

<sup>781</sup> "6BF Red particles on flax no Cargille oil ©Kohlbeck" on

<https://www.facebook.com/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater#!/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater>, cf. "6BF Remounted in Cargille type A Immersion oil 1.515. Red particles have turned black and exuded a yellow substance. ©Kohlbeck" on

<https://www.facebook.com/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater#!/photo.php?fbid=10201282418765168&set=oa.10151506022204822&type=1&theater>;

<http://www.holyshroudguild.org/drmccrone6.html>; different photos, more directly comparable, of the same phenomenon on the 6BF tape are online at [http://www.holyshroudguild.org/uploads/2/7/1/7/2717873/3561958\\_orig.jpg](http://www.holyshroudguild.org/uploads/2/7/1/7/2717873/3561958_orig.jpg) and in the slide show on [http://hollyshroudguild.org/dr-raes-problematic-threads\\_2.html](http://hollyshroudguild.org/dr-raes-problematic-threads_2.html)

<sup>782</sup> Bracaglia, Dr Raes problematic threads, part 2 [http://hollyshroudguild.org/dr-raes-problematic-threads\\_2.html](http://hollyshroudguild.org/dr-raes-problematic-threads_2.html)

<sup>783</sup> Maloney, Presentation Ohio 2008, <http://www.shrouduniversity.com/osucon08/audio/paulmaloney.mp3> from 19:41 (also transcribed in Marino and Prior, Chronological History..., 2008, <http://shroud.com/pdfs/chronology.pdf> p. 3-4 and on [http://hollyshroudguild.org/dr-raes-problematic-threads\\_2.html](http://hollyshroudguild.org/dr-raes-problematic-threads_2.html)); these slides seem to be owned by the Holy Shroud Guild now: [http://hollyshroudguild.org/dr-raes-problematic-threads\\_2.html](http://hollyshroudguild.org/dr-raes-problematic-threads_2.html)

<sup>784</sup> Nitwoski, Criteria for the Authentication..., 1986, <http://hollyshroudguild.org/dr-nitowski-new.html>, 2<sup>nd</sup>

Scribdocument, p. 19

<sup>785</sup> Heller and Adler, A Chemical..., 1981, Table 1, TOM 49.

<sup>786</sup> Schwartz, Mapping of..., <http://www.shroud.com/maptap2d.htm>

<sup>787</sup> McCrone, Judgement Day..., 1999, p. 93

<sup>788</sup> Fanti, Analysis of micro-particles..., 2012, Fig. 15-19; Villarreal and Villarreal, A new look..., 2012 presentation slide 25 of <http://www.shroud.com/pdfs/villarrealvppt.pdf>, and text on <http://www.shroud.com/pdfs/villarrealvtxt.pdf>

copies by touching them with the real Shroud,<sup>789</sup> but these paint pigments would not have stuck to the Shroud while wet.

Yet another possibility is that some of the red particles on 3AB and 3BC and perhaps even some of those on Raes fibers are heme-madder lake particles, as seems indicated by the observations during MOLE experiments and after mounting the red particles of 6BF in Cargille oil (see 2.1.2.6)<sup>790</sup>. The red particles on fibers of the Raes corner, as photomicrographed by Fanti (vacuumed dust), and those of the main Shroud, as photomicrographed by McCrone (sample 3CB) and by Kohlbeck (sample 6BF – lance wound area),<sup>791</sup> do look alike. The metal atoms of aluminium oxide ( $Al^{3+}$ ) and acid heme ( $Fe^{3+}$ ) have the same electrical charge and therefore would probably give about the same reddish color to the madder when complexed with it. Blood deposits which abraded – probably methemoglobin crystals and heme aggregates – from their original locations, could have been transposed all over the Shroud, even to the Raes corner. McCrone, who interpreted both the blood and image chromophore as red ochre, wrote “I have looked pretty carefully at about a dozen of the tapes & find plenty of red particles all like the blood(?) on 3-CB. The amount of degree of aggregation varies considerably. There is a great deal on 3AF (fingers), 3EF (wrist), 3CB, & 6AF (both right side wound). There seems to be less on purely body image (no blood) tapes but there is some, e.g., 1FB right calf – lots of small red particles.”<sup>792</sup> Tape sample 1FB is called “Image: Blood on Scourge Mark” in Schwartz’ tape sample map.

McCrone also wrote “I had also observed that the red ochre particles are not lying loosely on the fibers but must be dislodged with a fine needle. They seem to be “glued” to the fiber surface. A thin paint layer would be the only way to duplicate their good dispersion as single particles and their attachment to the fiber surfaces.”<sup>793</sup> Two other ways would be the complexing of dry airborne dust particles to wet madder dye and the complexing/adsorption of abraded heme aggregates and methemoglobin crystals to dry madder dye.

#### 4.2.7.2. Chemical testing procedure depending on microscopical observation and location

Only if at least 15 more or less identical looking particles were present on samples of a specific location on the cloth, they were considered a particle “class” or “type” and then chemically analysed as such: “Therefore, we have arbitrarily set a minimum threshold of 15 specimens of a particular set of characteristics to constitute a class of fibrils and particles typical of a specific location on the cloth. These classes, their “locations”, and microscopic characteristics are given in Table 2.”<sup>794</sup> These characteristics were “mainly color and surface appearance under phase contrast microscopy”.<sup>795</sup> Also later, before FTIR testing, certain fibers “were removed from the tape, and their identity as to type verified by the methods previously described in the chemical study”<sup>796</sup>. In this regard, Heller calls the difficulty to tell red particles apart a “Catch-22 situation”: how is one to classify and count particles before they are actually tested, and how is one to test a particle type without having gathered enough

<sup>789</sup> Ford, *The Shroud of Turin’s...*, 2000, p. 17

<sup>790</sup> Bracaglia, Dr Nitowski’s..., <http://holyshroudguild.org/dr-nitowski-new.html> and <https://www.facebook.com/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater#!/photo.php?fbid=10201282418765168&set=oa.10151506022204822&type=1&theater>, and the slide show on [http://holyshroudguild.org/dr-raes-problematic-threads\\_2.html](http://holyshroudguild.org/dr-raes-problematic-threads_2.html)

<sup>791</sup> “6BF Red particles on flax no Cargille oil ©Kohlbeck” <https://www.facebook.com/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater#!/photo.php?fbid=10201282400324707&set=oa.10151506022204822&type=1&theater>; Figure 6 of McCrone, *The Shroud of Turin: Blood or artist’s pigment?*, [http://www.mcric.org/CMSuploads/the\\_microscope\\_%20shroud.pdf?PHPSESSID=80273de2627d22c16744a4492a689496](http://www.mcric.org/CMSuploads/the_microscope_%20shroud.pdf?PHPSESSID=80273de2627d22c16744a4492a689496)

<sup>792</sup> McCrone, *Judgement Day...*, 1999, Notebook entry for Jan. 2, 1979, p. 83

<sup>793</sup> McCrone, *Judgement day...*, 1999, p. 100

<sup>794</sup> “Therefore, we have arbitrarily set a minimum threshold of 15 specimens of a particular set of characteristics to constitute a class of fibrils and particles typical of a specific location on the cloth. These classes, their “locations”, and microscopic characteristics are given in Table 2.” (Heller and Adler, *A Chemical...*, 1981, TOM 37)

<sup>795</sup> Adler, *The Shroud fabric...*, 2000, TOM 119

<sup>796</sup> Adler, Selzer and Deblase, *Futher Spectroscopic...*, 2002, TOM 94, referring to Heller and Adler, *A Chemical...*, 1981

microparticles to perform the test on?<sup>797</sup> Besides, if red aluminum oxide madder lake particles were present on all types of locations – non-image (3AB), bloodstain (3CB, 6BF), waterstain (Raes corner), and scorch (Raes corner) – they had no chance to become a type of a specific location anyway.

#### 4.2.7.3. “Blood” also meaning heme/heme madder lake

Heller wrote about “red spots” that “most of them” “were blood”, but this quantitative estimation (“most”) was only made by seeing how many of the red spots that were microspotted with hydrazine dissolved: “We knew that a microdrop of hydrazine would let us know rapidly if a particle was blood. If it was not, we would collect it for assay”; Heller and Adler were “harvesting” non-dissolving “red dots” for further testing – if these seemed a ‘type’ – and apparently did not test all hydrazine-dissolved dots for both a hemochromagen color and protein as well.<sup>798</sup> But, as “A number of tests on the Spanish linen fibrils showed that 97% hydrazine (N<sub>2</sub>H<sub>4</sub>) served as the best reductant with the additional convenient property of solubilizing even strongly denatured or aged samples”<sup>799</sup>, this hydrazine would also have reduced and dissolved the heme – the iron-containing porphyrin of denatured blood – of free or abraded heme aggregates, and probably also heme(III)-madder lakes.<sup>800</sup> Oddly, McCrone wrote that “None of the red image-area particles are soluble in hydrazine” and also that “None of the particles in blood-image areas dissolve in hydrazine”, but this probably were not conclusions from actually performed hydrazine tests – note that he did not use the Simple Past “were soluble” or “dissolved” –, but conclusions from his microscopic observations concerning refractive index differences during focusing.<sup>801</sup> Heller and Adler’s hemochromagen color test that was done

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<sup>797</sup> Heller, Report on..., 1983, p. 190-191

<sup>798</sup> “First, we determined which of the red spots were blood. We proved that most of them were, because (1) they dissolved in hydrazine, (2) produced hemochromagen, a pale pink material, and (3) gave a positive test for protein. Iron oxide does none of these things. Nor does any other metal oxide or salt.” Heller, Report on..., 1983, p. 191

<sup>799</sup> Heller and Adler, A Chemical..., 1981, TOM 31; Note that the team of Frache (G. Frache, E. Mari Rizzati, E. Mari, Relazione conclusiva sulle indagini d'ordine ematologico praticate su materiale prelevato dalla Sindone, in La S. Sindone. Ricerche e studi della Commissione di Esperti nominata dall'Arcivescovo di Torino, Card. Michele Pellegrino, nel 1969, suppl. *Rivista diocesana Torinese* (1976)

[http://it.cathopedia.org/wiki/Studi\\_scientifici\\_sulla\\_Sindone#cite\\_note-19](http://it.cathopedia.org/wiki/Studi_scientifici_sulla_Sindone#cite_note-19)), which had examined a bloodstained thread taken from the Shroud in 1973, reported in 1976 that “the pigmented encrustations did not pass into solution in the solvents, acids and the alkalis we used.” (English quote in McCrone, Judgement Day..., 1999, p. 11). According to McCrone they used “sulphuric acid” before looking for porphyrin fluorescence, “sodium carbonate solution” before looking for a hemochromagen spectrum, and – McCrone quoting a translation of the report – “extractive incubation in alkaline solution” before performing thin-layer chromatography with “a solvent system made up of a mixture of methanol, acetic acid and water (proportions 90:3:7)”, all giving negative results (no fluorescence, hemochromagen color, or fluorescence/benzidine color/hydrogen peroxide color, respectively) (McCrone, Judgement day..., 1999. p. 10). This apparent inability to dissolve the red encrustations might mean that had tried to dissolve heme-madder lake.

<sup>800</sup> Biochemist Berry: “If there are biomedical people on the team, it goes without saying that one uses mild and chemically-inert reagents (detergents, organic solvents etc ) to weaken associations. Adler’s resort to hydrazine to solubilize blood should frankly never have happened. That was a failure on the part of Adler, technically (he especially should have known one does not use a chemically-reactive substance to free the molecules one is trying to isolate) and [...]. No haematologist, biochemist or physiologist would have employed hydrazine in an extraction procedure”

<http://shroudofturinwithoutallthehype.wordpress.com/2013/11/27/sturp-got-its-priorities-entirely-wrong-result-no-real-scientific-insights/>

“Hydrazine is a chemist’s sledgehammer – not what you need in a physiological milieu. ... The deployment of ferocious agents like hydrazine was frankly incomprehensible. One simply does not do that in biochemistry and physiology.”

<http://shroudofturinwithoutallthehype.wordpress.com/2013/11/11/heres-what-alan-d-adler-should-have-done-to-investigate-the-porphyrins-of-those-anomalous-shroud-bloodstains/>

“Adler had to resort to heroic measures simply to get the stuff into solution, i.e. using hydrazine”

<http://shroudofturinwithoutallthehype.wordpress.com/2012/07/20/no-dear-yannick-adler-and-heller-did-not-prove-it-was-real-blood-on-the-shroud-of-turin-even-if-some-real-blood-is-now-present/> ; Perhaps, of heme aggregates stuck to madder, the actual heme-madder interface surface, where heme is complexed to madder, would not have dissolved, but in larger aggregates, the bulk of the heme would have, and this would be consistent with Heller’s description that “most of” the red dots were dissolving spots.

<sup>801</sup> McCrone, Judgement day..., 1999, p. 166 and 168, respectively; “Blood or any low refractive particles show a dark center under the same focusing conditions. On examining thousands of red image particles on the Shroud tapes, I saw no low refractive index red particles except rose madder particles and a few red silk fibers (from the Shroud wrapping cloth).

with hydrazine on individual fibrils with red dots was positive,<sup>802</sup> but would not only have been positive on (met)hemoglobin but also on heme. Their “positive test for protein”, on the other hand, would not have been positive on mere heme, but this test, the Biuret-Lowry test, had been done on fibrils from “a bloodstain area”<sup>803</sup> – which could have contained protein-loaded serum and the protein hemoglobin beside heme and heme-madder lakes – and most probably wasn’t done again on the microspotted red dots after they had dissolved in hydrazine. So, some of the dissolving red dots may, besides methemoglobin, just as well have been heme aggregates and perhaps abraded heme-madder lake fragments. The red particles on not specified fibers that were dissolved by proteases, on the other hand, may have been dry methemoglobin or dry heme particles that had abraded from smoothly red-coated fibrils of bloodstain areas (see 2.1.2.7).

#### 4.2.7.4. Occasional aluminum madder lake

If the red dots did not dissolve in hydrazine, they were collected “for assay” = further testing, but no systematic description is given of the results of such an assay of the thus collected red dots. Heller’s book first tells about the harvesting of non-dissolving red dots and then simply continues by telling about the gathering of red iron-oxide dots: the “several tapes” from which “red iron-oxide dots” were isolated<sup>804</sup> – probably called and gathered as such because the particles were birefringent – may have been some of the waterstain margin tapes 2AF, 2BF, 3BB, 3FB, 9BF, and 9CF that Heller and Adler had. So, that these particles were uncontaminated pure iron-oxide, i.e., without aluminum, doesn’t mean that some of the other red particles that had already been gathered “for assay” from other areas were not aluminum-madder lakes.

Note, that not only McCrone, but also Heller and Adler determined a “class” or “particle type” only by how they saw them. In fact, Heller and Adler did see madder particles, but apparently not enough on a single location, or too much in all locations, to consider them a “type” or “class”; Adler wrote that madder rose particles were a serious type of contaminant but “excluded” from testing: “A somewhat more serious type of contaminant is the occasional appearance of materials that can be clearly identified as artistic pigments such as rose madder or cinnabar, etc. ... For a given tape, an arbitrary minimum threshold of 15 specimens of a particular type of visually identifiable characteristics (mainly color and surface appearance under phase contrast microscopy) was set to constitute a class of fibers of particles assignable to a specific location on the cloth to be subjected to chemical testing. ... Carrying out this prescription excluded all the various types of contaminants discussed above”.<sup>805</sup> Madder lake or madder rose is indeed not listed in Table 2 of their article. Then, after this exclusion of madder rose, Heller said they only “examined every particle type” and “could not corroborate” McCrone’s observation on madder rose.<sup>806</sup> This procedure and phrasing means that they could not refute it either, especially as McCrone did not specify how many “madder” particles he had seen on samples 3CB and 3AB and how they looked. Ford wrote “McCrone believes he saw merely “a few particles” of rose madder pigment”<sup>807</sup>, and, in his book on the Shroud, McCrone defined “madder” as “1,2-dihydroxyanthraquinone·Al(OH)<sub>3</sub>” and writes “The ratio of the number of red ochre particles to the number of other extraneous colored particles is at least 1,000:1. Most of the red ochre particles are very tiny, less than 1 micrometer ... in diameter. Since the extraneous but rare particles may be of interest, I will list them: silk, ... pigments (madder, orpiment, yellow ochre,

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None of the red image-area particles are soluble in hydrazine.” (McCrone, Judgement day..., 1999, p. 166, underlining is McCrone’s)

<sup>802</sup> Heller, Report on..., 1983, p. 165

<sup>803</sup> This protein test was done in Colorado Springs (Heller, Report on..., 1983, p. 159-160, 163-164), before Heller and Adler took the tape samples to Connecticut and did further chemical investigations (Heller, Report on..., 1983, p. 168).

<sup>804</sup> From the not-dissolving dots, it seems that only iron-oxide dots were considered a ‘type’ and therefore tested further. “As I was harvesting red dots.... We began to gather microscopic red iron-oxide dots. ... I isolated iron-oxide particles from several tapes ... We examined every particle type we could find and tested it chemically” (Heller, Report on..., 1983, p. 195-196). After the description of the testing of the red iron-oxide particles, nothing is said about a second type of red dots, which would have been tested.

<sup>805</sup> Adler, The Shroud fabric..., 2002, TOM 119

<sup>806</sup> Heller, Report on..., 1983, p. 196

<sup>807</sup> Ford, The Shroud of Turin’s..., 2000, p. 10, refers to McCrone and Skirius, Light Microscopical..., 1980.

azurite), ... etc.”, and also “On examining thousands of red image particles on the Shroud tapes, I saw no low refractive index red particles except rose madder particles and a few red silk fibers (from the Shroud wrapping cloth)” and “I have observed small quantities of other pigments (madder, ...). ... They are present only as rare and only incidental single particles as one would expect if the cloth had been exposed to the atmosphere in an artist’s studio.”<sup>808</sup>

#### 4.2.7.5. Birefringent iron oxide a “type” typical of water stain margins and charred blood areas

Heller and Adler, who considered it a consistent explanation that “while non-birefringent red particulates are heme containing materials, the birefringent red particles are  $\text{Fe}_2\text{O}_3$  by both chemical and microscopic tests”, also wrote “We have been able to identify  $\text{Fe}_2\text{O}_3$  primarily in the water stain margins and charred blood areas indicating that it only constitutes a very small percentage of the total iron forms found on the Shroud” – the three iron forms found include, beside the heme bound form and the iron oxide, also the “cellulose bound chelated form” mentioned in 4.2.1.5 above.<sup>809</sup> The “Birefringent red particulate coated fibrils” are listed as class number 7 in their table 2 of Classes of sample objects tested.<sup>810</sup>

#### 4.2.7.6. Conclusion

The conclusion is that occasional aluminum oxide-madder lake particles were observed on main Shroud samples but neither tested by McCrone nor by Heller and Adler, and that the predominant red particles/dots in bloodstain areas were called “blood” and, more precisely, “heme containing materials”<sup>811</sup> by Heller and Adler, and may have been a mixture of heme aggregates and methemoglobin crystals.

#### 4.2.8. Pyrolysis/Mass Spectrometry

According to Rogers, Pyrolysis/Mass Spectrometry (PMS) did not detect lignin in any of the PMS-tested Shroud samples: these were single fibers from the main Shroud – although from not even one non-image non-scorch tape sample –, and also “Raes fibers” = “ample material from the Raes sample” (the Raes area is a light scorch area), and material from the main Shroud’s ‘Zina thread’, which was labelled as “image – yes”.<sup>812</sup> On the other hand, Rogers wrote “Mass 131 appeared at much higher temperatures in all of the spectra, but those are in the ranges where cellulose, lignin, and hemicelluloses are decomposing. The spectrum gets very complex at those higher temperatures.”<sup>813</sup> Furthermore, he reported that there was no “accurate, absolute sample temperature” available for microfibers in PMS.<sup>814</sup> Monomeric lignin yields signals at  $m/z$  180, 194, 210, and lignin dimers have at least 18 molecular fragments in the  $m/z$  250-350 mass region,<sup>815</sup> where also the signals of “n- fatty acids, unsaturated fatty acids, alkanes, alkenes, ..., alkyl aromatics and n- alkyl diesters” are to be found, especially those of alizarin: ruberythric acid that has lost its primeverose is an alizarin ion and

<sup>808</sup> McCrone, Judgement day..., 1999, p. 33 and 85 and 166 and 188

<sup>809</sup> Heller and Adler, A Chemical..., 1981, TOM 42

<sup>810</sup> Heller and Adler, A Chemical..., 1981, TOM 50

<sup>811</sup> Heller and Adler, A Chemical..., 1981: “the presence of blood on the Shroud” (TOM 41) and “heme containing materials” (TOM 42).

<sup>812</sup> Rogers, A Chemist’s..., 2008, p. 57, Rogers, Pyrolysis/Mass Spectrometry..., 2004, table on p. 2, and Rogers, Studies on..., 2005, p. 192

<sup>813</sup> Rogers, A Chemist’s..., 2008, p. 57

<sup>814</sup> Rogers, Studies on..., 2005, p. 192

<sup>815</sup> Marche et al., *Geodema* 2003, Chemical changes during composting of a paper mill sludge–hardwood sawdust mixture, <http://www.sciencedirect.com/science/article/pii/S0016706103001083>; Diné 2001, [http://books.google.nl/books?hl=nl&lr=&id=AvtidWy9HUgC&oi=fnd&pg=PA329&dq=marche++dimeric+lignin+\(m/z+&ots=uVqbHnMlie&sig=tylhWRi4er64JPTkPp4Ryj0l-VI](http://books.google.nl/books?hl=nl&lr=&id=AvtidWy9HUgC&oi=fnd&pg=PA329&dq=marche++dimeric+lignin+(m/z+&ots=uVqbHnMlie&sig=tylhWRi4er64JPTkPp4Ryj0l-VI)  
[http://books.google.nl/books?hl=nl&lr=&id=AvtidWy9HUgC&oi=fnd&pg=PA329&dq=marche++dimeric+lignin+\(m/z+&ots=uVqbHnMlie&sig=tylhWRi4er64JPTkPp4Ryj0l-VI#v=onepage&q&f=false](http://books.google.nl/books?hl=nl&lr=&id=AvtidWy9HUgC&oi=fnd&pg=PA329&dq=marche++dimeric+lignin+(m/z+&ots=uVqbHnMlie&sig=tylhWRi4er64JPTkPp4Ryj0l-VI#v=onepage&q&f=false) p. 335

has a signal at  $m/z$  239.3, and when alizarin-glycoside has lost its glucose, its signal is at  $m/z$  239.1.<sup>816</sup>

Dyestuffs such as alizarin and purpurin were not mentioned in the results of the Shroud's PMS-analysis at all, not even in the results of the ample material from the Raes sample, even though the dye had been found by wet microchemical analysis on Raes threads.<sup>817</sup> This means that the PMS method that was used probably was not capable of detecting these carbohydrate dyestuffs. Rogers' PMS paper reads "We wanted to see traces of materials that were not carbohydrates. ... Since we desired detection sensitivity rather than high resolution, we used a machine with moderate resolution, chemical ionization, and high sensitivity. ... It did not detect any unexpected pyrolysis fragments that indicated any Shroud materials other than carbohydrates."<sup>818</sup> So, perhaps there were PMS signals of pyrolysis products of lignin or vanillin or alizarin or purpurin that were either not "unexpected" or not recognized as such.

After all, the fluorescence spectra of the Shroud's non-image, image, and scorch areas have the characteristics of lignin/vanillin fluorescence: its shape and maximum near 435 nm under 365 nm excitation corresponds to lignin's fluorescence maximum at 435 under 337 nm excitation, and vanillin's fluorescence maximum at ca. 425 nm under 360 nm excitation<sup>819</sup> (see 4.2.3. and fig. 4.34).

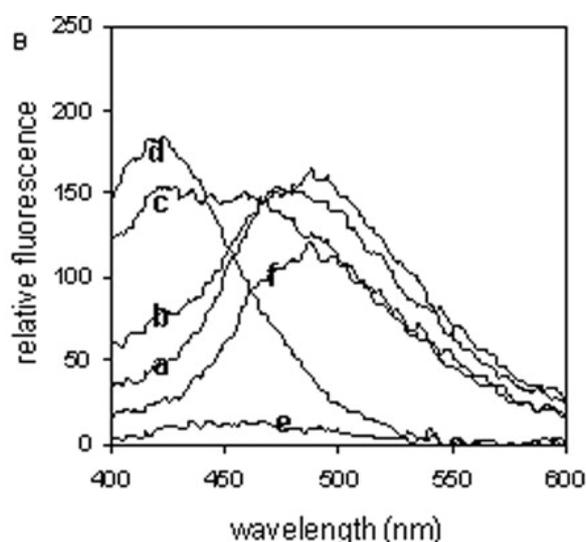


Fig. 4.34. Fluorescence emission spectrum of a vanillin solution (curve d) under 360 nm UV excitation. ©American Society for Biochemistry and Molecular Biology<sup>820</sup>

### 4.3. Not pectin or microbial bioplastic coating

Mottin has suggested, that the background fluorescence of the Shroud might be due to the presence of pectic substances not removed by primitive retting methods.<sup>821</sup> Adler and Heller indeed found

<sup>816</sup> Perla et al., red., Organic Mass Spectrometry in Art and Archeology, 2009, [http://books.google.nl/books?hl=nl&lr=&id=46klmqOb\\_gAC&oi=fnd&pg=PA3&dq=Rubia+tinctorum+xylose+arabinose&ots=IurMD4C3OX&sig=vn4Uuiyp4hMrNYAAweKgxQGtZKk#v=onepage&q=Rubia%20tinctorum&f=false](http://books.google.nl/books?hl=nl&lr=&id=46klmqOb_gAC&oi=fnd&pg=PA3&dq=Rubia+tinctorum+xylose+arabinose&ots=IurMD4C3OX&sig=vn4Uuiyp4hMrNYAAweKgxQGtZKk#v=onepage&q=Rubia%20tinctorum&f=false) p. 370

<sup>817</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 17-20; Rogers, Studies on..., 2005, p. 191-192

<sup>818</sup> Rogers, Pyrolysis/Mass Spectrometry..., 2004, p. 1

<sup>819</sup> curve d of Plot B of Fig. 3 <http://www.jbc.org/content/279/32/33492/F3.large.jpg> of <http://www.jbc.org/content/279/32/33492.full>, with original caption: "Fig. 3: [...] The excitation wavelength was 360 nm. [...] For comparison, the fluorescence emission spectra of 200  $\mu$ M vanillin (spectrum d), [...] are shown in B" <http://www.jbc.org/content/279/32/33492.full>

<sup>820</sup> This research was originally published in Journal of Biological Chemistry. Robert H. H. van den Heuvel, Willy A. M. van den Berg, Stefano Rovida, Willem J. H. van Berkel. Laboratory-evolved Vanillyl-alcohol Oxidase Produces Natural Vanillin. *Journal of Biological Chemistry*. 2004; Vol. 279: 33492-33500. © the American Society for Biochemistry and Molecular Biology. (Cf. [http://www.jbc.org/site/misc/Copyright\\_Permission.xhtml](http://www.jbc.org/site/misc/Copyright_Permission.xhtml))

pectins by wet chemistry and indications for it by FTIR spectroscopy (see 4.1.3.5),<sup>822</sup> but pectins are not auto-fluorescent: of the components of the cell walls of a linen fiber only lignin is fluorescent (light blue).<sup>823</sup> Garza-Valdes and Mattingly hypothesized that the whole Shroud is coated by a bioplastic coating produced by microorganisms.<sup>824</sup> Rogers commented that the required proteins, sulfur compounds and photosynthetic pigments are missing on the Shroud (referring to wet chemistry and PMS results), and that therefore “There is no significant amount of bioplastic polymers on the main part of the Shroud.”<sup>825</sup>

#### 4.4. Not Saponaria

Below is table with a survey of a number of striking similarities between Shroud properties and madder properties, which contrast with Saponaria properties (table 9). It is presented to evaluate Rogers’ hypothesis that assumes that Saponaria (also called struthium by Rogers) is the cause of the unusual background fluorescence of the Shroud – he wrote that “the fluorescence evidence” suggested the use of this herb, and that this herb could “explain” the fluorescence.<sup>826</sup> Also Adler wrote: “The background cloth shows a light greenish yellow emission not typical of other known old linen cloths and perhaps suggesting the presence of some type of thin coating of a fluorophore on the original linen”.<sup>827</sup> Each property will be addressed below.

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<sup>821</sup> Adler, *The Nature of...*, 1999, p. 4 TOM 106, referring to Mottin, *Actes du III Symp. Sci. Inter. Nice, CIELT, Paris* (1997).

<sup>822</sup> “Pectinase, and also the cellulase (but much more slowly than the pectinase) showed positive action against the non-image and radiocarbon fibers and did nothing with the image fibers in the same time period. It would appear that Mottin’s hypothesis is correct, pectic substances are present, but the matter should still be confirmed by spectral analysis.” Adler, *The Nature of...*, 1999, p. 4-5 (TOM 107)

<sup>823</sup> “Histology of Plant Extracellular Matrix” ascribes no fluorescence to pectin, but to lignin only (a light blue fluorescence) [http://www.cas.muohio.edu/~meicenrd/ANATOMY/Ch4\\_Histology/lab4.html](http://www.cas.muohio.edu/~meicenrd/ANATOMY/Ch4_Histology/lab4.html); and for a study of “Interaction of various pectin formulations with porcine colonic tissues” pectins had to be made fluorescent artificially in order to be able to observe the pectins’ behaviour in the colonic tissues (“Fluorescence-labeled pectins were prepared by the conjugation of fluoresceinamine to the molecules of P-25, P-94, and P-N by Belder’s method [17].” LinShu Liu e.a., *Biomaterials*, 2005, p. 5908, <http://ddr.nal.usda.gov/bitstream/10113/37497/1/IND44306122.pdf>; fluorescence of lignin: “The cell walls of kenaf phoem fibers are composed of cellulose and noncellulosic substances such as hemicelluloses, pectins, and lignins [10 ... . Lignin in the fiber cells is readily detected with ultraviolet light since the aromatic ring fluoresces blue [13], and is predominantly found in secondary cell walls that begin to form after cell expansion has ceased.” B.G. Aire, K. Stevens. et al, *Viscoelastic Properties of Kenaf Bast Fiber in Relation to Stem Age*, *Textile Research Journal*, Vol 79(11): 973–980, <http://www.lane-ag.org/pubs/kenaf/231386-WEBBER.pdf>, p. 974

<sup>824</sup> Garza-Valdes, *The DNA of God?*, 2001, chapter 5

<sup>825</sup> Rogers and Arnoldi, *Scientific Method...*, 2002, p. 25-27, and Rogers, *Frequently Asked Questions...*, 2004, #18

<sup>826</sup> Rogers, *A Chemist’s...*, 2008, p. 39-40

<sup>827</sup> Adler, *Chemical and Physical...*, 2002, TOM 13

	Shroud	madder	Saponaria
acidi-chromism	non-image: acid (conc. H <sub>2</sub> SO <sub>4</sub> ): bright yellow (Heller 199-200) Raes thread: acid (conc. HCl): bright yellow (Rogers SM 17-19)	acid: bright yellow	acid: deep brown  slightly acidic: saponarin: acid pH 5.6: colorless
	non-image, no addition: pale yellow	slightly acidic: pale yellow	base: pale yellow (H&A) (saponarin: base pH 7.9: yellow saponarin: dilute alkali: golden yellow apigenin: from pH 2 to 12: shifts from pale to intense yellow color)
	blood areas: base (hydrazine test): pink hemochromagen-like color non-image: base (calcium tests): violet test color	base: pink to violet/blue	
fluorescence	cloth under <u>long wave UV</u> (335-375 nm): weak green-yellow (M&P), not typical of other known old linen (H&A) non-image fiber: strong green yellow (GarzaV 34)	acid, under <u>long wave UV</u> (377 nm): 485-585 nm (green yellow)	base: on old linen under <u>short wave UV</u> : pale yellow-green (apigenin of saponarin and saponaretin at acidic pH: 440 nm (blue) and at neutral and alkaline pH: 470 nm (blue) under long wave UV (357 nm); saponins are not auto-fluorescent)
UV-vis	partly red-stained fibril: 450 nm absorbance peak (TOM 31)	acid: 450 nm absorbance peak	scorched and bleached on starched linen: 420 nm relative reflectance peak = absorbance dip (fluorescence contribution)
sugars	non-image: negative Seliwanoff's test for ketoses, no clear positive Bial's reagent test for pentoses or furfurals	sucrose (a ketose, not a pentose) probably fermented; In the Bial's reagent, a xylosyl containing madder component may not have been hydrolysed sufficiently to free the test-color-producing free xylose	after hydrolysis: pentoses (xylose); in the Bial's reagent, a xylosyl containing Saponaria component may not have been hydrolysed sufficiently to free the test-color-producing free xylose
solubility	cold water: no effect on fluorescence warm water: shift from green yellow to blue fluorescence	alizarin/purpurin: cold water: not easily soluble; carried by retrograded starch, which is warm water soluble	saponins: easily soluble in cold and warm water
iodine color	Raes fibers: red (Kohlbeck – HSG) lance wound blood fiber: iodine azide: reddish background, but not tested for starch (Rogers) lance wound sample 6BF (Kohlbeck): starch?	madder: probably no iodine color madder on twice retrograded starch: red	saponarin: blue and soluble saponarin on twice retrograded starch: blue solution around red fiber
effect on chelated iron	chelated iron detached and migrated to waterstain margins by cold water	acid: detaches weakly chelated iron	base (for pale yellow color and green yellow fluorescence): does not detach chelated iron
effect on image formation	green-yellow fluorescent weave areas: 'print' areas blue fluorescent weave areas: "no-print" areas	acid: catalyses acid oxidation = coloration absence: no acid oxidation catalysation = less image	base (for pale yellow color and green yellow fluorescence): yields colourless oxidation. absence: more coloration = more image
lake colour with Al <sup>3+</sup> and Ca <sup>2+</sup>	main Shroud: occasional red Al <sup>3+</sup> -madder lakes (rose madder = Al <sup>3+</sup> -madder lakes) Raes/C14: occasional red and blue madder lakes	red Al <sup>3+</sup> - and blue Ca <sup>2+</sup> -lakes	apigenin (in saponarin): yellow Al <sup>3+</sup> lakes
heme complex colour	different kinds of bloodstains: same pinkish red color	pinkish red (pink lake plus brown heme)	brown gypsogenic acid and flavonoid heme complexes
bloodstain reflectance spectra	distinct 630 nm absorbance peak no prominent Soret band	acidic madder dye plus acidic blood yields acid heme dimers with 630 nm abs. peak and no Soret band	base Saponaria plus acidic blood yield neutral or alkaline heme dimers without 630 nm abs. peak and with prominent Soret band

Table 9. Comparison of properties of the Shroud, madder and Saponaria

#### 4.4.1. Acidichromism – not Saponaria

Shroud non-image fibers are pale yellow, and also slightly acidic madder is pale yellow (see 4.2.1), and Heller and Adler reported that a linen cloth washed with an alkaline Saponaria extract also looks

pale yellow.<sup>828</sup> This pale yellow color of alkaline-Saponaria-treated cloth corresponds with the yellow color of its flavonoid saponarin in alkaline media (in slightly acidic media it is colorless).<sup>829</sup> Besides the fluorescence, this is the only similarity between the Shroud and a Saponaria washed cloth. Concentrated sulfuric acid renders Saponaria on linen deep brown: “samples of such Saponaria treated cloth ... immediately turn deep brown on treatment with concentrated sulphuric acid, thereby providing a simple test for such Saponaria treatment”.<sup>830</sup> Would xylene/toluene have washed off a Saponaria residue of non-image fibers when they were freed from the sticky-tape adhesive? It probably would not have removed all of it, at least not any sugars, as sugars are polar molecules (water-soluble) and xylene/toluene is non-polar<sup>831</sup>; Rogers searched for Saponaria sugars on washed non-image fibers (see 4.4.4). Anyway, both xylene/toluene-washed non-image fibers of the Shroud and madder are straw yellow after treatment with a strong acid, viz. concentrated sulphuric acid in the case of xylene/toluene-washed non-image fibers,<sup>832</sup> and concentrated hydrochloric acid in the case of madder dyed Raes fibers, which corresponds to the known acidichromism of madder (4.2.1.3. Indirect dye tests). Also the similar colors developed by the Shroud and madder in alkaline media have been discussed in 4.2.1.3. (Indirect dye tests).

#### 4.4.2. Fluorescence – not quite Saponaria

Just as the visible color, also the fluorescence of the Shroud (see 4.2.3. and 4.2.4.) and a Saponaria washed cloth are only similar if the used Saponaria solution is alkaline. Heller and Adler reported that 300-year-old Spanish linen saturated with an alkaline (1% NaOH) Saponaria solution (and then twice rinsed with distilled water) emitted a “pale yellow-green fluorescence under short wave UV”; the fluorescence color of the untreated linen was not reported.<sup>833</sup> UV photographers use the term “short wave UV” for UV radiation that extends from 200 to 280 nm,<sup>834</sup> so, the excitation that produced the yellow-green fluorescence of the Saponaria-treated old linen, was not the same as the 335-375 nm excitation used for Shroud fluorescence photography.<sup>835</sup> Therefore, the produced fluorescence colors can not be directly compared.

It is known that apigenin, the aglycone of Saponaria’s flavones saponarin and saponaretin, at an acidic pH of 2, has a maximum fluorescence at 440 nm (in the blue) under 357 nm excitation and, at a neutral pH of 8, as a monoanion, fluoresces stronger but still at ca. 470 nm (blue region) and even still does so, with a stronger and slightly differently shaped spectrum, as a di-anion at an alkaline pH of 12 (fig. 4.35).<sup>836</sup>

<sup>828</sup> “Saponaria officinalis. Specimens of this plant ... extracted with 1% NaOH, and the extracts were then used to prepare Spanish linen Saponaria treated controls.”, “Specific Spanish linen controls ... 5) a) Saturated with Saponaria extract for 1 hour, then rinsed twice with distilled water – Resembles Shroud pale yellow fibrils, show pale yellow-green fluorescence under short wave UV” (Heller and Adler, A Chemical..., 1981, TOM 38, 51)

<sup>829</sup> saponarin, dilute alkali: golden yellow (Barger, 1906, Saponarin, a New Glycoside, Coloured Blue with Iodine, <http://pubs.rsc.org/en/content/articlepdf/1906/ct/ct9068901210>); saponarin: slightly alkaline pH 7.9: yellow

<http://en.wikipedia.org/wiki/Saponarin>; saponarin, slightly acidic pH 5.6: colorless

<http://en.wikipedia.org/wiki/Saponarin>; apigenin, saponarin’s aglycone, turns from a pale to intense yellow color in a pH changing from 2 to 12 (Favaro et al., Acidichromism..., 2007, 710)

<sup>830</sup> Heller and Adler, A Chemical..., 1981, TOM 38

<sup>831</sup> cf. <http://www.sciencedirect.com/science/article/pii/0021961475901597> and “non-polar organic solvents such as the aromatics benzene and toluene.” <https://en.wikipedia.org/wiki/Toluene>

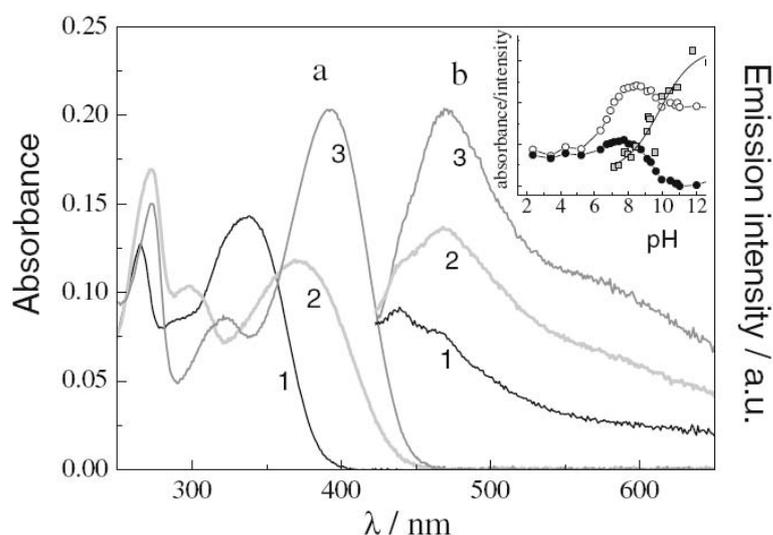
<sup>832</sup> Heller, Report on..., 1983, p. 199-200

<sup>833</sup> Heller and Adler, A Chemical..., 1981, TOM 51

<sup>834</sup> [http://en.wikipedia.org/wiki/Ultraviolet\\_photography](http://en.wikipedia.org/wiki/Ultraviolet_photography)

<sup>835</sup> Miller and Pellicori, Ultraviolet fluorescence..., 1981, p. 71

<sup>836</sup> “Then we prepared saponaretin (II) by acid hydrolysis of its natural 7 -O-glucoside, saponarin, extracted from Saponaria officinalis, and it was shown to be identical with the main product of 4 -benzylapigenin or apigenin C-glucosylation” Chopin, 1971, [http://link.springer.com/chapter/10.1007/978-3-642-65136-6\\_6](http://link.springer.com/chapter/10.1007/978-3-642-65136-6_6); “Absorption (a) and fluorescence ( $\lambda_{exc} = 357$  nm, isosbestic point) (b) spectra of AP at pH values that correspond to the neutral form (1, pH = 2), to the monoanion (2, pH = 8) and to the di-anion (3, pH = 12) in methanol–water (1/2 v/v) solutions. *Insert:* spectrophotometric [ $\lambda_{analysis} = 273$  (open circle) and 298 (filled circle) nm] and fluorimetric [ $\lambda_{exc} = 357$  nm and  $\lambda_{em} = 472$  nm (filled square)] titration curves”, AP = apigenin, fig. 3 in Favaro et al., Acidichromism..., 2007, <http://link.springer.com/article/10.1007/s10895-007-0222-0?no-access=true>



**Fig. 3** Absorption (a) and fluorescence ( $\lambda_{\text{exc}}=357$  nm, isobestic point) (b) spectra of AP at pH values that correspond to the neutral form (1, pH=2), to the monoanion (2, pH=8) and to the di-anion (3, pH=12) in methanol–water (1/2 v/v) solutions. *Insert*: spectrophotometric [ $\lambda_{\text{analysis}}=273$  (open circle) and 298 (filled circle) nm] and fluorimetric [ $\lambda_{\text{exc}}=357$  nm and  $\lambda_{\text{em}}=472$  nm (filled square)] titration curves

*Fig. 4.35. Absorption and fluorescence of apigenin (AP) at pH 2, 8 and 12* ©Springer Science + Business Media, LLC 2007<sup>837</sup>

It is also known that saponarin, an apigenin glycoside of Saponaria, is fluorescent at a twin peak of ca.440 nm and 470 nm (both blue) under unspecified excitation<sup>838</sup> (in a methanol extract of unknown pH, where the apigenin neutral molecule and its mono-anion probably are in an equilibrium). Saponins, the washing molecules, are not auto-fluorescent.<sup>839</sup> If alkaline apigenin flavonoids were the main fluorophores of the Shroud that made the color of its fluorescence noticeably untypical of other old linen cloths, their relatively strong fluorescence might also be expected to be quite noticeable at 470 nm. Yet, the raw fluorescence scan of the Shroud's background (fig. 4.25) peaks at about 435 nm under 365 nm excitation and doesn't show a peak or shoulder at ca. 470 nm or only a very, very small one.<sup>840</sup> That the shoulder in the Shroud's raw fluorescence scan at ca. 540 nm, in the green range, is in the expected range for acidic madder dye, has already been discussed in 4.2.3.

<sup>837</sup> With kind permission from Springer Science+Business Media: *Journal of Fluorescence*, Vol 17, 2007, pp. 707-714, G. Favaro, C. Clementi, A. Romani, V. Vickackaite, figure 3. DOI: 10.1007/s10895-007-0222-0, <http://link.springer.com/article/10.1007/s10895-007-0222-0?no-access=true>

<sup>838</sup> This figure with several spectra, <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-3040.2002.00942.x/full#f9> of Corevic et al., 2002, <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-3040.2002.00942.x/full>, has in the title bar: Fluorescence (QSEU) for the two right hand curves in the left hand figure. They represent the fluorescence of two segments of a barley leaf, of which the peaks in the logFER at 274 and 336 nm – as in the absorbance spectrum of this methanolic extract – are called “characteristic of saponarin (an apigenin glycoside), the major flavonoid derivative in barley” (Blume & McClure 1979; Liu et al. 1995). These are comparable with the absorption and fluorescence spectra of apigenin in methanol/water, in Fig. 3 (see fig. 4.35 above), and the absorption and fluorescence spectra of apigenin in methanol, in Fig. 1, of Favaro et al., *Acidichromism...*, 2007 <http://link.springer.com/article/10.1007/s10895-007-0222-0#>

<sup>839</sup> “The saponins in official saponin drugs are mainly triterpene derivatives, with a smaller number of steroids. ... Detection – 1. Without chemical treatment – With the exception of glycyrrhetic acid (from *Liquiritiae radix*), no saponins are detectable by exposure to UV-254 nm or UV-365 nm.” Wagner et al., 1984, *Saponin Drugs*, [http://link.springer.com/chapter/10.1007/978-3-662-02398-3\\_10#page-1](http://link.springer.com/chapter/10.1007/978-3-662-02398-3_10#page-1)

<sup>840</sup> Gilbert and Gilbert, *Ultraviolet-visible reflectance...*, 1980, fig. 5 <http://imagebank.osa.org/getImage.xqy?img=cCF6ekAubGFyZ2UsYW8tMTktMTItMTkzMC1nMDA1>; the effective instrument bandwidth being 8 nm (p. 1933)

#### 4.4.3. UV-vis absorbance – not Saponaria

Pellicori reported on relative reflectance curves, in fig. 5 of his article and in its caption, that “Top curve is for linen treated with saponaria glucoside (note 13: Rogers, personal communication) and shows a fluorescent contribution <450 nm. Bottom curves are for scorch on this linen before and after baking.”<sup>841</sup> Also this phenomenon is in accordance with (only neutral and alkaline) apigenin properties: for these pH regions, its absorbance and fluorescence emission curves overlap in the 400-450 nm region (fig. 4.35), and thus allow for a fluorescence contribution to a reflectance spectrum, especially one with an 8 nm spectral resolution, as Pellicori’s spectra below 500 and above 600 nm.<sup>842</sup> The Saponaria treated sample, investigated by Pellicori, was apparently received from Rogers, who had got it from Kate Edgerton, who, after starching and washing with Saponaria, had “ironed the samples with a “warm iron” which colored the cloth and changed the Saponaria glycosides” – probably by breaking (pyrolysing) them into aglycones and free sugars –, after which “the samples were bleached to remove most of the color by soaking in 3.5% hydrogen peroxide”.<sup>843</sup> The change of the Saponaria glycosides by both ironing and bleaching, may have changed their spectral properties, so, unfortunately, there can be no direct comparison with Shroud spectra. Yet, spectroscopically, the glycoside saponarin and its aglycone apigenin do not differ much: not just the fluorescence peaks, but also the absorbance peaks of barley saponarin (in methanol at 274 and 336 nm), coincide well with the absorbance peaks of apigenin (in methanol at 275 and 333 nm).<sup>844</sup> Also the isoflavone genistein and a genistein glycoside do not differ in absorbance maximum, reciprocally, and the flavone luteolin and a luteolin glycoside do not differ much in their absorbance maximum either.<sup>845</sup> The relative reflectance curve for Pellicori’s Saponaria-treated and scorched and bleached ‘Edgerton’ linen, compared to not-Saponaria-treated linen, also produced by Edgerton, has been published from 350 to 750 nm.<sup>846</sup> It is flat above 470 nm, sharply decreases from 375 to 350 nm – corresponding to a saponarin/apigenin absorbance peak near 336 nm and to the steep absorbance increase of (probably neutral or monoanion forms of) saponarin from 375 nm to 336 nm<sup>847</sup> –, but has a broad peak at ca. 420 nm. Such a 420 nm reflectance peak is not seen in the absolute Shroud background reflectance spectra.<sup>848</sup> At 450 nm, one partly red-stained Shroud fiber, examined in transmission, even showed an absorbance peak, which is the opposite of a reflectance peak, but does correspond to acidic madder’s absorbance peak at 450 nm (see 2.1.2.1).

#### 4.4.4. Sugars – no Saponaria evidence

Rogers reported that Saponaria extract, after hydrolysis, contains the sugars galactose, glucose, arabinose, xylose, fucose, rhamnose, and glucuronic acid.<sup>849</sup> Any ketoses among these sugars give a positive Seliwanoff’s test for ketoses and furfural, and the sugars arabinose and xylose are pentoses that give a positive Bial’s test. Roger’s book says, probably referring to xylene/toluene-washed

<sup>841</sup> Pellicori, Spectral properties..., 1980, p. 1917

<sup>842</sup> Favaro et al., Acidichromism..., 2007, fig. 3 = fig. 4.35 above; Pellicori, Spectral properties..., 1980, p. 1915

<sup>843</sup> Rogers and Arnoldi, Scientific Method..., 2002, p. 33

<sup>844</sup> The dotted line in the right hand plot of this figure <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-3040.2002.00942.x/full#f9> of Corevic et al., 2002, <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-3040.2002.00942.x/full>, represents the absorbance of a methanolic barley leaf extract, and shows peaks at 274 and 336 nm, which are called “characteristic of saponarin (an apigenin glycoside), the major flavonoid derivative in barley”. The absorption spectrum of apigenin in methanol/water has absorbance peaks at ca. 275 and 337 nm, and the absorption spectrum of apigenin in methanol, has absorbance peaks at ca. 275 and 333 nm (Fig. 1 and fig. 3 of Favaro et al, Acidichromism and, 2007 <http://link.springer.com/article/10.1007/s10895-007-0222-0#>)

<sup>845</sup> Gulmini, Identification of..., 2013, table 1

<sup>846</sup> Pellicori, Spectral properties..., 1980, Fig. 5

<sup>847</sup> see the dotted absorbance curve in the right hand plot of fig. 9 of Cerovic et al., 2002 <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-3040.2002.00942.x/full#f9>

<sup>848</sup> Gilbert and Gilbert, Ultraviolet-visible reflectance..., 1980, fig. 6

<sup>849</sup> Rogers, A Chemist’s..., 2008, p. 39; Rogers and Arnoldi, Scientific Method..., 2002, p. 6 refers to “V. Ya Chirva, P. K. Kintya, and G. Lazur’evskii, “Triterpene glycosides from Saponaria officinalis,” Khim. Prir. Soedin. 5 (1969), pp. 59-60.”).

background fibers: “The Seliwanoff’s reagent also gives a red color ... background fibers gave no color.”; “In order to make a more detailed analysis for possible flax impurities and/or sugars from *Saponaria officinalis* ... I made some Bial’s reagent (orcinol, con. HCl and FeCl<sub>3</sub>). It gives a bright Kelly green color with pentose sugars or furfural. I could not get a clear positive test for pentoses from Shroud samples”.<sup>850</sup> However, perhaps the xylene/toluene solvent had washed glycosides off of main Shroud fibers, or the concentrated HCl could not be heated enough and/or not long enough and therefore did not split off the xylose moiety, which is needed in a free form in order to give a positive test color. Certain HP-TLC plates containing lucimin, i.e., a primeverose glycoside that contains a xylosyl, were heated at 110 °C for 5 minutes to develop a test color with “orcinol spray reagent”, containing orcinol and concentrated H<sub>2</sub>SO<sub>4</sub>.<sup>851</sup> This kind of heating probably is not possible in a microspot well, as used for microscopical Shroud fiber examination.

A water-alcohol madder root extract contains sucrose,<sup>852</sup> which is a disaccharide consisting of glucose and fructose, which is/are not a pentose, so would not have given a positive Bial’s reagent test. A sufficient concentration of sucrose would have given a positive Seliwanoff’s test,<sup>853</sup> but most or all of madder’s sucrose may have been fermented by microbes in the extract before it was applied as a dye. Taking into account that a water extract of madder root contains potent antimicrobial agents against fungi and insects and some, but not all, yeasts, and is not active against many bacteria,<sup>854</sup> among which *E. coli*, a lactic acid bacterium (see 3.3.), it is explicable that a fresh madder extract rapidly starts fermenting spontaneously: on a dyeing procedure that employs water of 80 °C for extracting the colourants from crushed madder root, first, after which the roots are sieved out and the textile fiber is added, it is said: “When you finish dyeing, throw the liquor and madder roots away, if possible on the compost, as the liquid ferments and the roots go mouldy very quickly.”<sup>855</sup> In an unheated water extract (as by the ‘Nest Rubio’ technique, see also 2.4.2.2. above), ferments would have even more opportunity, as microbes are not eliminated from the liquid by heat and the extraction procedure takes much longer. Fermentation does not deteriorate the quality of the dye, but, on the contrary, is one of the procedures used deliberately to enhance the color of the extract: “Alizarin and pseudopurpurin are present in the root as glycosides (sugars) and treatments with acid or by fermentation were devised to hydrolyse these sugars to increase the quantity of free alizarin and purpurin available.”<sup>856</sup> “Roots of the madder plant are dried, crushed and hulled. ... The roots are boiled in weak acid to dissolve the dye, and fermented (in which the glycosides [...] hydrolyze to anthraquinones).”<sup>857</sup> Besides spontaneous or deliberate fermentation, also the addition of vinegar, e.g. from sour wine, to render the reddish root extract slightly acidic and thus pale yellow, may have given a fermentation by the acetic and lactic acid bacteria that are present in many vinegars.<sup>858</sup> If the

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<sup>850</sup> Rogers, A Chemist’s... , 2008, p. 39 and 40

<sup>851</sup> “In addition to lucimin and its epimer (S)-epilucumin, the primeverose moiety is restricted to a rare group of cyanogenic glycosides ... The acetone:MeOH (9:1, v/v) eluate from the silica column was concentrated under N<sub>2</sub>, resuspended in CHCl<sub>3</sub>:MeOH (2:1, v/v), and fractionated on aluminiumbacked Silica gel 60 HP-TLC plates (... ) developed in solvent A (CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O, 65:35:4, v/v). Cyanogenic glycosides were visualised with orcinol spray reagent (20 mg mL<sup>-1</sup> orcinol monohydrate in EtOH: conc. H<sub>2</sub>SO<sub>4</sub>:H<sub>2</sub>O; 75:10:5, v/v) and development for 5 min at 110 °C. Orcinol detects carbohydrate.” (p. 48) Miller et al., *Phytochemistry* 67 (2006) 43-51

[http://www.researchgate.net/publication/7459186\\_Potential\\_cancer\\_chemopreventive\\_activity\\_of\\_simple\\_isoquinolines\\_1-benzylisoquinolines\\_and\\_protoberberines/file/d912f505baef814c3c.pdf#page=43](http://www.researchgate.net/publication/7459186_Potential_cancer_chemopreventive_activity_of_simple_isoquinolines_1-benzylisoquinolines_and_protoberberines/file/d912f505baef814c3c.pdf#page=43);

[http://en.wikipedia.org/wiki/Bial%27s\\_test](http://en.wikipedia.org/wiki/Bial%27s_test)

<sup>852</sup> V.V.S. Murti, T.R. Seshadri, S. Sivakumaran, A Study of Madder, the Roots of *Rubia tinctorum* Linn., *Indian Journal of Chemistry*, Vol. 8, Sept. 1970, pp. 779-782.

<sup>853</sup> “Seliwanoff’s test ... Sucrose gives a positive test as it is a disaccharide consisting of fructose and glucose.”

<http://en.wikipedia.org/wiki/Seliwanoff>

<sup>854</sup> Kalyoncu et al., Antimicrobial Activity of Common Madder (*Rubia tinctorum* L.), *Phytotherapy Research* 20/6, 490-492 (2006), <http://onlinelibrary.wiley.com/doi/10.1002/ptr.1884/abstract>

<sup>855</sup> [http://wildcolours.co.uk/html/madder\\_dyeing.html](http://wildcolours.co.uk/html/madder_dyeing.html)

<sup>856</sup> [http://cameo.mfa.org/wiki/Rose\\_madder](http://cameo.mfa.org/wiki/Rose_madder)

<sup>857</sup> [http://footguards.tripod.com/06ARTICLES/ART33\\_madder.htm](http://footguards.tripod.com/06ARTICLES/ART33_madder.htm)

<sup>858</sup> In winemaking “Malolactic fermentation occurs when lactic acid bacteria metabolize malic acid and produce lactic acid and carbon dioxide. This is carried out either as an intentional procedure in which specially cultivated strains of such bacteria are introduced into the maturing wine, or it can happen by chance if uncultivated lactic acid bacteria are present. ... Volatile acidity test verifies if there is any steam distillable acids in the wine. Mainly present is acetic acid (the

dye was extracted in advance and a sour solution containing lactic acid bacteria was added to render the extract a yellow dye, which was then kept for some time before it was used to dye the Shroud, original free madder sucrose would have been a prime target of fermentation.<sup>859</sup>

Madder root extract also contains the colorant ruberythric acid, which is the glycoside of alizarin and the disaccharide primeverose, consisting of glucose and xylose.<sup>860</sup> Primeverose can be split off from ruberythric acid by the enzyme primeverosidase, also called beta-glucosidase and primeverase,<sup>861</sup> but, even when split off, does not give a positive Seliwanoff's test for ketoses, as neither glucose nor xylose is a ketose. The reason why it did not give a clear positive Bial's test on fibers from the main Shroud, may have been, as in the case of Saponaria, that either the xylene/toluene solvent had washed it off of fibers, and/or the concentrated HCl test solution was not heated enough or not long enough and therefore did not split off (enough) xylose sugar.

#### 4.4.5. Solubility – not Saponaria

Saponins are washing molecules and very soluble in both cold and warm water. Yet, the insides of the large pre-1532 diamond-shape waterstains of the Shroud are not remarkably different from the unstained background in fluorescence.<sup>862</sup> This shows that saponins are not the fluorophores that give the Shroud its yellow-green fluorescence that is untypical of other aged linen.<sup>863</sup> The small hot-water stains from the 1532 AD fire did change the fluorescence from yellow-green to dark blue (see 4.2.4. above), which means that the untypical fluorophore is only removable by warm water, not by cold water. Alizarin, the main chromophore and a fluorophore of madder, is “slightly to sparingly soluble” in cold water,<sup>864</sup> but more soluble in warm water.<sup>865</sup> Also retrograded starch is only soluble

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dominant component of vinegar), but lactic, butyric, propionic, and formic acid can also be found. ... Without the use of sulfur dioxide, wines can readily suffer bacterial spoilage no matter how hygienic the winemaking practice.”

<http://en.wikipedia.org/wiki/Winemaking>; “In addition to acetic acid, fruit vinegars often contain citric, malic, lactic, and tartaric acids and may also include phenolics, some of which are produced as a result of fermentation.” Shahidi et al., 2008, <http://apjcn.nhri.org.tw/server/APJCN/17/s1/380.pdf>; lactic acid is a “major” organic acid in wine vinegars: “The method was successfully validated and enables the reliable separation of major organic acids in wine vinegars (ie tartaric, citric, malic, lactic and acetic acid) in about 25 min.” Morales et al., 1998,

<http://www.sciencedirect.com/science/article/pii/S002196739800572X>

<sup>859</sup> “Lactic acid fermentation is a biological process by which glucose, fructose, and sucrose are converted into cellular energy and the metabolite lactate. ... In *homolactic fermentation*, one molecule of glucose is ultimately converted to two molecules of lactic acid. *Heterolactic fermentation*, in contrast, yields carbon dioxide and ethanol in addition to lactic acid, in a process called the phosphoketolase pathway.” [http://en.wikipedia.org/wiki/Lactic\\_acid\\_fermentation](http://en.wikipedia.org/wiki/Lactic_acid_fermentation)

<sup>860</sup> Ruberythric acid is one of the “colour components present in plants of the Rubiaceae family” and contains “6-O-β-D-xylopyrosyl-D-glycosyl”, Perla et al., *Organic Mass Spectrometry in Art and Archaeology*, [http://books.google.nl/books?id=46klmqB\\_gAC&printsec=frontcover&dq=organic+mass+spectrometry+in+art+and+archaeology&hl=nl&sa=X&ei=IQEU6fAH8OU0AX7l4DwBg&ved=0CD0Q6AEwAA#v=onepage&q=rubia%20inctoru&f=false](http://books.google.nl/books?id=46klmqB_gAC&printsec=frontcover&dq=organic+mass+spectrometry+in+art+and+archaeology&hl=nl&sa=X&ei=IQEU6fAH8OU0AX7l4DwBg&ved=0CD0Q6AEwAA#v=onepage&q=rubia%20inctoru&f=false) p. 370-371, fig. 13.2.

<sup>861</sup> <http://en.wikipedia.org/wiki/Primeverosidase>; On monotropitin it is said: “The glucoside, from whichever source obtained, is hydrolysed by the same enzyme variously described as gaultherase, betulase, or primeverase, which also occurs in *Monotropa*, giving methyl salicylate and the glucoxylose primeverose.” Haas et al., 1928, [http://www.archive.org/stream/introductiontoch01haas/introductiontoch01haas\\_djvu.txt](http://www.archive.org/stream/introductiontoch01haas/introductiontoch01haas_djvu.txt)

<sup>862</sup> In fig. 5 p. 77 and fig 8 p. 81 and fig. 9 p. 82 of Miller and Pellicori, *Ultraviolet Fluorescence...*, 1981, the inside of the large waterstain is similar to the background in fluorescence; in fig. 6 p. 78 and fig. 7 p. 80, the boundaries of the large waterstain seem to fluoresce a bit more yellow. In fig. 10 p. 83 (ventral feet area) all fluorescence colors are generally different from the those in the other photos and the large waterstains have very absorbing borders, while also parts of the insides seem more absorbant than the background; Miller and Pellicori say on the large waterstains that “the one at D-22 has an unusually wide absorbing boundary” (p. 83).

<sup>863</sup> Adler, *Chemical and Physical...*, 2002, TOM 13 (see 4.2.4. above)

<sup>864</sup> <http://en.wikipedia.org/wiki/Alizarin>

<sup>865</sup> “the solubility and mass-transfer coefficient of alizarin increases with an increase in hydrotrope concentration and system temperature” (Prakash et al., 2010 [http://www.tandfonline.com/doi/full/10.1080/00986440903155998#\\_UxBZG2eA2Uk](http://www.tandfonline.com/doi/full/10.1080/00986440903155998#_UxBZG2eA2Uk)); “In this paper, we investigate subcritical water extraction of anthraquinones using alizarin or 1,2-dihydroxyanthraquinone (Figure 1) as a standard compound. ... The release of anthraquinones from the roots can be seen readily by observing the yellow color of the extracts. Figure 3 shows the extracts obtained from extraction with ambient water, subcritical water, and ambient ethanol, respectively. As shown here, subcritical water shows a positive result as the extract appears yellow compared to the

in hot water,<sup>866</sup> so, a madder dye on a retrograded starch coating would not have been removed by cold water – as in the cold-water-stained and then lightly scorched Raes corner that still contains madder dye, retrograded starch and cold-water-soluble starch gum (= scorched starch) –, but by hot water only, as seems to be the case on the Turin Shroud (cf. 4.1.2. and 4.2.4. above).

#### 4.4.6. Color with iodine – not Saponaria

Also the flavonoid glycoside saponarin of Saponaria is cold water soluble<sup>867</sup>. It gives a blue color with iodine, because of which it has been erroneously called “soluble starch”.<sup>868</sup> A bubbling iodine azide reagent produced “a reddish background” around Shroud fibers from the lance wound area.<sup>869</sup> If Saponaria saponarin was present on a retrograded starch coating – also after washing with xylene/toluene – in such way that it gave the Shroud a pale yellow visible color and an untypical fluorescence, a blue background due to dissolved saponarin and iodine would be expected around a more reddish fiber due to retrograded starch, which contains mostly amylopectin and double-helix amylose, which turn red with iodine.<sup>870</sup> Saponarin has not been reported as a constituent of madder.<sup>871</sup> If madder was present on a retrograded starch coating, any remaining madder would not dissolve in the aqueous iodine azide solution and probably would not give a blue or red color with iodine either, but the bubbling iodine azide reagent may have brought some of the starch in suspension, giving the background a reddish color.

#### 4.4.7. Effect on chelated iron – not Saponaria

The Saponaria solution that would have given the Shroud its pale yellow color and a green yellow fluorescence, would have been an alkaline solution (see 4.4.1. and 4.4.2. above). If the Shroud with a residue of this alkaline Saponaria solution would have been wetted again by a neutral fluid, the new solution in the Shroud would also be alkaline. An alkaline solution cannot detach iron that is

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ambient water extraction. This is expected, because the increase in water temperature increases the solubility of the dissolve organic compound.” (Shotipruk et al., 2004, p. 1873, [http://www.researchgate.net/publication/8151403\\_Pressurized\\_hot\\_water\\_extraction\\_of\\_anthraquinones\\_from\\_the\\_roots\\_of\\_Morinda\\_citrifolia/file/3deec526316ed78935.pdf](http://www.researchgate.net/publication/8151403_Pressurized_hot_water_extraction_of_anthraquinones_from_the_roots_of_Morinda_citrifolia/file/3deec526316ed78935.pdf) , here roots of *Morinda citrifolia* were extracted)

<sup>866</sup> [http://en.wikipedia.org/wiki/Resistant\\_starch](http://en.wikipedia.org/wiki/Resistant_starch) “RS3 Resistant starch that is formed when starch-containing foods are cooked and cooled ... Occurs due to retrogradation, which refers to the collective processes of dissolved starch becoming less soluble after being heated and dissolved in water and then cooled.”; See fig. 1, 2, 3, and 4 in which R<sub>T</sub> is retrograded starch, of Fernández-Martin et al., 2008,

[http://www.researchgate.net/publication/233885487\\_Pressurization\\_of\\_some\\_starches\\_compared\\_to\\_heating\\_Calorimetric\\_thermo-optical\\_and\\_X-ray\\_examination/file/72e7e51b8e22d2a185.pdf](http://www.researchgate.net/publication/233885487_Pressurization_of_some_starches_compared_to_heating_Calorimetric_thermo-optical_and_X-ray_examination/file/72e7e51b8e22d2a185.pdf) or <http://www.sciencedirect.com/science/article/pii/S096399690800094X>

<sup>867</sup> Saponarin is one of the water-soluble constituents of *Nerviliae fordii* (Lu et al., 2010, [http://en.cnki.com.cn/Article\\_en/CJFDTOTAL-SZGY201012023.htm](http://en.cnki.com.cn/Article_en/CJFDTOTAL-SZGY201012023.htm) )

<sup>868</sup> “Apart from starch, there is saponarin (first wrongly called “soluble starch”), which with iodine furnishes a blue sol of an electronegative lyophobic colloid” Barger et al., 1915,

<http://pubs.rsc.org/en/content/articlepdf/1915/ct/ct9150700411> ; “When iodine is adsorbed by starch, saponarin, basic lanthanun acetate, cholalic acid, etc., blue substances are obtained.” Dhar, 1925, <http://pubs.acs.org/doi/abs/10.1021/j150257a004>

<sup>869</sup> Rogers, Comments On..., 2001, p. 13-14

<sup>870</sup> “Starch ... One of its components, amylose, dissolves in water to give a clear blue color with iodine. The other dissolves only in hot water to form a paste, and it gives a violet color with iodine. Some of it should have remained after the stiff cloth was washed immediately after manufacture” (Rogers, Comments on, 2001, p. 13-14). Only single-helical amylose can include the iodine ions in such a way that it colors blue

([http://braukaiser.com/wiki/index.php?title=Carbohydrates#Reaction\\_with\\_iodine](http://braukaiser.com/wiki/index.php?title=Carbohydrates#Reaction_with_iodine)). Completely retrograded amylose has formed double helixes with other amylose molecules or amylopectin molecules: “Although amylose is soluble in the hot gelatinized starch mixture, it tends to become insoluble in the cooled mixture. This phenomenon is called retrogradation and it occurs when the amylose chains bind together in helical and double helical coils. Retrogradation affects the texture of the food product and it also lowers the digestibility of the product.” (<http://www.encyclopedia.com/topic/starch.aspx> )

<sup>871</sup> Singh et al., 9, 10-Anthraquinones..., 2004

covalently bound (= chelated) to linen.<sup>872</sup> Yet, the Shroud seems to contain iron that is covalently bound to the linen and that in the big waterstains was detached from the linen and migrated to the waterstain margins; acid madder dye in the coating would have yielded the slight acidity of the water needed to free this iron from the linen (see 4.2.1.5).

#### 4.4.8. Effect on image formation – not Saponaria

Similarly, a residue of an alkaline Saponaria solution would not be a catalyst for the acid oxidation that apparently colored the image fibers of the Shroud straw yellow; Heller even said that base/alkaline oxidation does not produce a color at all.<sup>873</sup> So, the absence of alkaline Saponaria on certain weave areas could only have had a positive effect on the acid oxidation that formed the color. Yet, in certain weave areas that fluoresce blue – and that therefore seem to miss the fluorescence contribution of a coating – there is less image: they were called ‘no print’ areas; the lack of acidic madder and starch in these areas could explain this phenomenon (see 4.2.4.2).

#### 4.4.9. Lake colour with Al<sup>3+</sup> and Ca<sup>2+</sup> – not Saponaria

Apigenin, the aglycone of saponarin of Saponaria, forms yellow complexes (lakes) with aluminum. Favaro et al. say on the absorbance of apigenin (AP): “In the case of AP (Fig. 5), a new band increased at 382 nm, which was assigned to formation of an Al<sup>3+</sup>-AP complex.”<sup>874</sup> Their fig. 5 shows that the absorbance band is broad and extends to 450 nm, in the blue, and that there is no other absorbance band above 450 nm, which means that the complex looks yellow. This is in accordance with their observation that for apigenin “deprotonation and chelation induce spectral changes which are qualitatively parallel”, and that deprotonation changes the apigenin colour “from pale to intense yellow”.<sup>875</sup> They also say that apigenin is “less prone to give complexes with calcium than with aluminum” and that “weld lakes containing calcium salts are less stable than those on hydrated alumina.” This means that, if the Shroud has a Saponaria residue, the observation of yellow aluminum-apigenin lakes might be expected. Yet, no yellow lakes were reported as present on the Shroud, only occasional yellow ochre pigments.<sup>876</sup>

#### 4.4.10. Heme-complex colour – not Saponaria

*Saponaria officinalis* has saponins of the quillaic acid type and of the gypsogenic acid type,<sup>877</sup> both not aromatic. Quillaic acid does not bind iron, but gypsogenic acid is very similar to a saponin of alfalfa root - medicagenic acid -, which does bind iron in insoluble complexes; gypsogenic acid has

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<sup>872</sup> “Preparation of Chelates ... chelates were prepared by addition of the ferric nitrate to a solution of the trisodium salt of the chelating agents. Final pH was attained by adding dilute base as NaHCO<sub>3</sub> to the solution with vigorous stirring with the use of a Vortex mixer. It is important to emphasize that many of the ferric chelates are highly unstable systems. Attempts to use base more concentrated than the iron often leads to precipitates, particularly at ratios of citrate to iron of 1.0. All chelate solutions were taken to a final pH of 7.5 in a Tris buffer, 5 x 10<sup>-3</sup> M. All buffer solutions were equilibrated with the ambient air by aeration to assure a concentration of dissolved bicarbonate ion sufficient for the formation of the metalloprotein complex.” (Bates, Billups, and Saltman, The Kinetics and Mechanism of Iron(III) Exchange between Chelates and Transferrin, *The Journal of Biological Chemistry*, 1967, 242:2810-2815, <http://www.jbc.org/content/242/12/2810.full.pdf>)

<sup>873</sup> Heller, Report on ..., 1983, p. 200

<sup>874</sup> Favaro et al., Acidichromism..., 2007, p. 711

<sup>875</sup> Favaro et al., Acidichromism..., 2007, p. 711-712 and p. 710

<sup>876</sup> McCrone, Judgement day..., 1999, p. 85

<sup>877</sup> Zhonghua Jia et al., Major Triterpenoid Saponins from *Saponaria officinalis*, 1998, <http://pubs.acs.org/doi/abs/10.1021/np980167u>; Kazuo Koike et al., New Triterpenoid Saponins and Sapogenins from *Saponaria officinalis*, 1999, <http://pubs.acs.org/doi/abs/10.1021/np990311r>; Zhonghua Jia, Saponarioside C, the First  $\alpha$ -D-Galactose Containing Triterpenoid Saponin, and Five Related Compounds from *Saponaria officinalis*, 1999, <http://pubs.acs.org/doi/abs/10.1021/np980434w>

only one OH group more than medicagenic acid.<sup>878</sup> Gypsogenic acid is also very similar to corosolic acid, of the plant *Brachyotum microdon*: gypsogenic acid has a second carboxyl group (COOH) instead of a second OH group.<sup>879</sup> Corosolic acid forms complexes with human heme, most probably by a bond between the iron atom of heme and the carboxyl group (COOH, or rather COO<sup>-</sup>) of corosolic acid, for corosolic acid replaces Cl<sup>-</sup> in hemin (Cl-heme complexes).<sup>880</sup> The carboxyl group of acetic acid (CH<sub>3</sub>-COOH) is a high spin 6-coordinated ligand of human hemoglobin, and the complex is brown, as is usual for high spin 6-coordinated heme complexes<sup>881</sup>; an acetate-hemoglobin complex and acid methemoglobin A, which also is brown, indeed have similar absorbance spectra.<sup>882</sup> This means that the gypsogenic acid saponins of *Saponaria officinalis* probably form brown complexes with human hemoglobin and heme.

Alcohol, which has a saturated OH group (ethanol is CH<sub>3</sub>-CH<sub>2</sub>-OH), forms a “six-coordinate high-spin” ferric heme complex,<sup>883</sup> which is brown, just as acid methemoglobin. So, also the saturated OH groups of *Saponaria*'s glycosyls might perhaps form brown six-coordinate high-spin complexes with heme. Also saturated carbonyl groups (C=O) of the glycosyls in the saponins would not give a red heme complex,<sup>884</sup> but probably a brown one. Phenols, such as pyrogallol, also form brown

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<sup>878</sup> “Alfalfa root saponins and ammoniated glycyrrhizin, but not digitonin, quillaja and alfalfa leaf saponins, complexed zinc and iron, but not magnesium. It is proposed that one mechanism of action whereby saponins suppress growth is by the formation of insoluble saponin-mineral complexes.” (West, 1978 <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1978.tb15309.x/abstract>) ; “quillaja sapogenin” is “quillaic acid” (<http://www.chemspider.com/Chemical-Structure.91986.html>); “medicagenic acid 3-O-glucopyranoside, previously isolated from alfalfa roots” ... “the compound differing just with 2-OH substitution from gypsogenic acid glycosides” (Gumnicka et al., 1998, <http://pbsociety.org.pl/journals/index.php/asbp/article/download/asbp.1998.008/608>); gypsogenic acid structure: [http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=25202058&loc=ec\\_rcs](http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=25202058&loc=ec_rcs) ; medicagenic acid structure: <http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=65048>

<sup>879</sup> gypsogenic acid structure: [http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=25202058&loc=ec\\_rcs](http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=25202058&loc=ec_rcs); corosolic acid structure: [http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6918774&loc=ec\\_rcs](http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6918774&loc=ec_rcs) ; heme-binding corosolic acid: Gimenez et al., 2012, [http://www.bolivianchemistryjournal.org/QUIMICA%202012%20PDF/7\\_FERRIPROTOPORPHYRIN\\_BIOCRYSTALLIZATION.pdf](http://www.bolivianchemistryjournal.org/QUIMICA%202012%20PDF/7_FERRIPROTOPORPHYRIN_BIOCRYSTALLIZATION.pdf)

<sup>880</sup> Gimenez et al., Application of ferritoporphyrin biocrystallization inhibition test to find antiparasitic compounds in the flora of the “Zongo Valley” – Bolivia, 2012, [http://www.bolivianchemistryjournal.org/QUIMICA%202012%20PDF/7\\_FERRIPROTOPORPHYRIN\\_BIOCRYSTALLIZATION.pdf](http://www.bolivianchemistryjournal.org/QUIMICA%202012%20PDF/7_FERRIPROTOPORPHYRIN_BIOCRYSTALLIZATION.pdf)

<sup>881</sup> A. Boffi, co-author of “Crystal structure and ligand binding properties of the truncated hemoglobin from *Geobacillus stearothermophilus*”, 2007, <http://www.sciencedirect.com/science/article/pii/S0003986106003560>, personal communication on human hemoglobin, May 3, 2013; Fig. 3 C, the electron density map of “iron-bound acetate ligand” of trHb, in Ilari et al, shows how one of acetate's O-atoms is bound to the iron atom of the heme (Ilari et al., 2007, <http://www.sciencedirect.com/science/article/pii/S0003986106003560>); Also Adler implied that a high spin ferric hemoglobin is brown (Adler, The origin and..., 1986, TOM 60-61

<sup>882</sup> Fig. 5 A, “... The spectrum of the 100% acetate bound ferric adduct (dashed line) ...” of trHb, <http://ars.els-cdn.com/content/image/1-s2.0-S0003986106003560-gr5.gif>, in Ilari et al., 2007 (<http://www.sciencedirect.com/science/article/pii/S0003986106003560>), shows a spectrum that is similar to that of acid methemoglobin (cf. Kearse and Heimbürger, The Shroud Blood Science..., 2012, figures on p.8, or Austin and Drabkin, 1935, <http://www.jbc.org/content/112/1/67.short> p. 70 (p. 4 of pdf file) ); acid heme and acid methemoglobin are brown: “the colour of the coagulum being brown like that of acid haematin” (Meldrum, 1931, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1260779/pdf/biochemj01118-0060.pdf> )

<sup>883</sup> “We report herein the structure and dynamic equilibria for the six-coordinate high-spin ferric porphyrins with amine and alcohol as the ligands. These are expected to be appropriate models for aquomet-Hb and met-Mb, since (i) the axial ligands of the heme moiety are structurally analogous in both model and protein systems, and (ii) the present aquomet-Mb model complex is selectively and stably formed with various combinations of amine and alcohol derivatives in solution.” Morishima et al., 1979, <http://pubs.acs.org/doi/abs/10.1021/ja00527a047>

<sup>884</sup> “Related to this, I had also considered that a functional group on the cloth may interact with the porphyrin ring, a CO group, such as a carbonyl group, which are prevalent in carbohydrates (glycan coating as a result of *Saponaria*), but the structural biologists tell me the electron imprint would appear much different to the hemoglobin molecule than carbon monoxide. Just another thought.” Comment by K. Kearse, May 29, 2013, <http://shroudstory.com/2013/05/29/a-guest-posting-by-yannick-clment-two-quotes-about-the-blood/#comment-35281> ; carbon monoxide gas (CO) is a heme ligand that forms red hemoglobin complexes (HbCO): see the absorbance spectra of HbO<sub>2</sub> (red oxygen-rich blood) and HbCO and metHb in [http://biomedicaloptics.spiedigitallibrary.org/data/Journals/BIOMEDO/24849/JBO\\_17\\_9\\_090901\\_f001.png](http://biomedicaloptics.spiedigitallibrary.org/data/Journals/BIOMEDO/24849/JBO_17_9_090901_f001.png)

complexes with heme,<sup>885</sup> so, even the heme imprint of unsaturated OH of Saponaria's apigenin, a polyphenol, corresponds to a brown heme. However, as apigenin is a flavonoid dyestuff and would be deprotonated in the bond, its heme complex would probably be more yellow than that of colourless phenols (see 4.4.9). This means that the heme imprints of Saponaria's carboxyl and carbonyl and saturated (alcohol) and unsaturated (phenol) OH groups all correspond to a brown heme, and that the more yellow color of any apigenin complexes would only make the overall color a bit more yellow, but not red or pink. Yet, the color of the blood stains on the Shroud is not brown but pinkish-red. Madder's anthraquinones are polyphenols that are also dyes: they turn pink when complexed to iron,<sup>886</sup> as probably is the case in alizarin- and purpurin-heme complexes (see 2.1.1.3,4).

Rogers wrote "Saponaria is hemolytic, which could explain why the old blood stains are still red on the Shroud. We found that Saponaria was used in clinical chemistry to hemolyze blood for laboratory analysis. The process releases the hemoglobin. Hemoglobin is quite stable, and an observation of red blood would suggest either painting or hemolysis. Diane Soran (deceased) of Los Alamos tested hemolysis on Saponaria-washed cloth before we went to Turin. The blood stays red. It is still red on those 24-year old samples."<sup>887</sup>

This text has some peculiarities. It says that hemoglobin is quite stable, which is not correct. When blood is on a surface outside the body it dries and its hemoglobin oxidizes and loses water and turns into brown hemichrome within a few days,<sup>888</sup> which would then denature to free heme, of which the iron eventually separates from the porphyrin and turns black. Also, for Saponaria to keep the blood permanently red, it needs to form a very stable complex with the heme iron. But if it does that, Saponaria doesn't seem suitable as a first-step agent in clinical blood analysis. Besides, if Saponaria forms stable red complexes with ferric iron (as madder anthraquinones do), it would rather be a dye that can be mordanted red, instead of a soap. Also, Rogers seems to assume that hemolysis alone keeps blood red, which is not correct, for also released hemoglobin ages to brown hemichrome by drying.<sup>889</sup> If this "red" of Rogers' assumption and of Diane Soran's bloodstained Saponaria-washed cloth, just refers to the color of hemichrome or of the high-spin heme complexes described above, it is brown. Because another text of Rogers calls the Saponaria-bloodstains "red" in comparison with the "black" of ordinary old bloodstains,<sup>890</sup> this "red" might very well simply be brown. Unfortunately, Rogers did not publish photographs of these stains.

#### 4.4.11. Relative reflectance of bloodstains – not Saponaria

As said above, the Saponaria solution that would have given the Shroud its pale yellow color and a green yellow fluorescence, would have been an alkaline solution (see 4.4.1. and 4.4.2. above). If acidic post-mortem blood got on the Shroud, its acidity (ca. pH 6, see 5.1) would have been diminished or canceled by the base properties of the Saponaria residue. This means that the

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<sup>885</sup> Cornell University College of Veterinary Medicine, Red Maple Toxicosis, <http://ahdc.vet.cornell.edu/Sects/Toxic/redmaple.cfm>, Fig. 1 and 3 and text

<sup>886</sup> "Madder forms a bright red color when precipitated on an amorphous hydrated alumina substrate, such as alumina trihydrate. Tin, chromium, and iron mordants can produce purple, brown, and pink colors, respectively." <http://cameo.mfa.org/wiki/Madder>

<sup>887</sup> Rogers, An Alternate Hypothesis..., 2001, <http://www.shroud.com/pdfs/rogers10.pdf> p. 2

<sup>888</sup> The fractions of three different heme compounds found in a 7-days-old bloodstain, was 49% hemichrome, 16% methemoglobin, and 35% oxyhemoglobin; further ageing makes the hemichrome fraction increase at the expense of oxyhemoglobin. (Bremmer and Aalders, <http://www.slideshare.net/rolfok/ageing-bloodstains>, slide 16; cf. Bremmer et al., Age estimation of blood stains by hemoglobin derivative determination using reflectance spectroscopy, 2011, <http://www.sciencedirect.com/science/article/pii/S037907381000383X>)

<sup>889</sup> S. Maričić, Greta Pifat, V. Pravdić, Hydration of Haemoglobin and its Reversible Oxygenation, *Berichte der Bunsengesellschaft für physikalische Chemie* Volume 68, Issue 8-9, pages 787–793, October 1964, <http://onlinelibrary.wiley.com/doi/10.1002/bbpc.19640680827/abstract>

<sup>890</sup> "Saponaria is hemolytic, which could explain why the old blood stains on the cloth are still red. Diane Soran ... tested hemolysis on Saponaria-washed cloth before we went to Turin. The blood is still red on those 25-year-old samples. Controls are black." (Rogers and Arnoldi, *Scientific Method...*, 2002 p. 5; cf. Rogers, *A Chemist's...*, 2008, p. 19)

methemoglobin and heme on the Shroud would have been about neutral or alkaline, and the bloodstains would have yielded UV-vis reflectance spectra without a 630 nm band, but with a ca. 607 nm band and a more prominent Soret band.<sup>891</sup> Yet, relative reflectance spectra of the Shroud's bloodstains show a quite distinctive 630 nm band and no prominent Soret band, which can be explained by the presence of madder dye and an acidic heme-madder lake (see 2.1.2.2).

All of these properties of the Shroud make the presence of a *Saponaria* residue very improbable.

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<sup>891</sup> Austin and Drabkin, 1935, <http://www.jbc.org/content/112/1/67.short> Fig. 1, p. 70; “the specific absorption band of alkaline haematin (607 m $\mu$ ).” <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2164191/pdf/brmedj04008-0003.pdf>; See fig. 2.5 above, representing Fig 4. D : UV-vis absorbance of aqueous heme  $\pi$ - $\pi$  dimer at pH 6.029 (solid line) and at pH 9.669 (dotted line) and Fig. 4.F : UV/Vis absorbance of heme  $\mu$ - $\alpha$ - $\alpha$  dimer, induced in a aqueous pyridine in 0.1 M NaOH, showing a ca. 610 nm band, of Villiers et al, *Speciation and structure...*, 2007

## 5. FORMATION MECHANISMS

That very different sources of blood produced the same pinkish color on the Shroud,<sup>892</sup> and the same reflectance spectra, indicates that the Shroud's madder coating made the blood's heme bind to madder phenols in all of these cases, to form the same pinkish madder lake. The sources of blood would have been

- small of back: flowing post-mortem blood flowed as blood rivulets across and through the cloth
- lance wound area: wettish clotted post-mortem blood, possibly mixed with a clear fluid, made a penetrating imprint
- wrist and arms, forehead and hair, back of head, feet: wettish clotted post-mortem blood on the body, made penetrating imprints as stamps on the cloth
- scourges: dry clotted pre-mortem blood, made surface imprints of the open skin parts after hyperfibrinolysis remoistened and acidified only these parts of the clots, (many) hours after burial.

### 5.1. Post-mortem heme dimer formation – further acidification, hemolysis and heme adsorption by dyed cloth – powder formation and abrasion

There may have been three causes of acid denaturation of the blood:

- severe pre- and postmortem acidemia of the body
- acidic madder dye on the Shroud (its acidity washed away where much liquid blood gathered and drained away through the Shroud)
- denaturing and adsorbing madder phenols on the Shroud

The condition in which a person has acidic blood (acidemia) may arise from strenuous physical exercise and cramps (causing acidosis = acidic body fluids, e.g. lactic acidosis),<sup>893</sup> and/or from respiratory distress and/or dehydration/hypovolemic shock.<sup>894</sup> In a dying person with a severe case of acidic blood, the blood pH may be extremely low, especially after death, as the blood pH drops further after death: “Blood pH falls after death, and also sometimes before death, e.g. in patients who undergo prolonged resuscitation, or who die from respiratory or renal failure.”<sup>895</sup> Inside a dead human body, a blood pH as low as 5.5 has been found, 20 hours after death.<sup>896</sup> In rats that in 1-2 hours died from oxygen deficiency, the blood pH dropped from the normal 7.4 to 6.8, immediately after death, and to 6.6, three hours after death.<sup>897</sup> If a person has died with severe acidemia – resulting from strenuous exercise, cramps, respiratory failure for six hours and

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<sup>892</sup> Farey, in an online comment in January 2014, called them “fairly uniform pinkish stains”, <http://shroudstory.com/2014/01/21/cat-among-the-pigeons/#comment-74465>

<sup>893</sup> “Lactic acidosis is a physiological condition characterized by low pH in body tissues and blood (acidosis) accompanied by the buildup of lactate ... The condition typically occurs when cells receive too little oxygen (hypoxia), for example, during vigorous exercise.” ( [http://en.wikipedia.org/wiki/Lactic\\_acidosis](http://en.wikipedia.org/wiki/Lactic_acidosis))

<sup>894</sup> <http://en.wikipedia.org/wiki/Acidosis> [http://en.wikipedia.org/wiki/Hypoxia\\_\(medical\)](http://en.wikipedia.org/wiki/Hypoxia_(medical))

<sup>895</sup> Ferner, Post-mortem clinical pharmacology, *British Journal of Clinical Pharmacology*, 2008, 66 (4), pp 430-443 <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2125.2008.03231.x/full>

<sup>896</sup> Clarke's Analytical Forensic Toxicology, Pharmaceutical Press, May 1, 2013, <http://books.google.co.uk/books?id=UmWCCDcmqEC&pg=PA349&dq=blood+pH+postmortem&hl=nl&sa=X&ei=o-4AUrbVPMHnswb1w4HYAQ&ved=0CF4Q6AEwBw#v=onepage&q=blood%20pH%20postmortem&f=false> p. 349

<sup>897</sup> Takeichi et al., Fluidity of Cadaveric Blood After Sudden Death: Part I Postmortem fibrinolysis and plasma catecholamine level, *The American Journal of Forensic Medicine and Pathology* 1984, 5 (3) pp 223-228 ([http://journals.lww.com/amjforensicmedicine/Citation/1984/09000/Fluidity\\_of\\_cadaveric\\_blood\\_after\\_sudden\\_death\\_.6.aspx](http://journals.lww.com/amjforensicmedicine/Citation/1984/09000/Fluidity_of_cadaveric_blood_after_sudden_death_.6.aspx)) and Takeichi et al., Fluidity of Cadaveric Blood After Sudden Death: Part III – Acid-Base Balance and Fibrinolysis, *The American Journal of Forensic Medicine and Pathology* 1986, 7 (1) pp 35-38, ([http://journals.lww.com/amjforensicmedicine/abstract/1986/03000/fluidity\\_of\\_cadaveric\\_blood\\_after\\_sudden\\_death\\_.7.aspx](http://journals.lww.com/amjforensicmedicine/abstract/1986/03000/fluidity_of_cadaveric_blood_after_sudden_death_.7.aspx))

dehydration/hypovolemic shock (such as Jesus probably suffered)<sup>898</sup> – blood pH could easily be below 6.6. A blood pH 6.19 has been found in a person within 1¼ hour after a fatal cardiac arrest, which may be considered a cause of both metabolic and respiratory (CO<sub>2</sub>-induced) acidemia.<sup>899</sup> This person's blood pH would have fallen a bit further three hours after death, e.g. to approximately pH 6.1. If an effect of severe acidemia before the cardiac arrest is added, the blood pH of such a person could be lower than 6.1 three hours after the cardiac arrest. The difference in blood pH between normal and severely acidotic living persons, for instance because of “prolonged agonal states”, is sufficient to distinguish the two “within the first five hours postmortem”.<sup>900</sup> In two acidotic patients, the cardiac blood pH (left ventricle) was 6.09 4¼ hours after death, and 6.03 nearly 6 hours after death, respectively; in another acidotic patient this blood pH was about 6.22 3 hours after death.<sup>901</sup>

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<sup>898</sup> “Experimental ... A common complaint was a feeling of chest rigidity and leg cramps between 10 and 20 minutes into suspension.” (Zugibe, Forensic and Clinical..., 2000, <http://e-forensicmedicine.net/Turin2000.htm>); “The results would cause a significant degree of trauma with impending shock (traumatic shock) and fluid loss and impending hypovolemic shock (fluid loss shock), the latter resulting from the various sweating episodes, and from the fluid accumulation around the lungs (pleural effusion) from the scourging. ... These episodes of unrelenting pains added to the pains of the chest wall from the scourging would greatly increase the state of traumatic shock and the excessive sweating induced by the ongoing trauma and by the hot sun, would cause a increase in the degree of hypovolemic shock. The pathophysiological events that occur as a result of these events leading to death are those of traumatic and hypovolemic shock.” (Zugibe, Ibid.); “This method of nailing led to breathing impairment: with the arms raised at an approximately 15 degree angle causing the ribcage to expand, the lungs had difficulty expiring, reducing air flow. In addition to this, each deep breath the Man took to speak or to catch his breath will have put a strain on the lower limbs causing him intense pain.” (Vatican Insider on Injury article of Bevilacqua et al, 2014, <http://vaticaninsider.lastampa.it/en/world-news/detail/articolo/sindone-shroud-sudario-33948/>); the “fluid accumulation around the lungs (pleural effusion)”, mentioned by Zugibe, would probably cause respiratory failure as well: “Animal experimentation by Daniels and Cate<sup>[22]</sup> showed that blows to the chest in animals resulted in rupture of the air spaces in the lung (alveoli) and spasms of the air tubes (bronchi). Moreover the term "traumatic wet lung" refers to the accumulation of blood, fluid and mucus from severe trauma (injury) to the chest. This would be manifested several hours after the scourging.” (Zugibe, Forensic and Clinical..., 2000, <http://e-forensicmedicine.net/Turin2000.htm>) and “Pleural effusion is excess fluid that accumulates between the two pleural layers, the fluid-filled space that surrounds the lungs. Excessive amounts of such fluid can impair breathing by limiting the expansion of the lungs during ventilation.” ([http://en.wikipedia.org/wiki/Pleural\\_effusion](http://en.wikipedia.org/wiki/Pleural_effusion)); “According to the authors of the *Injury* article, the serum stains, which are separate to the stains of blood that came from the chest and were probably caused by the stabbing with a spear after he had died, were formed as a result of bleeding in the lungs. This bleeding will have started before the crucifixion, after the violent fall which caused the cross to fall onto the Man's shoulders. ... Restricted breathing and the presence of the haemothorax which put pressure on the right lung were not enough to bring about death by asphyxia. ... The four experts say the fall and/or the flagellation have caused not only a pulmonary contusion but also a cardiac contusion.” (<http://vaticaninsider.lastampa.it/en/world-news/detail/articolo/sindone-shroud-sudario-33948/page/1/>); “But one of the soldiers pierced his side with a spear, and at once there came out blood and water.” (Gospel of John 19:34)

<sup>899</sup> Ding-Kuo Chien et al., 2010, Survival Prediction of Initial Blood pH for Nontraumatic Out-of-hospital Cardiac Arrest Patients in the Emergency Department, *International Journal of Gerontology* 4 (2010) 171-175 <http://www.sgecm.org.tw/db/Jour/2/201012/2.pdf>

<sup>900</sup> “Blood pH has been shown to decrease in proportion to the time of death. The value obtained is dependent of the site of the sample. A small study has shown postmortem pH from a cardiac sample fell from 7.0 at the time of death to 5.5 at 20 hours after death.<sup>11</sup> However, the difference in pH between normal and severely acidotic individuals is sufficient to distinguish the two within the first five hours postmortem.<sup>3</sup> Plasma lactic acid increases postmortem; it is significantly increased within the first postmortem hour and progressively increases to between 50 and 75 times the normal antemortem values.<sup>2</sup> Vitreous lactic acid also increases but only doubles (from 80-160 to 210-260 mg/dL) at 20 hours. Values are low in sudden cardiac death and are high in cases of prolonged agonal states.<sup>39</sup>” Weeden, Siebert and Prahlow, *Postmortem Chemistry*, Taylor and Francis Group, 2012, [http://www.elliottlakeinquiry.ca/exhibits/pdf/09242-DMQ\\_E000003331.pdf](http://www.elliottlakeinquiry.ca/exhibits/pdf/09242-DMQ_E000003331.pdf)” p. 240 / p. 6 in online pdf, in which 3 = Sturmer WQ, 2006. Chemical considerations, in *Medicolegal Investigation of Death*, 4<sup>th</sup> ed., ed by W.U. Spitz, Springfield, IL, Charles C Thomas, pp. 128-148, and in which 11 = Sawyer WR, Steup DR, Martin BS, Forney RB, Cardiac blood pH as a possible indicator of postmortem interval. *Journal of Forensic Sciences*, 1988 Nov; 33(6):1439-44. <http://www.ncbi.nlm.nih.gov/pubmed/3204346>

<sup>901</sup> Straumfjord, J.V., and Butler, J.J., Evaluation of antemortem acid-base status by means of determining the pH of postmortem blood. *Am. J. Clin. Pathol.*, 23:165, 1957, 1957 Aug;28(2):165-70, <http://www.ncbi.nlm.nih.gov/pubmed/13458141> ; “The pH of blood from the upper extremities was higher than that of blood from the left ventricle of the heart, which in turn was higher than that from the right ventricle.” – its table 1 shows six cases, in which blood from the right ventricle had a 0.03 to 0.29 lower pH, and another six cases, in which blood from the antecubical vein (arm) had a 0.00 to 0.36 higher pH than that from the left ventricle (Ibid. p. 166-168).

Acidosis may also result in methemoglobinemia.<sup>902</sup> But also without antemortem methemoglobinemia, postmortem blood may have an unexpectedly high methemoglobin level: no relationship was found between antemortem and postmortem levels.<sup>903</sup> When postmortem blood leaves the body, the oxygen in the air will start oxidizing its hemoglobin to methemoglobin.

In a solution of 0.02 M ionic strength, which is much smaller than physiologic ionic strength 0.154 M,<sup>904</sup> acid denaturation of human methemoglobin occurs in the pH region 4-5: if an acid methemoglobin solution (pH 4.9, Soret  $\epsilon = 17.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ) is brought into a pH 4.40 buffer solution of 25°C, the denatured fraction is about 40%, measured by comparing the Soret absorbance in transmission of native and completely denatured methemoglobin.<sup>905</sup> At a lower temperature the curve representing the denatured fraction vs pH, is shifted to higher pH's, e.g. at 15 °C 40% denaturation is seen at pH 4.56.<sup>906</sup> Furthermore, a higher ionic strength stretches the acid-denaturation pH range: at 0.2 M ionic strength and 24.9 °C, acid denaturation (in HCl) is active at pH values from at least pH 3.0 to pH 6.6.<sup>907</sup> Assuming that small effects of temperature and ionic strength are linear, acid methemoglobin that has a temperature of 20 °C, an ionic strength of ca. 0.2 M and a pH 6.1 (as blood could have, three hours after the death of a severely acidemic person<sup>908</sup>), would have a denatured fraction of about 6.8%.<sup>909</sup> This means that a small fraction of detached heme is present in the blood.

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<sup>902</sup> “Exposure to oxidizing drugs or chemicals, systemic acidosis (a generalized, abnormal increase in body fluid acidity), diarrhea, [...] can all result in methemoglobinemia.” Agency for Toxic Substances and Disease Registry, 2011, <http://www.atsdr.cdc.gov/toxfaqs/tfacts204.pdf>

<sup>903</sup> “Postmortem methemoglobin has been studied and expressed as a percentage of total hemoglobin. No relationship was found between recorded levels and the time of death or autopsy findings. Postmortem concentrations were deemed not to be reliable indicators of methemoglobinemia in the antemortem state.<sup>210</sup> ... 210. Reay, D.T., Insalaco, S.J., and Eisele, J.W.: Postmortem methemoglobin concentrations and their significance. *J. Forensic Sci.*, 29 (4): 1160-1163, 1984.” Sturmer WQ, 2006. Chemical considerations, in *Medicolegal Investigation of Death*, 4<sup>th</sup> ed., ed by W.U. Spitz, Springfield, IL, Charles C Thomas, pp. 128-148.

<sup>904</sup> Villiers et al., *Speciation and structure...*, 2007, p. 103

<sup>905</sup> Steinhardt and Hiremath, *A Comparison of ...*, 1967. “pH 4.9”: p. 1295; 40% decrease: *Ibid.* fig. 3; 100% denaturation here seems to mean 100% loss of the Soret absorbance difference between native and denatured, for the native minus denatured difference spectrum is smaller than the native spectrum (cf. their fig. 11 (1 mm path-length) and fig. 12 (5 mm pathlength)). In 1966, Steinhardt et al. published the absorbance spectra of both native and completely denatured horse carboxyhemoglobin, in which the denatured Soret absorbance is about 1/3 of the native one (Steinhardt, Polet and Moezie, Acid Denaturation of Horse Carboxyhemoglobin in the Absence of Oxygen, *The Journal of Biological Chemistry*, Vol. 241, No. 17, Sept 1966, pp. 3988-3996, <http://www.jbc.org/content/241/17/3988.full.pdf>); in the same 1966 article they also published a curve in which the denatured fraction of horse methemoglobin ranged from 0 to 100% (fig. 6), and they republished this curve in their 1967 article in its fig. 5, which also shows the denatured fraction of human methemoglobin.

<sup>906</sup> Steinhardt and Hiremath, *A Comparison of ...*, 1967

<sup>907</sup> Fig. 7 of Steinhardt and Hiremath, *A Comparison of ...*, 1967, which caption says “0.3 M KCl”, actually shows an acid binding-vs-pH plot from pH 3.0 to 7.0 for “0.2 M” ionic strength, as the text repeatedly says, viz. at p. 1295, 1298. As an “increase in acid-binding groups ... accompanies the spectroscopic changes” and ... “it can be concluded that unmasking of individual prototropic groups and changes affecting the binding of the prosthetic group occur simultaneously”, this figure 7, representing the pH dependence of acid binding in the pH 3.0 to 6.6 range, also represents pH dependence of spectroscopic changes of the Soret absorbance in the same pH range.

<sup>908</sup> “The water content and pH of a postmortem blood sample may also differ significantly from physiological ranges.

The water content of postmortem blood was observed to range from 60 to 90% [50] and [51].” G. Skopp, Preanalytic aspects in postmortem toxicology, *Forensic science international*, Volume 142, Issues 2–3, 10 June 2004, Pages 75–100, <http://www.sciencedirect.com/science/article/pii/S0379073804001021>. Assuming physiological ionic strength (0.154 M) inside a red blood cell in normal blood, a 60-90% water content in post-mortem blood would correspond to an ionic strength of 0.169 – 0.216 M. As blood pH in rats dropped from 6.8 to 6.6 in 3 hours, and 0.03 in the third hour (Takeichi et al., 1986), a human post-mortem pH drop from 6.19 to 6.1 from 1 ¼ to 3 postmortem hours seems a reasonable estimate.

<sup>909</sup> As pH dependence of Soret absorbance/acid-binding is also similar in acetate and HCl solutions (in fig. 4 of Steinhardt and Hiremath 1967, Cl<sup>-</sup> pH-stat datapoints match acetate buffer datapoints), and as both the 0.02 M acetate curve and the 0.2 M HCl titration curve show a bend in their higher pH part (fig. 5 and fig. 7), it seems that the 17% Soret absorbance decrease/fraction denatured at the bend in the acetate curve is a good estimate of the acid binding decrease at the bend in the 0.2 M titration curve, viz. at pH 5.2; this is corroborated by the corresponding “little denaturation” at pH 4.4 and the “partial denaturation” at pH 3.6 (fig. 10). As 10 degrees Celsius cooling to 15 °C makes the 25 °C Soret absorbance-vs-pH curve shift to 0.16 higher pHs (fig. 3; and “ $\Delta H$  changes sign at a temperature near 15°”

If acid heme has detached from the protein inside a severely acidic red blood cell, it is expected by one of the discoverers of the aqueous  $\pi$ - $\pi$  dimer, to form exactly this dimer – even taking into account that also chloride and a high concentration of proteins is present inside the red blood cell – and to aggregate.<sup>910</sup> Aggregation makes the heme less active as peroxidation catalyst.<sup>911</sup> The dimers probably would not precipitate at the inner surface of the red blood cell wall, as this wall consists of a phospholipid bilayer that has its hydrophilic heads at its two surfaces, towards both the extracellular fluid and the cytoplasm.<sup>912</sup> This means that the surfaces of the cell wall attract water molecules that would hinder the binding of the hydrophobic heme dimers<sup>913</sup> to the wall.

If such partly acid-denatured blood leaves the cold dead body, it may first lose most of its potassium-rich (hyperkalemic) serum by serum draining from the clot along a vertical cold body surface<sup>914</sup> or through the filter effect of a tight waterresistant coated cloth/shroud – lying on a wooden burial bier or on a waterabsorbing subcloth –, if a drop of blood had fallen on it or ran across it. If acid blood/hemolysate of pH ~6.1 got on an insoluble layer of acid yellow madder dye having protonated alizarin and purpurin and some deprotonated purpurin (see 4.2.1.), this pH would deprotonate OH groups of both alizarin ( $pK_{a1}$  = ca. 6.6) and purpurin ( $pK_{a1}$  = ca. 4.7),<sup>915</sup> which deprotonation alone would already turn the surface of the insoluble dye coating from yellow to red. Also, the deprotonation of the dyestuffs would make the wet blood material more acidic, making its pH drop from 6.1 to perhaps 5, thus increasing the denaturation and the fraction of acid heme dimers, perhaps to about 20%.<sup>916</sup> If an acidic red blood cell at the surface of a clot on the body – or at the bottom of a filtered drop/rivulet on the cloth – is hemolysed by contact with madder root saponins<sup>917</sup> in the cloth's coating, its cytoplasm (which then is its hemolysate) would leave the cell and its heme dimers would be adsorbed onto the madder phenols on the cloth<sup>918</sup> and probably form red/pink-colored complexes with the cloth's madder hydroxyanthraquinones: the deprotonated OH groups of the now

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p. 1297), also the acid binding-vs-pH curve may shift to 0.16 higher pHs with 10 degrees C and thus to 0.08 higher pHs at 20 °C. This would result in a curve that connects 17% change to 0% from pH 5.28 to pH 6.68, and therefore a 6.8% change at pH 6.1.

<sup>910</sup> T.J. Egan, co-author of Villiers et al., Speciation and structure of ferriprotoporphyrin IX in aqueous solution: spectroscopic and diffusion measurements demonstrate dimerization, but not  $\mu$ -oxo dimer formation, *Journal of Biological Inorganic Chemistry* (2007) 12: 101-117 <http://link.springer.com/article/10.1007%2Fs00775-006-0170-1>, and of Asher et al., Speciation of Ferriprotoporphyrin IX in Aqueous and Mixed Aqueous Solution Is Controlled by Solvent Identity, pH, and Salt Concentration, *Inorg. Chem.*, 2009, 48 (16), pp 7994–8003 <http://pubs.acs.org/doi/abs/10.1021/ic900647y>, personal communication, July 25, 2013

<sup>911</sup> “UV/Vis spectroscopy of AH aggregates at acidic pH, low GSH concentrations and chloroquine suggests a shift of AH aggregates toward the less aggregated state, more active as peroxidation catalyst.” <http://www.sciencedirect.com/science/article/pii/S0006295201005585>, 2001 (AH =  $\alpha$ -hematin = the OH-ligated human heme, which would be H<sub>2</sub>O-ligated at acidic pH; chloroquine is an antimalarial drug).

<sup>912</sup> [http://en.wikipedia.org/wiki/Cell\\_membrane](http://en.wikipedia.org/wiki/Cell_membrane)

<sup>913</sup> “Given the hydrophobic nature of Fe(III)PPIX” Villiers et al., Speciation and structure..., 2007, p. 106

<sup>914</sup> As may be inferred from the experiments of Lavoie, Blood on the Shroud... Part II, 1983, described above in 2.3.2.

<sup>915</sup> Miliani et al., Acidichromic effects..., 2000, table 5

<sup>916</sup> Cf. fig. 7 of Steinhardt and Hiremath, A Comparison of..., 1967: “Fig. 7 presents the titration curve ... in 0.2 M KCl at 24,9°”

<sup>917</sup> *Rubia tinctorum* root has froth number 154 (the leaves have froth number 200), which means that also the root contains saponins which create a froth (Table 5 of Dengre et al., Comparative studies of *Rubia cordifolia* Linn. and *Rubia tinctorum* Linn (Rubiaceae), 1993, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3336544/pdf/ASL-13-165.pdf>); Henry, 1989, [http://link.springer.com/chapter/10.1007/978-3-642-73617-9\\_24#page-1](http://link.springer.com/chapter/10.1007/978-3-642-73617-9_24#page-1); see 2.4.2.2.

<sup>918</sup> “An earlier hypothesis suggested that the thermodynamically limiting step of spontaneous heme crystallization is the solubility of heme from its acid amorphous aggregate [26] (Alcohols induce beta-hematin formation via the dissociation of aggregated heme and reduction in interfacial tension of the solution, Huy et al. 2007.), [27]. Different chemical and physical factors ... suggest that increased dissolution of insoluble heme aggregates is a key parameter that would modulate  $\beta$ H formation.” Stiebler et al., 2010,

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0012694>; madder dye contains phenols (see 3.3), and probably even alcohol from spontaneous fermentation of the root extract (see 3.3); “phenol and water are immiscible, two phases form” (<http://bitesizebio.com/articles/the-basics-how-phenol-extraction-works/>); phenol is absorbed to hydrophobic minerals (Water Remediation by Adsorption of Phenol onto Hydrophobic Modified Clay, <http://link.springer.com/article/10.1007%2Fs11270-008-9863-0>); “phenol adsorbs organic compounds” <http://pharmabook.net/en/protivomikrobye--protivoparaz/antiseptiki-i-dezinficiruyusch/carbolic-acid.html>

red dye stuffs would be free to form red complexes (madder lakes) with the  $\text{Fe}^{3+}$  ion of the heme, as in the well known ferric ion test for phenols.<sup>919</sup> The madder phenols of the cloth would probably first have bound the already present acid heme dimer aggregates of the hemolysate, before denaturing some of the present methemoglobin and binding its acid heme dimers, and only lastly would perhaps have oxidized any remaining hemoglobin to methemoglobin, and denatured the methemoglobin to acid  $\pi$ - $\pi$  dimers, which would precipitate on the cloth as well. As acid denaturation at physiological ionic strength is very fast,<sup>920</sup> the first  $\pi$ - $\pi$  dimer adsorbance to the cloth would shift the acid-denaturation equilibrium in the solution towards more acid denaturation of the remaining intact methemoglobin in the hemolysate, and thus toward more acid  $\pi$ - $\pi$  dimer formation and precipitation. Thus, all of the phenol surface that was touched by hemolysate, would be red and largely covered with an acid  $\pi$ - $\pi$  dimer precipitate. As phenols are ligands to heme,<sup>921</sup> the resulting complex would simply be a brown heme precipitate if the phenols were not a dye.<sup>922</sup> But in the case of yellow madder dye phenols, the bound heme would be the dye's mordant and form a red madder-mordant complex, a red madder lake.

As the hemolysate dries and loses 90% of its water, its pH would drop another unit, for example from pH 5 to pH 4, which would also induce more acid denaturation. As also the ionic strength slowly increases in drying, there is a possibility that from a certain moment  $\mu$ -oxo dimers would be formed instead of acid  $\pi$ - $\pi$  dimers.<sup>923</sup>

Anyway, through the initial adsorbance, an amorphous insoluble acid  $\pi$ - $\pi$  dimer precipitate would be formed on the cloth very quickly, leaving any remaining intact acid methemoglobin free to denature or crystallize to a powder in the subsequent drying process. If the blood clot on the body (containing some broken and still intact red blood cells in its fibrin mesh) and the cloth are finally separated, the powder layer of crystalline acid methemoglobin (and perhaps some crystalline  $\mu$ -oxo dimer) between a dried clot and the cloth's  $\pi$ - $\pi$  dimer precipitate would probably be disrupted and then be largely abraded from the precipitate in further handling of the cloth. This disruption of an acid methemoglobin powder layer might explain the observation that "No broken fibers were found under the blood clots" of the Shroud.<sup>924</sup> This bloodstain composition also holds for the acid blood that got onto the cloth in liquid form, lost its serum and surplus blood as it drained through the waterresistant cloth to, for instance, the bare wood of a burial bier (cf. Luke 7:14) or a liquid-absorbing burial subcloth<sup>925</sup>, and of which remaining red blood cells dried and oxidized and hemolysed on the cloth

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<sup>919</sup> "If the phenol is water soluble, add a few drops of 2.5% aqueous ferric chloride solution to a 3% aqueous solution of the phenol. A deep red, green, or blue color is positive." Chickos, Garin and D'Souza, University of Missouri-St. Louis, <http://www.umsl.edu/~orglab/experiments/UNKEXP.html> on <http://www.umsl.edu/~orglab/>; cf. Heller and Adler, A Chemical..., 1981, Table 7, TOM 54: "Tests employed .... Species: phenols – Method: a) nitrous acid b) ferric ion"

<sup>920</sup> "at 0.2 ionic strength ... at this high ionic strength, the denaturation rate is so great that pH-stat experiments are impracticable unless the rate is decreased. Advantage has been made of the stabilizing effect of combining ferrihemoglobins with cyanide to observe the full course of the reaction at an ionic strength of 0.2" (Steinhardt and Hiremath, A Comparison of..., 1967, p. 1295).

<sup>921</sup> "The  $k_2$  values are strongly sensitive to the nature of the ligand. Reactivity decreases in the order nitrite > azide > thiocyanate > cyanate > cyanide > phenol > fluoride > chloride > bromide (Tables I and II). This order suggests that effectiveness in promoting autooxidation is related to nucleophilicity as a ligand except that cyanide seems out of order in terms of relative nucleophilicity." WA Wallace, RA Houtchens, JC Maxwell, WS Caughey, Mechanism of autooxidation for hemoglobins and myoglobins. Promotion of superoxide production by protons and anions. *Journal of Biological Chemistry*, Vol 257, No. 9, pp, 4966-4977, 1982, <http://www.jbc.org/content/257/9/4966.full.pdf>, p 4969.

<sup>922</sup> "the precipitates obtained in the earlier experiments (air present) were dark brown" (Steinhardt et al., Oxygen Carbonylhemoglobin in the Absence of Oxygen, 1966, <http://www.jbc.org/content/241/17/3988.full.pdf>); A simple phenol such as tannic acid and gallic acid, when added to equine blood, gives it a dark brown color: Fig. 1 of "Red Maple Toxicosis" of the Cornell University College of Veterinary Medicine, <http://ahdc.vet.cornell.edu/Sects/Toxic/redmaple.cfm>

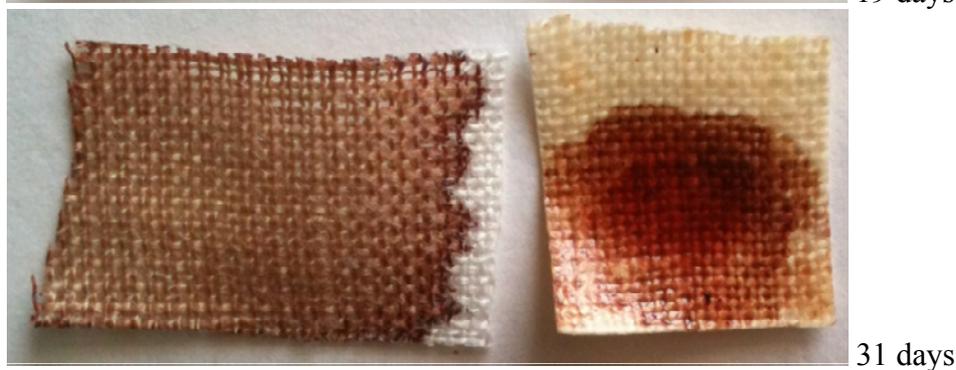
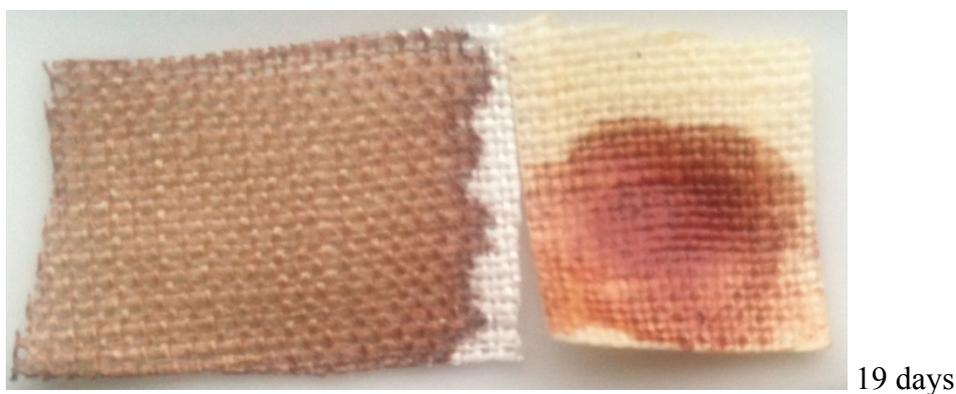
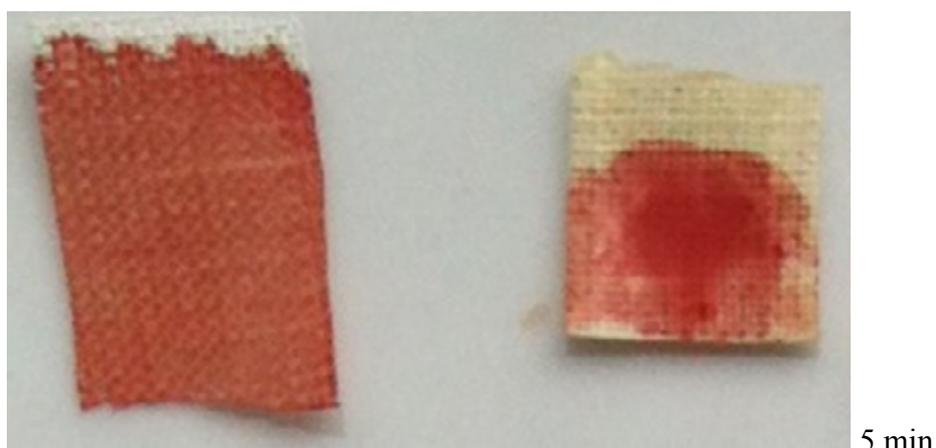
<sup>923</sup> In a NaCl solution of 1.2 M ionic strength " $\mu$ -oxo dimer formation is likely to occur" (Villiers et al., Speciation and Structure..., 2007, p. 115)

<sup>924</sup> "A78) No broken fibers were found under the blood clots (N. Svensson 2005). ... SVENSSON N. Shroud Science Group communication 2005." Fanti et al., Evidences for..., 2005, <http://www.shroud.com/pdfs/doclist.pdf>

<sup>925</sup> "Sometimes funerary biers or stretchers were made with textiles, sometimes they were simple leather mattresses filled with twigs ... formerly, they would carry out the rich in a state bed or fancy litter called *darghesh* (דָּרְגֵשׁ) and the poor

to form a largely abradable stain of dry red blood cells (with hardly any fibrin mesh), acid methemoglobin (crystals), amorphous acid heme dimer precipitate and heme-madder lake. The amorphous insoluble heme precipitate would remain on the cloth longest, and the  $\pi$ - $\pi$  heme dimer-madder lake would simply be a part of the cloth's madder coating.

That blood is able to form stains on starched and madder-dyed linen that are pinkish and more colorfast than bloodstains on pure linen, has already been shown in experiments represented in fig. 2.46 and 2.47. Another experiment, in which fresh whole blood was acidified by adding an aqueous vinegar solution of pH 5.5 - 6 and then was dropped on pure and starched-(not-washed)-and-madder-dyed linen (and then smeared out on the dyed piece), showed that the stain on the pure linen turned brownish pink but the stain on the dyed linen remained a bit more red (fig. 5.1). Here must be noted that these new stains of artificially acidified – and thus diluted and contaminated – fresh normal blood can not directly be compared to old stains of authentic postmortem acidic blood. Also the simple weave of this linen is different from the weave of the Shroud.





Wood lamp, photo with L-42 filter

*Fig. 5.1. Staining pure and starched-and-madder-dyed linen by dropping and smearing artificially acidified blood on it. Photos 5-10 min and 19 and 31 days after staining. ©AvdH*

#### *Rogers on thermal denaturation of blood on the Shroud*

The Shroud evidence list of 2005, i.e., of after Rogers's death says: "A61) The blood on the TS is not denatured. Therefore both the image-formation mechanism and the 1532 fire did not involve processes that would denature the blood (Rogers 2004)", in which the reference is to "ROGERS R. Shroud Science Group communication 2003, 2004".<sup>926</sup> In this communication, Rogers probably did not make a statement about denaturation by acids, but only stated that the blood was not denatured by simple heating (pyrolysis), a conclusion he had drawn – with referral to the image-formation process and the fire – on account of the appearance of "a low-temperature emission of hydroxyproline" in Pyrolysis/Mass Spectrometry of the Zina thread's red spots.<sup>927</sup> Heller and Adler did speak of a denatured methemoglobin species on the Shroud.<sup>928</sup>

## **5.2. Blood drying on the body**

### *Side*

The large bloodstain on the Shroud side area could be authentic post-mortem blood of a crucified person such as Jesus, who was stabbed in the side after death while still on the cross (John 19:32-34). The blood that flowed from the wound would start to form clots (a fibrin mesh holding the red blood cells) on the wound and on the intact cold skin beneath it. The cold, acid and hyperkalemic blood plasma, containing much of the blood's potassium, would start to be exuded from the clot and could flow to the margin of the lowest parts of the blood clots while on the cold skin. From there, plasma may have drained away from the body. At the margin of the drying red clot, the remaining serum would be drying to form yellow/orange halos around the red clots on the skin. The X-ray Fluorescence (XRF) measurement carried out inside the area of the red side blood did not show a potassium signal. As the Gospel says that, besides blood, also water came out of Jesus' side (John 19:34), the drying of the side blood may have taken longer than that of post-mortem blood that got out later, without water. Besides, new blood may have come out of the side wound when the body was taken from the cross and laid on the shroud, later.

<sup>926</sup> Fanti et al., Evidences for..., 2005, <http://www.shroud.com/pdfs/doelist.pdf>

<sup>927</sup> Rogers, A Chemist's..., 2008, p. 56-58

<sup>928</sup> "Thermodynamically the latter fibrils would be expected to show the spectrum of a fully oxidized denatured met-hemoglobin, i.e., a so-called perturbed acid met-hemoglobin", and "In our opinion the spectral data taken in aggregate are positive in confirming the presence of perturbed acid met-hemoglobin species on the Shroud." (Heller and Adler, Blood on the Shroud..., 1980, TOM p. 30-31; see Heller, Report on..., 1983, p. 144-147, and 2.1.1.1.)

### *Wrist and arms, head and foot*

Also the bloodstains on the Shroud areas of the wrist and arms, head, and toes could be authentic post-mortem blood of a crucified person such as Jesus, for some blood would have come out at and after the removal of the nails and crown of thorns and may have fallen from the crown of thorns at its removal.<sup>929</sup> Also this blood would start to form clots on the wounds and on the intact cold skin, and would start to exude serum which could flow to the the lowest parts of the blood clots while on the cold skin, where it could form yellow/orange serum halos. Note that on the wrist and arms and toes areas, no XRF measurements were made, so nothing can be said of the potassium level; at the lateral margin of one of the faint vertical bloodstains in the hair image of the head, an XRF measurement was made, and the measured iron level was not significantly higher than in the neighbouring, definitely bloodless, areas. So, the measured lack of a potassium signal at this margin need not mean there wasn't any potassium in this actual bloodstain.

Scripture says Jesus was wrapped in a linen cloth first and only then laid in the tomb. The Gospel of Matthew says of Joseph of Arimathea: "He went to Pilate and asked for the body of Jesus. Then Pilate ordered it to be given to him. And Joseph took the body, and wrapped it in a clean linen shroud, and laid it in his own new tomb, which he had hewn in the rock; and he rolled a great stone to the door of the tomb, and departed", and Luke says "Then he took it down and wrapped it in a linen shroud, and laid him in a rock-hewn tomb, where no one had ever yet been laid," and Mark even says that Joseph of Arimathea, while "taking him down, wrapped him in the linen shroud."<sup>930</sup> There may have been some time between the taking from the cross and the covering of the body with the upper half of the shroud, though, for John 19:38-42 has a verse about Nicodemus coming to bring a large weight of spices ("Nicodemus also ... came bringing a mixture of myrrh and aloes, about a hundred pounds' weight." vs. 39) between two verses about the taking of the body. The first verse probably speaks of the taking away (the verb *αἶρω*) from the cross by Joseph of Arimathea, as in his request to Pilate ("Joseph of Arimathea ... asked Pilate that he might take away (*αἶρη*) the body of Jesus, ... he came and took away (*ἤρην*) his body" vs. 38) and the second speaks of the taking of the body (the verb *λαμβάνω*) by both Joseph of Arimathea and Nicodemus, probably right before they laid down the body on the lower half of the Shroud ("They took the body of Jesus, and bound it in linen cloths with the spices, as is the burial custom of the Jews" vs. 40). During the placing of the spices, the bloodstains on the body may have had some opportunity to dry, before the upper half of the shroud was laid over the body. This may also have been the case at the soles of the feet (at least the toes), for the soles needn't have rested completely flat on the lower half of a horizontal shroud.

Once the body was lying horizontal for burial and was covered with the Shroud, the hemolysate, released from the clot on the horizontal body by the Shroud's saponins (see 2.4), would have been sucked up to the reverse side of the Shroud through the interstices of the weave by capillary action. The side stain is also visible on the reverse side of the Shroud.<sup>931</sup> The Shroud 2.0 application, showing the photos made by Haltadefinizione of the observe side of the Shroud, shows that at the side bloodstain both warp and weft threads are pinkish (fig. 5.2).<sup>932</sup> This is also the case in the forehead bloodstain (fig. 2.19). That the short weft threads (in the furrows of the weave) look a little bit darker than the long warp threads, even in the Haltadefinizione photos, is probably mostly due to shadow created by the lighting. The photos show that the frays at the border of the burn holes throw large shadows on the backing cloth visible through the burn holes (fig. 5.2).

<sup>929</sup> Zugibe, *The Man on...*, 1989; Wesselow, *The Sign*, 2012, end of chapter 10.

<sup>930</sup> Matt 27:58-60 Luke 23:53 Mark 15:46

<sup>931</sup> <http://shroud.wikispaces.com/PROPERTIES>

<sup>932</sup> "The lighting system was especially designed to filter any harmful radiation to the cloth, but most of all special attention was given to the calibration of the spectrum, which helped faithfully reproduce the actual color and fabric of the Shroud image." (<http://www.haltadefinizione.com/en/the-shroud>).



Fig. 5.2. Side: bloodstain, scorch, frays, burnhole with backing cloth. Photo by HALTADEFINIZIONE® IMAGE BANK - Copyright Arcidiocesi di Torino<sup>933</sup>

The contact of the shroud with the ‘half-dry’ blood clots may have formed the layered interface between shroud and blood clot, as described above, consisting of madder lake at the surface of the Shroud, amorphous acid heme dimer precipitate next, then acid methemoglobin crystals, then lysed erythrocytes – out of and in the fibrin mesh of the clot –, then the rest of the blood clot on the body. Where serum halos were still wet enough they would have transferred directly to the Shroud, as seems the case at least in the fluorescent halo at the tip of the wrist blood and perhaps at some margins of the side blood (see 2.2).

When the shroud and body were separated, the interface between shroud and blood clots was disrupted, probably mainly at the level of the acid methemoglobin powder and lysed cells. These cells and crystals and eventually also most heme aggregates could have gradually abraded in ageing and folding, exposing the unbroken pinkish madder-lake-stained fibers.

### 5.3. Rivulets running across the Shroud

#### *The small of the back*

Also here the bloodstains can be explained by the presence of authentic post-mortem blood of Jesus. In the area of the small of the back there are bloodstains that look like small neat rivulets of blood, without smudging. As these stains appear in a region where there is hardly any image of the back, it seems that in this area there was no contact between the Shroud and the body whose image is on the Shroud. This means that the blood rivulets we see probably ran across the Shroud and not across the body. This is confirmed by two of STURP’s observations. One is on sample 3EB, from one of the bloodstains at the small of the back: “Microscopic observation of blood flecks of sample 3EB showed specular reflection: the blood went onto the surface as a liquid”.<sup>934</sup> This entry A64 of the 2005 evidence list, referring to Rogers’ notebook, doesn’t say whether or not red blood cells were seen, and whether the specular reflection was from red or golden yellow fibers. The other observation is on some bloodstain areas, labeled “BLOOD FLOW. In these areas, the fluid seems to

<sup>933</sup> Used, with permission, from the Shroud 2.0 application made by Haltadefinizione, downloadable from <http://www.haltadefinizione.com/en/the-shroud>.

<sup>934</sup> “(Rogers 1978).” Here, the reference is to “ROGERS R. Research Notebood: Shroud, October 1978”, Fanti et al., Evidences for..., 2005, <http://www.shroud.com/pdfs/doclist.pdf>

have flowed along contours of the cloth such as folds. The clearest area of this happening is in the region corresponding to the area of the small of the back above the buttocks.”<sup>935</sup> This can be explained by the position of the hands as pictured on the Shroud. When the body had been laid in this position on the lower half of the Shroud, which probably was on a wooden burial bier, some post-mortem blood would have started to flow down from the wrist wounds along the underside of the forearm to the region of the hips and the elbows and perhaps around the underside of the lower back. Dependent of the thickness and amount and ‘running speed’ of the blood, some blood would have fallen from the body in drops, and dripped on the lower half of the Shroud. From where the drop touched the Shroud it may have been induced to run across the waterresistant Shroud by the irregular movement of the burial bier in the carrying of the bier from the cross to the tomb: if a rectangular flat wooden burial bier was carried on the shoulders of the bearers (the Jewish "kattafim" = shoulderers<sup>936</sup>) from Mount Golgotha to the garden tomb which “was close at hand” (John 19:42 RSV), it would have rocked/wobbled a little, making the drops of blood fall from the body at different positions and then run to one direction on the Shroud and then to another. Together the drops could have made the irregular but neat blood rivulets pattern we see now, which probably extended to beyond the right and left sides of the body, as artistically depicted on the fourteenth-century French Lirey pilgrim’s badge. Now, burn holes of the 1532 fire are at the Shroud locations where the off-image blood rivulets from the elbow and hip region towards the lateral sides of the Shroud would have been.

No XRF measurements were made in the area of the small of the back, so nothing can be said about the potassium level in these bloodstains. Where there was enough liquid, blood or hemolysate probably drained through the Shroud via the interstices of the weave (cf. fig. 2.19), as most parts of the rivulets are visible on the reverse side of the Shroud.<sup>937</sup> Any surplus liquid was probably absorbed by the wooden bier beneath the Shroud. Remaining red blood cells on the surface of the Shroud would have formed pinkish madder lake stains by hemolysis by saponins and then heme adsorption to madder dye on the surface, as described above. The body probably was not picked up from the bier, but bier and body were probably carried into the sepulcher together and laid on a stone platform there together. The Jewish custom was that, whatever funerary bier had been used, it had to be buried as well because it had become unclean from the contact with the dead body.<sup>938</sup> Of the sepulchral chamber of a Palestinian rock tomb, “the porch on the fourth side was large enough to afford room for the bier and the visitors”.<sup>939</sup> Jesus’ burial on the evening before the Sabbath was a provisional one that the women wanted to complete on Sunday morning with their spices for anointing the body (Mark 16:1-2 Luke 24:1).

After the separation of body and Shroud somehow took place, the dry lysed cells could have abraded from the Shroud in handling.

### *Back of the head*

When Jesus’ body was laid on the shroud, the head may still have been bent toward the chest, as on the cross in rigor mortis. The blood that flowed from the puncture wounds of the back of the head, after removal of the crown of thorns, would first have flowed down into the hair and then into the shroud. The very costly pure nard ointment which was poured over Jesus’ head “two days before the Passover”, as an anointment “beforehand for burying” (Mark 14:3-9; cf. Matt 26:6-13), seems indeed to have still been on his hair during burial for it seems to have impregnated the Shroud first, as the bloodstains of the back of the head show peculiar small lighter circles.<sup>940</sup>

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<sup>935</sup> Jumper et al., *A comprehensive...*, 1984, p. 460

<sup>936</sup> Jewish Encycloedia, Burial <http://www.jewishencyclopedia.com/articles/3842-burial#anchor9>

<sup>937</sup> see the photo of the reverse of the Shroud at the bottom of this page: <http://shroud.wikispaces.com/PROPERTIES>

<sup>938</sup> “funerary biers or stretchers ... whatever they were made of, they were finally buried, because they were unclean by coming into contact with the dead body.” Grossi, *Jewish Shrouds...*, 2012, note 48 p. 9

<sup>939</sup> Jewish Encyclopedica, Burial – The Family Sepulcher <http://www.jewishencyclopedia.com/articles/3842-burial#anchor10>

<sup>940</sup> See Shroud Scope at

<http://www.sindonology.org/shroudScope/shroudScope.shtml?zl=11&image=3&lon=3358.0&lat=11507.0>

#### 5.4. Pools of wet blood – brown bloodstains

The ultimate tips of a few bloodstains that look like rivulets that flowed either across the Shroud or across the body look brown while the rivulet itself looks red. Beside the dorsal right foot, the small area at the outer edge of the outer bloodstain that is more brown than the rest of the stain (cf. fig. 2.1), is darker in fluorescence. The brown and, in some places, pinkish off-elbow stain<sup>941</sup> is not on the fluorescence photograph, but the trail leading to it seems just as dark as the bloodstains on the arm. At the small of the back, the brown side tip of the dense blood at the end of a blood rivulet is lighter than the dense red tip beside it in visible light, but just as dark in the fluorescence photo (fig. 5.3). So, these brown stains are not mainly serum, which would have been lighter in fluorescence. Perhaps at these ultimate tips there was a small pool of wet blood, which clotted in the Shroud before most of its liquid could drain away through the Shroud to the bier. This clot in the Shroud would contain red blood cells in a fibrin mesh, and therefore would retain most of these cells, in contrast to stains mainly made by hemolysate of clots on the body and of thin filtered rivulets. The contents of these cells would not get into contact with madder dye and therefore would denature to brown hemichrome or heme, just as blood in ordinary bloodstains does.



Fig. 5.3. Comparison of a small of the back area in visible light and in UV light (fluorescence). Left: visible light photo (ME 03) ©1978 Mark Evans Collection, STERA, Inc.<sup>942</sup>; right: fluorescence photo (mirrored) ©Biocommunications Association, Inc.<sup>943</sup>

#### 5.5. Scourge marks

##### 5.5.1. Very faint – not dense – not chemically tested – no spectra

The scourge marks, both the scratch- and smear-like ones and the dumbbell-shaped ones, are as pinkish as the Shroud's larger bloodstains, but very faint (fig. 5.4 and 5.5) and therefore different from the dense blood in some of the rivulets in the small of the back area.

<sup>941</sup> As seen in the Haltadefinizione photo in the Shroud 2.0 application

<sup>942</sup> Used from the [www.shroud.com](http://www.shroud.com) photo gallery <http://shroud.com/gallery/index.htm>, <http://shroud.com/gallery/pages/ME-03.htm#>, with permission from STERA, Inc.

<sup>943</sup> Image reprinted (and mirrored) from Miller & Pellicori, *Journal of Biological Photography*, Vol 49, No 3, 1981, p. 71-85, with permission from the publisher, the Biocommunications Association, Inc.



*Fig. 5.4. Streak-like pink scourge mark on right calve at location 1FB of STURP's tape sample map. Photo by HALTADEFINIZIONE® IMAGE BANK - Copyright Arcidiocesi di Torino<sup>944</sup>*



*Fig. 5.5. Dumbbell-shaped scourge mark at the back, between shoulder blades (image area). Photo by HALTADEFINIZIONE® IMAGE BANK - Copyright Arcidiocesi di Torino<sup>945</sup>*

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<sup>944</sup> Used, with permission, from the Shroud 2.0 application made by Haltadefinizione, downloadable from <http://www.haltadefinizione.com/en/the-shroud>. Note that for the Haltadefinizione photography “The lighting system was especially designed to filter any harmful radiation to the cloth, but most of all special attention was given to the calibration of the spectrum, which helped faithfully reproduce the actual color and fabric of the Shroud image.”

<sup>945</sup> Used, with permission, from the Shroud 2.0 application made by Haltadefinizione, downloadable from <http://www.haltadefinizione.com/en/the-shroud>. Note that for the Haltadefinizione photography “The lighting system was especially designed to filter any harmful radiation to the cloth, but most of all special attention was given to the calibration of the spectrum, which helped faithfully reproduce the actual color and fabric of the Shroud image.”

Micrograph ME 06, made by photographer Evans of STURP in 1978,<sup>946</sup> is a part of the dense blood of the ‘dragon head’ at the tip of the upper rivulet in the small of the back area, photographed in micrograph ME 03<sup>947</sup>, as is shown by comparison of the photos in fig. 5.6. This dense blood does not belong to a scourge mark.<sup>948</sup> The original STURP notes on the sample sites also shows the drawing of a round mark, labeled “micro”, around the tip of the upper blood rivulet that runs from the man’s left side to almost the middle of his back and ends there, and no round mark around a scourge mark in the small of the back area.<sup>949</sup> Recently, also Latendresse, who developed the online Shroud Scope, located ME-06 at the tip of this blood rivulet, and noted that the grid location D15, assigned to ME-06 and provided by Schwartz, is obviously wrong.<sup>950</sup>

The only sample from a scourge mark that is mentioned in the sample list published by Schwartz, viz. sticky tape sample 1FB = “Image: Blood on Scourge Mark”, from one of the calves, did not belong to the set of samples consigned to Heller and Adler by Rogers for the testing of the presence of blood.<sup>951</sup> Also, none of STURP’s reflectance, UV-fluorescence or X-ray fluorescence spectra was obtained from a scourge mark area.

Table 1 of Heller and Adler, says “4CB Scourge blood image, middle back” (B = Back = dorsal half of the Shroud) (A Chemical, TOM 49), but Schwartz’ map and list of the dorsal tape samples (<http://www.shroud.com/maptap2d.htm>) says “20 Light scorch (Approx. Test Point) (4C)”, and the original STURP notes (in Nitowski, Criteria for Authentication..., 1986, Scibd document on <http://holyshroudguild.org/dr-nitowski-new.html>) say “4C BACK : intersection ½” width ; 1.0 kg”. The original STURP map in this same document also shows the locations of 4A (in scorched fold), 4B (in dark scorch beside fold and lower than 4A), 4C (intersection scorch/non-image beside 4B) and 4D (image and perhaps scourge beside 4C). Samples 4A, 4B and 4D, are not even mentioned in the sample list as published by Schwartz. Has sample 4CB been mixed up with sample 4D, or were only their names mixed up, and therefore described as “Scourge blood image” by Heller and Adler, who got the tapes from Rogers? Did they actually see red spots on it? Or red coated fibers? And did they test any of this red?

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<sup>946</sup> <http://shroud.com/gallery/pages/ME-06.htm>

<sup>947</sup> <http://shroud.com/gallery/pages/ME-03.htm>

<sup>948</sup> Heimburger, who labelled the ME 06 micrograph “Fig.8-1: ME 06. Dense blood, scourge marks of back. X 32 ... Fig.8-2: ME 06. Dense blood, scourge marks of back. X 32. Detail”, and “Dense blood stains (Scourge mark on back). ME-06. 32× © Mark Evans” (Heimburger, Mark Evans’..., 2013, p. 13-14; Heimburger, A detailed..., 2008, p. 2), is mistaken.

<sup>949</sup> Nitowski, Criteria for authentication..., 1986, [www.holyshroudguild.org/dr-nitowski-new.html](http://www.holyshroudguild.org/dr-nitowski-new.html), 2<sup>nd</sup> Scibd document of Sr. Damian Nitowski, p. 16

<sup>950</sup>

<http://www.sindonology.org/shroudScope/shroudScope.shtml?zl=3&image=3&lon=1028&lat=1724&photomicro=t&popup=ME-06+371+25>); “The grid locations marked with an asterisk (e.g., D15\*) have been considered at odd with the description of their photomicrograph”, Latendresse, <http://www.sindonology.org/photomicrographs.shtml>

<sup>951</sup> <http://www.shroud.com/maptap2d.htm>; Heller and Adler, A Chemical..., 1981, Table 1, TOM 49. Table 1 of Heller and Adler, says “4CB Scourge blood image, middle back” (B = Back = dorsal half of the Shroud) (A Chemical, TOM 49), but Schwartz’ map and list of the dorsal tape samples (<http://www.shroud.com/maptap2d.htm>) says “20 Light scorch (Approx. Test Point) (4C)”, and the original STURP notes (in Nitowski, Criteria for Authentication..., 1986, Scibd document on <http://holyshroudguild.org/dr-nitowski-new.html>) say “4C BACK : intersection ½” width ; 1.0 kg”. The original STURP map in this same document also shows the locations of 4A (in scorched fold), 4B (in dark scorch beside fold and lower than 4A), 4C (intersection scorch/non-image beside 4B) and 4D (image and perhaps scourge beside 4C). Samples 4A, 4B and 4D, are not even mentioned in the sample list as published by Schwartz. Has sample 4CB been switched with sample 4D, before it was consigned to Heller and Adler, or was only its designation switched and therefore described as “Scourge blood image” by Heller and Adler, who got the tapes from Rogers? Did they actually see red spots on it? Or red coated fibers? And did they test any of this red?

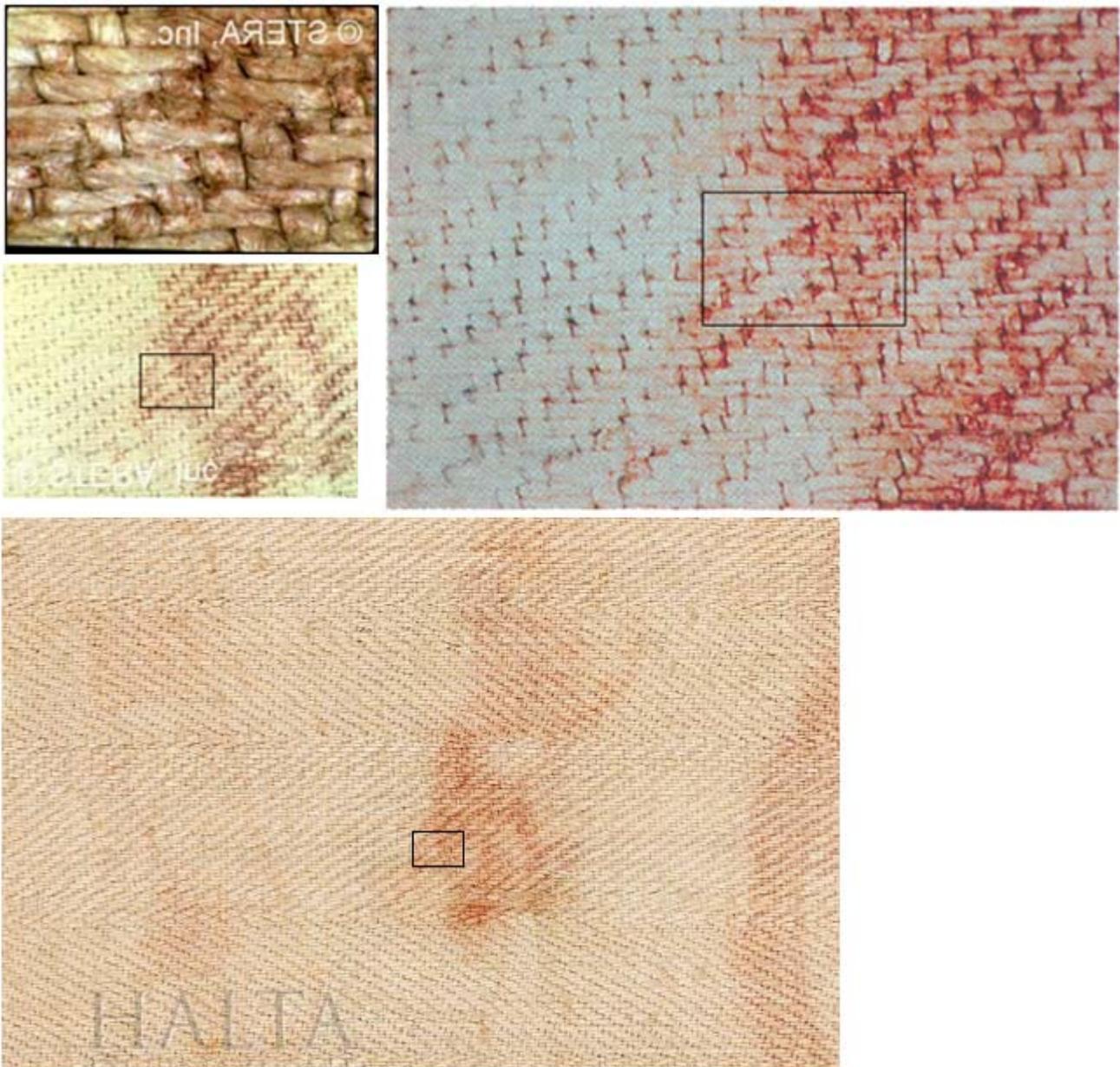


Fig. 5.6. Same dense bloodstain at the tip of a bloodrivulet in the small of the back area<sup>952</sup>, with the black rectangle indicating the same cloth area in all photos. Top left (ME 06, adapted) and middle left (ME 03, adapted): ©1978 Mark Evans Collection, STERA, Inc.<sup>953</sup>; top right (part of ME 03 as published by McCrone<sup>954</sup>, adapted): © 1990 ACS<sup>955</sup>; bottom left, photo (adapted) showing faint pinkish dumbbell-shaped scourge mark, and dense bloodstain and another blood rivulet: Photo by HALTADEFINIZIONE® IMAGE BANK - Copyright Arcidiocesi di Torino<sup>956</sup>. Composition: ©AvdH

<sup>952</sup> compare <http://www.sindonology.org/shroudScope/shroudScope.shtml?zl=11&image=4&lon=15912.0&lat=3003.0>

<sup>953</sup> Used and adapted from the Shroud.com photo gallery, <http://shroud.com/gallery/pages/ME-03.htm#> and <http://shroud.com/gallery/pages/ME-06.htm#> with permission from STERA, Inc.

<sup>954</sup> “Red blood-image area from small of back; photomicrograph by Mark Evans, 22 x.” McCrone, *The Shroud of Turin...*, 1990, p. 79

<sup>955</sup> Adapted with permission from W.C. McCrone, *The Shroud of Turin: blood or artist’s pigment?*, *Accounts of Chemical Research*, 1990, Vol 23, Issue 3, pp. 77-83, DOI: 10.1021/ar00171a004,

<http://pubs.acs.org/doi/abs/10.1021/ar00171a004?prevSearch=A.D.%2BAdler&searchHistoryKey=>, Copyright 1990 American Chemical Society.

<sup>956</sup> Used, with permission, from the Shroud 2.0 application made by Haltadefinizione, downloadable from <http://www.haltadefinizione.com/en/the-shroud>. Note that for the Haltadefinizione photography “The lighting system was especially designed to filter any harmful radiation to the cloth, but most of all special attention was given to the calibration of the spectrum, which helped faithfully reproduce the actual color and fabric of the Shroud image.”

<http://www.haltadefinizione.com/en/the-shroud>

### 5.5.2. No fluorescent serum scratches or serum borders

As already noted in paragraph 2.2.2, in the UV-fluorescence photos of Miller and Pellicori, there are no light scratches on the calves, only dark scratches. So, these dark scratches are not transposed serum, but rather dark blood products or images of elevated stripes.

Miller and Pellicori wrote on the dorsal midsection area (buttocks to shoulder blades): “Many scourges have fluorescing bordering areas”<sup>957</sup>, but these lighter borders around scourge marks are visible in both in UV and white light. This is in contrast to what certain other authors have said, viz. that all scourge marks, and even all blood marks, have fluorescent borders that are not seen in white light.<sup>958</sup> The lighter borders around scourge marks in both UV and white light probably result from a tenting effect in the body-image formation (see 2.2.3). If these borders were pure serum, they would have been golden yellow or orange in white light.

Schwalbe and Rogers reported that “The u.v.-fluorescence photographs of Miller and Pellicori [14] reveal an interesting effect in several nominal image areas where “blood” flows are present: the dark “blood” regions are partially surrounded by margins that appear to fluoresce as intensely as do the non-image background areas. Weaver [3] has published four of these photographs along with drawings to illustrate the “light-colored” margin locations at the side wound, the “nail” wound in the wrist, and the “blood” flow at the right foot on the dorsal image. (At other “blood” locations, such as the flow across the back and the left dorsal foot region, no fluorescent margins are seen.) This unexpected effect is most clearly visible under the special conditions described above, and it is unfortunate that this observation was made only after the direct examination period.”<sup>959</sup>

### 5.5.3. Only dorsal scourge marks on reverse side

The online photo of the reverse side (non-image side) of the Shroud does not seem to show scourge marks of the ventral half, but does seem to show some of the scourge marks of the dorsal half, for instance at location 1FB at one of the calves, although the color is unclear.<sup>960</sup> This may be an indication that the pinkish scourge marks got on the Shroud as a minimal quantity of liquid, which only at the dorsal side of the body flowed a bit more abundantly because of gravity and/or the dorsal half of the Shroud was pressed flat and thinner by the weight of the body.

### 5.5.4. Hyperfibrinolysis caused pink imprints but no smears before image formation

The presence of many scourge marks of the same dumbbell shape suggests they were caused by one and the same kind of object and that their bloodstains were not smeared. A possible explanation for these similar unsmearred dumbbell-shape bloodstains is that a dumbbell-shaped scourge (e.g. a simple form of a Roman flagrum, cf. fig. 5.7<sup>961</sup>) created small wounds in the skin.

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<sup>957</sup> Miller and Pellicori, *Ultraviolet fluorescence...*, 1981, p. 79

<sup>958</sup> Heller says on a number of UV photos that he had seen: “At the margin of each scourge mark there was a pale white fluorescence that could not be seen in white light.” (Report on..., 1983, p. 185) and Adler says: “Also the border of every blood mark shows the typical yellowish fluorescence of the serum exudate ring around scabs as expected for clot retractions transfer marks, thus confirming the medical forensic analysis and the observations of Barbet (cf., Figure 2).” (Adler’s Fig. 2 = UV photo of upper part of frontal body image with side bloodstain; Adler, *Chemical and Physical...*, 2002, TOM 14) and “The bloodmarks on the Shroud are all depressed in the centres, raised on the edges, and in the ultraviolet photography we can see around all of these a halo of the exuded serum.” (Adler, *The origin and...*, 1986TOM 60). Note that Miller and Pellicori summarized on the blood: “Blood UV: Highly absorbing. No color. Fluorescing borders apparent around some areas.” (Miller and Pellicori, *Ultraviolet fluorescence...*, 1981, Table 1, p. 79)

<sup>959</sup> Schwalbe and Rogers, *Physics and Chemistry...*, 1982, p. 39 ([3] = “K.F. Weaver, *Nat. Geogr. Mag.*, 157 (1980) 730.” <http://ngm-beta.nationalgeographic.com/archive/science-seeks-to-solve-the-mystery-of-the-shroud/>)

<sup>960</sup> Shroud Science Group, <http://shroud.wikispaces.com/PROPERTIES>

<sup>961</sup> A woodcut of a Roman scourge as depicted on a coin and as a separate object shows that it looks like two or three leather strips with many bones: “The cut below represents a scourge taken from a bas-relief of the statue of Cybele in the Museum of the Capitol at Rome, and fully justifies the epithet of Horace (l.c.), horrible flagellum.” (Dictionary of Greek and Roman Antiquities, 1848-1953, p. 540

<http://books.google.co.uk/books?id=QIQJAAAQAAJ&printsec=frontcover&dq=Dictionary+of+Greek+and+Roman+A>



Fig. 5.7. “Tomb portrait of a gallus with equipment including cymbals, tympanum, double flute and a whip of knuckle bones. C2nd CE, Rome. Capitoline Museum, Rome.”<sup>962</sup> (Public domain)

Forensic pathologist Zugibe states that blood from such small wounds would have flowed all over the body.<sup>963</sup> In Jesus’ case, the scourging by the Romans took place in the morning (before 12 AM =

[ntiquities&hl=nl&sa=X&ei=mlfUd6BNYqRtObb\\_YHAAw&ved=0CEcQ6AEwAw&q=knotted&f=false#v=snippet&q=knotted&f=false](http://www.romanreligion.org/content/373/) ). A photo of this bas-relief of a statue of Cybele in the Museum of the Capitol is online and shows three lashes with 7-9 bones each and the caption says “Tomb portrait of a gallus with equipment including cymbals, tympanum, double flute and a whip of knuckle bones. C2nd CE, Rome. Capitoline Museum, Rome

(<http://www.romanreligion.org/content/373/> photo: <http://www.romanreligion.org/content/wp-content/uploads/GallusRelief.jpg> ). The 1842 edition of “A Dictionary of Greek and Roman antiquities” says: “FLAGRUM, dim. FLAGELLUM (μαστιξ) ... The whip used to punish slaves was a dreadful instrument (horribile flagellum, Hor. l.c.), knotted with bones or heavy indented circles of bronze (αστραγαλωτη = ‘astragaloote , Athen. iv, 38), or terminated by hooks, in which case it was aptly denominated a scorpion (Isid. l. c.; 2Chr x, 11.) The infliction of punishment with it upon the naked back of the sufferer (Juv. l.c.) was sometimes fatal (Hor. Sat. i. 2. 41), and was carried into execution by a class of persons, themselves slaves, who were called lorarii. It appears that there was another class that submitted to be thus whipped for hire. (Festus, s.v. Flagatores.) A slave who had been flogged was called flagrio (... ) which of course became a term of mockery and contempt.” (ed. W. Smith, p. 424 ,

<http://books.google.co.uk/books?id=w1vVPbrHidAC&printsec=frontcover&dq=Dictionary+of+Greek+and+Roman+Antiquities&hl=nl&sa=X&ei=4ErUcSnKsaitAbQioCYDg&ved=0CC8Q6AEwAA&q=flagrum&f=false#v=snippet&q=flagrum&f=false> ).

The Perseus Greek Word Study Tool has “αστραγαλωτός , ή, όν, **A.** made of “αστραγαλοι, μάστιξ” *Crates Com.35, Plu.2.1127c*; “ίμάς” *Posidon.9.*” and “αστραγαλος [ρα^], ό, (v. όστέον) ... **IV.** pl., αστραγαλοι *knucklebones used as dice or a game played with dice, ... V.* ή εκ των αστραγαλων μάστιξ *scourge of strung bones, Luc. Asin.38*; cf. αστραγαλωτός.” (<http://www.perseus.tufts.edu/hopper/morph?l=a%29stragalwth%2F&la=greek#lexicon> and <http://www.perseus.tufts.edu/hopper/morph?l=a%29stra%2Fgaloi&la=greek&can=a%29stra%2Fgaloi0&prior=o/n#lexicon> )

; cf. Perseus on FLAGRUM: “the flagellum was the worst ( ... ). It was a “knout” or “cat,” with lashes of knotted cord, or even wire; ... , it might be loaded with knuckle-bones ( ... ), or other cruel aggravations ... Some flagella found at Herculaneum consist of several short chains with knobs of metal at the end, attached to a short handle. Rich, who figures one of them, thinks that this sort was the flagrum, the other the flagellum; other writers treat flagrum and flagellum as equivalent”

<http://www.perseus.tufts.edu/hopper/text?doc=Perseus:text:1999.04.0063:entry=flagrum-cn> ; cf. a first-century heavy indented bronze element of a Roman flagrum at <http://www.delcampe.net/page/item/id.225634625,var.element-de-FLAGRUM-romain-en-bronze.language.E.html>

<sup>962</sup> text: S. Green, University of Leeds, <http://www.romanreligion.org/content/373/>; figure:

[http://en.wikipedia.org/wiki/Galli#mediaviewer/File:Relief\\_of\\_Archigallus.jpg](http://en.wikipedia.org/wiki/Galli#mediaviewer/File:Relief_of_Archigallus.jpg)

<sup>963</sup> “Such injuries are only seen at autopsy after gently washing the wounds otherwise there would be blood all over the body from these wounds obscuring the patterned impressions.” Zugibe, *Forensic and Clinical...*, 2000

“the sixth hour” John 19:14) and his death by crucifixion in the middle of the afternoon (at about 3 PM = “the ninth hour” Matt 27:46.50), so the blood from the scourges would have been dry at the burial in the beginning of the evening (Matt 27:57-60 Mark 15:42-43 Luke 23:53-54). Zugibe wrote that “no one would argue that the scourge wounds were made and clotting begun several hours prior to death. Moreover, most forensic experts agree that the Man of the Shroud shows evidence of rigor mortis because of the bent knees and absence of a neck, therefore indicating that the crucified was dead for some time before being taken down from the cross. Thus, according to the studies of Lavoie's group, these *perfectly defined wounds* should not have transferred at all.”<sup>964</sup>

Fibrinolysis is a physiological process which breaks down the fibrin mesh of a blood clot that is in contact with fibrinolytic plasma, either inside a blood vessel or in a wound or *in vitro*; ordinary fibrinolysis in a living person takes place several days after the clotting of the blood clot, which allows wounded skin to heal before any blood clots are broken down.<sup>965</sup> Scourge wounds that are covered with dry blood clots of less than two days old would not yet have a closing tissue layer that completely separates the clot from the plasma of the internal blood: “About two or three days after the wound occurs, fibroblasts begin to enter the wound site, marking the onset of the proliferative phase even before the inflammatory phase has ended.<sup>[36]</sup> ... Fibroblasts begin entering the wound site two to five days after wounding as the inflammatory phase is ending, and their numbers peak at one to two weeks post-wounding.<sup>[19]</sup> ... Granulation tissue functions as rudimentary tissue, and begins to appear in the wound already during the inflammatory phase, two to five days post wounding, and continues growing until the wound bed is covered. Granulation tissue consists of new blood vessels, fibroblasts, inflammatory cells, endothelial cells, myofibroblasts, and the components of a new, provisional extracellular matrix (ECM)... One of fibroblasts' most important duties is the production of collagen.<sup>[37]</sup> Collagen deposition is important because it increases the strength of the wound; before it is laid down, the only thing holding the wound closed is the fibrin-fibronectin clot, which does not provide much resistance to traumatic injury.<sup>[20]</sup> ... The formation of granulation tissue into an open wound allows the reepithelialization phase to take place”.<sup>966</sup>

As wound closure by new tissue only starts to take place two days after wounding, the plasma of the dead scourged Jesus, in these two first days, would have had access to the blood clots on the broken skin of the scourges, but not to the dried blood that had flowed across the intact skin and that had lost its humidity by drying.

Also in a corpse fibrinolysis usually does not seem to reliquify clots until several days after the clotting.<sup>967</sup> Yet, in persons with trauma, hyperfibrinolysis may start before death: “acquired disturbance of fibrinolysis (Hyperfibrinolysis), is not uncommon. Many trauma patients suffer from an overwhelming activation of tissue factor and thus massive hyperfibrinolysis. ... It could lead to

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<sup>964</sup> Zugibe, *The Man of ...*, 1989 (emphasis his)

<sup>965</sup> “Plasmin is produced in an inactive form, plasminogen, in the liver. Although plasminogen cannot cleave fibrin, it still has an affinity for it, and is incorporated into the clot when it is formed. Tissue plasminogen activator (t-PA) and urokinase are the agents that convert plasminogen to the active plasmin, thus allowing fibrinolysis to occur. t-PA is released into the blood very slowly by the damaged endothelium of the blood vessels, such that, after several days (when the bleeding has stopped), the clot is broken down. This occurs because plasminogen became entrapped within the clot when it formed; as it is slowly activated, it breaks down the fibrin mesh.”

<http://www.bionity.com/en/encyclopedia/Fibrinolysis.html#Physiology> ; fibrinolysis in vitro:

<http://in.answers.yahoo.com/question/index?qid=20100103114156AA3Xi7N> ;

<sup>966</sup> [http://en.wikipedia.org/wiki/Wound\\_healing#Fibroplasia\\_and\\_granulation\\_tissue\\_formation](http://en.wikipedia.org/wiki/Wound_healing#Fibroplasia_and_granulation_tissue_formation), 36 = Falanga V. (2005).

Wound Healing. American Academy of Dermatology (AAD),

[http://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(05\)67700-8/fulltext](http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(05)67700-8/fulltext) , 19 = de la Torre J., Sholar A.

(2006). Wound healing: Chronic wounds, <http://emedicine.medscape.com/article/1298452-overview#aw2aab6b3>, stating: “Two to three days after wounding, fibroblasts migrate inward from wound margins over the fibrinous matrix established during the inflammatory phase. During the first week, fibroblasts begin producing glycosaminoglycans and proteoglycans, the ground substance for granulation tissue, as well as collagen, in response to macrophage-synthesized bFGF and TGF-β, as well as PDGF.”

<sup>967</sup> “After death the blood generally clots slowly and remains clotted for several days. In some cases, however, fibrin and fibrinogen disappears from blood in a comparatively short time and the blood is found to be fluid and incoagulable soon after death.” Müllertz, Fibrinolytic Activity of Human Blood after Death, *Acta Physiologica Scandinavica* 27, 1953, <http://onlinelibrary.wiley.com/doi/10.1111/j.1748-1716.1953.tb00942.x/abstract>

massive bleeding if not diagnosed and treated early enough.”<sup>968</sup> “Critically ill trauma patients suffer from a complex coagulopathy with multiple aetiologies, including overwhelming activation of tissue factor, consumption of coagulation factors and platelets, haemodilution, hypothermia, metabolic acidosis, massive transfusions, and hyperfibrinolysis.”<sup>969</sup> In 2012, Bergeron reported the possibility that shock and trauma-induced coagulopathy contributed to Jesus’ death.<sup>970</sup> Besides, the power of the fibrinolytic activity of post-mortem plasma, expressed by the level of fibrinogen degradation products (FDP) in the plasma, also depends on the acidity of the blood: a study with rats showed that blood pH 6.8 is a critical value for the onset of post-mortem hyperfibrinolysis: “The pH value decreased very rapidly after death in all rats, regardless of cause of death. It decreased most in rats that died of O<sub>2</sub> deficiency, being 7.360 (in living animals), 6.789 (immediately after death), and 6.506 (8 h after death). ... Regardless of cause of death, the lower the blood pH, the higher the serum FDP level increase. ... In the vascular perfusion system, the lower the pH of the perfusate, the higher the fibrinolytic activity. Fibrinolytic activity was markedly elevated when the pH was below 6.800. ... Increase in fibrinolytic activity in the acidotic state was observed not only in cadaveric blood but also in experiments using the perfusion technique *in vivo*. ... we conclude that the fluidity of cadaveric blood is due to fibrinolysis activated by a) the release of plasminogen activator as a specific reaction through the receptors on the vascular wall for various vasoactive materials that increase during the agonal period; and b) the leakage of plasminogen activator due to enhanced permeability and to degeneration or necrosis of the cell membrane as a result of excessive acidosis after death”.<sup>971</sup> The data of this study, as represented in fig. 5.8, show that the acidity of the blood increases with post-mortem time and that the acidity and its corresponding fibrinolytic activity which were reached 3 hours after death, depends on the cause of death, with a pH of 6.8 (the limit for compatibility with life in mammals<sup>972</sup>) being the threshold for the start of hyperfibrinolysis, and fatal oxygen deficiency for 1-2 hours inducing the highest fibrinolysis level in this test.<sup>973</sup> Another study on cadaveric blood found that “the fibrinolytic level is higher in venous blood than in arterial blood”.<sup>974</sup>

So, it is not unreasonable to assume that, although the clots on Jesus’ scourge wounds probably were dry at the burial on Friday evening – which can partly explain why there are no smears to the many similar dumbbell shaped bloodstains –, they may have got just enough gel-like by hyperfibrinolysis from within, while the body rested in the grave, to make a neat imprint on the Shroud at Sunday morning. The severe acidity of the blood even before death because of the crucifixion and the increased acidity at burial (e.g. pH 6.1), which started the formation of acid heme dimers that could form pink madder lake in the big bloodstains (see 5.2), would only have increased further after burial. This early onset and increase of blood acidity would have corresponded to an earlier and elevated fibrinolytic activity – probably a hyperfibrinolytic activity –, which could eventually have remoistened and acidified the blood clots directly on the scourge wounds just enough and just in time

<sup>968</sup> [http://en.wikipedia.org/wiki/Fibrinolysis#Role\\_in\\_disease](http://en.wikipedia.org/wiki/Fibrinolysis#Role_in_disease) refers to Tieu BH, Holcomb JB, Schreiber MA, Coagulopathy: Its pathophysiology and treatment in the injured patient. *World J Surg* 2007;31:1055-64

<sup>969</sup> Levrat et al., 2008 <http://bj.oxfordjournals.org/content/100/6/792.long>

<sup>970</sup> Bergeron, J.W., The crucifixion of Jesus: Review of hypothesized mechanisms of death and implications of shock and trauma-induced coagulopathy, *Journal of Forensic and Legal Medicine*, 19 (3) 2012, <http://www.deepdyve.com/lp/elsevier/the-crucifixion-of-jesus-review-of-hypothesized-mechanisms-of-death-hM58CJPlou?key=elsevier> p. 116

<sup>971</sup> Takeichi et al., Fluidity of Cadaveric Blood After Sudden Death: Part III: Acid-Base Balance and Fibrinolysis\*, *American Journal of Forensic Medicine & Pathology*, 1986 [http://journals.lww.com/amjforensicmedicine/abstract/1986/03000/fluidity\\_of\\_cadaveric\\_blood\\_after\\_sudden\\_death\\_7.a.spdx](http://journals.lww.com/amjforensicmedicine/abstract/1986/03000/fluidity_of_cadaveric_blood_after_sudden_death_7.a.spdx)

<sup>972</sup> “Blood pH values compatible with life in mammals are limited to a pH range between 6.8 and 7.8.” <http://en.wikipedia.org/wiki/Acidemia>

<sup>973</sup> The methods of sacrifice of the rats is described in part I of the series: Takeichi et al., 1984, [http://journals.lww.com/amjforensicmedicine/Citation/1984/09000/Fluidity\\_of\\_cadaveric\\_blood\\_after\\_sudden\\_death\\_6.aspx](http://journals.lww.com/amjforensicmedicine/Citation/1984/09000/Fluidity_of_cadaveric_blood_after_sudden_death_6.aspx)

<sup>974</sup> Takeichi et al., 1984, [http://journals.lww.com/amjforensicmedicine/Citation/1984/09000/Fluidity\\_of\\_cadaveric\\_blood\\_after\\_sudden\\_death\\_6.aspx](http://journals.lww.com/amjforensicmedicine/Citation/1984/09000/Fluidity_of_cadaveric_blood_after_sudden_death_6.aspx)

before evident putrefaction of the body set in (Baima Bollone, referring to experiments by Rodante, assumed that normal postmortem fibrinolysis only set in 40 hours after death,<sup>975</sup> which is also about the time when putrefaction was assumed to start according to Brillante<sup>976</sup>, and which is the time before which the body must have separated from the Shroud as there are no signs of putrefaction on it). This acid moist bloodmaterial could then get hemolysed and form the same pinkish madder lake, before image formation, as in the other, earlier bloodstains. That the scourge wounds became only slightly wet again (gel-like) after burial is also consistent with the apparent lighter imprints of ventral scourge wounds than of dorsal scourge wounds. Apparently, body and Shroud separated before prolonged hyperfibrinolysis would have caused larger quantities of blood to seep down from the dorsal wounds (also at the back of the head and from the feet and perhaps wrists), which would have destroyed the distinct dumbbell pattern of the pinkish scourge marks and the moderate blood loss pattern at the back of the head. That the dumbbell pattern is still intact and has no apparent smears on both halves of the Shroud, also after separation of body and Shroud, means that this separation apparently did not smear the moist scourge wound clots and thus would have taken place in an extremely delicate way. The separation of the presumably dry blood and dry bloodstains on intact skin outside scourge or other wounds apparently did not cause fibers to break either, as Svensson reported in a private communication to the Shroud Science Group in 2005 that “No broken fibers were found under the blood clots”.<sup>977</sup> Here it is not clear whether by “the blood clots” is meant blood clots on the Shroud or the blood clots that would have been on the body, staining the Shroud. It seems to be the latter, for there hardly are any blood clots on the Shroud, as even red blood cells were hardly found on it (see 2.4). The later observation that “no broken crusts” are evident on the Shroud, seemingly done by Bucklin,<sup>978</sup> is even harder to interpret as there seem to be no intact crusts on the Shroud either.

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<sup>975</sup> “The Syracuse doctor Sebastiano Rodante has shown with experimental research in the catacombs of Syracuse that it is possible to obtain hematic images transferred onto cloths, such as those of the first type, when the contact between the skin and the cloth is maintained for less than 36-40 hours. After 40 hours the phenomenon of fibrinolysis begins which determines the liquefaction of the coagulates. Rodante thus deduces that if the Shroud remained in contact with the corpse for a longer time the presence of more or less diffuse stains would have been obtained.” Baima Bollone, *The Forensic Characteristics...*, Proceedings Turin 2000, p. 216-217.

<sup>976</sup> “the body of the Man remained in the TS for less than 40 hours, because no signs of putrefaction can be found.” Brillante, Fanti and Marinelli, *Bloodstains characteristics...*, p. 1

<sup>977</sup> “A78) No broken fibers were found under the blood clots (N. Svensson 2005). ... SVENSSON N. Shroud Science Group communication 2005.” Fanti et al., *Evidences for...*, 2005, <http://www.shroud.com/pdfs/doclist.pdf>

<sup>978</sup> Fanti, *List of evidences...*, 2010, A78 <http://www.acheiropoietos.info/proceedings/FantiListWeb.pdf>, referring to Bucklin, Robert. "The Shroud of Turin: A Pathologist's Viewpoint." *Legal Medicine Annual* (1982): 33 – 9. Ottesen Søvik wrote “I could not find this being said anywhere in Bucklin’s article, even with slow and careful reading” (Ottesen, *The Shroud of Turin – A Critical Assessment*, 2013-2014, [https://www.academia.edu/7236952/The\\_Shroud\\_of\\_Turin\\_-\\_A\\_Critical\\_Assessment](https://www.academia.edu/7236952/The_Shroud_of_Turin_-_A_Critical_Assessment))

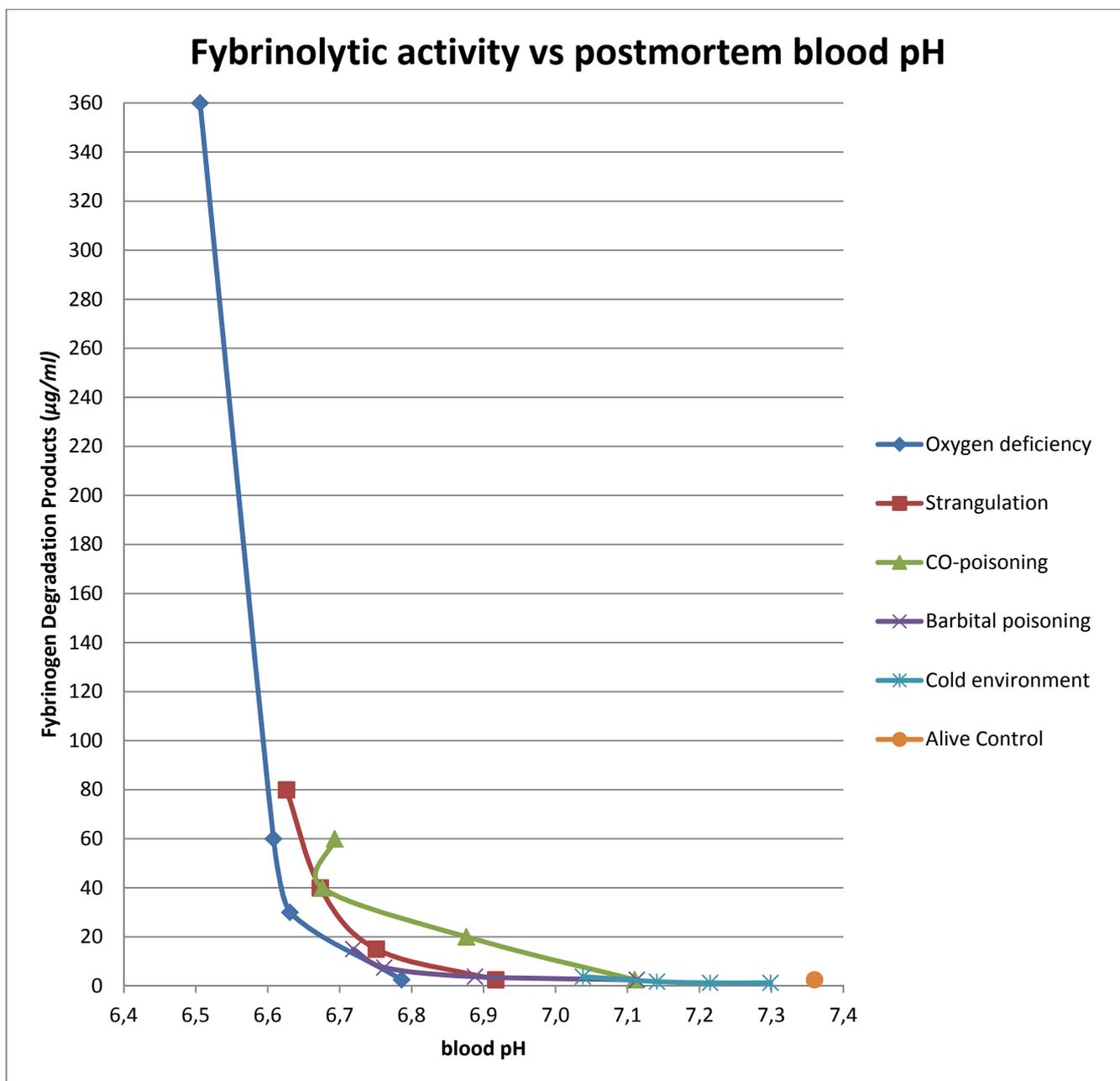


Fig. 5.8. Decreasing blood pH (= increasing blood acidity), 0, 1, 3, and 8 hours after a sudden death by various causes, versus the increasing concentration of Fibrinogen Degradation Products. Data from a study on rats by Takeichi et al., 1986<sup>979</sup>, except the 3-hours values of 'Oxygen deficiency' and 'Cold environment' (these are interpolated from Takeichi's values) and the 360 µg/ml value at 8 h after death by oxigen deficiency (this is the middle of the range given by Takeichi). ©AvdH

#### 5.5.4.1. No serum transfer

Pure serum can not fibrinolyse, as, by definition, it does not contain fibrinogen or fibrin in the first place. Any dried serum that had been extruded from the scourge blood on the skin, would be at the bottom of the blood flow on the skin and would probably not get wet again as a separate golden yellow liquid during fibrinolysis, as it only could get wetted by the reliquified red bloodclots on the wounds. So, no separate serum halos would be expected around transfers of fibrinolysed scourge clots. This is in accordance with the observations of Schwalbe and Rogers<sup>980</sup> (see 5.5.2).

<sup>979</sup> Takeichi et al., Fluidity of Cadaveric Blood Afer Sudden Death: Part III: Acid-Base Balance and Fibrinolysis, [http://journals.lww.com/amjforensicmedicine/abstract/1986/03000/fluidity\\_of\\_cadaveric\\_blood\\_after\\_sudden\\_death\\_7.a.spx](http://journals.lww.com/amjforensicmedicine/abstract/1986/03000/fluidity_of_cadaveric_blood_after_sudden_death_7.a.spx)

<sup>980</sup> Schwalbe and Rogers, Physics and Chemistry..., 1982, p. 39

## 5.5.5. Other ways of scourge mark transfer

### 5.5.5.1. Scourge blood never dried or got remoistened? - no

Forensic pathologists say that bleeding wounds as small as the scourge marks and as many as depicted on the Shroud, would have covered the living man with blood all over.<sup>981</sup> If the blood never coagulated and kept running because of some kind of a blood coagulation disorder,<sup>982</sup> then the scourge blood (and other blood) would have been all over the image of the Shroud, not in the distinct pattern that is seen on the Shroud now. If dried blood from the scourge wounds got wet again because of moisture in the atmosphere or in the cloth, also then the scourge blood would have got wet all over the body and thus would have been transferred all over the Shroud, not in the present dumbbell and streak pattern. So, it is unlikely that original scourge blood was transferred in this way.

### 5.5.5.2. Scourge blood dried and was washed off and scourge wounds issued post-mortem blood? – probably not

A possible mechanism for the formation of distinct scourge wound imprints is that the body was gently washed before it was laid on the Shroud. This washing would have to have removed only the dried scourge blood on the skin outside the scourge wounds, while leaving the clots inside the scourge wounds intact and only wetting them. If the washing removed the clots, post-mortem blood would have started to seep from the opened dorsal wounds and, at least from scourge wounds that had no contact with the bier, would have flowed in indistinct patterns on the Shroud (as at the back of the head) from the moment the body was laid on the Shroud on Mount Golgotha to some moment later when the blood stopped flowing. Besides, if the clots were removed from the wounds by washing, the issued post-mortem blood would probably not have formed elevated convex protuberances above the ventral skin surface for lack of blood pressure from a living heart, and some of the apparent ‘tenting’ in image formation could not have taken place here. Rotation/bending of the body during washing would have disturbed formation of any new dumbbell-shaped protuberant wet clots as well. Also, the washing would have to have left all of the post-mortem blood – from the head, side, wrists and feet – on the body, as this was prescribed by the Jewish law for victims of a violent death.<sup>983</sup>

Evidence that seems to contradict a washing is the presence of aragonite limestone dirt – congruent with a Jerusalem type – in the areas of the images of the feet, left knee and nose,<sup>984</sup> and the detection of relatively high levels of Na and Cl in particles vacuumed from the reverse side of the Shroud, possibly from sweat.<sup>985</sup> Zugibe wrote that Rogers wrote an email of 12/05/03 about the lack of

<sup>981</sup> “Such injuries are only seen at autopsy after gently washing the wounds otherwise there would be blood all over the body from these wounds obscuring the patterned impressions.” Zugibe, *Forensic and Clinical* . . . , 2000

<sup>982</sup> cf. <http://shroudstory.com/2013/04/04/blood-clotting-and-the-strange-case-of-brother-hirudo/#comment-29308> and <http://www.uptodate.com/contents/pathogenesis-and-etiology-of-disseminated-intravascular-coagulation>

<sup>983</sup> Grossi, *Jewish Shrouds* . . . , 2012, note 47; unfortunately, this paper incorrectly states that “The Shroud of Turin doesn't carry selvages nor hems on the short ends” (p. 3), for the Shroud does have rolled hems on the short edges (cf. Heimburger, Cotton in Raes . . . , 2009, Fig. 15, <http://www.shroud.com/pdfs/thibault7part3.pdf>; “Dr. Flury-Lemberg found the cloth's finishing, at its hems, and in the joining seam to have been done using an unusual type of stitching very nearly invisible on one side, and as such closely resembling that of ancient Jewish textiles as found at Masada, the Jewish palace-fortress that was overthrown by the Romans in AD 73, never to be occupied again” (Wilson, ‘The Turin Shroud – past, present and future’, Turin, 2-5 March, 2000 – probably the best-ever Shroud Symposium, *BSTS Newsletter* 51, June 2000, <http://www.shroud.com/pdfs/n51part2.pdf>).

<sup>984</sup> “A79) Earthy material (limestone composed of aragonite with strontium and iron) was found on the feet of TS Man (Kohlbeck 1986, Nitowski 1986, 1998, Antonacci 2000). Earthy material was also found in correspondence with the nose and the left knee (Pellicori, Spectral properties, 1981).” Fanti et al., *Evidences for* . . . , 2005); “reflectance spectroscopy . . . By the time the Gilberts had reached one knee, all the spectra were alike, except for the heel. . . . Sam Pellicori . . . looked at it carefully under full magnification . . . Deep into and between the threads dirt particles could be seen.” Heller, *Report on* . . . , 1983, p. 112; Villarreal and Villarreal, *A new look* . . . , 2012, <http://www.shroud.com/pdfs/villarrealvppt.pdf> comparison of spectra of “Jerusalem limestone” and “Limestone found on Shroud fibers” at slides 24 and 25;

<sup>985</sup> “Recent unpublished data from the vacuumed “dust” (by Riggi Di Numana) coming from specific areas of the TS have been furnished to the authors. Some of the reddish particles have the characteristics of blood (SEM and Raman spectroscopy). These preliminary studies seem to confirm the presence of hemoglobin and iron as well as the biological (not mineral) origin of these particles. A very low level of potassium is confirmed. Unexpected relatively high levels of

evidence of liquid sweat on the Shroud,<sup>986</sup> but Jesus' sweat may have been just as dry as the blood of the scourge wounds at the time of burial. On the other hand, the detected Na and Cl on the reverse side may also result from a layer of salt crystals on which Jews used to lay their dead to retard decomposition of the body before washing and anointing and burying it.<sup>987</sup> Some of the aragonite dirt was found on the left knee image, and the left knee (and more clearly the right knee) also shows a few faint pinkish bloodstains that could be imprints of scourge wounds (fig. 5.9). So it seems at least the knees weren't washed in a way that removed both the dry blood flows on the knees and the dirt. If a superficial washing removed only the dry blood flows and the superficial dirt but not the dirt deeper in the skin (or the clots on the wounds), then this remaining deeper dirt would not have been transferred upward into the Shroud either. So, a washing scenario for the transferral of the scourge marks seems unlikely.

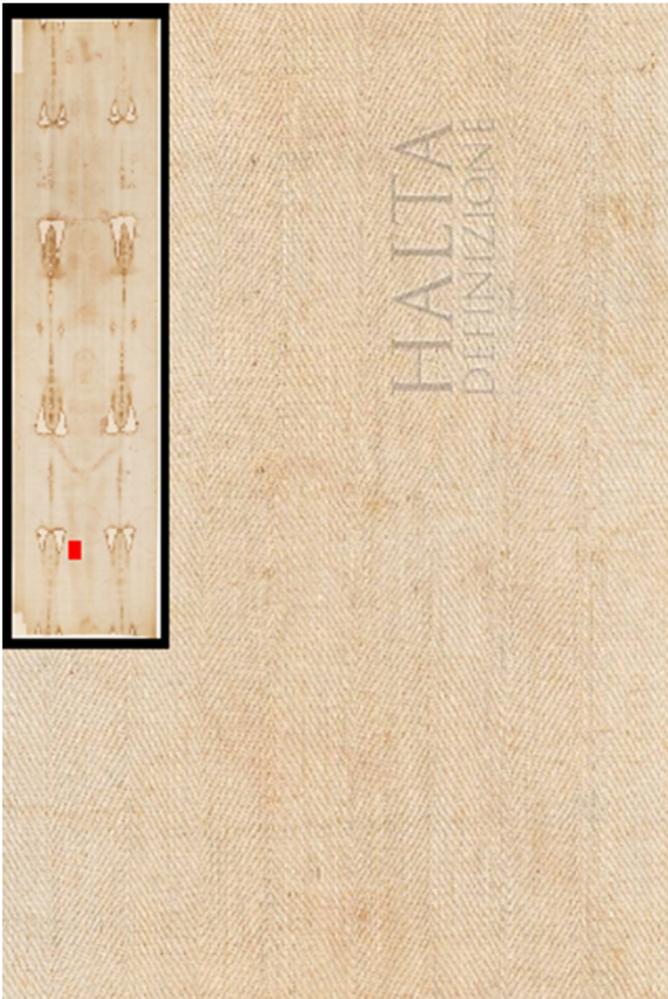


Fig. 5.9. Left knee area. Photo by HALTADEFINIZIONE® IMAGE BANK - Copyright Arcidiocesi di Torino<sup>988</sup>

Na and Cl were found in these particles which are interpreted as possibly coming from sweat (personal communication 2011 with Prof. Giulio Fanti)". Svensson and Heimburger, Forensic aspects..., 2012, p. 2530 (p. 8 of pdf)

<sup>986</sup> "He argues that residues of squalene and other components of sweat, which would have been carried into the cloth by surface-tension effects in the liquid phase, were absent from the Shroud. (Shroud Science mail 12/5/03)." Zugibe, The Crucifixion of Jesus, Completely Revised and Expanded: A Forensic Inquiry, 2005, p. 220, <http://books.google.co.uk/books?id=naV1ua58ZQ8C&printsec=frontcover&dq=zugibe+crucifixion&hl=nl&sa=X&ei=p mHIUIysNOyY0QXV7IGIAw&ved=0CC0Q6AEwAA&q=liquid%20sweat&f=false#v=snippet&q=liquid%20sweat&f=false>

<sup>987</sup> "the body placed upon sand or salt on the floor to retard decomposition, metal or glass being put upon the navel to prevent swelling. Then the body was washed and anointed with aromatic unguents, and wrapped in linen clothes." Jewish Encyclopedia, Burial, <http://www.jewishencyclopedia.com/view.jsp?artid=1607&letter=B&search=burial>

<sup>988</sup> Used, with permission, from the Shroud 2.0 application made by Haltadefinizione, downloadable from <http://www.haltadefinizione.com/en/the-shroud>. Note that for the Haltadefinizione photography "The lighting system was

### **5.5.5.3. Some scourge marks from swellings: welts=stripes or elevated dry blood/serum crusts – possibly some**

Evidence C36 of the 2005 evidence list says “The scourge marks are part of the image and primarily not caused from blood coming out of the wounds (Hoare 1994). (HOARE R., "The Turin shroud is genuine", Souvenir Press, London 1994)".<sup>989</sup> This body-image character may be the property of some scourge marks, but certainly not all. Photos by Haltadefinizione show that the color of many scourge marks is pink, not brown or yellow (fig. 5.4, 5.5 and 5.6). Note that for the Haltadefinizione photography “The lighting system was especially designed to filter any harmful radiation to the cloth, but most of all special attention was given to the calibration of the spectrum, which helped faithfully reproduce the actual color and fabric of the Shroud image.”<sup>990</sup> Via Haltadefinizione photos, Latendresse showed there are bloodstains next to some body images of elevated blood rivulets or welts of the skin on the forearm, which would be consistent with the tridimensional form of a real arm covered with a shroud.<sup>991</sup>

### **5.5.5.4. Scourge blood is painted on – probably not**

This mechanism is discussed in the chapter 6 “Other red color hypotheses” for the bloodstains in general. As the color of the scourge marks is about as pinkish as the color of the larger bloodstains, they probably have the same origin as well.

## **5.6. Blood smears from hands of buriers**

The few smear-like pinkish bloodstains located in areas that seemingly did not have body-cloth contact at the moment of image formation, such as at the back of a knee, or just beside a dorsal leg, or just above a dorsal shoulder, may have been smears of Jesus’ post-mortem blood from the bloody hands of the men who had taken Jesus from the cross and laid Him on the dorsal half of the Shroud and bent his arms towards his body and then perhaps moved his legs towards each other again as well. The large blood rivulets or smears beside the dorsal feet may be due to such relocation movements of the legs which made the pool of blood gathered at the heels of the feet drop on or smear the cloth. At the moment the body was laid and perhaps partly relocated on the Shroud, the clots on the scourge wounds probably were still dry and thus could not make smears during such relocation movements.

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especially designed to filter any harmful radiation to the cloth, but most of all special attention was given to the calibration of the spectrum, which helped faithfully reproduce the actual color and fabric of the Shroud image.”

<http://www.haltadefinizione.com/en/the-shroud>

<sup>989</sup> Fanti et al., Evidences for..., 2005

<sup>990</sup> <http://www.haltadefinizione.com/en/the-shroud>

<sup>991</sup> Latendresse, <http://www.sindonology.org/>, scroll down to the March 28, 2013 entry at about the middle of the page; see also the color difference in Shroud Scope

<http://www.sindonology.org/shroudScope/shroudScope.shtml?zl=5&image=3&lon=1290.0&lat=6777.0>

## 6. OTHER RED COLOR HYPOTHESES

### 6.1. Authentic blood

#### 6.1.1. Blood of a living, crucified person

Bloodstains formed by alkaline or normal, not acid, blood on the skin of a living person, which stains somehow got acidic later, while already in dry deposits on a madder-dyed cloth

- could not form a translucent acid methemoglobin crystal
- could not form acid heme dimers, which lack a distinct Soret absorption band
- could not form bloodstains that lack potassium
- could not have naturally stained the cloth in the pattern of unsmearred scourge marks. If the body of the person whose blood is on the Shroud did not die before it left the Shroud, there would not have been (enough) hyperfibrinolysis to wet the scourge wound clots before it left the Shroud, and therefore the pattern of unsmearred scourge marks could not have formed on the Shroud.

There have already been published a few hypotheses regarding the red blood on the Shroud, which are based on the assumption of the presence of authentic blood of a crucified person who was dead for a few hours, and whose blood therefore would have been acidic. These are discussed below.

#### 6.1.2. Bilirubin

Adler hypothesized that the blood plasma of the man of the Shroud had both a high bilirubin level and a high hemolysate level, while still in the body. He suggested that the color of the mix of this “yellow-orange” bilirubin and “orangy-brown” hemolysate (“methemoglobin in its para-hemic form”) and plasma is red, and that this mix was exuded from drying blood clots outside the body and caused the red stains on the Shroud.<sup>992</sup> He described a “very good fit” of the Shroud bloodglob FTIR spectra with the FTIR spectrum of a simulated clot, having a digitally (theoretical) increased bilirubin pattern.<sup>993</sup> He also wrote he found a “good agreement” between the UV-vis absorbance of a serum-bilirubin-methemoglobin mix and the spectra of the Shroud bloodstains and Shroud blood specimens<sup>994</sup>; Marino commented that a duplicate of the “redness” of the Shroud’s bloodstains was achieved by Adler by making/simulating a mix having a bilirubin level that was 500 times the normal physiological level.<sup>995</sup>

##### 6.1.2.1. Not red – no separate serum – green serum – too much bilirubin

Obvious objections to this hypothesis are that a mix of yellow-orange and orangy-brown liquid (e.g. paint) is not pinkish red but orange-brown. So, the red bloodstains on the Shroud would have to have been orange-brown to correspond to Adler’s hypothesis. Besides, if the bilirubin and hemolysate levels in the plasma were both high in the body, also the separate yellow serum-coated fibers, found on the Shroud, should have been orange-brown: no distinction between yellow and red stains would have been possible. And even assuming that the separate yellow serum for some reason had a much lower hemolysate level, it still would have to have had a high bilirubin level in Adler’s hypothesis, as bilirubin is present in plasma/serum, not in red blood cells, as bilirubin is a product of the spleen and is bound to serum albumin which renders it soluble in blood plasma/serum for transportation to the

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<sup>992</sup> Alder, *The origin and nature...*, 1986, TOM 61

<sup>993</sup> Adler, *Updating recent...*, 1996, TOM 84

<sup>994</sup> Adler, *Updating recent...*, 1996, TOM 84

<sup>995</sup> “But McCrone was not the only one to puzzle over the color of “blood” on the Shroud. Adler, himself puzzled over it and with experimentation told me that he could duplicate the “redness” of the blood by assuming that the Man of the Shroud was a victim of blood trauma where the bilirubin was some 500 times concentrated.” Comment of J. Marino, <http://shroudstory.com/2013/05/25/an-important-and-highly-informative-guest-posting-by-paul-maloney/#comment-34815>

liver.<sup>996</sup> But serum with a high bilirubin level, viz. five times the physiological level, is greenish-yellow, as noted in Goldoni's experiments,<sup>997</sup> and not golden yellow as seen on the Shroud. The Shroud's golden yellow 'serum' fibers were indeed not tested for bilirubin. Goldoni noted that a five times elevated bilirubin level already constitutes yellowing of the sclera and skin, and he did not test a higher level because "he did not feel to postulate an intense jaundice either in the Christ of the Gospels or in the Man of the Shroud."<sup>998</sup> This means that Adler's unpublished UV-vis match with an exudate simulacrum/simulation having a 500 times elevated bilirubin level, is way out of the range that could be postulated for Christ or the man of the Shroud. Also biochemist Berry wondered how the prolonged trauma of Christ, even including the scourging, could have induced severe jaundice.<sup>999</sup> Fanti reported that "Using Raman analysis, G. Moscardi (2008) detected the limited presence of Bilirubin in red crusts coming from the TS".<sup>1000</sup>

### 6.1.2.2. No published FTIR match

FTIR spectrometry was done on "two blood globs (particles unattached to fibers) from the lance wound area" and on "two serum coated fibers from the edge of the lance wound"<sup>1001</sup>, and also on a "traumatic clot exudates simulacrum"<sup>1002</sup>, made by "mixing three drops of whole blood (finger stick) with three drops of a bilirubin/human albumin diagnostic standard (Sigma Chemical)"<sup>1003</sup>. Adler wrote that "The pattern match of the simulated clot seems only fair, but spectral analysis utilizing the computer software shows that reducing the protein pattern and increasing the bilirubin pattern makes a very good fit to the blood sample pattern. Conversely, the mineral simulated blood pattern is a complete mismatch except for the presence of protein. Bilirubin is clearly required to obtain a proper spectral match."<sup>1004</sup> FTIR spectrum plots of Shroud "blood globs" (fig. 2.22, or online<sup>1005</sup>) are very different from the FTIR spectrum plots of Shroud "serum" fibers (fig. 2.40, or online<sup>1006</sup>). An FTIR spectrum labelled "simulated" is shown in Adler, Selzer and DeBlase's fig. 10 (fig. 2.25, or

<sup>996</sup> <http://en.wikipedia.org/wiki/Bilirubin#Metabolism>

<sup>997</sup> "Dr. Goldoni stated that in his tests he did not exceed more than five times the physiological concentration of bilirubin because "he did not feel to postulate an intense jaundice either in the Christ of the Gospels or in the Man of the Shroud." In fact, already at such a concentration limit, we have "a yellowish colour of the sclera and a yellowing of the skin" Moreover, also the blood serum "shows a peculiar greenish-yellow colouring"." Goldoni, *The Shroud of Turin...*, 2008, <http://www.ohioshroudconference.com/papers/p04.pdf> p. 2, footnote 3

<sup>998</sup> Goldoni, *The Shroud of Turin*, <http://www.ohioshroudconference.com/papers/p04.pdf> p. 2, footnote 3

<sup>999</sup> "I suspect it takes a lot more than amputation, or even "trauma" to elevate bilirubin. It's understandable that should happen in foot strike injury where the red cells are subject to mechanical stress and undergo haemolysis, but to read some of the Shroud literature, most emanating from Alan D Adler – a man with a limitless imagination – one could be forgiven for thinking that any prolonged trauma induces severe jaundice. That's news to me... What could be the mechanism? Scourging, maybe, but if that is primarily damage to superficial blood capillaries, would not most of the damaged red blood cells simply bleed out, rather than return to the venous circulation (how could they if the capillary vessels are damaged?)" Berry, <http://shroudofturinwithoutallthehype.wordpress.com/2012/10/23/ok-so-there-is-bilirubin-in-the-shroud-bloodstains-or-so-we-are-told-but-how-much-precisely/#comment-996>

<sup>1000</sup> Fanti, *Body image formation...*: Discussion, 2008, p. 17, <http://www.ohioshroudconference.com/papers/p15.pdf>, referring to "Moscardi G.: "Analysis by Raman Microscopy of powder Samples drawn from the Turin Shroud", III Prize, Poster Session, International Conference on the Shroud of Turin "Perspectives of a Multifaced Enigma", Columbus - Ohio August 14-17, 2008." (p. 25)

<sup>1001</sup> Adler, Selzer and DeBlase, *Further spectroscopic...*, 2002, TOM 94.96-97

<sup>1002</sup> Adler, *Updating recent...*, 1996, TOM 82-84

<sup>1003</sup> Adler, Selzer and DeBlase, *Further spectroscopic...*, 2002, TOM 94

<sup>1004</sup> Adler, *Updating recent...*, 1996, TOM 84

<http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMIcq90QWto4GAAw&ved=0CDEQ6AEwAA#v=snippet&q=only%20fair&f=false>

<sup>1005</sup> fig. 9 of Adler's TOM 101

<http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMIcq90QWto4GAAw&ved=0CDEQ6AEwAA#v=snippet&q=clot%20controls&f=false>

<sup>1006</sup> fig. 8 of Adler's TOM 100, visible online via scrolling back from p. 101

<http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMIcq90QWto4GAAw&ved=0CDEQ6AEwAA#v=snippet&q=clot%20controls&f=false>

online<sup>1007</sup>). The caption of the figure, which also shows a “bilirubin”, a “hemoglobin”, and a “dried blood” spectrum, reads “Fig. 10 Typical FTIR absorbance patterns of simulated exudate blood clot controls”, but it doesn’t say whether the “simulated” spectrum is a measured spectrum of a physical mixture simulating a clot or a digitally altered/simulated spectrum with a digitally reduced pattern of some protein and a digitally increased bilirubin pattern. It also doesn’t say whether the pattern of the hemoglobin protein or of the albumin protein or of both proteins were reduced, despite the hypothesized high level of albumin-bound bilirubin in the Shroud blood – “only the hemolyzed haemoglobin goes out along with the serum albumin which binds the bilirubin.”<sup>1008</sup> Also, no numbers are given for the ‘fitting’ bilirubin and “protein” concentrations.

Anyway, there is no obvious “very good fit” of the published “simulated” FTIR spectrum with the Shroud’s blood glob spectra; the shape and wavenumbers of blood glob spectra diverge more from those of the “simulated” spectrum than from those of the red madder lake spectra (cf. fig. 2.22 and 2.25 and table 3 in 2.1.2.3).

### 6.1.2.3. No published UV-vis absorbance match

In 1986, Adler wrote about “micro-spectrum photometry on the non-birefringent red-coated fibrils from the Shroud. It was obvious that the spectrum it produced did not match the spectrum of methemoglobin, at least as it is given in the standard references, which is a solution spectrum of blood. But in a film hemoglobin there is a confirmation [sic] change; it no longer remains in the “met” form but goes to the para-hemic form. ... You now mix bilirubin which is yellow-orange with methemoglobin in its para-hemic form which is an orangey-brown and you get blood which has a red color. In fact, we have been able to simulate this spectrum in the laboratory by the process described above.”<sup>1009</sup>

The mentioned “process” probably is the creation or simulation of a mixture with “an extraordinarily high bilirubin count, almost as high as the methemoglobin” in which “serum albumin ... binds the bilirubin”<sup>1010</sup> (cf. “a traumatic blood clot exudate (whole blood diluted with bilirubin-enriched human albumin)”<sup>1011</sup>). A physical “traumatic clot exudates simulacrum”<sup>1012</sup> was made by “mixing three drops of whole blood (finger stick) with three drops of a bilirubin/human albumin diagnostic standard” and subjected to both “FTIR-analysis” and “UV-VIS analysis”<sup>1013</sup>, without specification of reflectance or transmission mode. Although Adler says he had been able to simulate “this spectrum”, without specifying whether he means a Shroud blood spectrum or the hypothesized “red color” spectrum of a mix of yellow-orange and orangey-brown, still, the UV-vis absorbance spectrum of the physical simulated clot, having a strong Soret peak at ca 415 nm, is very different from both the UV-vis spectrum of the reddish Shroud fiber (strong peak at ca. 450 nm) and the UV-vis spectra of the Shroud bloodstains (no strong peak in the 400-450 nm region<sup>1014</sup>): compare the UV-vis spectrum of

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<sup>1007</sup> fig. 10 of Adler’s TOM 101

<http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMIcq90QWto4GAAw&ved=0CDEQ6AEwAA#v=snippet&q=clot%20controls&f=false>

Note that in fig. 2 of TOM 83, the FTIR spectrum of a bloodglob has been switched with that of a serum coated fiber: cf. fig. 1 of TOM 83 and cf. the figures 8 and 9 of TOM 100-101

<sup>1008</sup> “only the hemolyzed haemoglobin goes out along with the serum albumin which binds the bilirubin.” Adler, *The origin and nature...*, 1986, TOM 61

<sup>1009</sup> Alder, *The origin and nature...*, 1986, TOM 61; “methemoglobin in its para-hemic form” is an outdated expression for hemichrome (see 2.1.1.3 -2, citing S. Maričić et al., Hydration of Haemoglobin and its Reversible Oxygenation, *Berichte der Bunsengesellschaft für physikalische Chemie* Volume 68, Issue 8-9, pp. 787–793, Oktober 1964, p.790, <http://onlinelibrary.wiley.com/doi/10.1002/bbpc.19640680827/abstract> )

<sup>1010</sup> Alder, *The origin and nature...*, 1986, TOM 61

<sup>1011</sup> Adler, *Updating recent...*, 1996, TOM 82-84; cf. Adler, Selzer and DeBlase, *Further spectroscopic...*, 2002, TOM 97, 101

<sup>1012</sup> Adler, *Updating recent...*, 1996, TOM 82-84

<sup>1013</sup> Adler, Selzer and DeBlase, *Further spectroscopic...*, 2002, TOM 94

<sup>1014</sup> Pellicori wrote “The Shroud curve shows a pronounced band at 630 nm, a general band beginning near 525 nm, and no suggestion of a Soret band” (Pellicori, *Spectral properties...*, 1980, p. 1916).

the simulated clot (online fig. 12 of TOM 101<sup>1015</sup>), with the UV-vis spectra in fig. 2.2 (= fig. 2 of TOM 31, also online<sup>1016</sup>) and fig. 2.3.<sup>1017</sup> Adler wrote in 1996 “The clot simulation is in good agreement with the previously reported spectra of Shroud blood specimens and that taken from the on-site examination of the blood images on the whole cloth matching the observed peaks at 420+450+520+580+630 nm. Increasing the amount of bilirubin in this simulacrum will also improve the fit as with the FTIR data”.<sup>1018</sup> Here, it is not clear whether “the observed peaks” are of either the “clot simulation” (which doesn’t show an absorbance peak at 520 nm but at ca. 540 nm) or one of the Shroud’s partly red-stained fibrils (which, for instance, doesn’t show a peak at 420 nm but at 450 nm (fig. 2.2.A<sup>1019</sup>) or the brown-red crystal (which didn’t show a peak at 420 nm but at 405-410 nm<sup>1020</sup>) or the mean of the Shroud’s cloth stains (which plot is more or less flat from 380 to 520 nm, see fig. 2.3) or a combination of them.

In 1981, Heller and Adler also wrote that “In the previous spectral work(14) it was noted that a peak does appear both in the whole cloth reflection studies and in microspectrophotometry of the tape samples at about 450 nm. This is quite typical of bilidienes and similer bile pigment structures”.<sup>1021</sup> Note, that the strong absorbance band at 450 nm, appearing in their fig. 2 A from a partly red-stained fibril (here fig. 2.2.A), had been interpreted by Heller and Adler as a Soret band in its accompanying text of 1980, for it says “All these fibrils showed intense Soret (400-450 nm) absorption”.<sup>1022</sup>

In 2000, Adler wrote about a hypothetical type of blood, containing haptoglobin-hemoglobin aggregates and bilirubin-albumin complexes, but here he did not say the appearance and properties of the simulation match those of the red Shroud blood but match those of “this class of test objects”, without specifying this any further.<sup>1023</sup>

#### 6.1.2.4. Ehrlich’s reagent test very unspecific for bilirubin

As already discussed above, in 2.5.5., the Ehrlich’s reagent test used by Adler is very unspecific for bilirubin, as phenols and other substances also give the test color. This was made clear, in connection with the Shroud, by Berry in 2012-2013.<sup>1024</sup>

<sup>1015</sup>

<http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMlCq90QWto4GAAw&ved=0CDEQ6AEwAA#v=snippet&q=clot%20controls&f=false> choose p. 101, see fig. 12

<sup>1016</sup>

[http://books.google.nl/books?id=J2jBnDN3VxMC&pg=PA30&lpg=PA30&dq=spectral+data+of+the+Shroud+blood&source=bl&ots=zenDjCt69-&sig=WmPZiDBpkxQp61QSpVy4ZjVddk&hl=en&sa=X&ei=F3djUc-uOYmi8gTL1oCIBw&redir\\_esc=y#v=onepage&q=spectral%20data%20of%20the%20Shroud%20blood&f=false](http://books.google.nl/books?id=J2jBnDN3VxMC&pg=PA30&lpg=PA30&dq=spectral+data+of+the+Shroud+blood&source=bl&ots=zenDjCt69-&sig=WmPZiDBpkxQp61QSpVy4ZjVddk&hl=en&sa=X&ei=F3djUc-uOYmi8gTL1oCIBw&redir_esc=y#v=onepage&q=spectral%20data%20of%20the%20Shroud%20blood&f=false) (scroll down to page 31).

<sup>1017</sup> The statement in Adler, Selzer and DeBlase’s 2002 article, Further Spectroscopic..., that “The peak position pattern and relative overall absorbance ratio of the blue to red region of the spectrum of traumatic clot exudates sample is in excellent agreement with the previously reported spectra of Shroud blood specimens and also the spectra taken from the examination of the whole cloth blood images”, is an exaggeration.

<sup>1018</sup> Adler, Updating recent..., 1996, TOM 84

<sup>1019</sup> Heller and Adler, Blood on the Shroud..., 1980, Fig. 2. A

[http://books.google.nl/books?id=J2jBnDN3VxMC&pg=PA30&lpg=PA30&dq=spectral+data+of+the+Shroud+blood&source=bl&ots=zenDjCt69-&sig=WmPZiDBpkxQp61QSpVy4ZjVddk&hl=en&sa=X&ei=F3djUc-uOYmi8gTL1oCIBw&redir\\_esc=y#v=onepage&q=spectral%20data%20of%20the%20Shroud%20blood&f=false](http://books.google.nl/books?id=J2jBnDN3VxMC&pg=PA30&lpg=PA30&dq=spectral+data+of+the+Shroud+blood&source=bl&ots=zenDjCt69-&sig=WmPZiDBpkxQp61QSpVy4ZjVddk&hl=en&sa=X&ei=F3djUc-uOYmi8gTL1oCIBw&redir_esc=y#v=onepage&q=spectral%20data%20of%20the%20Shroud%20blood&f=false) (scroll down to page 31).

<sup>1020</sup> Heller, Report on..., 1983, p. 146-147

<sup>1021</sup> Heller and Adler, A Chemical..., 1981, TOM 39 (14 = Heller and Adler, Blood on the Shroud of Turin, 1980)

<sup>1022</sup> Heller and Adler, Blood on the Shroud..., 1980, TOM 30-31

<sup>1023</sup> “When such blood is shed and then clots, the exudate will contain these protein bound complexes with an expected range in a non-uniform color from red to orange, but any intact cells will remain in the clot. A simulation of such a traumatic blood exudate prepared from laboratory chemicals as a control matches the appearance and properties of this class of test objects.” Adler, Chemical and physical..., 2002, TOM 2

<sup>1024</sup> Berry on his blog <http://shroudofturinwithoutallthehype.wordpress.com/2014/01/25/10-reasons-for-thinking-that-the-shroud-of-turin-does-not-show-a-crucified-man-more-likely-a-barbecued-knight-templar/> and <http://shroudofturinwithoutallthehype.wordpress.com/2013/11/25/mr-barrie-schwartz-president-of-ster-a-inc-please-stop-proselytizing-incomplete-prematurely-abandoned-narrative-driven-so-called-science/> and in a comment elsewhere <http://shroudstory.com/2013/04/04/blood-clotting-and-the-strange-case-of-brother-hirudo/#comment-30257>

The test seemingly was not done on golden yellow serum coated fibers and the redder red coated fibers – even though bilirubin on the Shroud would be a serum and plasma ingredient –, but only on olive brown shards (replica casts of the fibers), orange globs, and more orange colored red coated fibers<sup>1025</sup>. During their investigation, Heller and Adler had 22 sticky-tape samples, of which 6 are described as blood samples.<sup>1026</sup> In his book, Heller describes how they got the idea there might be bilirubin, and how then the Erlich's reagent test was done on olive brown shards. Right after describing this test, he writes: "By this time we had used up most of the blood and serum fibrils"; then, after describing how an immunological test could be done, he continues "We decided to use one of the remaining serum-coated fibrils for the test".<sup>1027</sup> So, it is unclear whether golden yellow coated fibers were actually tested for bilirubin, or reserved for immunological testing.

The observed blue spots on the olive brown shards, red coated fiber samples, and orange globs, might have resulted from a reaction with the phenols in the madder dye coating that would be accessible on a fractured or particulate coated fiber (on the "Red coated fibrils" "Coating varies from smooth to fractured to particulate appearance"<sup>1028</sup>), and was possibly abraded with the shards and globs from the linen fibers. Note that the yellow colored 'Ghosts' easily break off of "all superficial linen fibers".<sup>1029</sup> Besides, the madder moiety of madder lake still is a phenol as well, for it retains at least one of its free hydroxyl groups.<sup>1030</sup> So, bilirubin needn't have been present in these samples at all.

#### 6.1.2.5. Not light-stable

"Bilirubin is degraded by light. Blood collection tubes containing blood or (especially) serum to be used in bilirubin assays should be protected from illumination."<sup>1031</sup> That bilirubin is not light-stable was said by Adler himself in 1999 in the context of the conservation of the Shroud,<sup>1032</sup> and was signalled again by Berry in 2012-2013<sup>1033</sup>.

#### 6.1.2.6. Bilirubin-rich blood turns brown

In 2010, Svensson's article reported about an experiment that was done with blood from a person who had jaundice for several months and toxic liver failure, and it showed photographs of both normal bloodstains and the bilirubin-rich bloodstains (both red immediately after sampling, and both brown after one week and after four months).<sup>1034</sup> In 2012, Svensson and Heimburger wrote the experiment could not be used as definitive proof against Adler's hypothesis, for the following reasons: "1) the blood used in this work is whole blood and not exudates of blood (hemolyzed

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<sup>1025</sup> Heller and Adler, A Chemical..., 1981, TOM 39

<sup>1026</sup> Heller and Adler, A Chemical..., 1981, Table 1, TOM 49

<sup>1027</sup> Heller, Report on..., 1983, p. 186-188

<sup>1028</sup> Heller and Adler, A Chemical..., 1981, Table 2, TOM 50

<sup>1029</sup> Fanti et al., Evidences for..., 2005, fact A3, <http://www.shroud.com/pdfs/doclist.pdf>

<sup>1030</sup> cf. the structure of an Al<sup>3+</sup>:alizarin 1:2 complex in fig. 2 <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0039914007005309-gr2.gif> of Claro et al., 2008

<sup>1031</sup> [http://en.wikipedia.org/wiki/Bilirubin#Blood\\_tests](http://en.wikipedia.org/wiki/Bilirubin#Blood_tests)

<sup>1032</sup> "Bilirubin, which is found in the bloodstains on the Shroud, is NOT light-stable. Every time it's illuminated, whether by ambient light, fluorescent light, photographic imaging lighting or even a flashbulb, the clock runs down to the time when the bilirubin will change in color. Further, the effects of these light-induced chemical changes on the adhesion of bilirubin to the linen is not known and could be detrimental." (Adler in Richmond 1999, cited by Walsh, The Recent Renovation of the Turin Shroud, <http://www.shroud.com/restored.htm#walsh> )

<sup>1033</sup> Berry in e.g. his blog posts <http://shroudofturinwithoutallthehype.wordpress.com/2012/08/23/the-pope-calls-it-an-icon-written-with-blood-i-say-its-an-elaborate-medieval-con-still-being-promoted-for-all-its-worth/> and <http://shroudofturinwithoutallthehype.wordpress.com/2012/10/23/ok-so-there-is-bilirubin-in-the-shroud-bloodstains-or-so-we-are-told-but-how-much-precisely/> and <http://shroudofturinwithoutallthehype.wordpress.com/2012/06/27/the-turin-shroud-spotlight-on-a-particular-mark-stain-some-call-it-blood-i-call-it-clack/> and <http://shroudofturinwithoutallthehype.wordpress.com/2012/10/23/ok-so-there-is-bilirubin-in-the-shroud-bloodstains-or-so-we-are-told-but-how-much-precisely/#comment-2082> and <http://shroudofturinwithoutallthehype.wordpress.com/2012/07/15/blood-grouping-the-shroud-of-turin-like-trying-to-sort-apples-from-oranges-in-the-dark-wearing-boxing-gloves/#comment-364>

<sup>1034</sup> Svensson, Medical and forensic aspects..., 2010, p. 4, and fig. 11, 12 and 13

<http://www.acheiropoietos.info/proceedings/SvenssonWeb.pdf>

methemoglobin in Adler's hypothesis) and 2) in Adler's hypothesis, bilirubin is mainly non-conjugated (or free) bilirubin, while the bilirubin in this experiment is mostly composed of conjugated bilirubin."<sup>1035</sup> These remarks on Adler's hypothesis seem not quite correct, for 1) Adler himself used a mix of whole blood and albumin-bound bilirubin for his FTIR and UV-vis 'matching' experiments, in which the hemoglobin in the red blood cells would oxidize to methemoglobin quickly, and the bilirubin would not bind to (met)hemoglobin anyway, even if both were in the serum (= 'clot exudate'),<sup>1036</sup> and 2) Adler wrote that, in his hypothesis, the bilirubin in the clot exudate is albumin-bound bilirubin, not "free" bilirubin.<sup>1037</sup> In his description of the physiology, Adler did not tell that the spleen forms the bilirubin from hemoglobin and binds it to albumin and sends it to the liver, where the liver detaches the bilirubin from albumin and binds it to glucuronic acid, making it soluble in water.<sup>1038</sup> The term "conjugated bilirubin" used by Heimburger and Svensson probably refers to this glucuronic acid-bound bilirubin, as is done in medical blood tests.<sup>1039</sup> How the blood of Adler's hypothesis could have such a high albumin-bound bilirubin level, seemingly even higher than the glucuronic-acid-bound bilirubin level, is not specified by Adler.

### 6.1.2.7. Fluoresces green

Pure bilirubin has a green "intrinsic bilirubin (triangles) fluorescence (525 nm emission under 467 nm excitation)"<sup>1040</sup> and 1:1 mixture of bilirubin and serum albumin also fluoresces green under blue excitation (e.g., 528 nm emission under 487 nm excitation or ca. 535 nm emission under 468 nm excitation)<sup>1041</sup>, while human serum albumin fluoresces in the ultraviolet and blue (ca. 340-365 nm peak emission under 295 nm excitation).<sup>1042</sup> As also the maximum absorbance (probably coinciding with the maximum excitation) of bilirubin is near 450 nm<sup>1043</sup>, in the visible blue, bilirubin fluoresces

<sup>1035</sup> Svensson and Heimburger, Forensic aspects..., 2012, p. 2520-21

<sup>1036</sup> "You see, bilirubin curls up on itself through internal hydrogen bonding – which explains its surprisingly low solubility in aqueous buffers and why it has to be transported in the bloodstream attached to serum albumin. (So any association with haem would require wresting it first off albumin)." Berry, blogpost <http://shroudofturinwithoutallthehype.wordpress.com/2012/06/27/the-turin-shroud-spotlight-on-a-particular-mark-stain-some-call-it-blood-i-call-it-clack/>;

<sup>1037</sup> "But a torture, scourging and crucifixion leading to shock – that would produce a tremendous hemolysis. In less than 30 seconds, the hemolyzed haemoglobin will run through the liver building up a very high bilirubin content in the blood. If that blood then clots, the exudate forms, and all the intact cells with haemoglobin stay behind, only the hemolyzed haemoglobin goes out along with the serum albumin which binds the bilirubin." Adler, The origin and nature..., 1986, TOM 61

<sup>1038</sup> "The heme is then turned into unconjugated bilirubin in the reticuloendothelial cells of the spleen. This unconjugated bilirubin is not soluble in water, due to intramolecular hydrogen bonding. It is then bound to albumin and sent to the liver. ... In the liver bilirubin is conjugated with glucuronic acid by the enzyme glucuronyltransferase, making it soluble in water." <http://en.wikipedia.org/wiki/Bilirubin#Unconjugated> .28.22Indirect.22.29 ; Glucuronic acid-bound bilirubin does not contain albumin, see [http://product.lookchem.com/info/7195302/27071-67-6-Bilirubin-glucuronide-Shanghai-Pengteng-Fine-Chemical-Co-Ltd-27071-67-6.html#U2kTiWe\\_mUk](http://product.lookchem.com/info/7195302/27071-67-6-Bilirubin-glucuronide-Shanghai-Pengteng-Fine-Chemical-Co-Ltd-27071-67-6.html#U2kTiWe_mUk) and [http://en.wikipedia.org/wiki/Bilirubin\\_digluconide](http://en.wikipedia.org/wiki/Bilirubin_digluconide)

<sup>1039</sup> [http://en.wikipedia.org/wiki/Bilirubin#Blood\\_tests](http://en.wikipedia.org/wiki/Bilirubin#Blood_tests)

<sup>1040</sup> Zucker et al., 2011, <http://www.jlr.org/content/42/9/1377/F5.expansion.html> of <http://www.jlr.org/content/42/9/1377.abstract>

<sup>1041</sup> Fig. 1 of fluorescence of serum albumin-bilirubin mixtures – bottom curve is zero bilirubin – <http://ars.els-cdn.com/content/image/1-s2.0-S0141813099000562-gr1.gif> and such a mixture under 467 nm plotted against pH in fig. 5 <http://ars.els-cdn.com/content/image/1-s2.0-S0141813099000562-gr5.gif> of Athar 1999, Use of fluorescence enhancement technique to study bilirubin–albumin interaction,

<http://www.sciencedirect.com/science/article/pii/S0141813099000562> ; Fig. 6 of human serum albumin (HSA)-bilirubin fluorescence – also here, the bottom curve probably represents zero bilirubin – <http://ars.els-cdn.com/content/image/1-s2.0-S0968089604002767-gr6.gif> of Trynda-Lemiesz 2004, Paclitaxel–HSA interaction. Binding sites on HSA molecule, <http://www.sciencedirect.com/science/article/pii/S0968089604002767>

<sup>1042</sup> Human serum albumin (a) fluoresces ca. 365 nm (peak wavelength) with a fluorescence tail until ca. 450 nm, at  $\lambda_{ex} = 295$  nm (fig. 1 <http://ars.els-cdn.com/content/image/1-s2.0-S0927776512007187-gr1.jpg> in Li et al., 2013, <http://www.sciencedirect.com/science/article/pii/S0927776512007187>); in another study it fluoresces ca. 340 nm under  $\leq 295$  nm excitation (Naveenraj et al., 2010, <http://www.sciencedirect.com/science/article/pii/S0731708510002207> fig. 3.(b)A)

<sup>1043</sup> absorbance spectrum of bilirubin: <http://www.bioblab.cn/uploads/1/Image/20090606031136124.jpg> or [http://www.npsg.uwaterloo.ca/data/skin/bilirubin\\_extinction.png](http://www.npsg.uwaterloo.ca/data/skin/bilirubin_extinction.png)

green under ordinary white visible light. This is probably the reason why the serum with a bilirubin level five times the physiological level, made by Goldoni, already showed “a peculiar greenish-yellow colouring”.<sup>1044</sup> If the Shroud bloodstains and serumstains had the 500 times elevated bilirubin-albumin level as hypothesized by Adler, the Shroud’s serum coated fibers would look green instead of golden yellow, and its bloodstains would fluoresce greenish instead of the reddish fluorescence color seen on fluorescence photographs of Shroud bloodstains (see 2.1.2.4).

All of this means that there is a lot of evidence against the presence of a high bilirubin level in the Shroud blood, and it precludes that bilirubin is the cause of the red color of its reddish bloodstains.

### 6.1.3. Prior UV-irradiation

Goldoni described experiments with linen (“treated with an aqueous solution of aloe, myrrh and artificial sweat”) which had acquired blood stains from contact with artificial blood clots (with added “calcium salts for the re-coagulation”) of whole human blood containing at least three different, increasing amounts of bilirubin: a normal physiological concentration, two times, and five times this concentration; then each bloodstain type was subjected to three types of treatment: no treatment, ageing for 10 hours in a stove at 120 °C, and exposure to UV light (365 nm) for 6 hours while “kept under continuous observation”; only the third treatment (UV light plus apparently ordinary light for continuous observation) had the effect that “the blood stains of all decals, after 6 hours of irradiation, took a bright red colour (Fig. 1).”<sup>1045</sup> Apparently, also the bloodstain with the normal bilirubin concentration took a bright red color. Whether this color was also visible in only ordinary white light – without added 365 nm UV light or sunlight –, and also weeks or months after the experiment, is not specified. Fanti published a photo of bloodstains, with the caption “Figure 15. Experiments with UV light on bloodstains made by C. Goldoni to reproduce the specific redness of the TS bloodstains” and commented: “In addition, C. Goldoni (2008) did experiments with human blood samples (Figure 15) and found that samples previously exposed to UV light show a particular redness similar to the one detected on the TS blood”.<sup>1046</sup> Goldoni reported to have said “In fact, it is known that the irradiation of blood with ultraviolet light transforms normal and pathological quantities of bilirubin in similar compounds such as lumirubin and isolumirubin.”<sup>1047</sup>

Lumirubin is indeed the main photoproduct formed from bilirubin during irradiation with UV light<sup>1048</sup>, and also during light therapy, especially by the blue visible light in it (“462+/-3 nm ... gives optimal lumirubin formation”), and lumirubin fluoresces blue (“typical fluorescence peak of lumirubin around 415 nm (410-420 nm)”) under UV-irradiation (“excitation wavelength of 315 nm”).<sup>1049</sup> This blue fluorescence might give (otherwise brown) bloodstains a temporary red-shift while observed under UV+white light, for a red stain reflects blue, yellow and red, but no green<sup>1050</sup>, and a brown stain reflects all colors with an increasing intensity from (almost no) blue to (a lot of) red<sup>1051</sup>. In only ordinary white light, the lumirubin would probably produce no, or only very little, fluorescence (“The emission peak of the pure lumirubin in aqueous solution (phosphate buffer) is at 415 nm while the main excitation peak is at 315 nm” and the phenomenon is called “UV excitable

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<sup>1044</sup> Goldoni, *The Shroud of Turin...*, 2008, p. 2, footnote 3

<sup>1045</sup> Goldoni, *The Shroud of Turin...*, 2008

<sup>1046</sup> Fanti, *Body image...*: Discussion, 2008, p. 17, <http://www.ohioshroudconference.com/papers/p15.pdf>

<sup>1047</sup> Goldoni, *The Shroud of Turin...*, 2008, p. 4

<sup>1048</sup> Bacci et al., UV excitable fluorescence of lumirubin, *Journal of Photochemistry and Photobiology B: Biology*, Volume 3, Issue 3, June 1989, pp. 419–427, DOI: 10.1016/1011-1344(89)80046-6, <http://www.sciencedirect.com/science/article/pii/1011134489800466>

<sup>1049</sup> Grass et al., Formation of lumirubin during light therapy in adults, *Journal of Biological Sciences* 4 (3): 357-360, 2004, [http://www.meduniwien.ac.at/user/wolfgang.wyskovsky/homepage/h\\_texte/Grass/Lumirubin\\_Light\\_Therapy.pdf](http://www.meduniwien.ac.at/user/wolfgang.wyskovsky/homepage/h_texte/Grass/Lumirubin_Light_Therapy.pdf)

<sup>1050</sup> see the absorbance spectra representing various visible colors

[http://www.vernier.com/images/innovate/food\\_coloring\\_spectra.jpg](http://www.vernier.com/images/innovate/food_coloring_spectra.jpg)

<sup>1051</sup> cf. the absorbance of (brown) melanins

<http://biomedicaloptics.spiedigitallibrary.org/data/Journals/BIOMEDO/22416/027801jbo2.jpeg>

fluorescence of lumirubin”<sup>1052</sup>) and therefore probably give no, or only a minimal, red-shift to brown bloodstains.

That some type of blood, with or without elevated bilirubin level, has locally and permanently become redder in ordinary light by temporary local UV irradiation, e.g. by a UV irradiation mainly from one side of the Shroud, accompanying the image formation process, seems improbable also for another reason: there are red bloodstains in non-image areas of the observe side, and also the reddish bloodstains on the almost imageless reverse side of Shroud are the same color as the red bloodstains in the image areas of the observe side.<sup>1053</sup> If UV light of sunlight or blue light of indoor light produced lumirubin from bilirubin, during expositions of the Shroud in the past, it is not easy to explain the presence of brown (parts of) bloodstains on various parts of the Shroud, right beside red bloodstains (see 5.4).

This last argument also applies to Jackson’s hypothesis that the bloodstains were naturally formed on the Shroud by contact with the blood, but were chemically altered by the same process that altered the chemistry of the image fibers while the cloth collapsed through a dematerialized body.<sup>1054</sup> In the small of the back area, within the body contour, there is a red blood rivulet with a brown tip (fig. 5.3), and also outside the dorsal foot image there is a red blood trail/rivulet with a brown tip (fig. 2.1). Besides, if the dorsal image is a contact image, as proposed in Jackson’s cloth collapse hypothesis,<sup>1055</sup> the blood stains on the reverse side of the dorsal half of the Shroud would probably not have been altered by the image formation process. Yet, the bloodstains on the dorsal reverse look just as pinkish as on the ventral reverse.<sup>1056</sup>

#### 6.1.4. CO-ligand from carbon monoxide gas

It has been conjectured that authentic blood has stayed red because of CO gas binding to it, for example during a fumigation or other burial ritual or other event liberating carbon monoxide and smoke. Carbon monoxide is a ligand to hemoglobin, giving the complex HbCO a red color comparable to the bright red color of HbO<sub>2</sub>, as their absorbance spectra are almost similar; but their spectra are very different from the methemoglobin spectrum<sup>1057</sup> and Shroud bloodstain spectra. Kears commented that the CO, ligated to hemoglobin, would exchange out within a few days at the most when exposed to the atmosphere, and that he believes CO is not permanently affixed to free heme either.<sup>1058</sup>

#### 6.1.5. Saponaria-treated cloth

The presence of a residue of Saponaria extract on the Shroud has already been discussed above, also in relation to the effect of Saponaria on hemoglobin and blood color (see 4.4). The many observations discussed there show that Saponaria is most probably not present on the Shroud in a level that would give the Shroud its atypical fluorescence, and even if it was present, it would not keep the blood red, but rather keep it brown.

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<sup>1052</sup> Bacci et al., UV excitable fluorescence of lumirubin, *Journal of Photochemistry and Photobiology B: Biology*, Volume 3, Issue 3, June 1989, pp. 419–427, DOI: 10.1016/1011-1344(89)80046-6,

<http://www.sciencedirect.com/science/article/pii/1011134489800466>

<sup>1053</sup> see a photo of the reverse side on the Shroud Science Group wiki <http://shroud.wikispaces.com/PROPERTIES>

<sup>1054</sup> Jackson, An Unconventional Hypothesis ..., 1991, <http://theshroudofturin.blogspot.nl/2012/01/john-p-jackson-unconventional.html>, paragraph E 3.

<sup>1055</sup> Ibid. D 3

<sup>1056</sup> see a photo of the reverse side on the Shroud Science Group wiki <http://shroud.wikispaces.com/PROPERTIES>

<sup>1057</sup> [http://biomedicaloptics.spiedigitallibrary.org/data/Journals/BIOMEDO/24849/JBO\\_17\\_9\\_090901\\_f001.png](http://biomedicaloptics.spiedigitallibrary.org/data/Journals/BIOMEDO/24849/JBO_17_9_090901_f001.png)

<sup>1058</sup> Kears, comment <http://shroudstory.com/2013/05/29/a-guest-posting-by-yannick-clment-two-quotes-about-the-blood/#comment-35281>

## 6.2. Painted-on bloodstains

If the bloodstains were painted on the cloth, they would have been painted when the body image was already visibly there, for the bloodstains are present in the correct locations for the representation of the two body sides of a crucified person. If the bloodstains were on the cloth before the image was, as is necessary for red madder lake formation on yellow madder dye before this madder dye would be degraded in image formation, all painted-bloodstain hypotheses are false.

Also the realistic appearance of the bloodstains, both from an artistical point of view, as indicated by De Wesselow<sup>1059</sup> and others, and from a medical point of view, as indicated by Barbet and later by Baima Bollone<sup>1060</sup>, makes a painted-blood hypothesis very unlikely. Below, a few chemical-physical features of a few feasible ‘bloodpaints’ are discussed and shown to be incompatible with the features of the Shroud bloodstains.

### 6.2.1. ‘Cured’ blood paint – NO or CO

A possibility for explaining the red color of the Shroud bloodstains is that they were painted with blood that had been ‘cured’ by ligands that give it a (pinkish) red color, such as CO (carbon monoxide), CN<sup>-</sup> (cyanide)<sup>1061</sup>, and NO (nitric oxide). Yet, these ligands do not yield the typical absorbance peak of acid methemoglobin (or acid heme dimers) at ca. 630 nm,<sup>1062</sup> a peak observed in the Shroud bloodstain UV-vis spectra.<sup>1063</sup> HbCO doesn’t show any absorbance peak in this region.<sup>1064</sup> “Cyanomethemoglobin does not absorb at 630 nm”<sup>1065</sup> and free cyanide probably was unavailable in the Middle Ages anyway.<sup>1066</sup> Nitrosoheme (NO ligated to heme) has no absorbance peak in the 600-700 nm region either (fig. 6.1), turns brown by photooxidation in light and oxygen, and its FTIR spectra (fig. 6.2 and 6.3) do not resemble the FTIR spectra of the Shroud blood globs (fig. 2.23).<sup>1067</sup>

<sup>1059</sup> De Wesselow, *The Sign*, 2012, chapter 10

<sup>1060</sup> “P. Barbet 18 noted that the TS blood has the aspect of blood clots formed on the skin. Those blood clots have a pale internal zone, sharp evident edges and a pale halo of serum; are depressed in the centers, raised on the edges. (18 Pierre Barbet: “La prova della autenticità della Sindone nelle sue macchie di sangue”, *Sindon*, Quaderno No. 14-15, Dicembre 1970, pp. 21-43).” Brillante et al, *Bloodstains characteristics...*, 2002, p. 5, 8; “the stains in some places such as the (sic) on the forehead and in the hair .... The borders of these stains are more coloured than their centres.” Baima Bollone, *The Forensic Characteristics...*, Proceedings Turin 2000, p. 215-216

<sup>1061</sup> “Pink mauve discoloration of the face, neck and front of the chest in a man who died after swallowing calcium cyanide. This curious coloration is due to the presence of cyano-haemoglobin in the blood.”

<http://www.google.com/imgres?start=353&hl=nl&biw=1024&bih=543&tbn=isch&tbnid=1qyvQHglblBumM:&imgrefurl=http://forum.codoh.com/viewtopic.php%3Ff%3D2%26t%3D7320%26start%3D15&docid=BSjSPt1xHXEWFm&imgurl=http://www.nazigassings.com/images/Gresham/GAGreshamfig37p57wCaption.jpg&w=609&h=504&ei=egiNUfO6JojTPJzhgOAO&zoom=1&iact=rc&page=18&tbnh=172&tbnw=190&ndsp=20&ved=1t:429,r:69,s:300,i:211&tx=65&ty=81>

<sup>1062</sup> “Acid methemoglobin has a characteristic absorption peak at 630-631 nm” (A Mansouri, AA Lurie, *Methemoglobinemia*, *American Journal of Hematology*, 1993,

<http://onlinelibrary.wiley.com/doi/10.1002/ajh.2830420104/abstract>), 5<sup>th</sup> find at <http://scholar.google.nl/scholar?hl=nl&q=absorption+acid+methemoglobin&lr>

<sup>1063</sup> “a pronounced band at 630 nm” (Pellicori, *Spectral properties...*, 1980, p. 1916); cf. fig. 2.3

<sup>1064</sup> [http://biomedicaloptics.spiedigitallibrary.org/data/Journals/BIOMEDO/24849/JBO\\_17\\_9\\_090901\\_f001.png](http://biomedicaloptics.spiedigitallibrary.org/data/Journals/BIOMEDO/24849/JBO_17_9_090901_f001.png)

<sup>1065</sup> US patent “Blood substitute for carrying oxygen”, <https://www.google.com/patents/US4001200>

<sup>1066</sup> It was first obtained by heating the pigment Prussian blue, which was only synthesized from iron, carbon, and nitrogen for the first time around the year 1706 <http://en.wikipedia.org/wiki/Cyanide> - [http://en.wikipedia.org/wiki/Prussian\\_blue](http://en.wikipedia.org/wiki/Prussian_blue) - <http://en.wikipedia.org/wiki/Ferrocyanide>

<sup>1067</sup> Fig. 1 and fig. 4 of Soltanizadeh et al., 2012, <http://pubs.acs.org/doi/abs/10.1021/jf300023s>, full text with figures at [http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file\\_pubwdet/role\\_of\\_globin\\_moiety\\_in\\_the\\_chemical\\_structure\\_of\\_curing\\_pigment\\_0.pdf](http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file_pubwdet/role_of_globin_moiety_in_the_chemical_structure_of_curing_pigment_0.pdf) cf. Fig. 9 of Adler, Selzer and DeBlase, *Further spectroscopic...*, 2002, TOM 101 <http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMlcq90QWto4GAaw&ved=0CDEQ6AEwAA#v=snippet&q=clot%20controls&f=false>

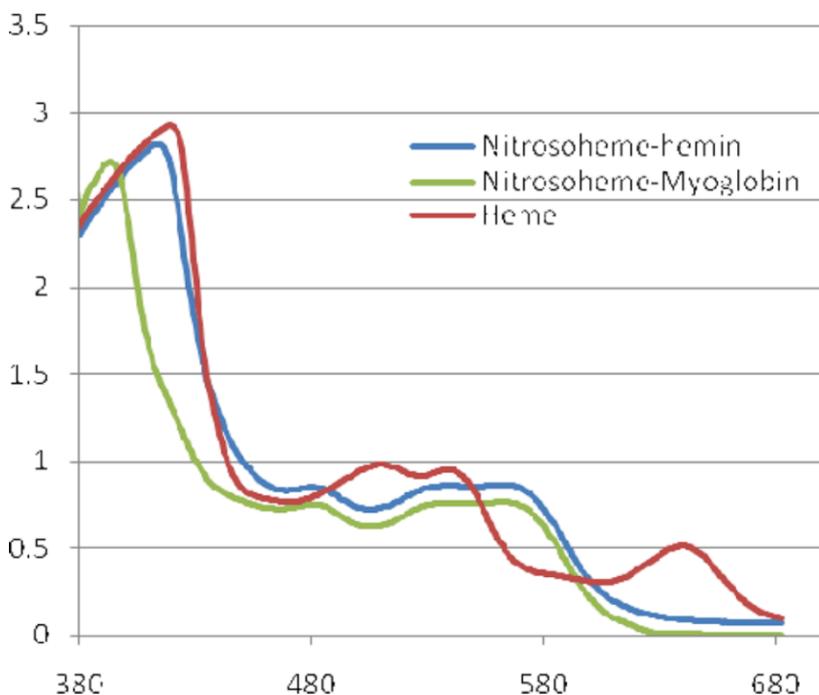


Fig. 6.1. Absorbance of heme and of nitrosoheme made of hemin and myoglobin, respectively ©ACS<sup>1068</sup>

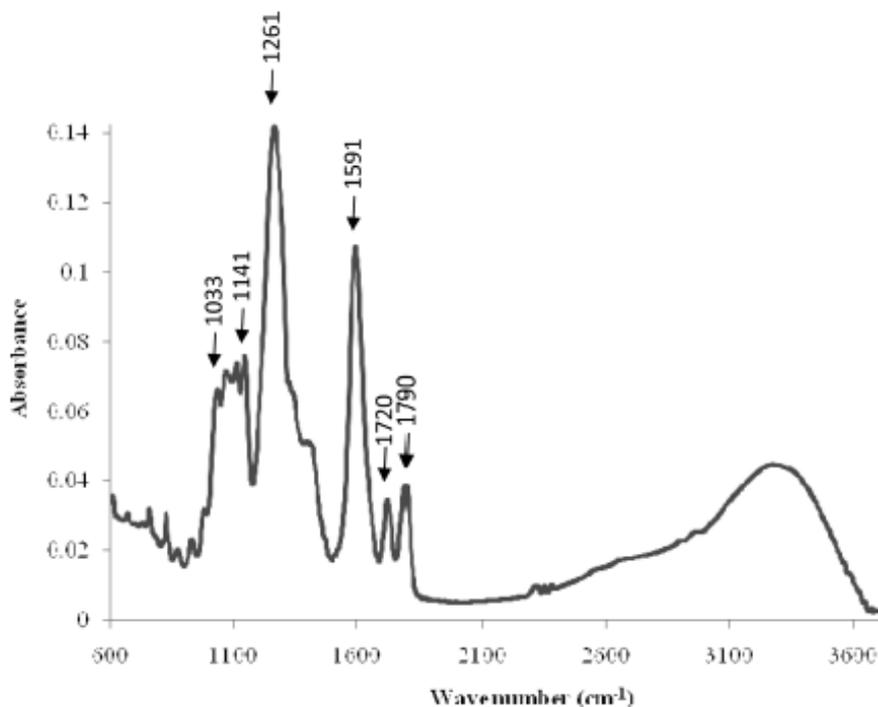


Fig. 6.2. FTIR spectrum of nitrosoheme made of hemin ©ACS<sup>1069</sup>

<sup>1068</sup> Reprinted and adapted with permission from Nafiseh Soltanizadeh and Mahdi Kadivar, Role of Globin Moiety in the Chemical Role of Globin Moiety in the Chemical Structure of Curing Pigment, *Journal of Agricultural and Food Chemistry*, May 1, 2012, 60. pp. 4718-4724, Copyright 2012 American Chemical Society, Fig. 1, [http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file\\_pubwdet/role\\_of\\_globin\\_moiety\\_in\\_the\\_chemical\\_structure\\_of\\_curing\\_pigment\\_0.pdf](http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file_pubwdet/role_of_globin_moiety_in_the_chemical_structure_of_curing_pigment_0.pdf)

<sup>1069</sup> Reprinted and adapted with permission from Nafiseh Soltanizadeh and Mahdi Kadivar, Role of Globin Moiety in the Chemical Role of Globin Moiety in the Chemical Structure of Curing Pigment, *Journal of Agricultural and Food Chemistry*, May 1, 2012, 60. pp. 4718-4724, Copyright 2012 American Chemical Society, Fig. 4, [http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file\\_pubwdet/role\\_of\\_globin\\_moiety\\_in\\_the\\_chemical\\_structure\\_of\\_curing\\_pigment\\_0.pdf](http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file_pubwdet/role_of_globin_moiety_in_the_chemical_structure_of_curing_pigment_0.pdf)

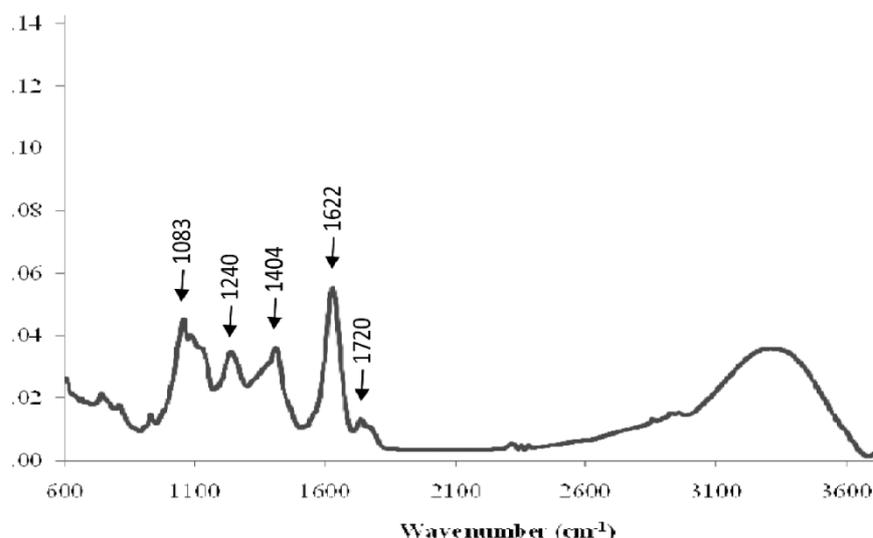


Fig. 6.3. FTIR spectrum after photooxidation of nitrosoheme made of hemin ©ACS<sup>1070</sup>

Also, in the case of ‘cured’ blood, it is problematic how heme dimers with a reduced Soret absorption band would have been formed from (met)hemoglobin. The ‘cured’-blood-paint hypotheses do not easily explain the lack of red blood cells and the lack of a potassium signal in X-ray fluorescence either (see 2.3 and 2.4, above). Berry hypothesized that the ‘blood paint’ may have been the gut contents of a medicinal leech that has sucked whole human blood, of which it has excreted the serum and potassium and digested the cell membranes and the globin protein and has ‘cured’ the remaining heme with nitrites or gaseous NO.<sup>1071</sup> That a medicinal leech produces such ‘cured’ heme can not be proven or ruled out for lack of available research, although one study showed that feeding in medicinal leeches enhances bacterial digestion and the excretion of NH<sub>3</sub> (ammonia gas).<sup>1072</sup>

Other objections are that the ‘cured’-blood-paint hypotheses do not explain the presence of an acid methemoglobin crystal on the Shroud<sup>1073</sup> and that they do not explain the reddish looking fluorescence of the Shroud bloodstains (see 2.1.2.4, above), assuming the ‘cured’ blood was painted on a madderless cloth; hemoglobin, under excitation < 300 nm, only fluoresces near 330 nm, in the ultraviolet.<sup>1074</sup>

<sup>1070</sup> Reprinted and adapted with permission from Nafiseh Soltanizadeh and Mahdi Kadivar, Role of Globin Moiety in the Chemical Role of Globin Moiety in the Chemical Structure of Curing Pigment, *Journal of Agricultural and Food Chemistry*, May 1, 2012, 60. pp. 4718-4724, Copyright 2012 American Chemical Society, Fig. 6, [http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file\\_pubwdet/role\\_of\\_globin\\_moiety\\_in\\_the\\_chemical\\_structure\\_of\\_curing\\_pigment\\_0.pdf](http://soltanizadeh.iut.ac.ir/sites/soltanizadeh.iut.ac.ir/files/file_pubwdet/role_of_globin_moiety_in_the_chemical_structure_of_curing_pigment_0.pdf)

<sup>1071</sup> Berry, <http://shroudofturinwithoutallthehype.wordpress.com/2012/08/04/its-still-looking-good-for-leech-digesta-as-the-source-of-blood-on-the-shroud-of-turin/> and <http://shroudofturinwithoutallthehype.wordpress.com/2013/03/02/shroudie-alert-day-12-time-now-to-write-that-long-overdue-letter-to-the-royal-society/>

<sup>1072</sup> “1. In *Hirudo*, feeding results in a dramatic rise of the metabolic rate as indicated by O<sub>2</sub> consumption, excretion of nh<sub>3</sub> and elimination of ions and water, each showing a characteristic time-course.

2. Proteolytic activity is absent in the anterior alimentary system and unusually low in the intestine. In the latter it increases upon feeding and, after reaching a maximum, slowly decreases again.

3. The diverticules contain potent protease inhibitors which were not detected in the intestine.

4. Respiration, NH<sub>3</sub> excretion and the rise of proteolytic activity are inhibited by the antibiotic kanamycin.” (Zebe et al., 1986, <http://www.sciencedirect.com/science/article/pii/0300962986900411>); “Kanamycin (also known as kanamycin A) is an aminoglycoside bacteriocidal antibiotic.” <http://en.wikipedia.org/wiki/Kanamycin>

<sup>1073</sup> Heller, Report on..., 1983, p. 144-7 (quote in note in 2.1.1.1. above, and online at [http://books.google.fr/books?ei=dNPnUbiBLoL\\_PKercAH&hl=fr&id=o9gIK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&q=acid+methemoglobin](http://books.google.fr/books?ei=dNPnUbiBLoL_PKercAH&hl=fr&id=o9gIK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&q=acid+methemoglobin) and [http://books.google.fr/books?ei=dNPnUbiBLoL\\_PKercAH&hl=fr&id=o9gIK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&q=hit+400](http://books.google.fr/books?ei=dNPnUbiBLoL_PKercAH&hl=fr&id=o9gIK7LcmHUC&dq=heller+report+on+the+shroud+of+turin&q=hit+400) )

<sup>1074</sup> “Fig. 3. Effect of DMY on fluorescence spectra of HHb (*T* = 296 K, pH 7.4). (a) 5.0 μM HHb; (b-h) 5.0 μM HHb in the presence of 2.5, 5, 7.5, 10.0, 12.5, 15, 17.5 μM DMY; (i) 17.5 μM DMY.” (HHb = human hemoglobin) <http://ars.els->

A general objection against all painted-blood hypotheses is that they do not explain the presence of separate serum on the Shroud (see 2.2), such as probably present in the fluorescence halo at the tip of the wrist bloodstain (unless the ‘blood paint’ was used to touch up authentic bloodstains). When, as during painting, a drop of fresh blood is left to dry on a horizontal cloth with interstices through which it reaches the reverse side, as on the Shroud, no separate serum ring would be produced around the bloodstain (see fig. 6.4: in pure linen on a glass plate, such a stain does not change shape after very rapidly attaining its maximum shape); the serum would rather drain down to the reverse side. On a vertical cloth, painted-on blood would drain down inside the cloth, and if any serum borders formed (which does not happen in relatively loosely woven pure linen, see fig. 6.5), they would probably all be on the same side of the bloodstains, instead of on different sides, such as on the Shroud lateral of the wrist bloodstain, and seemingly above and beside and beneath the chest bloodstain.

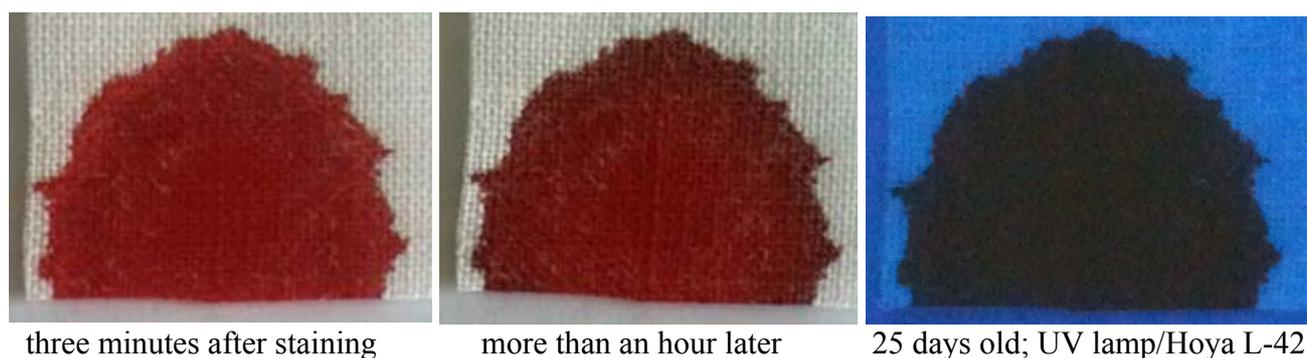
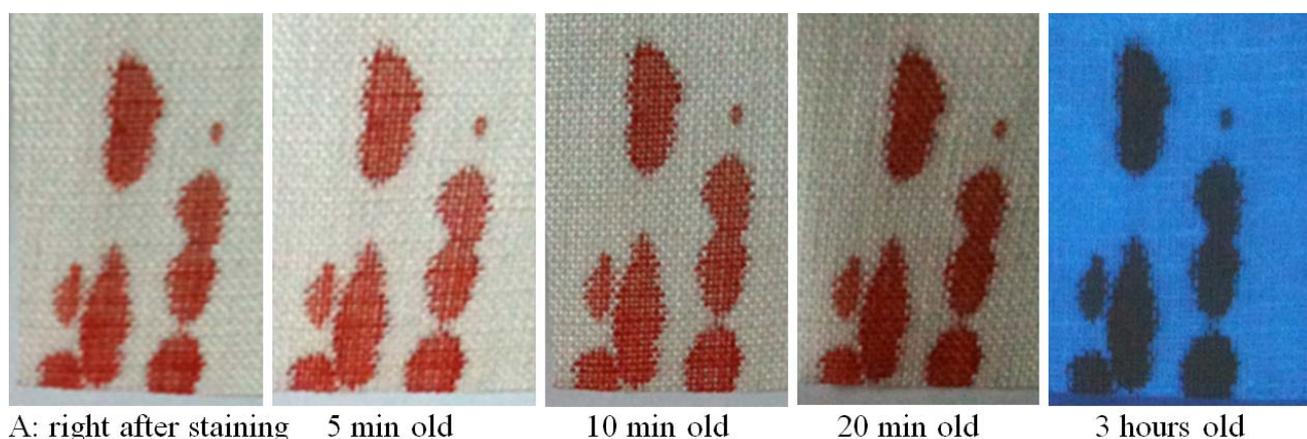


Fig. 6.4. Fresh whole blood fallen on horizontal linen does not produce a serum halo. ©AvdH



[cdn.com/content/image/1-s2.0-S1386142512002260-gr3.jpg](http://ars.els-cdn.com/content/image/1-s2.0-S1386142512002260-gr3.jpg) in Chen et al., 2012, <http://www.sciencedirect.com/science/article/pii/S1386142512002260> ; “Fig. 3. Fluorescence spectra of Hb (12.2  $\mu\text{M}/\text{heme}$ ). From bottom to top, the concentration of CTAB: 0,  $2.0 \times 10^{-4}$ ,  $6.0 \times 10^{-4}$ ,  $1.0 \times 10^{-3}$ ,  $1.0 \times 10^{-2}$  mol/L. Inset in figure shows the fluorescence intensity of Hb (12.2  $\mu\text{M}/\text{heme}$ ) ( $\lambda_{\text{em}} \approx 334$  nm) with CTAB.” <http://ars.els-cdn.com/content/image/1-s2.0-S0141813005002448-gr3.gif> in Liu et al, 2005, <http://www.sciencedirect.com/science/article/pii/S0141813005002448>; cf. “Fig. 2. UV-vis spectrum and fluorescence spectrum of Hb.  $c_{\text{Hb}}$  (mol L $^{-1}$ ):  $5 \times 10^{-6}$ , (1) UV-vis spectrum of Hb, (2) fluorescence spectrum of Hb. (a) Hb/H $_2$ O system, (b) Hb/niosome system.” <http://ars.els-cdn.com/content/image/1-s2.0-S0927775706005693-gr2.gif> in Liu et al., 2007, <http://www.sciencedirect.com/science/article/pii/S0927775706005693>

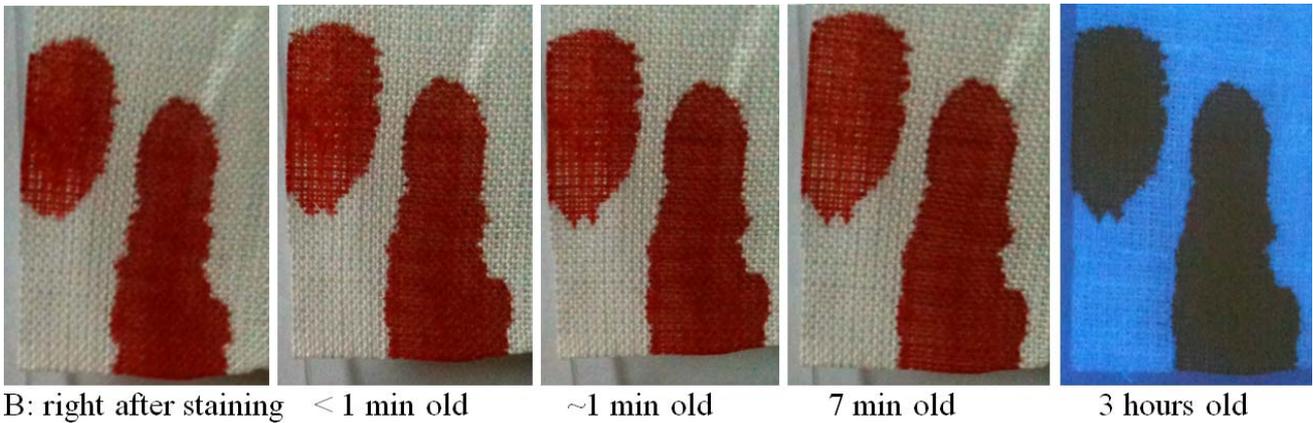


Fig. 6.5. Vertical linen stained with fresh whole blood, at 23-24 °C, 60-70% rel. humidity. A: linen at ~85° from table, blood falling past cloth. B: linen ~70° from table, bloody fingertip briefly touching one point of the cloth (left), blood falling on cloth (right). Last photos: Wood lamp and Hoya L-42 filter. ©AvdH

### 6.2.2. Iron oxide particles in protein binder

McCrone claimed that iron oxide particles in a protein binder were used to paint both the body image and the bloodstains.<sup>1075</sup> As already discussed in 2.4, the size of red iron oxide particles (= red ochre) and the size of the Shroud's linen fibers preclude that the observed smoothly red colored fibers (see for instance fig. 2.49) were stained by iron oxide particles in a yellowish binder. "Both red ochre and vermilion are about one micron (1/25,00<sup>th</sup> inch) in diameter"<sup>1076</sup>, while the diameter of Shroud linen fibers is 10-20 micron.<sup>1077</sup>

Also the iron oxide hypothesis does not explain the presence of separate serum and an acid methemoglobin crystal and some red blood cells, nor the 630 nm absorbance peak of bloodstains (a comparison of the absorbance spectra of iron oxide and the Shroud bloodstains is in Rogers book<sup>1078</sup>), nor the presence of porphyrin species<sup>1079</sup> or the reddish fluorescence of the bloodstains, and the FTIR spectrum of iron oxide is totally different from the FTIR of Shroud blood globs.<sup>1080</sup>

Iron oxide particles do not dissociate into a yellow exudate and black particles after mounting in Cargille oil, as red particles on washed fibers from a blood area sticky-tape did<sup>1081</sup>, nor do they bubble up and turn black in MOLE experiments, as most red flecks from Shroud blood areas did<sup>1082</sup> (see 2.1.2.6).

### 6.2.3. Iron-madder lake

The elevated iron concentration in Shroud bloodstains, compared to non-image and image areas, found by X-ray fluorescence, allows the hypothesis of the presence of painted-on red iron-madder lake. Besides most of the general objections against paint hypotheses (presence of separate serum, acid methemoglobin crystal, 630 nm absorbance peak), painted-on iron-madder lake does not explain the presence of some red blood cells nor the presence of a porphyrin species producing porphyrin

<sup>1075</sup> e.g. McCrone, *The Shroud of Turin...*, 1990; McCrone, *Judgement day...*, 1999

<sup>1076</sup> McCrone, *Judgement Day...*, 1999, p. 167

<sup>1077</sup> Fanti et al., *Evidences for...*, 2005, A6

<sup>1078</sup> Rogers, *A Chemist's...*, 2008, p. 50, Fig. VIII-1

<sup>1079</sup> Heller and Adler, *Blood on the Shroud*, 1980, TOM 31

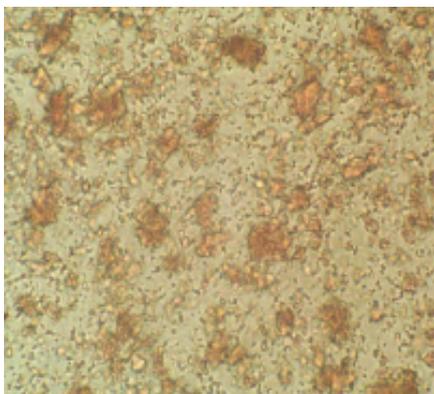
<sup>1080</sup> Adler, Selzer and DeBlase, *Further spectroscopic...*, 2002, Fig. 9 and 11, TOM 101, online at

<http://books.google.co.uk/books?id=J2jBnDN3VxMC&printsec=frontcover&dq=adler+orphaned+manuscript+chemical+and+physical+aspects&hl=en&sa=X&ei=3v6tUPjMIcq90QWto4GAAw&ved=0CDEQ6AEwAA#v=snippet&q=clot%20controls&f=false>

<sup>1081</sup> Bracaglia, Dr Nitowski's..., <http://holyshroudguild.org/dr-nitowski-new.html>

<sup>1082</sup> Rogers, *A Chemist's...*, 2008, p. 61

fluorescence,<sup>1083</sup> unless the paint was used to touch up real bloodstains. Yet, if a pure iron-madder lake was painted on cloth, its red fluorescence would not be quenched by heme aggregate deposits on top of it, and the painted bloodstains would probably fluoresce stronger than the non-image (yellow madder-dyed) background. This is not the case for the Shroud. Also, madder lake paint consists of particles in a binder (fig. 6.6) and therefore can not explain the presence of the smoothly purplish colored fibers from the side stain area (fig. 2.49). Painted-on madder dye, on the other hand, can not explain the presence of free blood globs, even yielding an FTIR spectrum resembling that of madder lake (fig. 2.21 and 2.22), unless it was painted on authentic heme stains.



*Fig. 6.6. Microscopic appearance of a madder lake in a paint medium in a painting at x500 mag ©WebExhibits of the Institute for Dynamic Educational Advancement (IDEA)<sup>1084</sup> (madder lake absorbs much binder, about 100% by volume<sup>1085</sup>)*

#### **6.2.4. Acid blood**

Acid blood or acid hemolysate, either authentic or painted-on, on an uncoated cloth would turn brown and black like any other blood.

#### **6.3. Survey red color hypotheses**

The following survey (table 10) shows that, of all bloodstain hypotheses, the acid heme-madder lake hypothesis explains the bloodstain characteristics best.

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<sup>1083</sup> Heller and Adler, Blood on the Shroud, 1980, TOM 31

<sup>1084</sup> <http://www.webexhibits.org/pigments/indiv/technical/madder.html>

(<http://www.webexhibits.org/pigments/intro/about.html>, with permission for non-commercial sharing, see <http://www.webexhibits.org/about/legal.html>); the madder page

<http://www.webexhibits.org/pigments/indiv/technical/madder.html> links to

<http://www.irug.org/ed2k/spectra.asp?file=IOD00201.DX>, where the FTIR “IOD00201 Madder, area 3” of the IRUG used to be (it is now online at <http://www.irug.org/jcamp-details?id=176>) (see fig. 2.21 above).

<sup>1085</sup> [http://www.essentialvermeer.com/palette/palette\\_madder\\_lake.html#.U84nJWe\\_mUk](http://www.essentialvermeer.com/palette/palette_madder_lake.html#.U84nJWe_mUk)

	<b>post-mortem acid heme-madder lake</b> on madder-and-starch coated Shroud on wooden board	<b>ante-mortem blood</b> on starch-and-madder coated Shroud on wooden board, later acidified	<b>bilirubin</b> and metHb in clot exudate = serum (authentic blood, no madder)	<b>Saponaria coating</b> (authentic blood on starch, no madder)	<b>UV/blue light irradiation</b> (authentic blood, no starch, no madder)	<b>painted-on NO-heme</b> (medicinal leech digest?, no starch, no madder)	<b>painted-on iron oxide</b> = hematite = Fe <sub>2</sub> O <sub>3</sub> (no madder)	<b>painted-on acid blood</b> (pH of authentic blood, no starch, no madder)	<b>painted-on iron-madder lake</b> (no starch, no madder dye)
correct locations and realistic appearance	+	- no scourge mark pattern	+	+	+	- unless painted after image formation	- unless painted after image formation	- unless painted after image formation	- unless painted after image formation
pink-red color	+	+/-?	- orange brown turns brown	- brown	-+ stable? also in ordinary light?	+/- pink-red turns brown	- red-brown	- turns brown	+
separate yellow serum	+	+	-	+	+	-	-	-	-
porphyrins	+	+	+	+	+	+	-	+	-
few red blood cells, at least some	+	+	+	+	- no saponin hemolysis, more clots	?	-	-no saponin hemolysis, more clots	-
no/little K <sup>+</sup>	+	-	- K <sup>+</sup> in exudate = serum	+	- plasma absorbed into uncoated TS	?	+	- plasma absorbed into uncoated TS	+
acid metHb crystal	+	-	-	+	- no saponin hemolysis	? if acid	-	? no saponin hemolysis	-
630 nm absorbance peak - cloth stain	+	+	+	+	? if acid	? if acid	-	+	-
FTIR bloodglobs	+	?	-+	-	?	-	-	-	+/- (no heme)
no ca. 400 nm Soret absorbance peak – cloth stain	+	-	-	+	? no saponin hemolysis, more clots, RBC's and metHb	?	+	? no saponin hemolysis, more clots, RBC's and metHb	+
450 nm absorbance - cloth stain	+	+	+/-	- only fluorescence contribution <400 nm	?	-	-+	-	-
450 nm absorbance peak - fiber	+	+	+	-	?	-	-	-	-
525+280 nm absorbance - cloth stain	+	?	-	-	?	-	-	-	+
weak red fluorescence	+	?	-	-	?	-	-	-	-+ stronger red fluorescence
red stains on observe and reverse side, brown stains beside red stains	+	+/-?	-	-	-?	?	-	?	-

Table 10. Survey of red-color hypotheses and their congruence with Shroud bloodstain features

## 7. BLOOD ON THE PETALON - NOT ON THE BEARD

Besides the already mentioned evidence for the use of a (wooden) burial bier for the trasferral of the enshrouded body (viz. the necessity of the use of a flat wobbling plane on which the Shroud lay when blood rivulets ran across the waterresistant Shroud and a water-absorbant substrate beneath it – see 2.3.4 and 5.3), there is another indication for an authentic first-century Jewish burial, and even for the burial of Jesus. “In Biblical times persons, especially of high rank, were arrayed at burial in the garments, ornaments, and weapons which they had worn in life ... To be buried without garments was considered a disgrace ... As a token of honor, it was customary to cast the most costly garments and ornaments upon the bier of a dear relative or friend ... (Ket. 8b; M. K. 27b).”<sup>1086</sup> Beneath the beard of the body image there seems to be the image of a flat, more or less oval object (fig. 7.1). Height displays based on the grey scale values of (the negative of) a Shroud photo show the flatness of this object and some smaller protruding shapes on its flat surface (fig. 7.2 and 7.3). It seems to be either a flat and rather angular and seemingly unconnected part of the beard – a kind of Zeus beard in two parts – or else a distinct object that lay on the chest of the body. The color photography of Haltadefinizione shows that on the lower edge of this object there is the pink color that also constitutes the scourge marks and bloodstains (fig. 7.4 and 7.5).<sup>1087</sup> This means that there was blood on the edge of the object.



Fig. 7.1. Photograph of the face of the Shroud, negative ©1978-2014 Barrie M. Schwartz Collection, STERA, Inc. (enhanced by Soons)<sup>1088</sup>

<sup>1086</sup> “... the Rabbis deprecated such practise (...). In fact, since funeral expenses became common extravagances and an object of alarm to the relatives, R. Gamaliel II. set the example by the order he gave for his own funeral, and thus introduced the custom of burying the dead in simple linen garments (...).”

([www.jewishencyclopedia.com/view.jsp?artid=1607&letter=B](http://www.jewishencyclopedia.com/view.jsp?artid=1607&letter=B)); Gamaliel II: [www.jewishencyclopedia.com/view.jsp?artid=52&letter=G](http://www.jewishencyclopedia.com/view.jsp?artid=52&letter=G); the Talmud: <http://www.sacred-texts.com/jud/t01/t0135.htm> and <http://www.sacred-texts.com/jud/t04/rab02.htm>; and <http://www.sacred-texts.com/jud/t04/rab03.htm>

<sup>1087</sup> See also the colors of the area in Shroud Scope, Durante 2002

<http://www.sindonology.org/shroudScope/shroudScope.shtml?zl=8&image=3&lon=2174.0&lat=10258.0>

<sup>1088</sup> Used from P. Soons, Three Hebrew Letters on Surface of Solid Object, fig. 6 on <http://shroud3d.com/findings/three-hebrew-letters-on-surface-of-solid-object>, with permission from STERA, Inc. and P. Soons.

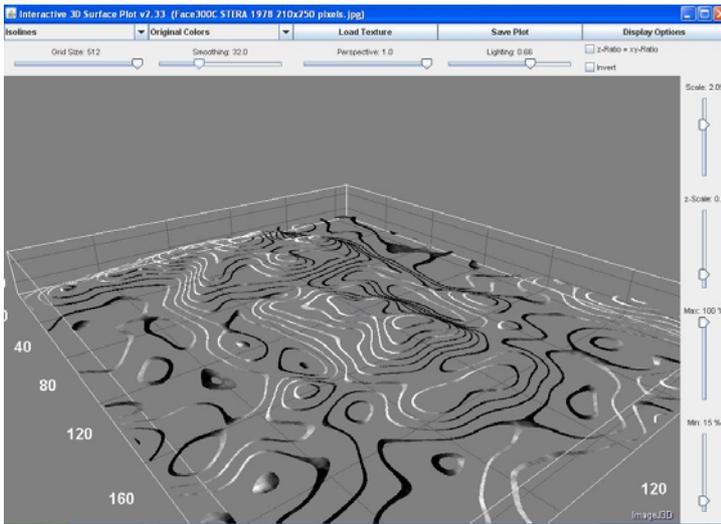


Fig. 7.2. Height display of isolines of gray scale values of negative photo of the Shroud face – settings set to show the overall flatness of the object beneath the chin (original Shroud photo<sup>1089</sup> ©1978 Barrie M. Schwartz Collection, STERA, Inc.; height display ©AvdH)

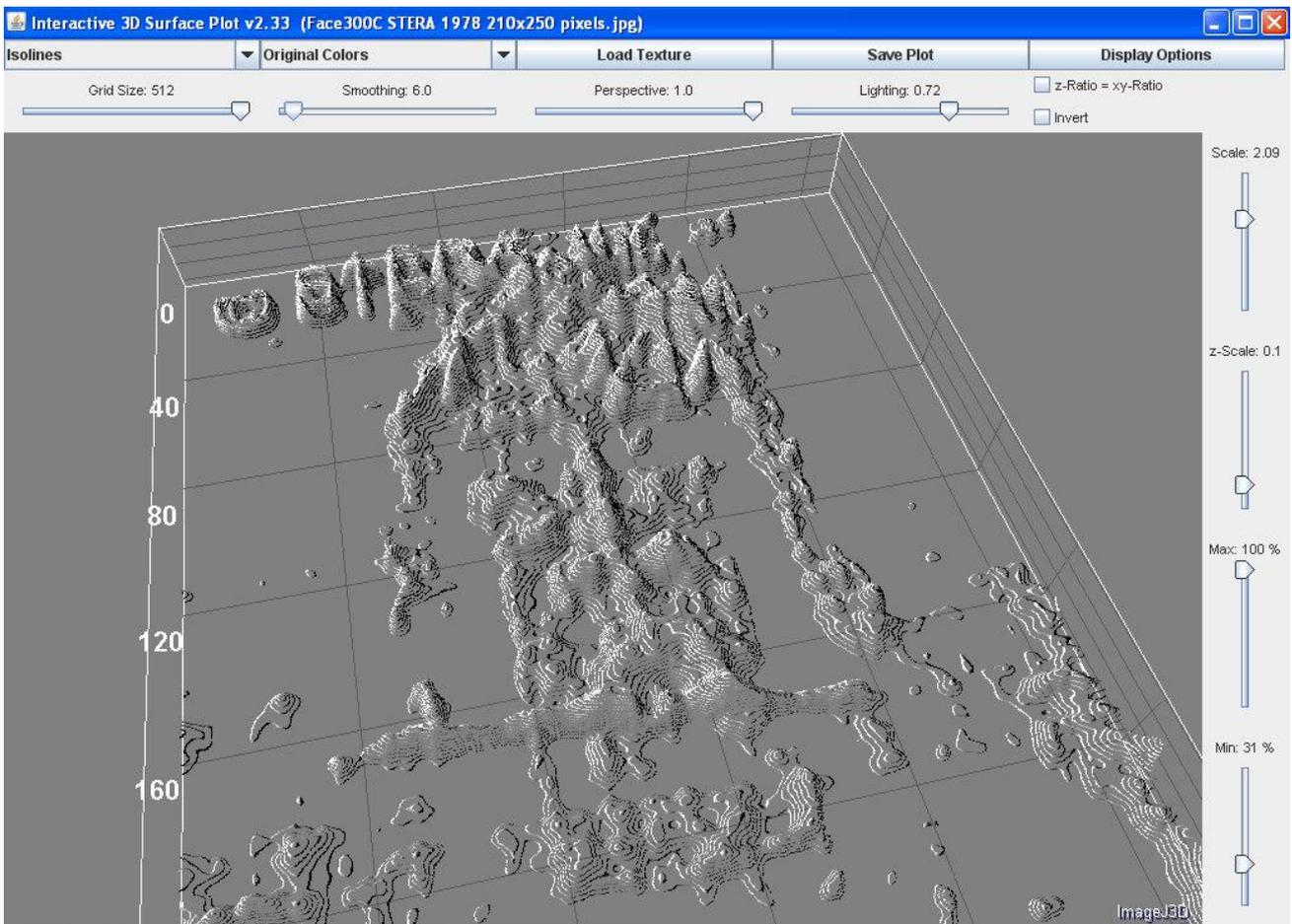
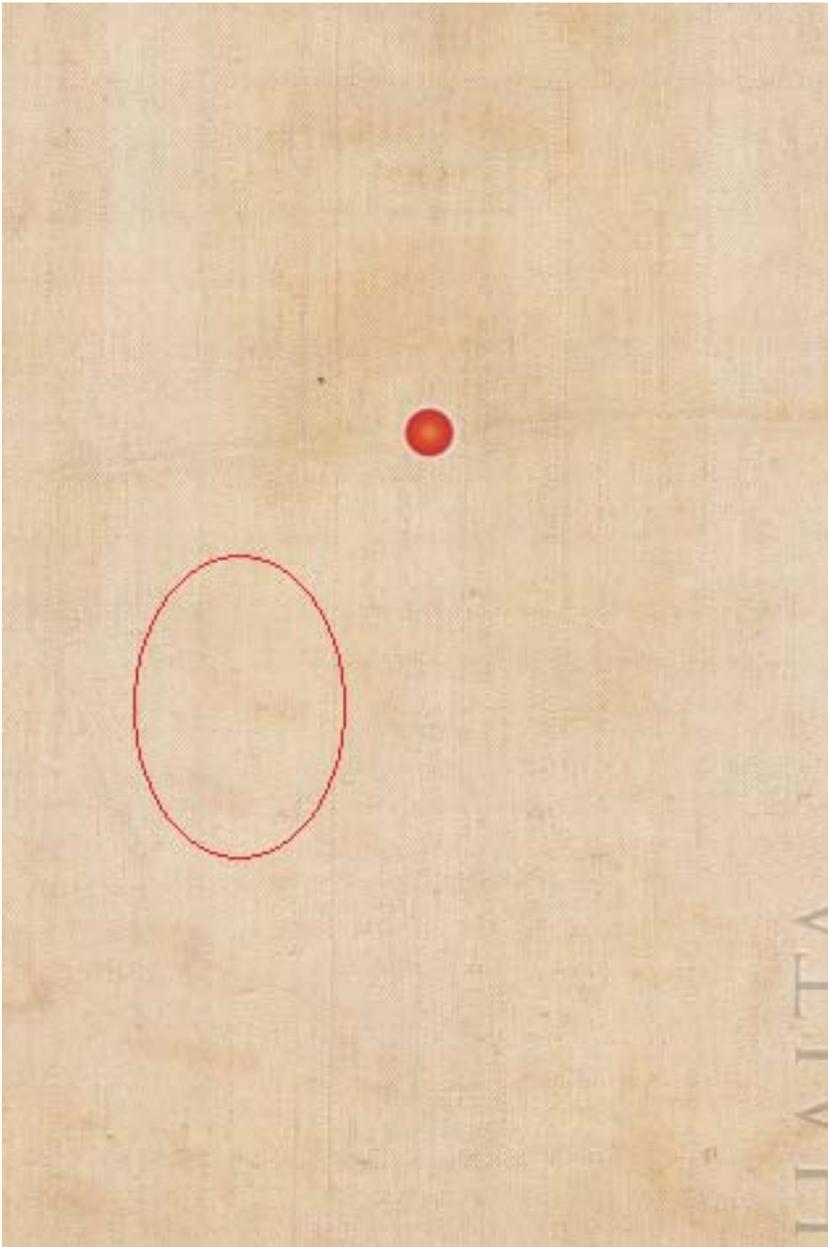


Fig. 7.3. Height display of isolines of gray scale values of negative of photo of the Shroud face – settings set to show the small protruding shapes on the flat surface of the object beneath the chin, looking like the Hebrew letters א נ נון = ayin aleph nun = 71 N. (original Shroud photo<sup>1090</sup> ©1978 Barrie M. Schwartz Collection, STERA, Inc.; height display ©AvdH)

<sup>1089</sup> <http://www.shroud.com/gallery/pages/Face300C.htm> on <http://www.shroud.com/gallery/index.htm>

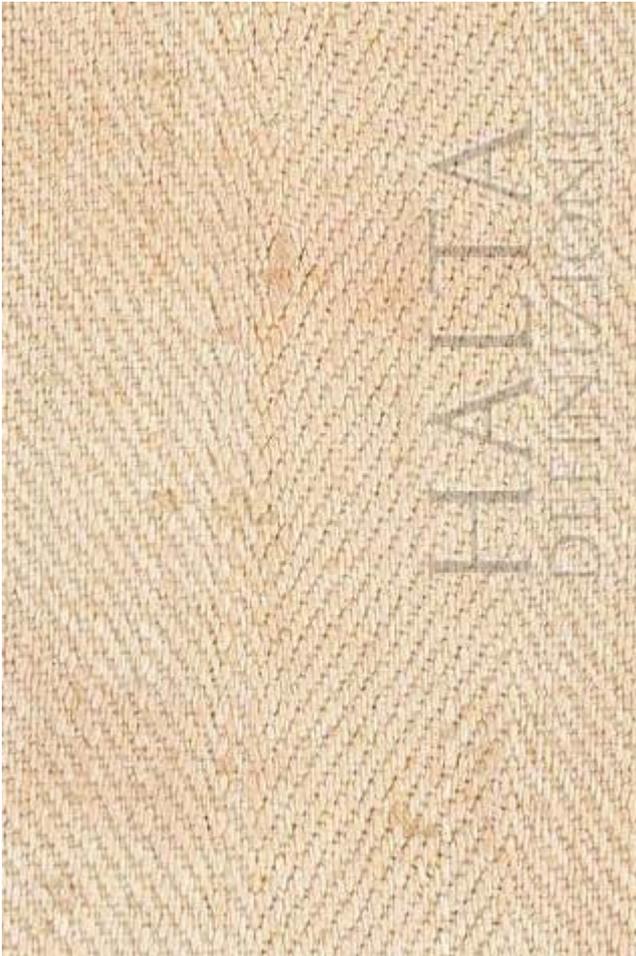
<sup>1090</sup> <http://www.shroud.com/gallery/pages/Face300C.htm> on <http://www.shroud.com/gallery/index.htm>



*Fig. 7.4. Beard, petalon-shape with pink lower edge, and chest with pink scourge marks on the Shroud (red oval line added by AvdH). Photo by HALTADEFINIZIONE® IMAGE BANK - Copyright Arcidiocesi di Torino<sup>1091</sup>*

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<sup>1091</sup> Used, with permission, from the Shroud 2.0 application made by Haltadefinizione, downloadable from <http://www.haltadefinizione.com/en/the-shroud>. Note that for the Haltadefinizione photography “The lighting system was especially designed to filter any harmful radiation to the cloth, but most of all special attention was given to the calibration of the spectrum, which helped faithfully reproduce the actual color and fabric of the Shroud image.” <http://www.haltadefinizione.com/en/the-shroud>



*Fig. 7.5. Pink blood on petalon (top middle) and pink blood of scourge mark on chest (bottom left). Photo by HALTADEFINIZIONE® IMAGE BANK - Copyright Arcidiocesi di Torino<sup>1092</sup>*

The question is whether this bloodstain, which looks like a smear, could have been present on the edge of the beard. There are blood rivulets on the image of the hair, but before image formation this blood may have been on the skin of the front and sides of the face.<sup>1093</sup> Baima Bollone wrote that the bloodstains on the forehead and hair have borders that are more coloured than the centres,<sup>1094</sup> which according to Barbet is typical for blood clots formed on the skin.<sup>1095</sup> The blood on the edge of the oval object has the aspect of a smear on a solid edge, not of blood soaked into the usually frayed and fluffy end of a beard. This seems evidence in support of the presence of a so-called ‘petalon’, a gold oval ornament of a member of the Jewish Great Sanhedrin between ca. 6 and 70 AD, engraved I X Y

<sup>1092</sup> Used, with permission, from the Shroud 2.0 application made by Haltadefinizione, downloadable from <http://www.haltadefinizione.com/en/the-shroud>. Note that for the Haltadefinizione photography “The lighting system was especially designed to filter any harmful radiation to the cloth, but most of all special attention was given to the calibration of the spectrum, which helped faithfully reproduce the actual color and fabric of the Shroud image.” <http://www.haltadefinizione.com/en/the-shroud>

<sup>1093</sup> Lavoie, Bonnie and Adler, Blood on the Shroud of Turin, Part III: The Blood on the Face, 1986, TOM 63-66. Reasons why there are no images of these blood trickles on the skin are 1. Most of the blood would have been sucked into the cloth, as the bloodstains in the hair image also are /seem to be present on the reverse of the Shroud (e.g. at Q-P 50 <http://shroud.wikispaces.com/PROPERTIES> ). So the remnants of these bloodtrickles on the face would not be very elevated from the skin. 2. The sides of the face are not imaged on the Shroud anyway, see <http://www.sindonology.org/shroudScope/shroudScope.shtml> . In 3D they would have been too far away from the cloth to produce an image (‘tenting’). So, also any remnants of bloodstains on the sides of the face would not produce an image. 3. The upper long trickle in the hair image would have been on the skin of the forehead/temple that would have been imaged where now the short upper blood trickle is on the Shroud. <http://www.sindonology.org/shroudScope/shroudScope.shtml>

<sup>1094</sup> Baima Bollone, The Forensic Characteristics..., Proceedings Turin 2000, p. 215-216

<sup>1095</sup> Brillante, Fanti and Marinelli, Bloodstain characteristics..., 2002, p. 5, 8

= the Hebrew letters ayin – aleph – nun ׀ ׀ ׀ = 70 1 N = seventy-one Nesiim = 71 rulers of the Great Sanhedrin.<sup>1096</sup> Joseph of Arimathea and Nicodemus, who buried Jesus, were members of this ruling council, and under the cross, the wet bloodstained hand of one of them could have laid the petal on the chest of Jesus, whom they considered to be the biblical Messiah, the everlasting high priest-king. The gifts of the extremely fine and costly temple mantle and the enormous amount of costly aloe and myrrh comply with such a gift of the petal for the burial of Jesus as a Jewish high priest-king. The image formation of such a gold object can not have taken place by putrefaction gas emanation (the already falsified Maillard reaction hypothesis<sup>1097</sup>), but may have taken place by means of partial electrical discharges (viz. through non-radiative chain reactions of collisions of electrons and atoms in a corona discharge, able to produce a latent image), which also could have formed the image of the body and the hair.<sup>1098</sup>

After the radiocarbon dating, performed in 1988 by three laboratories on pieces of a single sample taken from the Raes corner of the Shroud, Van Haelst statistically tested the dates reported in the Nature article. In the paper he presented in Rome in 1993,<sup>1099</sup> he concluded that “An X<sup>2</sup> test value 8.43 > 5.99 states that there is a SIGNIFICANT DIFFERENCE between the results of the 3 laboratories. From the X<sup>2</sup> test result, one can determinate the % significance level :  $2.718^{-(8.43/2)} = 1.3$  %. From this test, one may conclude, that the probability of obtaining, by chance alone, a scatter as high as that observed for the Shroud, is only 13 in 1000. Because we assume all radiocarbon dates to be correct, we must conclude, that the SMALL samples, taken at the same place, do not have the same radioactivity and are not REPRESENTATIVE for the Shroud.” Furthermore, he criticized the Nature article in that “The Arizona error was arbitrary enlarged from 17 to 31. The Wilson & Ward mean 689+16 was replaced by the UNWEIGHTED mean 691+31. The multiplying t-factor for 95% confidence was enlarged from 1.96 to 2.6. The claimed “at least 95 % confidence” for the medieval dating of the Shroud is NOT supported by statistical analysis.” Also the powerful F-test performed by Van Haelst, showed that the data reported in Nature are not consistent: “Such data should not be used in any further calculations”, he wrote, yet, “Without any new evidence one declares a non existent “95% confidence” to be “conclusive evidence”.”<sup>1100</sup> Also Van Haelst’s newest statistical analysis, based on Burr statistics, shows the inconsistency of the radiocarbon data.<sup>1101</sup> In 2010, Riani et al. published statistical evidence for the existence of a strong linear trend in the twelve measurements of the Shroud’s radiocarbon subsamples, which therefore can not be considered repeated measurements of a single unknown age.<sup>1102</sup> This suggests there is a systematic apparent-radiocarbon-age-affecting ‘contamination’ of some sort.

<sup>1096</sup> Hoeven, The seam and missing corners...., 2011, updated 2013, p. 17-20

<sup>1097</sup> Hoeven, Internal selvedge..., 2012

<sup>1098</sup> “CD acts at a chemical level with the linen fibers in such a way that color was produced as the fibers aged.”, “The image produced by CD is probably at first latent or weakly visible. Further heating lower than 200° C, exposition to sunlight or aging that dehydrates the polysaccharide layer, turns the image into a yellow to light brown color” (CD = Corona Discharge) Fanti et al., Body Image Formation Hypotheses Based on Corona Discharge, 2005, p. 9. (<http://www.dii.unipd.it/~giulio.fanti/research/Sindone/corona.pdf> ; Fanti, Body image formation hypotheses based on corona discharge: Discussion, 2008, <http://www.ohioshroudconference.com/papers/p15.pdf> ; Amoruso and Lattarulo, A physicochemical interpretation of the Turin Shroud imaging, 2012, [http://www.academicjournals.org/article/article1380797948\\_Amoruso%20and%20Lattarulo.pdf](http://www.academicjournals.org/article/article1380797948_Amoruso%20and%20Lattarulo.pdf) ; García-Macedo, Cansino and Valverde-Aguilar, Corona discharge to explain the image of the Turin Shroud: Experimental results, 2012, [http://www.academicjournals.org/article/article1380798163\\_García-Macedo%20et%20al.pdf](http://www.academicjournals.org/article/article1380798163_García-Macedo%20et%20al.pdf) ; Fanti, Hypotheses Regarding the Formation of the Body Image on the Shroud of Turin: A Critical Compendium, 2011, <http://www.shroud-enigma.com/challenge/paper/g-fanti-paper.html>

<sup>1099</sup> Van Haelst, Radiocarbon Dating The Shroud - A Critical Statistical Analysis, 1997, <http://www.shroud.com/vanhels3.htm> (emphasis by Van Haelst); video of part of the presentation: <https://www.youtube.com/watch?v=DIFBOZgmIhQ>

<sup>1100</sup> Van Haelst, Radiocarbon dating the Shroud of Turin – The Nature report, <http://www.shroud.com/vanhels5.pdf> p. 26 and 27

<sup>1101</sup> Van Haelst, Analyzing Radiocarbon data using Burr statistics, 2011, <http://www.shroud.com/pdfs/vanhelst8.pdf>

<sup>1102</sup> Riani et al., Carbon Dating of the Shroud..., 2010, <http://www.lse.ac.uk/statistics/research/RAFC04May2010.pdf>

In 2013, Fanti and Gaeta reported that the combination of age-dependent mechanical and spectrometric parameters, measured on linen fibers vacuumed from the Shroud, yielded a mean date of 33 BC with an uncertainty of plus or minus 250 years at 95% confidence level.<sup>1103</sup>

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<sup>1103</sup> Fanti and Gaeta, *Il mistero della Sindone*, 2013

## 8. CONCLUSION

The present analysis of available scientific data obtained from the Shroud of Turin and the results of a few experiments allow the conclusion that the best explanation, and a consistent one, for the peculiar pinkish redness of the bloodstains on the Shroud is that authentic acid blood of a dead crucified person stained an authentic Jewish madder-dyed temple mantle during and after an authentic Jewish burial procession of a person whose dead body formed an image on and disappeared from the Shroud in an extremely delicate way before putrefaction. This delicate and timely disappearance of the dead body and the presence of a bloodstained image of what seems to be a first-century Jewish ornament of a Sanhedrin member indicate that this person most probably was Jesus Christ.

That the Shroud is an authentic Jewish temple mantle is evidenced by the collective of microscopic, spectroscopic, UV-photographic and chemical data consistent with the presence of yellow unmordanted and fluorescing, and hemolysing and easily oxidizable, and preservative acid madder dye on top of a hot-water-washable and cold-water-resistant retrograded starch coating (as most clearly evidenced by the bluer fluorescence inside hot-water stains that are lighter in normal reflected light) on an uncomparable large cloth of extremely fine linen threads and fibers, spun in a Z-twist conforming to Jewish temple prerequisites, and woven on an ancient loom in a typical apparel weave and with a planned side seam, stitched at manufacture with two lines of overcast stitches typical for the sewing of a fringeless braid to a garment by the ancient Egyptians and sewn before the transverse hems were stitched across the seam by the same professional hand in a first-century Jewish type of stitching – characteristics that are typical for a Jewish priest's temple mantle that had a biblical Pharisaic enlarged border and that was not allowed to be washed but would be cut to pieces and burnt when worn-out or dirty. That xylene and toluene – the solvents used to isolate and clean the fiber samples of the Shroud – are each a solvent for madder dyestuffs, explains why madder dye was not found by wet-chemistry on main Shroud fibers but was readily found on whole threads from the Shroud's radiocarbon-dating corner, which is not a repair. That the yellowish Shroud also has a pinkish hue and the bloodstains are pinkish red, visibly confirms that the Shroud was dyed with a yellow madder dye that in certain lighting looks pinkish on linen and in the bloodstains was rendered definitely pinkish red by the blood. In turn, the madder dye stabilized the heme of the blood and preserved the cloth and the separate serum on it. Experiments showed that blood is able to form stains on starched and madder-dyed linen that are pinkish and remain so during the time that red bloodstains on pure linen turn brown.

That the Shroud was used in an authentic first-century Jewish burial procession is evidenced by a) the imprinting a few out-of-body-contour pink blood smears on the dorsal half of the Shroud, as could have happened when the dead crucified body was laid on the Shroud on a burial bier by wet bloodstained hands; b) a pink bloodstain on the edge of the image of what seems to be an oval flat engraved object, which therefore probably is not a beard but a first-century Jewish engraved ornament (*petalon*) of a Sanhedrin member, which was laid on the chest of the body by a wet bloodstained hand, e.g. of the Sanhedrin member Joseph of Arimathea who buried Jesus; c) blood rivulets that ran across the dorsal half of the starched and thus cold-water-resistant Shroud, probably while it was lying on the absorbing surface of a flat shoulder-carried bier, during the burial procession.

That the Shroud was stained by authentic acid postmortem blood is evidenced by the presence of an acid methemoglobin crystal and five consistent characteristics of acid postmortem blood ( $\text{pH} < 6.8$ , incompatible with life) in red stains: the presence of heme's clear Charge Transfer band and the lack of a Soret band in the absorbance spectra, the lack of potassium corresponding to separation of hyperkalemic serum, the apparent draining away of serum which hardly takes place on a warm living body but readily on a cold surface, and the apparent hyperfibrinolysis of the scourge wound clots. The Shroud has stains of potassium-poor pink acid heme-madder lake (as most clearly evidenced by the FTIR spectra of blood globs from the Shroud) and separate serum (evidenced by a.o. FTIR spectra and immunochemistry) – separated by blood clotting on a cold dead body on the ventral side and by filtering through the waterresistant cloth from blood that flowed onto the dorsal side – of,

apparently, a crucified person who had died with extreme acidemia (acid blood from e.g. cramping, dehydration, a long period of respiratory failure) and hyperkalemia (elevated serum potassium level corresponding to the acidity of the blood) a few hours before burial, allowing the red blood cells to lose more potassium and to acidify further and form acid heme dimers without Soret band. The Shroud also has a pattern of not-smearred pink imprints of scourge wound clots that apparently were dry at burial and only became wet after burial, which also corresponds to the already acidic antemortem blood further increasing in acidity after death, resulting in clot-wetting hyperfibrinolysis only after burial and, because of the pink color of the imprints irrespective of local image density, before madder-degrading body image formation and also before an extremely delicate disappearance of the dead body from the cloth that did not smear the wet imprints, before putrefaction.

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### Corrected errors

- p. 33, line 3, “from yellow to red” removed;
- p. 78, line 17, “18-19” replaced by “18-24”;
- p. 81, line 2, “*in a room at 18-19 °C and 60-70% rel. humidity*” added;
- p. 131, third line from botton, “that” replaced by “which”;
- p. 155, line 5, “Heller and” removed.

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