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TECHNICAL NOTE

Kenneth G. Wiggins;[1] *Julie-Ann Holness,*[1] *B.Sc.; and Bridget M. March,*[1] *B.Sc.*

The Importance of Thin Layer Chromatography and UV Microspectrophotometry in the Analysis of Reactive Dyes Released from Wool and Cotton Fibers

ABSTRACT: Samples of reactively-dyed wool and cotton were obtained from a range of dye manufacturers, dye distributors and the Forensic Science Service (FSS) Fibre Data Collection. The wool fibers were red in color and had previously been compared using comparison microscopy (CM), visible range microspectrophotometry (VS) and thin layer chromatography (TLC). The cotton fibers were blue and black in color and had not been previously compared. Red, blue and black fibers were chosen because they are often encountered in casework. The usage of reactive dyes to color fibers has increased over the last 10–15 years and these are often seen in casework. Before techniques were available that allowed reactively-dyed fibers to be compared using TLC only CM and microspectrophotometry were routinely carried out. Many laboratories, who had a microspectrophotometer, only had a visible range instrument. It was therefore important to see which techniques provide additional information, that gives greater individuality to fibers, to that obtained from CM. The color was released from the wool and cotton fibres using alkaline hydrolysis and a cellulase enzyme respectively. Many of the red wool samples were differentiated from each other using CM. More differentiation was found using VS and even more when ultraviolet range microspectrophotometry (UV) or TLC was used. Two samples could only be differentiated using TLC because CM, VS and UV failed to separate them. The black cotton samples were predominately differentiated using CM but VS allowed for further differentiation. With the samples used in this project UV and TLC failed to separate the samples further. The blue cotton samples benefited from the use of CM, VS and either UV or TLC to reduce the number of matching pairs. All techniques aided differentiation although with this set TLC and UV proved to be complementary techniques. Results demonstrate that TLC and UV both yield important information over and above that obtained from CM and VS. Although in some parts of the project TLC and UV are complementary if the concentration of the dye in the fiber is not sufficient for TLC or the scientist doesn't wish to 'destroy' the fiber UV would be of more use than TLC.

KEYWORDS: forensic science, criminalistics, reactive dyes, wool, cotton, comparison microscopy, visible light microspectrophotometry, UV microspectrophotometry, thin layer chromatography.

In the investigation of crime, the transfer of textile fibers is often used to discover whether there is a link between two people or a person and a crime scene. Additionally fibers found on objects used in the commission of a crime, such as a car and weapons, can also be significant (1–3).

Advances in DNA detection and interpretation over the last five or ten years lead many to believe that the use of traditional trace evidence types in crime investigation, including fibers, would cease or become severely restricted. However, as Grieve and Wiggins (4) pointed out, there are reasons why the sceptics have been proved wrong.

In 1996 Wiggins et al. (5) showed the value of TLC when a number of reactively dyed red wool samples were analysed and compared using a method of color extraction described by Crabtree et al. (6). After using CM and VS there were 15 matching pairs remaining, however these were reduced to four when the colored extracts were compared using TLC. These same red wool samples (31 in number) have been used to investigate the value of UV absorbance microspectrophotometry . Additionally, 29 black and 47 blue reactively dyed cotton samples were analysed and compared to see if UV absorbance microspectrophotometry and/or TLC (7) give additional information. This information would hopefully mean that samples could be differentiated from each other thus strengthening the evidential value in a similar casework situation.

The use of reactive dyes encountered in casework has increased dramatically over the last 10–15 years. As stated in the earlier paper, red (as a color) was chosen because the end result is often achieved by using a single component dye. Hence we are less likely to detect a second dye or shading colors. This could add to the problem of differentiating between similar colored samples of the same fiber type. Black and blue cotton were chosen, as they are two of the most common fiber/color types encountered in forensic textile fiber examination.

Materials

Thirty-one bulk red reactively-dyed wool samples, in the form of pattern cards, were obtained from five different manufacturers. Twenty-six of the black reactive dyed cotton samples were also

[1] The Forensic Science Service, London Laboratory, 109 Lambeth Road, London SE1 7LP, England.

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from pattern cards and three were from the FSS Fibre Data Collection. Finally, 42 of the blue reactive dyed cotton samples were from pattern cards supplemented with five samples from the Data Collection.

The cellulase (EC 3.2.1.4 from *Penicillium funiculosum,* activity 6.7 units/mg) used for digesting the cotton was purchased from Sigma Chemical Company. The TLC plates were Merck DC-Alufolien Kieselgel 60F254 (5.0 by 7.5 cm). The eluents were made up of mixtures of distilled water with n-butanol, ethanol, pyridine, propan-1-ol and ammonia (sp.gr 0.880) which were all General Purpose Reagent (GPR) grade. The methanol was HPLC grade and the XAM neutral improved white mounting medium was from British Drug Houses Limited (BDH). The sodium hydroxide and citric acid were GPR grade as were the sodium acetate, acetic acid, xylene, absolute alcohol and glycerol.

The buffer was 0.1 M sodium acetate made up to pH 5.0 with acetic acid (GPR grade). The enzyme solution was made up by dissolving 80 mg cellulase in 50 mL of buffer.

Experimental

The analytical pathway followed involved the use of comparison microscopy, UV and visible microspectrophotometry and finally TLC.

Comparison Microscopy

Fibers from each of the bulk samples were mounted on glass microscope slides in XAM. The comparison microscope used (E. Leitz (Instruments) Ltd.) consisted of two Orthoplan microscopes connected by a comparison bridge with a binocular head. White light illumination was from quartz iodine sources and UV from mercury vapor lamps.

Fibers were compared under transmitted white light and a broad band of UV and blue. The Leitz Ploempak system was used for all fluorescence examination. The magnification for all comparisons was ×100 and ×400.

UV and Visible Microspectrophotometry

Fibers were removed from the comparison microscopy slide, washed in xylene and absolute alcohol, dried and mounted on a quartz slide in glycerol. A dark, mid-color and pale fiber were selected to ensure that the range of dye uptake was covered. A Zeiss UMSP50/80 microspectrophotometer was used to obtain the absorbance spectra using the following parameters:

Wavelength range 240–730 nm (red wool) and
250–730 nm (blue and black cotton).
Step Interval 5.
Scan Average 20.
Number of Scans 1.
Bandwidth 5 nm.

The spectra from all samples within a block of colour were then compared to each other.

Wool Fiber Dissolution

A 1 cm thread of reactively dyed wool was placed in an Eppendorf tube with 100 μL of 0.75 M sodium hydroxide and incubated with inversion at 40°C for 24 h. After this time 66 μL of 0.3 M citric acid in methanol was added. The resulting solution was mixed and centrifuged at 7000 rpm for 5 min (6).

Cotton Fiber Dissolution

A 1 cm thread of cotton was removed from each sample and placed in an Eppendorf tube with 50 μL of 3 M sodium hydroxide. The tubes were placed in gripseal bags and placed in a box containing ice. The box was put into a refrigerator at 0°C. After 4 h the sodium hydroxide was removed from each sample and the fibers rinsed with 50 μL of 0.5 M acetic acid solution. Each sample was then washed twice using 150 μL of cellulase solution (80 mg cellulase in 50 mL 0.1 M sodium acetate buffer, pH 5). After the final wash 150 μL of cellulase solution was placed in the tube to digest the fiber. The tube was sealed and placed in a rotator in an oven at 45°C for 20 h. The samples were then removed and spun in a centrifuge at 7000 rpm for 5 min. Fifty μL of the solution was removed from each tube and placed in a new tube together with an equal volume of methanol. This solution was used for TLC.

Thin Layer Chromatography

Dye extracts were spotted 1 cm from the base of the TLC plate while warming on a hotplate to achieve a spot size of approximately 2 mm. To ensure the spots were fully dry, the plates were placed in an oven at 100°C for 5 min. This process was carried out in duplicate.

Wool

The wool extracts needed an initial elution (pre-run) of 2 mm in methanol/ammonia (13:7 v/v) to produce a sharp line origin. After drying, the samples were eluted in propan-1-ol, methanol, water and ammonia (6:3:1:4 v/v).

Cotton

The cotton extracts were eluted in two systems. The first plate was eluted using n-butanol, ethanol, ammonia, pyridine and water (8:3:4:4:6) and the second with n-butanol, ethanol, ammonia, pyridine and water (6:3:2:6:6).

All samples were eluted to a distance of approximately 2 cm above the origin. Elution was completed in covered glass beakers and the plates dried in a hot air stream. A standard dye was included on each plate as a means of monitoring eluent performance.

Results and Discussion

Red Wool

As previously stated in the paper by Wiggins et al. (5), 31 samples yielded 465 pair-wise comparisons. After comparison microscopy there were 68 matching pairs, which were reduced to 15 after visible microspectrophotometry. TLC reduced this number to four. Using UV microspectrophotmetry, the15 visible matches were reduced to the same four matching pairs as demonstrated when TLC was used.

The generic name and origin of the four matching pairs were compared (Table 1). The Society of Dyers and Colorists collect product information from dye developers and manufacturers, including information on the properties and constitution of dyes. Each unique dye is given a Color Index (CI) Generic Name and a Constitution Number (8). Two of the four matching pairs were eliminated from our results as they had identical Generic Names and Constitution Numbers—confirming the presence of identical dyes, i.e., samples 41 and 52 (both Reactive Red 158) and samples 42 and 58 (both Reactive Red 159). This left three samples (i.e., two matching

TABLE 1—*Matching pairs of red reactively dyed wool samples.*

Sample	CI Generic Name	Matching Sample	CI Generic Name
34	Reactive Red 147	41	Reactive Red 158
34	Reactive Red 147	52	Reactive Red 158
41	Reactive Red 158	52	Reactive Red 158
42	Reactive Red 159	58	Reactive Red 159

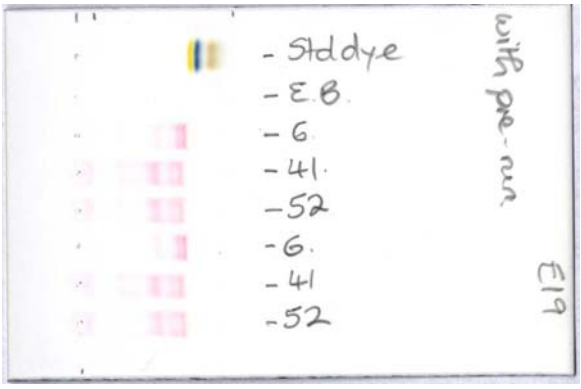

FIG. 1—*TLC plate of samples 6, 41 and 52.*

pairs) which were indistinguishable by any technique. The pairs that remained were: sample 34 positive to sample 41, and sample 34 positive to sample 52. The CI Generic Names were as follows: Sample 34—Reactive Red 147, Samples 41 and 52—Reactive Red 158. The manufacturers of these dyes were contacted but would not say whether Reactive Red 147 and 158 were the same dye. However, they did say that the dyes' different CI Generic Names relate to differing manufacturing processes, but could result in the same dye being produced.

There were three pairings 6–34, 6–41 and 6–52 that matched when comparison microscopy, visible and UV microspectrophotometry were used. However, TLC showed that sample 6 was clearly different to the other three samples. Sample 6 was Reactive Red 180, sample 34 was Reactive Red 147 and samples 41 and 52 were Reactive Red 158. An example of the differences seen when TLC was used to compare samples 6, 41 and 52 can be seen in Fig. 1.

Black Cotton

There were 406 pair-wise comparisons for the black cotton. After comparison microscopy 10 pairs matched. Visible microspectrophotometry reduced this to eight matching sets, but UV microspectrophotometry and TLC could not reduce the matches further. The matches were 1&8, 3&7, 11&19, 11&28, 19&28, 14&17, 14&29 and 17&29. Relevant sample information is listed in Table 2.

Blue Cotton

For the blue cotton samples there were 1081 pair-wise comparisons. After comparison microscopy 49 pairs matched. Visible microspectrophotometry reduced this to 19 with UV microspectrophotometry further reducing it to 16 matching pairs. TLC also reduced the 19 pairs matching after visible microspectrophotometry to the same 16 matching pairs identified after UV microspectrophotometry.

TABLE 2—*Matching black reactively-dyed cotton.*

Sample	Identification	CI Generic Name
1	Grey P-NR liquid 10	Unknown
8	Grey P-NR	Unknown
3	Black P-NBR liquid 40	Unknown
7	Black P-NBR	Unknown
11	Black B Hoechst Remazol	Reactive Black 5
19	Black B Sumitomo Sumifix	Reactive Black 5
28	Black F-B BASF Basilan F-B	Unknown
14	M/2520/93 SB/4	Unknown
17	Black EX conc. Sumitomo Sumifix	Unknown
29	Basilan Black F-DF BASF	Unknown

TABLE 3—*Matching blue reactively dyed cotton.*

Sample	Identification	CI Number
7	Nippon Kayaku Keyacelon Turquoise CN-2G	227
12	Nippon Kayaku Kayacion Turquoise E-A	71
17	Nippon Kayaku Kayacion Turquoise P-A	71
9	Nippon Kayaku Kayacelon Blue CN-MG	Unknown
10	Nippon Kayaku Kayacelon Blue CN-BL	216
11	Nippon Kayaku Kayacion Blue E-SE	237
28	Nippon Kayaku Kayacion Blue E-NB	212
23	ICI Procion Turquoise H-A	71
24	ICI Procion Turquoise MX-G	140
34	Sumitomo Sumifix Supra Turquoise Blue BGF	231
35	Sumitomo Sumifix Turquoise Blue G 150%	21
48	SW/586/93 DP/3	Unknown
49	DW/2636/93 PG/5	Unknown

The matches were 7&12, 7&17, 12&17, 9&10, 9&11, 9&28, 10&11, 10&28, 11&28, 23&24, 23&34, 23&35, 24&34, 24&35, 34&35, 48&49. Relevant sample information is listed in Table 3.

The matching samples shown in Table 3 fall into four discreet blocks. Looking at the CI Numbers (samples 12, 17 and 23 are all CI Number 71) it seems strange that the block containing samples 7,12 and 17 doesn't match the block containing samples 23,24,34 and 35. There is, however, a very simple explanation, samples 7,12 and 17 are paler in color than those in the other block and were ruled out at the comparison microscopy stage although the dyes are identical.

There were also three pairs that were positive after comparison microscopy and visible range microspectrophotometry but were eliminated both on TLC and UV range microspectrophotometry. An example of the difference seen in the UV range is shown in Fig. 2 for the paired samples 12 and 20. The difference can be clearly seen in the region around 270 nm.

Conclusion

This paper demonstrates the value of using TLC and/or UV range microspectrophotometry as well as comparison microscopy and visible range microspectrophotometry when comparing fiber samples. Although the set of black cottons examined in this project were not further differentiated when UV microspectrophotometry or TLC was used, these techniques helped with the differentiation of the red wool and the blue cotton. It is also important to note that if the concentration of dye in the fiber is not sufficient for TLC or the scientist does not wish/or is not allowed to "destroy" the fiber, a UV spectrum could usually be obtained allowing for the possibility of further differentiation.

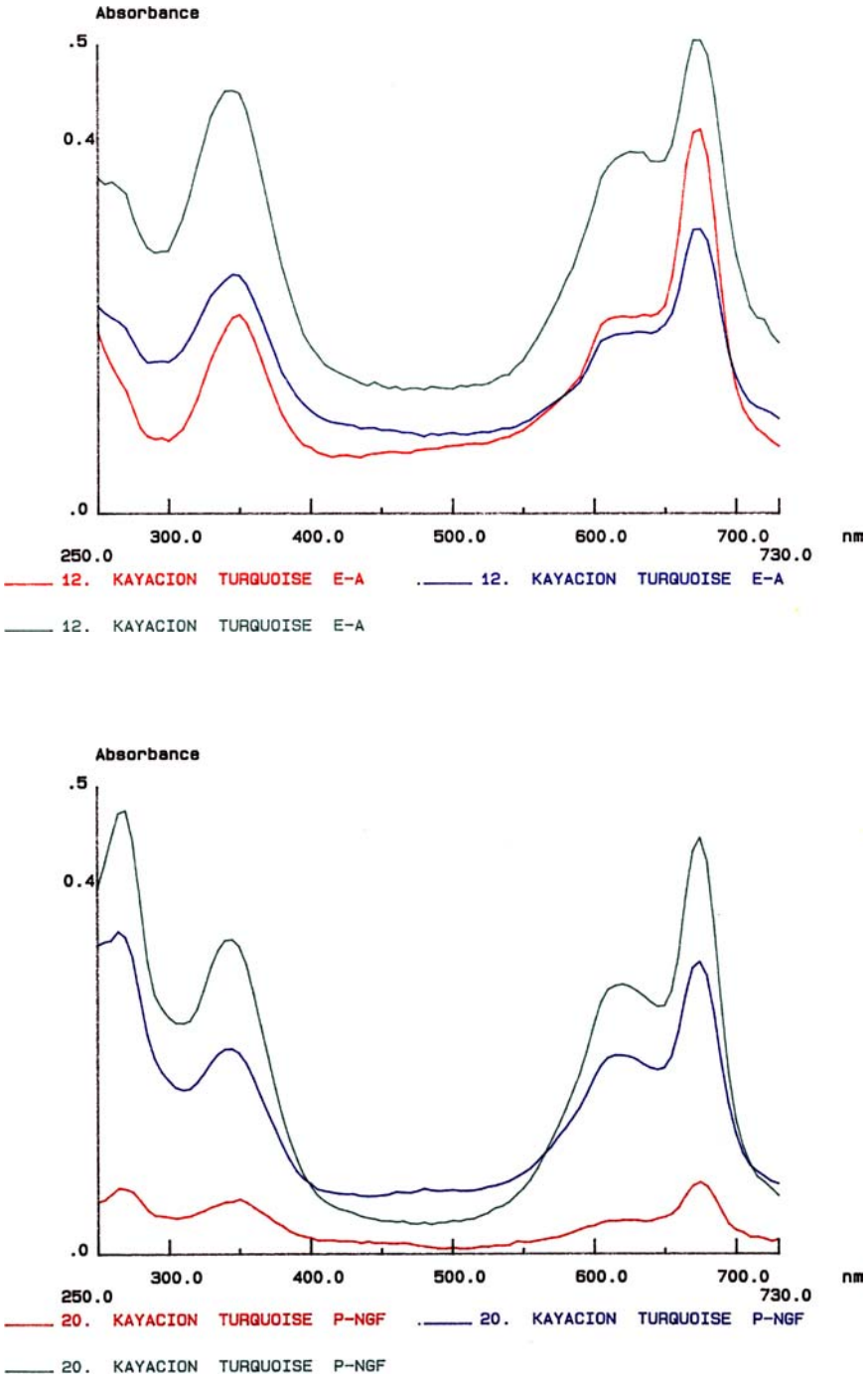

FIG. 2—*UV Absorbance spectra of samples 12 and 20.*

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Additional information and reprint requests:
Ken G. Wiggins
Fibers Consultant to R&D
Forensic Science Service
109 Lambeth Road
London
SE1 7LP
England