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## Microspectrophotometry (MSP) of Blood – An Update

**ABSTRACT:** Microscopic sized particles of suspected blood too small for classical serological testing can be encountered in forensic samples such as its presence on fiber and hair surfaces and as trace residues on larger objects. These samples may be of probative value and the classification of a material as blood may promote subsequent DNA typing. Microscopy and microspectrophotometry (MSP) are instrumental methods used to identify trace material as blood. Spectral absorbencies of blood in the visible and ultraviolet ranges and effects of aging are discussed.

**KEYWORDS:** Forensic Science, Microscopy, Microspectrophotometry, MSP, Blood

Microscopists are constantly attempting to identify the origin of small particles. Occasionally the presence of blood in the form of small particles or dried residue on a fiber or hair surface can be important evidence to an investigation. Chemical methods for confirming the presence of blood have been used in forensic science for many years. However, chemical methods are not generally applicable to microscopic sized samples and would be destructive in nature. A spectroscopic method would be an alternative to chemical methods.

The spectrophotometric identification of liquid blood is based in traditional liquid spectroscopy (1–3 & 5). In 1986, Kotowski and Grieve expanded the use of spectroscopy to identify small dried blood particles mounted on glass slides using a Nanometrics 10S microspectrophotometer (MSP) (4). Blood samples can be further differentiated as to oxyhemoglobin and methemoglobin forms. These forms can be detected using both liquid and MSP methods (1, 2 & 4). Oxyhemoglobin converts to methemoglobin upon aging. The absorbance values found by Fiori using liquid spectroscopy (1) are:

Oxyhemoglobin

576–578 nm, 540–542 nm, 412–415 nm, 330–340 nm and 270–280 nm

Methemoglobin

Methemoglobin is divided into acid (pH 5–6) and alkaline methemoglobin (pH > 10)

Acid ..... 630 nm, 576 nm, 540 nm, 500 nm, 404 nm, 275 nm

Alkaline..... 630 nm shifts to 600 nm, 576 nm and 540 nm become more pronounced.

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The data generated by Kotowski and Grieve (4) identify the spectral absorption maxima for blood as:

Major peak @ 421 nm (known as the “Soret” band)

Smaller peak @ 545 nm (known as the “ $\alpha$ ” band)

Smaller peak @ 581 nm (known as the “ $\beta$ ” band)

Kotowski and Grieve also noted that the aging process resulted in changes to the spectral absorbance values observed for “fresh” blood. Specifically, they noted a progressive tendency for peak intensities at 545 nm and 581 nm to diminish and at the same time the “ $\beta$ ” band shifting from 581 nm to approximately 574 nm. These changes indicated the formation of methemoglobin.

### **Background**

While conducting a routine fiber examination in a case, a green cotton fiber was recovered from a subject’s briefs and compared to the known green fabric of the victim’s underwear. The questioned fiber was consistent in all characteristics to the fibers of the known fabric and had several areas where a red–brown material was noted to have dried on the surface (see fig. 1). The victim’s panties had a large apparent blood stain in the crotch area.

The microscopic appearance of the red–brown material on the questioned fiber was consistent with dried blood. It would be significant to the case if a confirmation of blood could be established. Serological testing of such a small sample for the presence of blood is not feasible and if attempted would remove the material necessary for n–DNA analysis. Therefore, attempts to determine the presence of blood using microspectrophotometry was conducted.

### **Instrumentation and method of study**

A CRAIC Technologies QDI 2010 MSP was used to analyze the residue and perform validation work on known blood samples. Kotowski and Grieve had analyzed a number of potentially interfering substances such as bile, cosmetics, dyes, and pigments. They found none of these substances to interfere with the absorbance values associated with blood. This supports earlier work (1–3 & 5) that indicates the spectral absorbencies of blood are in fact unique to blood. It should be noted here that the spectroscopic characterization of blood is not species specific, that is, non–human blood will exhibit the same spectral characteristics as human blood. The Nanometrics 10S MSP used by Kotowski and Grieve was not able to perform spectral scans below 400 nm. Fiori (1) lists two absorbance bands in the ultraviolet region. One band is listed at approximately 330–340 nm and the second at 270–280 nm. The CRAIC system is capable of scanning from 240 nm to 850 nm.

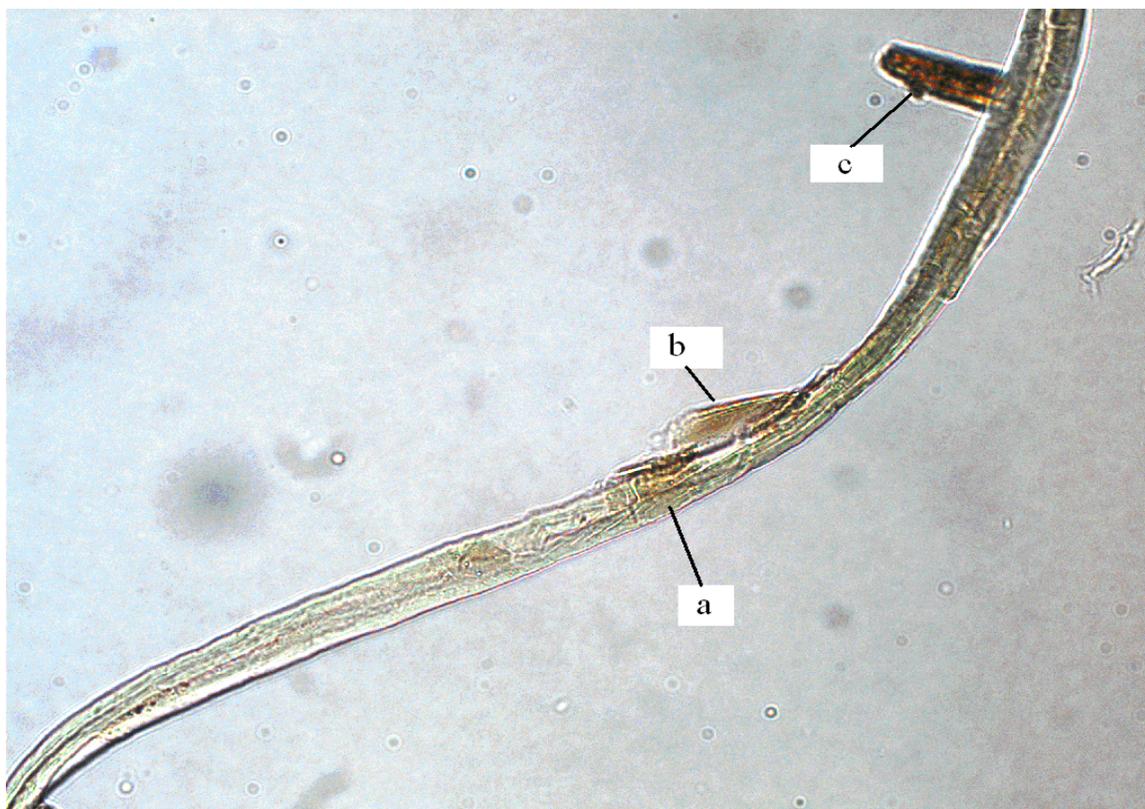


Fig. 1 Red-brown material dried on the surface of a green cotton fiber. a) material on surface of fiber. b) material extending outward from fiber c) larger material extending outward from fiber.

### **Creation of known blood test samples**

In order to create fibers with dried blood for use as a known reference sample, a cotton tipped swab was coated with human blood and allowed to air dry. Fibers with dried blood were mounted in Entellan and Permount mounting media on glass slides/cover glasses to duplicate the technique used for fiber examinations. Additional fiber samples were mounted in glycerin on quartz slides/cover glasses in order to create samples suitable for UV MSP examination. MSP scans were performed from 240 nm to 850 nm.

An aged blood sample was prepared by mounting a sample of fourteen year old dried blood in Entellan on glass slides/cover glasses. Freshly dried blood samples were also subjected to heat to simulate extreme conditions. Heated samples were prepared by exposing dried blood samples to 200 °C for 2 hours on a hotplate before mounting.

Blood samples were analyzed two ways: as an individual particle and as a residue on a fiber surface (see fig. 1 for example). When blood was present on the fiber surface, background scans were performed on non-blood areas of the same fiber. Light to moderately dyed fiber samples did not interfere with typical absorbencies of blood when fiber background scans were employed.

**Results**

The spectral absorbencies of blood did not vary significantly from fresh blood to aged blood. Similar data was obtained for the Entellan and Permount mounted samples. The Soret absorbance band generally ranges from 412–419 nm, the  $\alpha$  absorbance band ranged from 532–540 nm and the  $\beta$  absorbance band ranged from 554–570 nm. In addition, the UV absorbance band ranged between 350–365 nm and generally appeared as a shoulder on the Soret band. Occasionally this absorption will appear as a peak with a somewhat lower absorption around 347 nm. The UV absorption band around 285–290 nm was not observed on samples mounted on glass slides (see fig. 2). However, when mounted using glycerin and quartz slide/cover slip, the 285–290 nm absorption band generally appeared as a peak (see fig. 3). For large blood particles (c in Fig. 1), the 285–290 nm absorption band may appear as a shoulder on the side of the Soret band.

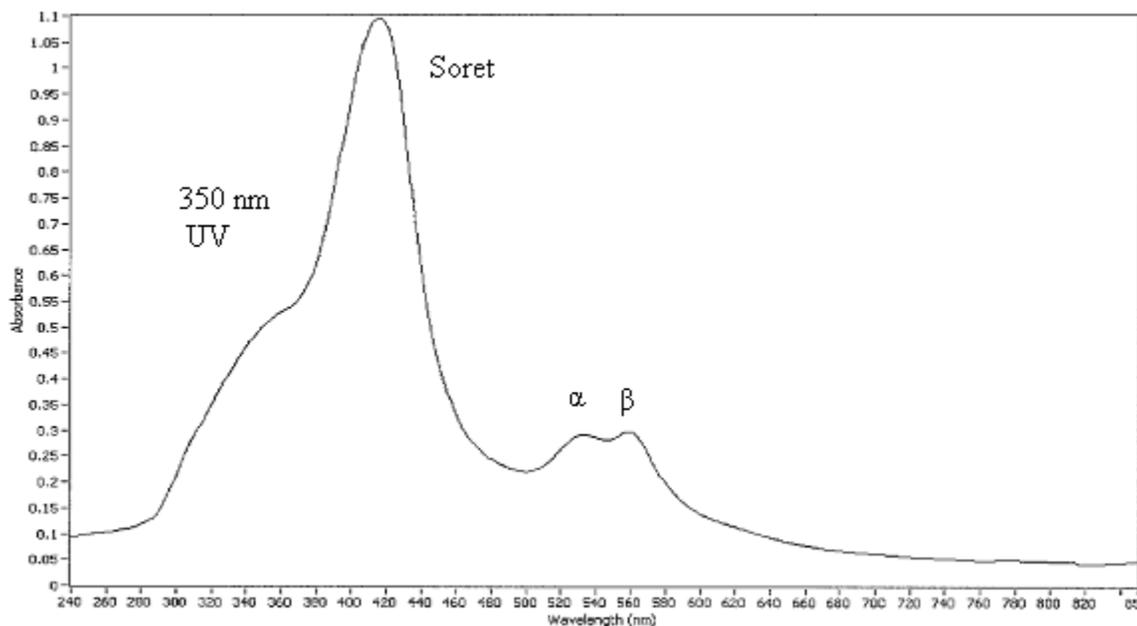


Fig. 2 MSP spectrum of blood 2 months old mounted in Entellan on glass slide.

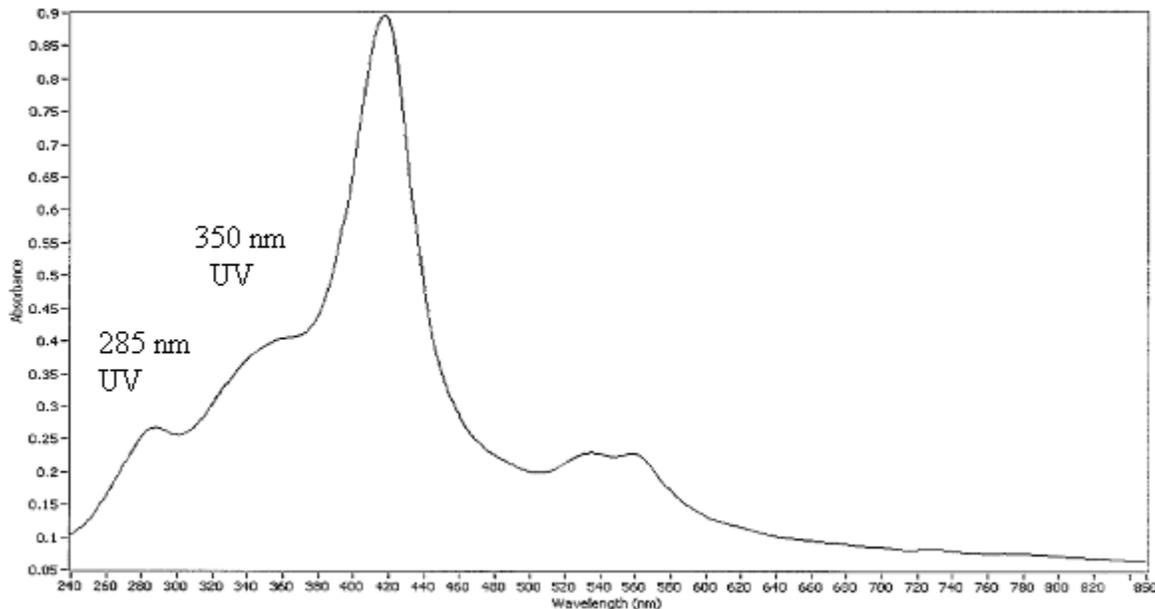


Fig. 3 MSP spectrum of blood 2 months old mounted in glycerin on quartz slide.

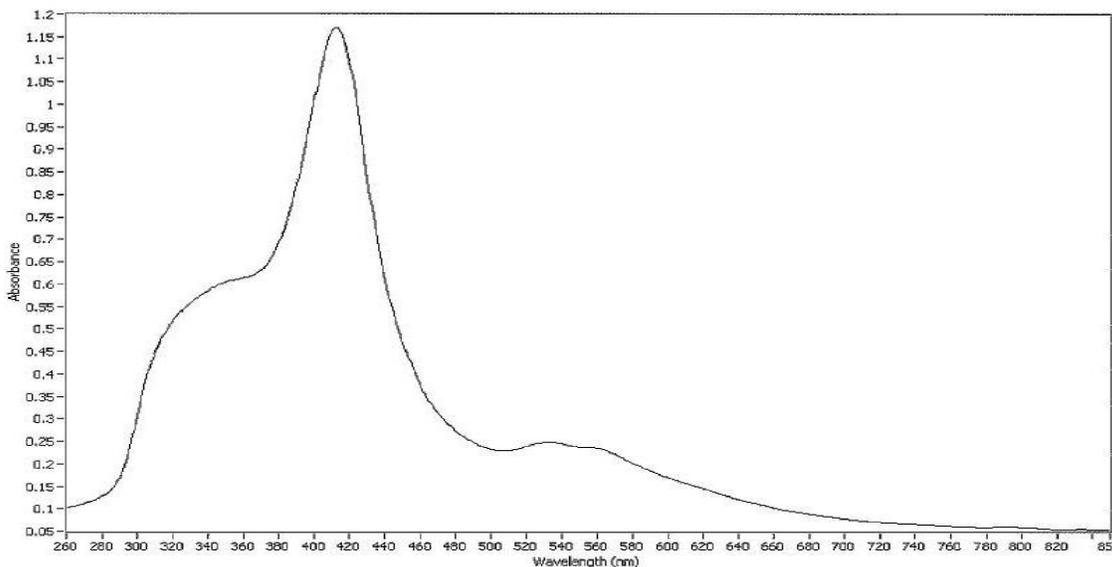


Fig. 4 MSP spectrum of 14 year old blood mounted in Entellan on glass slide.

**Conclusion**

The microscopic and microspectrophotometric characterization of microscopic sized blood samples is possible on samples too small for classical serological testing. Reliable characteristic spectra can be obtained on aged samples as well as samples

subjected to heat. The presence of the Soret,  $\alpha$  and  $\beta$  absorption bands serve as a confirmatory test for blood when coupled with a microscopical examination. For those analysts desiring a chemical change prior to the confirmation of a substances identity; Kotowski and Grieve (4) reference the use of the Takayama test as a chemical alteration method for blood which can be implemented on microscopic sized particles. Microspectrophotometry would be performed both prior to and following the test noting an absorbance shift.

The UV absorbance at 350–365 nm can be observed when the sample is mounted for normal microscopic examination on glass slides, however the lower UV peak cannot be observed unless a glycerin/quartz type mount is prepared. The observation of the two UV peaks could serve as additional verification for the presence of blood.

### References

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