TECHNICAL NOTE

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The Importance of Thin Layer Chromatography in the Analysis of Reactive Dyes Released from Wool Fibers

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ABSTRACT: Samples of reactively-dyed wool were obtained from a range of manufacturers and distributors and "digested" by alkaline hydrolysis to yield colored solutions. Results demonstrate that thin layer chromatographic analysis of reactive dyes yields important additional information, over and above that obtained from techniques such as comparison microscopy and visible light microspectrophotometry. Colored solutions obtained from single fibers were analyzed by thin layer chromatography (TLC) and reproducible results were obtained from a range of fiber lengths.

KEYWORDS: forensic science, criminalistics, reactive dyes, wool, single fibers, comparison microscopy, visible light microspectrophotometry, thin layer chromatography

In the investigation of crime, the transfer of textile fibers can be used to discover whether or not there is a link between two people, or a person, and a scene. Fibers found on objects used in crime, such as cars and weapons can also be of significance (1,2). In this laboratory, control and recovered fibers can be compared using microscopy, microspectrophotometry, infra-red spectroscopy, and TLC. However, many of the dyes currently in use for the coloration of wool are not extractable, i.e., they are reactive dyes (3,4).

Crabtree et al. (5) described a method in which reactively-dyed wool could be disrupted by alkaline hydrolysis to yield a colored solution which can be analyzed by TLC. Although the hydrolysis products are not true dye extracts, for the sake of convenience, we shall refer to them as extracts.

Bulk samples (small fiber tufts) of red reactively-dyed wool were compared to determine whether or not fiber dissolution and subsequent TLC would yield additional information to that generated by techniques already used in casework.

A range of single fibers selected from dye manufacturers color patten cards and casework samples were also tested using TLC after their dyes had been successfully extracted. This was to determine if results could be obtained from single fibers and to test the reproducibility of the system.

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Materials

Thirty one bulk red reactively-dyed wool samples in the form of color pattern cards were obtained from five different manufacturers. Red was chosen because the color is often achieved by using a single component dye.

Forty one 10-mm fibers were also selected from the pattern cards and an additional seven 6-mm fibers from casework garments. These 48 fibers were reactively-dyed wool and colored as follows: 23 red, 15 blue, 6 black, 2 orange, 1 green, and 1 brown. A range of lengths from a small number of these fibers was also analyzed.

The chemicals and other materials used were as follows: sodium hydroxide, citric acid, propan-1-ol, and ammonia (sp.gr 0.88) were all GPR grade. Methanol was Fisons HPLC grade. XAM neutral medium improved white was from BDH. The TLC plates were Merck DC-Alufolien Kieselgel 60F254 (5.0 by 7.5 cm).

Experimental Procedures

Comparison Microscopy

Fibers from each of the bulk samples were mounted in XAM. The comparison microscope used (E. Leitz [Instruments] Ltd)

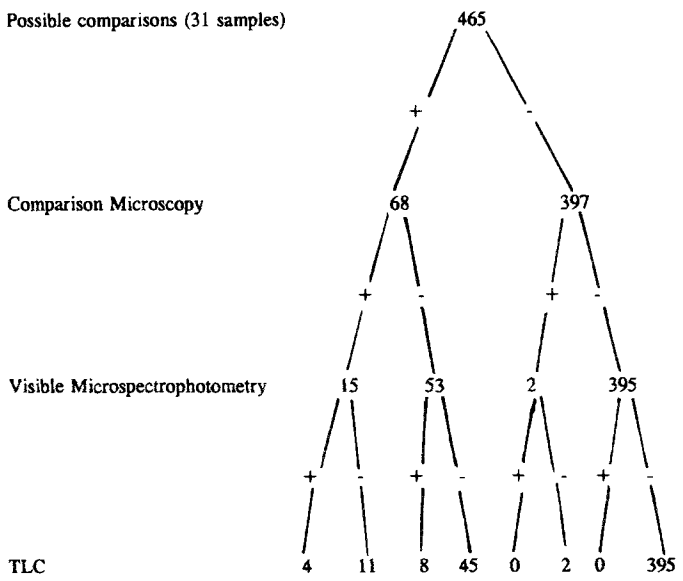

FIG. 1—*Results of the bulk samples investigation.*

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

Sample number	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

consisted of two Orthoplan microscopes connected by a comparison bridge with a binocular head. White light illumination was from quartz iodine sources and ultraviolet (UV) light from mercury vapor lamps.

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

Microspectrophotometry

The previously prepared slides were placed on the stage of a Zeiss UMSP50 microspectrophotometer and observed under a magnification of ×250. Using a bandwidth of 5 nm and a step interval of 5 nm, absorbance spectra were produced between 390 and 730 nm. Three spectra from different fibers in each sample were recorded and compared.

Wool Fiber Dissolution

Bulk Samples—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 then centrifuged at 7000 rpm for 5 min (5).

Single Fibers—The procedure used for single fibers was a scaled down version of that described above. A length of fiber was placed in a capillary tube that was sealed at one end, 3 μL of 0.75-M sodium hydroxide was added to the tube, sufficient to cover the fiber.

The open end of the capillary was sealed and the tube incubated with agitation at 40°C for 24 h. The tube was then opened and 2 μL of 0.3-M citric acid in methanol added. The resulting solution was mixed and then centrifuged at 7000 rpm for 5 min.

Thin Layer Chromatography

Dye extracts were spotted 1 cm from the base of the TLC plate while warming on a hot plate 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.

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

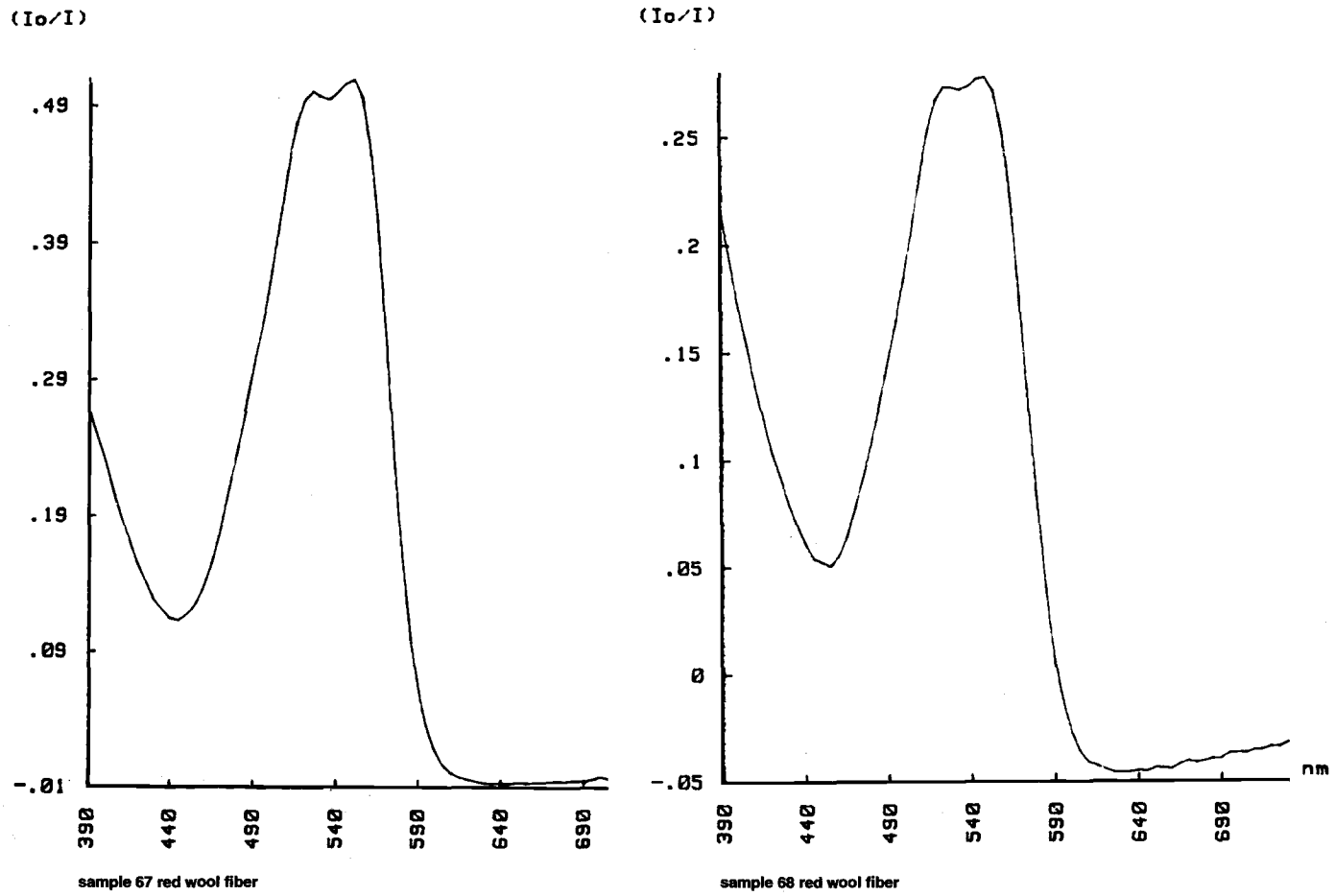

FIG. 2—*Samples that are positive after visible microspectrophotometry.*

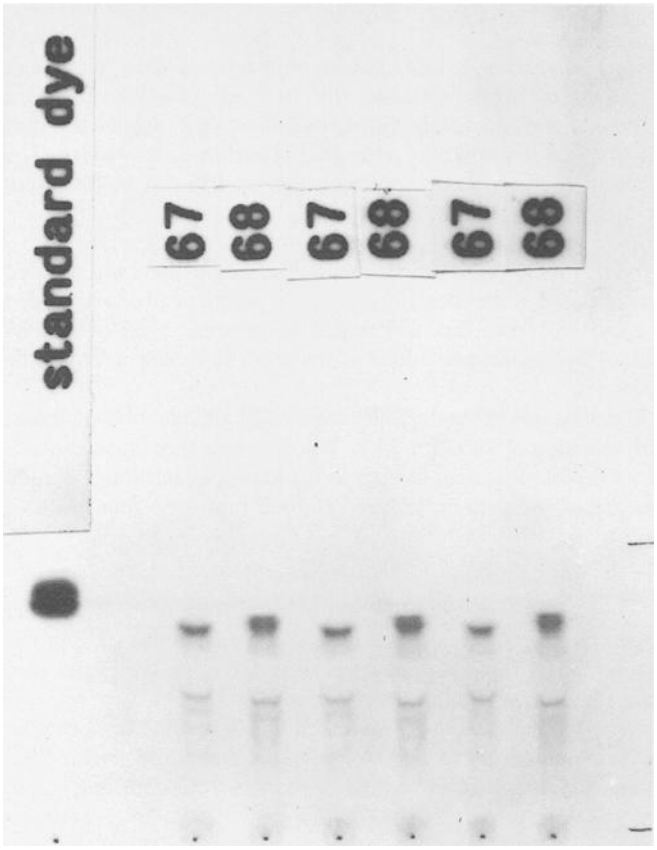

FIG. 3—*Samples that are negative after thin layer chromatography. Note: The solvent front was allowed to migrate to a distance of approximately 2 cm above the origin.*

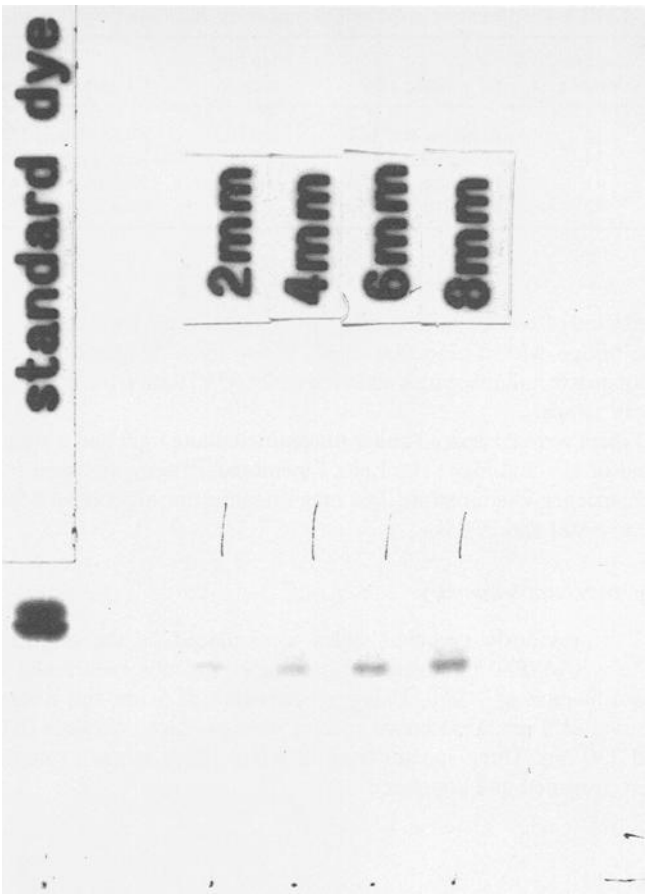

FIG. 4—*Thin layer chromatography of dye extracts from single fibers. Note: The solvent front was allowed to migrate to a distance of approximately 2 cm above the origin.*

and ammonia (6:3:1:4 v/v) to a distance of approximately 2 cm above the origin. This eluent was selected following extensive studies which showed it gave the best separation when a large number of samples were tested (5). Elution was completed in covered glass beakers and the plates dried in a hot air stream. A standard dye mixture was included on each plate as a means of monitoring eluent performance.

Bulk Samples Comparison

When sampling, care was taken to ensure that fibers selected represented the complete range of dye uptake across the sample. These bulk samples were compared to each other using comparison microscopy followed by visible microspectrophotometry, then TLC. If at any stage the samples did not match, the result was recorded as a negative comparison.

Single Fiber Analysis

The 41 10-mm fibers selected from the range of dye manufacturers' color pattern cards and seven 6-mm fibers collected from casework were tested using TLC after their dyes had been successfully extracted. Six strongly colored samples were selected again from the range of dye manufacturers. Single fibers of lengths 2-, 4-, 6-, 8-, and 10 mm were selected, their dyes extracted, and the resulting solution subjected to TLC. A further six 10-mm single fibers were selected and, after extraction, TLC was performed. This final procedure was repeated over six days to assess the consistency of results obtained.

Results and Discussion

Bulk Samples Comparison

A summary of the results is shown in Fig. 1.

There are a total of 465 possible pair-wise comparisons. After comparison/microscopy, there are 68 matching pairs and 397 negative (nonmatching pairs). After visible microspectrophotometry on these matching pairs, 15 still match and 53 do not. Thin layer chromatography of the dyes from these remaining 15 matching pairs reveals only four that still match. An example of samples which match after visible microspectrophotometry but are negative after TLC is shown in Fig(s). 2 and 3.

The chemical composition 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 (6). Two of the four were eliminated from our results as they had identical Generic Names and Constitution numbers—confirming the presence of identical dyes. This left three samples (i.e., two matching 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.

The results obtained show that visible microspectrophotometry and TLC are complementary techniques. Figure 1 shows that nine matching pairs that remained positive after spectrophotometry were eliminated after TLC was performed. Equally, eight samples that were negative at the spectrophotometry stage were positive after TLC. The dissolution of reactively-dyed wool and subsequent analysis of dye extracts by thin layer chromatography described by Crabtree et al. (5) is highly discriminating when used in conjunction with microscopy and visible microspectrophotometry.

Single Fiber Analysis

All 41 10-mm fibers selected from the range of dye manufacturers' color patten cards and the seven 6-mm fibers from casework yielded extracts which were successfully chromatographed.

The chromatography of the dyes from 4-, 6-, 8-, and 10-mm fibers was successful for all samples. However, only four of the 2-mm samples showed strong banding. Figure 4 shows examples of successful TLC results obtained from 2-, 4-, 6-, and 8-mm fibers. The bands from all the other fiber samples were clearly defined and well separated. The reproducibility studies also yielded good results. Very slight differences in the elution of the dyes were noticed and this was thought to be because a "pre-run" of exactly 2-mm is hard to achieve. This would not, however, be a problem in casework as suspect and control fiber extracts are chromatographed on the same plate.

Conclusion

This paper demonstrates that alkaline hydrolysis of reactively-dyed wool and TLC of the extract provide valuable additional information to that obtained using conventional techniques. It also demonstrates how dyes from single fibers encountered in casework can be analyzed using this technique. Although this new method is destructive, it is likely that in terms of evidential value, the benefits of chromatographic analysis will far outweigh the loss of the fiber. In most cases, only a proportion of transferred fibers need to be examined in this way, and the others will be available for re-examination if required.

Acknowledgments

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References

(1) Cook R, Wilson C. The Significance of finding extraneous fibers in contact cases. Forensic Sci Int 1986;32:267–73.
(2) Jackson G, Cook R. The significance of fibers found on car seats. Forensic Sci Int 1986;32:275–81.
(3) Lewis DM. Wool dyeing. England: Dyers' Company Publications Trust, 1992.
(4) Macrae R, Smalldon KW. The extraction of dyestuffs from single wool fibers. J Forensic Sci 1979;24:109–16.
(5) Crabtree SR, Rendle DF, Wiggins KG, Salter MT. The release of reactive dyes from wool fibers by alkaline hydrolysis and their analysis by thin layer chromatography. J Soc Dyers Colorists 1995;3:100–2.
(6) The Society of Dyers and Colorists. Color Index, 3rd Ed (Fourth Revision), Vol. 9 incorporating Vol. 5. Bradford, England 1992.

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