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The Extraction of Dyestuffs from Single Wool Fibers

The most important techniques of those currently used in Home Office forensic science laboratories for the analysis of fiber dyestuffs are thin-layer chromatography and visible absorption spectroscopy of dye solutions. To obtain useful discrimination 20 to 50 ng of each dye component must be extracted. In most fiber examinations the recovered fibers are between 2 and 10 mm in length and contain between 2 and 200 ng of dye, depending on the depth of dyeing. Consequently, dye extraction must be efficient if the techniques are to be applied in a reasonable proportion of cases involving fiber comparisons. This paper is concerned with the extraction of dyes from wool fibers.

The complex protein structure of wool can accept a wide range of dyestuffs. Acid dyes are the most important class currently used. The members of this class may be azo, anthraquinone, or triphenylmethane dyes, but they all contain sulfonic acid groups as the solubilizing moiety. Acid dyes can be further classified on the basis of their wet fastness properties and suitability for various dyeing processes into equalizing dyes, milling dyes, and supermilling acid dyes [1]. The increase in fastness of the milling dyes is a reflection of their lower aqueous solubility and is a direct consequence of the presence of fewer solubilizing groups in the molecule. Brightly colored woolen fashion garments are predominantly dyed with acid dyes.

The fastness of acid dyes on wool can be greatly increased by the use of chrome as a mordant. The mordant may be applied before, after, or during the dyeing, although at present the process of applying the chrome after dyeing, known as the "afterchrome process" [2], is the most widely used. Chrome dyes are extensively used for men's wear where their high fastness properties are valuable but their comparative dullness of hue is not a disadvantage.

In metal-complex dyes the metal component is incorporated during their manufacture so that a separate mordant process is not required. Metal-complex (1:1) dyes have fastness properties similar to the corresponding chrome dyes but give rise to brighter colors. Metal-complex (1:2) dyes also possess high fastness properties and are used extensively for light shades.

Wool contains a large number of reactive sites that can link covalently with reactive dyes. Triazine, vinyl sulfone, and acryloylamino dyes have all been applied to wool but as yet they are not widely used, probably because of the cost of the dyes and the high fastness that can be achieved with alternative materials.

Basic dyes are absorbed by wool and were previously of some importance. However, they were discarded when acid dyes of much better fastness were made available. Solubilized vat dyes were originally developed for use on wool but the poor diffusion of the oxidizing

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reagents through the substrate makes the use of these dyes difficult [3]. Indigo is the only vat dye now used to any extent on wool [4]. Natural dyes have been almost entirely superseded by synthetic products except for specialized applications. Logwood (Natural Black 1) is still used for dyeing both wool and silk.

Various procedures for dye classification [5-10] and the identification of specific dyes after extraction [11-14] are available in the general and textile literature. However, these schemes are not readily adapted for routine use in forensic science, where the samples may be limited to a single fiber. It was the aim of this work to produce a simple classification and extraction scheme for dyed wool fibers. The classes of dye finally selected for extraction studies were, in order of importance, acid, chrome, metal-complex, and reactive.

Experimental Procedure

Samples

Dyed wool fibers were taken from pattern cards supplied by the dye manufacturers. Details of the 19 representative samples selected for the extraction study are shown in Table 1.

Extraction Procedure

A single wool fiber approximately 1 cm long was teased from the pattern card with tweezers and placed in a capillary tube together with 5 μ l of solvent, and then the tube was sealed. Distilled water (5 μ l) was introduced into a second tube containing a fiber and this tube was used for color reference. After the extraction period the tubes were compared under a low-powered microscope ($\times 16$) and the efficiency of dye extraction assessed on the arbitrary ordinal scale 0 through 5, 0 corresponding to no extraction (equivalent to aqueous blank) and 5 to complete extraction.

TABLE 1—Dyed wool fibers used in extraction study.

Identification Code	Dye	Dye Class
A	Brilliant Sulfur Red B	acid ^a
B	Brilliant Alizarine Sky Blue BS	acid ^a
C	Acid Black 4B	acid ^a
D	Erio Red 3BL	acid ^b
E	Erio Blue BGL	acid ^b
F	Erio Black HA	acid ^b
G	Lanacron Black S-R	acid ^b
H	Omega Chrome Red GM	chrome ^a
I	Omega Chrome Brilliant Blue B	chrome ^a
J	Acid Alizarine Black R	chrome ^a
K	Irgalan Red 2GL	metal-complex ^b
L	Irgalan Blue FBL	metal-complex ^b
M	Irgalan Black RBL	metal-complex ^b
N	Irganol Brilliant Red BL	reactive/acid ^{b,c}
O	Irganol Brilliant Blue RLS	reactive/acid ^{b,c}
P	Drimalan Blue F-2GL	reactive ^a
Q	Drimalan Red F-2BL	reactive ^a
R	Lanasol Red 6G	reactive ^b
S	Lanasol Blue 3G	reactive ^b

^a Manufactured by Sandoz.

^b Manufactured by Ciba-Geigy.

^c Classified by manufacturer as acid dye but contains reactive groups that can bond to wool.

Nine solvents were selected which, at one time or another, have been used for dye extraction. The compositions of the solvents are shown in Table 2. The degree of extraction obtained with the nine solvents was assessed for each wool sample after 30 min at room temperature (20°C) and again after 15 min at 90°C.

Results and Discussion

The results (Table 3) demonstrate that none of the solvents efficiently extracted all the dye classes. Thus a single general extractant could not be found.

Extraction of Acid Dyes

The acid dyes (A to G) were efficiently extracted by all the basic solvents, as would be anticipated. At both temperatures the best results were obtained by using Solvent II (aqueous pyridine) and after 15 min at 90°C complete extraction was obtained in all cases.

Extraction of Chrome and Metal-Complex Dyes

Some of the chrome and metal-complex dyes were extracted with acidic solvents, such as dimethylformamide/formic acid, and some with basic solvents, such as aqueous pyridine. The addition of ethylenediaminetetraacetic acid (EDTA) as a chelating agent did not assist the extraction of metallized dyes and it is clear that members of this class are more resistant to extraction than the acid dyes (Table 3).

Oxalic acid is known to act as a chelating agent and cleaves the bonds between chromium and wool. After acid treatment the chromium-dye complex effectively remains attached to the fiber as an acid dye and can therefore be removed with basic solvents.

Samples containing chrome (H, I, J) and metal-complex dyes (K, L, M) were pretreated with 2% aqueous oxalic acid for 20 min, the acid was removed, and the fiber was extracted with aqueous pyridine, as previously described. The efficiencies of extraction before and after oxalic acid treatment are compared in Table 4. In all cases dye extraction increased after oxalic acid treatment and in some cases (Dye I) the effect was dramatic. Color changes were also noted during oxalic acid treatment (Table 4).

Extraction of Reactive Dyes

With the exception of Sample N the reactive dyes were totally resistant to extraction with all the usual solvents (Table 2). Additional extractions using dilute mineral acids and

TABLE 2—Composition of extracting solvents.

Identification Code	Solvent Components	Composition, v/v
I	dimethylformamide/formic acid	1:1
II	pyridine/water	4:3
III	dimethylformamide/water	2:1
IV	dimethylformamide/2% aqueous ammonia	1:1
V	dimethylformamide/4% aqueous ammonia/4% aqueous EDTA	2:1:1
VI	dimethylformamide/4% aqueous ammonia/4% aqueous 1,2-diaminoethane	2:1:1
VII	hexamethylphosphoramide/water	1:1
VIII	dimethyl sulfoxide/water	1:1
IX	dimethylformamide/trifluoroacetic acid	1:1

TABLE 3—Assessment of dye extraction from 19 wool samples using nine different solvents.^a

Dye	Solvent Used (30 min at 20°C)									Solvent Used (15 min at 90°C)								
	I	II	III	IV	V	VI	VII	VIII	IX	I	II	III	IV	V	VI	VII	VIII	IX
A	3	5	2	2	2	4	1	0	0	5	5	5	5	5	5	5	5	1
B	3	5	0	0	0	0	0	0	0	5	5	5	5	5	5	5	5	1
C	2	2	0	1	1	3	0	0	0	4	5	4	5	5	5	5	3	1
D	4	4	0	0	0	1	0	0	0	5	5	5	5	5	5	5	5	5
E	4	4	1	0	0	0	0	0	0	5	5	5	5	5	5	5	5	3
F	2	3	0	2	2	0	0	0	0	4	5	4	5	5	5	5	2	2
G	0	1	0	1	0	0	0	0	0	2	5	2	4	4	4	4	1	1
H	1	0	0	0	0	0	0	0	0	3	3	2	3	3	3	2	2	2
I	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
J	0	1	0	0	0	0	0	0	0	1	4	1	4	3	4	3	2	0
K	1	0	0	0	0	0	0	0	0	5	4	4	5	5	4	4	4	2
L	1	0	0	1	0	0	0	0	0	2	1	1	3	1	2	1	2	0
M	0	1	1	0	0	1	0	0	0	5 ^b	4	2	5	5	5	4	1	1
N	0	0	0	0	0	0	0	0	0	0	1	1	2	2	2	1	0	0
O	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

^a0 corresponds to no extraction and 5 to complete extraction.

^bColor change noted.

TABLE 4—Effect of oxalic acid treatment on extraction of chrome and metal-complex dyes from wool fibers using aqueous pyridine.^a

Dye	Extractions Without Treatment		Extraction with Treatment	
	30 min at 20°C	15 min at 90°C	30 min at 20°C	15 min at 90°C
H	0	3	2	4
I	0	0	3 ^b	5 ^b
J	1	4	2	5
K	0	4	3 ^c	5 ^c
L	0	1	2	3
M	1	4	3 ^b	5 ^b

^a0 corresponds to no extraction and 5 to complete extraction.

^bColor change to red during oxalic acid treatment.

^cColor change to yellow during oxalic acid treatment.

1,2-diaminoethane were therefore carried out as described earlier and the results are shown in Table 5.

The dilute acids are poor extractants for reactive dyes but treatment with 1,2-diaminoethane for 1 h at 90°C provided reasonable extraction, probably because of partial degradation of the wool. Certainly the dye extracts, heavily contaminated with proteinaceous material, are of little use for thin-layer chromatography. Most of the reactive dyes covalently bond to the basic amino acids in wool fibers, particularly to lysine, forming a bond very resistant to hydrolysis. Attempts to completely hydrolyze the protein at 90°C, to allow characterization of the lysine-dye adduct, resulted in decomposition of the dye [15].

Thus, although reactive dyes could be brought into solution, the extracts were suitable only for visible absorption spectrophotometry in the capillary tube [16].

Sequential Extraction Procedure

The results allowed the design of a sequential extraction procedure that provided efficient extraction for all the samples examined here; also, the stage at which extraction is obtained gives an indication of the class of dye present.

The fibers were initially extracted with pyridine/water (4:3 v/v) at room temperature and then heated at 90°C if required. Efficient extraction at an early stage indicates the presence of an acid dye. If the treatment is not successful the fibers are pretreated with oxalic acid and the process repeated. Improved extraction indicates the presence of a chrome or metal-

TABLE 5—Extraction of reactive dyes from six wool samples.^a

Dye	Solvent Used (1 h at 90°C)		
	10% Aqueous Hydrochloric Acid	10% Aqueous Sulfuric Acid	1,2-Diaminoethane
N	0	0	4
O	0	0	5
P	0	0	4
Q	1	1	4
R	0	0	5
S	0	0	3

^a0 corresponds to no extraction and 5 to complete extraction.

complex dye. Finally, if both procedures fail (efficiency of extraction scores less than 3 on the arbitrary scale), a reactive dye is indicated and extraction with 1,2-diaminoethane should be attempted. The results obtained with this three-step procedure are shown in Table 6.

General Discussion

A three-step extraction procedure has been developed for the major dye classes found on wool fibers. A detailed comparison of dye extracts from single fibers at various stages in the procedure should provide a useful means of fiber discrimination. In addition, some idea of the class of dye present may be obtained.

In subsequent work it has been found that more than 90% of dyed wool fibers encountered are readily extracted with aqueous pyridine, presumably because they contain acid dyes.

Although the overall proportion of wool fibers requiring oxalic acid treatment is small, chrome dyes are commonly found in men's suiting material and in black knitting wools. If the presence of chromium is suspected, it can be readily confirmed for any large sample available by using energy dispersive X-ray fluorescence spectrometry (Fig. 1). On occasion, for example with black reused wools, some dye components may be directly extractable with aqueous pyridine and additional ones after oxalic acid treatment. Thin-layer chromatograms obtained from three such samples are shown in Fig. 2. Thus a more specific means of characterization may be achieved in some cases.

The use of reactive dyes on wool appears to be limited, although increasing slowly, and so fibers containing these dyes are probably of greater evidential value.

TABLE 6—Extraction of dyes from various wool fibers using a three-step sequential procedure.^a

Dye	Step 1; 15 min Aqueous Pyridine at 90°C	Step 2; Oxalic Acid Pretreatment plus 15 min Aqueous Pyridine at 90°C	Step 3; 1 h 1,2-Diaminoethane at 90°C
Acid			
A	5	—	—
B	5	—	—
C	5	—	—
D	5	—	—
E	5	—	—
F	5	—	—
G	5	—	—
Chrome and metal-complex			
H	3	4	—
I	0	5	—
J	4	5	—
K	4	5	—
L	1	3	—
M	4	5	—
Reactive			
N	1	0	4
O	0	1	5
P	0	0	4
Q	0	0	4
R	0	1	5
S	0	0	3

^a0 corresponds to no extraction and 5 to complete extraction; — indicates that satisfactory extraction had been obtained in previous steps.

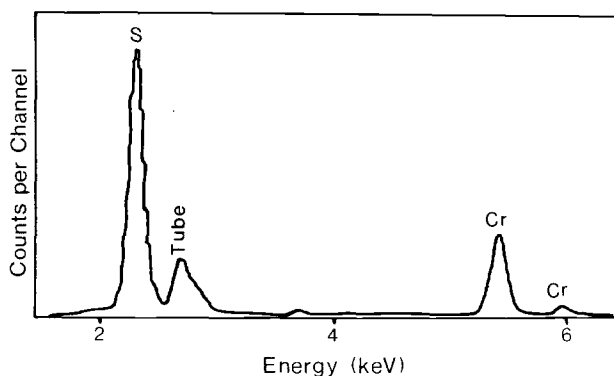


FIG. 1—X-ray fluorescence spectrum of a wool strand containing a chrome dye (unfiltered rhodium radiation, 35kV).

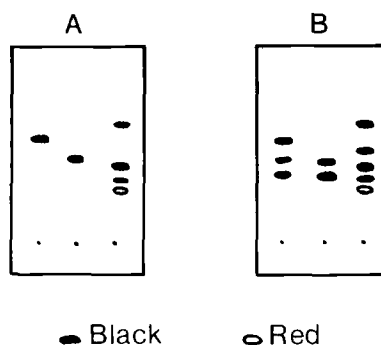


FIG. 2—Separation of dyes extracted from three different black wool fibers before (A) and after (B) oxalic acid treatment (for chromatographic conditions see Ref 17).

The extraction procedure described has been used to strip the dyes from a collection of wool fibers prior to their characterization by solution spectrophotometry and thin-layer chromatography. The results have been reported in another paper [17].

Summary

The efficient extraction of dyestuffs from fibers is a prerequisite for their analysis by thin-layer chromatography or solution absorption spectrophotometry. A sequential procedure has been described for stripping the dyestuffs from single wool fibers. The efficiency of extraction at each stage is both a useful property for fiber comparisons and indicates whether acid, metallized, or reactive dyes are present on the fiber.

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