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# The Characterization of Dyestuffs on Wool Fibers with Special Reference to Microspectrophotometry

Although synthetic fibers have achieved much greater importance in the textile industry over the years, wool still remains the fiber type most frequently encountered in Home Office forensic science laboratories.

It is not particularly difficult to locate wool samples from different sources that appear similar under microscopic examination, a method that has been employed for many years in the identification and comparison of fibers [*1,2*]. However, such a method may not discriminate between similar colors achieved by combinations of different dyestuffs. Dye mixtures yielding metameric colors may be distinguished by a number of other techniques, including microspectrophotometry of mounted fibers, visible absorption spectrophotometry of dye solutions, and thin-layer chromatography of dye extracts.

Microspectrophotometry was developed by Caspersson [*3,4*] for the location and identification of chemical constituents within biological cells. Later a more convenient reflecting microscope was used by other workers for biochemical studies [*5,6*]. Commercial microspectrophotometers have been used for the examination of textile fibers for a number of years in some European forensic science laboratories [*7,8*]. However, no procedure has been described for testing the spectra for significant differences. Neither has the discriminating power [*9*] of the technique been compared with those obtained from the conventional and much less expensive methods employing dye extraction.

Various approaches have been detailed in the textile literature for the identification of the application class of dyes on fibers and for their extraction into solution [*10-14*]. A three-step procedure suitable for the extraction and comparison of dyes from single wool fibers has recently been described by Macrae and Smalldon [*15*]. The subsequent dye solutions can then be examined by visible absorption spectrophotometry and thin-layer chromatography.

Solution absorption spectra can be recorded by using fairly simple capillary accessories that fit into conventional spectrophotometers [*16,17*]. Some of the problems that may be encountered with such an approach have been discussed by Smalldon and King [*18*].

A small volume of solution, previously used for spectrophotometry, can be spotted onto a thin-layer plate and the dye components separated by chromatography. Many systems have been reported in the general and textile literature for the chromatographic separation of fiber dyes [*19-23*], but few, if any, in the forensic science literature. Thus those laboratories employing thin-layer chromatography in fiber dyestuff comparisons tend to use a wide variety of chromatographic methods.

In this paper a procedure is developed for the interpretation of spectra obtained by microspectrophotometry, and the discriminating power [*9*] of this technique is compared

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with that for microscopy, solution spectrophotometry, and thin-layer chromatography by using a collection of visually similar dyed wool samples.

## Experimental Procedure

### *Samples*

The wool samples selected for study were from three distinct color groups found to be the most frequently encountered in a survey of case materials. All the samples within a color group were visually similar and were collected from members of staff, manufacturers' pattern cards, and operational case materials. The final collections comprised 12 red wools of Methuen color code 10A8 [*24*], 18 blue wools of color code 20E8, and 12 black wools.

### *Comparison Microscopy*

Microscopic examinations were carried out with a Leitz Orthoplan comparison microscope at a magnification of ×400. For comparisons under ultraviolet (UV) illumination the excitation filters BG38 and UG1 were used in combination, together with a K430 barrier filter.

Several fibers from each sample were mounted on microscope slides with XAM mounting medium (Hopkin & Williams, England). All samples within each color group were compared with one another under white light and then under UV light. For each comparison the operator recorded whether or not indistinguishable fibers were present.

### *Microspectrophotometry*

Fibers were mounted as previously described and spectra were recorded from two different fibers on each slide with a Shimadzu MPS-50L spectrometer, with the microscope attachment in the sample beam. The instrumental conditions were as follows:

| | |
|---|---|
| substage diaphragm | 100 μm |
| objective diaphragm | 20 μm |
| pen response | 1 |
| absorbance scale | 0–1.0 |
| spectral range | 400–700 nm |
| calibration standards | holmium and didymium glasses |
| scale expansion | 10 nm/cm chart |
| scan time | 135 s |
| objective | ×25 glass |
| eyepiece | ×10 glass |
| light source | tungsten |

Baseline correction was achieved by using the external compensating unit. A baseline was recorded for a blank portion of the slide on each sheet of chart paper, on which six spectra were later recorded. The absorbance values, after background subtraction, were tabulated for each spectrum at 10-nm intervals. The total absorbance under each curve, as found by summation, was recorded together with the proportion of the total absorbance found in each 10-nm interval. Spectral information in this form, for two fibers from each sample, was then used for data processing.

### *Dye Extraction*

A fiber approximately 1 cm in length was taken from the sample and placed in a 1.5-mm internal diameter glass capillary tube with 10 μl of solvent. The tube was sealed and extraction carried out by using the sequential procedure previously described [*15*].

*Solution Spectrophotometry*

The sealed capillary tube was tapped until the dye solution reached the end opposite the extracted fiber. An absorption spectrum was recorded for the visible region (380 to 770 nm) with a capillary cell holder, incorporating a beam condenser, fitted to a Beckmann Series 25 spectrophotometer [*17*]. Spectra were recorded for two different fibers from each sample, and data reduction was carried out as described for microspectrophotometry.

*Thin-Layer Chromatography*

The capillary tube was broken and the dye solution applied to the origin of the plate (Merck DC-Alufolien Kieselgel 60 $F_{254}$) to give a spot about 2 mm in diameter. The plate was warmed at 50°C on a hot plate in a stream of air during spotting and for about 10 min afterwards. When a suitable number of samples had been applied to the origin the plate was developed for 2 or 3 mm in methanol and then dried once more. The plate was suspended in a cylindrical tank (Fig. 1) for 5 min to allow equilibration with the solvent vapor. Development was then carried out for about 5 cm with the solvent system pyridine/amyl alcohol/10% aqueous ammonia (4:3:3 v/v). The developed plate was finally dried for 15 min as previously described.

## Results and Discussion

*Comparison Microscopy*

The results of the microscopic comparisons are shown in Tables 1 through 3. The blue Sample 12 was inhomogeneous and therefore only one of the blue shades present was used for further comparisons.

For the red and blue wools about half the comparisons of different samples using white light yielded no similar fibers. As might be expected less discrimination was obtained for the black wools. Despite the uneven fluorescence of some samples, UV microscopy considerably increased discrimination of the red and blue wools. In the case of the black wools only one sample was weakly fluorescent and so discrimination was not greatly enhanced by fluorescence microscopy for this color group.

*Microspectrophotometry*

Spectra recorded for one of the two fibers from the 12 red wool and 18 blue wool samples are shown in Figs. 2 and 3, respectively. The black wools absorbed so strongly that useful spectra could not be recorded.

Although the spectra were visually examined in some detail it was thought desirable to develop an objective procedure for spectral comparisons which would be suitable

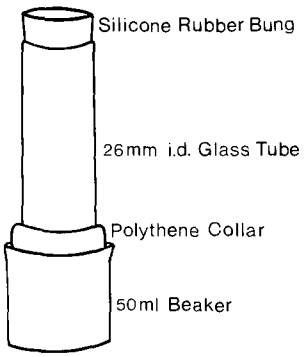


FIG. 1—*Tank used for development of thin-layer chromatography plates.*

TABLE 1—*Microscopic comparison of twelve visually similar red wools under white light and UV light.* [a]

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ... | D | D | UV | D | D | D | D | D | UV | D | UV |
| 2 | ... | ... | X | UV | D | X | D | D | X | X | UV | UV |
| 3 | ... | ... | ... | D | D | X | UV | D | X | X | UV | UV |
| 4 | ... | ... | ... | ... | D | D | D | D | UV | D | X | D |
| 5 | ... | ... | ... | ... | ... | D | X | UV | D | D | D | D |
| 6 | ... | ... | ... | ... | ... | ... | D | D | UV | UV | X | X |
| 7 | ... | ... | ... | ... | ... | ... | ... | D | D | D | D | X |
| 8 | ... | ... | ... | ... | ... | ... | ... | ... | D | D | D | D |
| 9 | ... | ... | ... | ... | ... | ... | ... | ... | ... | X | UV | UV |
| 10 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | UV | UV |
| 11 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | UV |
| 12 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

[a]D = distinguishable under white light and UV = distinguishable under UV light; X = indistinguishable samples.

TABLE 2—*Microscopic comparison of 18 visually similar blue wools under white light and UV light.* [a]

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ... | UV | D | X | D | X | D | UV | D | D | D | X | D | UV | D | UV | D | D |
| 2 | ... | ... | UV | X | UV | D | UV | X | D | UV | D | UV | D | UV | D | X | D | D |
| 3 | ... | ... | ... | D | X | UV | X | UV | D | UV | UV | UV | UV | UV | UV | X | X | D |
| 4 | ... | ... | ... | ... | X | X | D | UV | D | X | D | X | X | X | D | X | UV | D |
| 5 | ... | ... | ... | ... | ... | D | X | X | D | X | D | X | UV | UV | D | X | X | D |
| 6 | ... | ... | ... | ... | ... | ... | D | X | D | D | D | X | UV | X | D | X | X | UV |
| 7 | ... | ... | ... | ... | ... | ... | ... | X | D | X | D | X | UV | X | UV | X | X | D |
| 8 | ... | ... | ... | ... | ... | ... | ... | ... | D | X | D | UV | D | UV | D | X | X | D |
| 9 | ... | ... | ... | ... | ... | ... | ... | ... | ... | X | D | X | D | UV | D | D | D | D |
| 10 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | D | D | D | X | D | X | X | D |
| 11 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | X | D | X | D | X | X | D |
| 12 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | D | D | D | X | X | D |
| 13 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | UV | D | UV | UV | D |
| 14 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | D | X | X | D |
| 15 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | D | D | D |
| 16 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | D | D |
| 17 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | D |
| 18 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

[a] D = distinguishable under white light; UV = distinguishable under UV light; X = indistinguishable samples.

TABLE 3—*Microscopic comparison of twelve visually similar black wools under white light and UV light.*[a]

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ... | D | X | X | X | X | X | X | X | X | D | X |
| 2 | ... | ... | D | D | UV | D | UV | UV | D | D | D | D |
| 3 | ... | ... | ... | X | X | X | X | X | D | X | X | X |
| 4 | ... | ... | ... | ... | X | X | X | X | X | X | X | X |
| 5 | ... | ... | ... | ... | ... | X | X | X | X | X | D | X |
| 6 | ... | ... | ... | ... | ... | ... | X | X | X | X | X | X |
| 7 | ... | ... | ... | ... | ... | ... | ... | X | X | X | X | X |
| 8 | ... | ... | ... | ... | ... | ... | ... | ... | X | X | X | X |
| 9 | ... | ... | ... | ... | ... | ... | ... | ... | ... | X | X | X |
| 10 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | X | X |
| 11 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | X |
| 12 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

[a]D = distinguishable under white light; UV = distinguishable under UV light; X = indistinguishable samples.

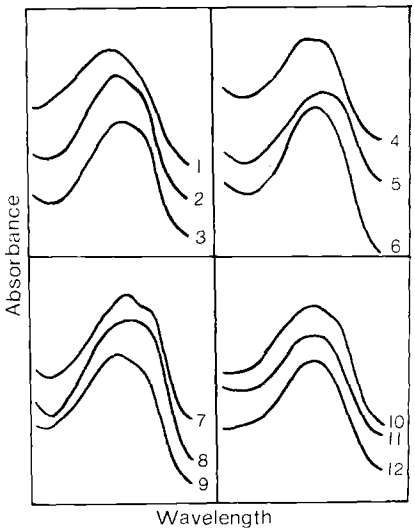


FIG. 2—*Spectra from microspectrophotometer for twelve visually similar red wool fibers.*

for computer processing. For the total absorption (area under the curve) the variation between duplicate samples was about the same as the variation between different samples, and so this parameter was not useful for discrimination purposes. This situation arises primarily because the samples are visually similar in color and because there are variations both in fiber thickness and in dye uptake. The normalization process (that is, the recording of the proportion of the total absorbance in each 10-nm interval) removes this component from the spectral data.

The normalized spectra for each pair of duplicates were compared to assess the within-sample variation by using the following test parameters:

(1) the difference in wavelength at corresponding peak maxima ($\lambda_{max}$);

(2) the sum of the squares of the differences in absorbance between corresponding data points;

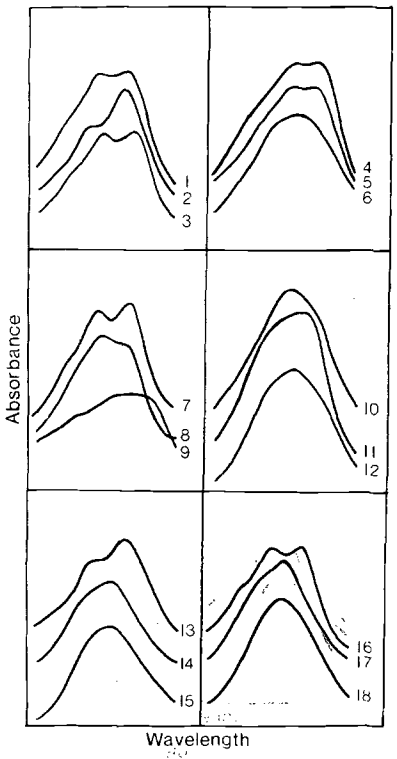


FIG. 3—*Spectra from microspectrophotometer for 18 visually similar blue wool fibers.*

(3) the sum of the absolute differences in gradient at corresponding data points; and
(4) the maximum difference between the cumulative absorbance distributions, obtained by successive addition of absorbance values across the spectra.

Values of $\lambda_{max}$ are used to record data in all forms of spectroscopy and sums of squares parameters are well known in statistical testing. The sum of the differences in gradient was examined to determine its sensitivity to minor differences in dye composition that merely lead to points of inflection in the spectra. The maximum difference in the cumulative distributions has recently been used by Dudley and Smalldon [25] for the comparison of the size distributions of soil particles.

The distribution of each test parameter was examined for all the duplicates in each color group and no outliers that would seriously affect subsequent analysis were detected. The calculations were then repeated for all the possible paired comparisons involving different samples within each color group. For example, in the group of blue wools 153 comparisons were made for each test parameter.

For the purposes of this comparative study any pair of different samples that produced a value of a test parameter outside the range found for the duplicates was considered to be discriminated with that particular parameter. Conversely, any pair that gave a value of the test parameter falling within the range found for duplicates was considered similar. For example, the largest difference between any corresponding $\lambda_{max}$ values for the duplicates was 5 nm, and therefore any pair of spectra from different samples showing larger differences in $\lambda_{max}$ values were said to be discriminated. The discriminating powers [9] obtained for the red and blue wools with the four test parameters are given in Table 4.

When $\lambda_{max}$ values alone were used the discriminating powers were relatively poor, although rather better values were obtained for the blue wools (0.87) than for the red wools (0.67) since spectra for the former color group often contained multiple peaks. The other parameters, which took band shape into account, produced higher discrimi-

TABLE 4—*The discriminating power of microspectrophotometry for red and blue wools as measured by using various test parameters.*

| Parameter | Red Wools[a] | Blue Wools[b] |
|---|---|---|
| $\lambda_{max}$ | 0.67 | 0.87 |
| Cumulative squares of differences | 0.89 | 0.97 |
| Cumulative difference in gradients | 0.85 | 0.97 |
| Maximum difference in cumulative spectra | 0.80 | 0.87 |
| Cumulative squares + $\lambda_{max}$ | 0.94 | 0.99 |
| Cumulative gradients + $\lambda_{max}$ | 0.92 | 0.99 |

[a] 66 paired comparisons examined.
[b] 153 paired comparisons examined.

nating powers. The maximum difference between the cumulative distributions was not a very powerful parameter in this application because the two spectra being compared often crossed several times and differences were alternatively accumulated and then cancelled out, thus producing a low maximum difference. When this parameter was used in the soil application [*25*] the distributions were simpler and showed only one peak. The sums of squares and the sums of gradient parameters produced good discriminating powers, with the former yielding marginally better values.

The distributions of the sums of squares of differences for duplicates and different samples of blue wool are shown in Fig. 4. The overlap is small and hence high discrimination is obtained. Some representative pairs of normalized spectra for blue wools are shown in Fig. 5 together with the values obtained for the sum of the squares of differences in each case. The third pair (*C*) are only just discriminated although their $\lambda_{max}$ values are quite different. It was generally apparent that the $\lambda_{max}$ parameter was discriminating different samples from the sum of squares parameter and both failed to discriminate some pairs of spectra that clearly looked different. However, when used in combination, these two parameters yielded excellent discriminating powers for both the red (0.94) and blue (0.99) wools, as shown in Table 4. The spectra that could not be discriminated by this combination of parameters were extremely similar by visual inspection. It was thus concluded that a satisfactory objective procedure had been developed for spectral comparisons and that reasonable estimates of the discriminating power of microspectrophotometry had been made for both the red and blue wools.

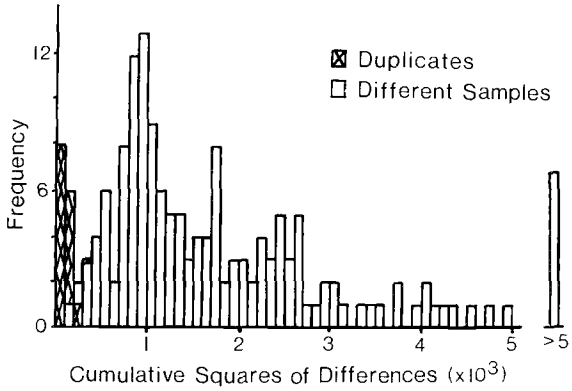


FIG. 4—*Distributions of sum of squares parameter for 18 duplicate pairs and 153 different pairs of blue wools.*

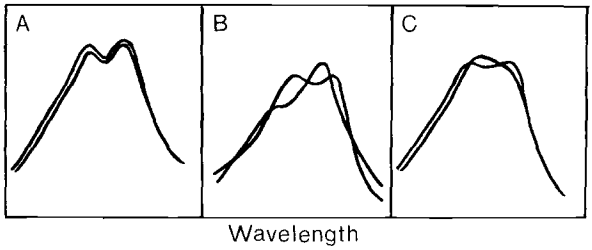


FIG. 5—*Comparison of normalized spectra for three pairs of blue wools;* (A) *pair of duplicates* (*sum of squares* = $3.23 \times 10^{-5}$), (B) *pair of very dissimilar samples* (*sum of squares* = $2.34 \times 10^{-3}$), *and* (C) *pair of barely discriminated different samples* (*sum of squares* = $4.59 \times 10^{-4}$).

*Dye Extraction*

The dyestuffs from all the red and blue wools were efficiently extracted in the first step using pyridine/water (4:3 v/v), thus indicating that acid dyes were present. However, seven of the black wools were less readily extracted and chromium was detected on the fibers with energy dispersive X-ray fluorescence spectrometry [*15*]. These seven black wools were therefore treated with oxalic acid prior to extraction with aqueous pyridine [*15*].

*Solution Spectrophotometry*

The capillary cell used for this work was designed at the Metropolitan Police Laboratory, London, and, unlike previous versions [*16*], employs a beam condenser [*17*]. The resultant spectra showed excellent resolution and major differences were observed within each color group. One of the blue wools was readily discriminated from the others because the dyes became colorless in the basic solvent used even though they were efficiently extracted. Other samples also showed considerable changes in color on extraction.

The discriminating power for this technique was evaluated for each fiber color using $\lambda_{max}$ and the difference in gradient parameter only. Owing to the use of nonstandard cells (that is, capillary tubes), baseline shifts could easily have been introduced into the spectra. These baseline shifts would have a serious effect on the squares of differences parameter and maximum difference parameter but would have no effect on $\lambda_{max}$ and, for small shifts, would have little effect on the difference in gradient parameter. Thus only these latter two parameters could be used to evaluate solution spectrophotometry (Table 5).

As with microspectrophotometry, $\lambda_{max}$ was not a very discriminating parameter when used alone, whereas the difference in gradient parameter gave excellent discrimination both when used alone and in conjunction with $\lambda_{max}$.

*Thin-Layer Chromatography*

The chromatographic system described showed good sensitivity with 1-cm lengths of fiber and produced excellent separations. A sketch of the plate obtained for the 18 blue wools is shown in Fig. 6 to demonstrate that many samples showed major differences in dye composition. Discriminating powers of 0.94, 0.99, and 0.94 were obtained for the red, blue, and black wools, respectively.

The chromatographic procedure was selected from more than 40 systems examined in the present study. Two other systems, in which the same plates were used, also yielded good results. The developing solvents for these two other systems were chloroform/methanol/water/0.88 ammonia (11:7:1:1 v/v) and *sec*-butyl alcohol/acetone/0.88 ammonia/water (5:5:2:1 v/v). The use of these two systems in addition to the one already

TABLE 5—*The discriminating power of solution spectrophotometry for red, blue, and black wools as measured by using various test parameters.*

| Parameter | Red Wools[a] | Blue Wools[b] | Black Wools[c] |
|---|---|---|---|
| $\lambda_{max}$ | 0.59 | 0.88 | 0.65 |
| Cumulative difference in gradients | 0.93 | 0.97 | 0.93 |
| Cumulative gradient + $\lambda_{max}$ | 0.97 | 0.98 | 0.93 |

[a] 66 paired comparisons examined.
[b] 135 paired comparisons examined (one sample heterogeneous).
[c] 55 paired comparisons examined (one sample gave poor duplicate spectra).

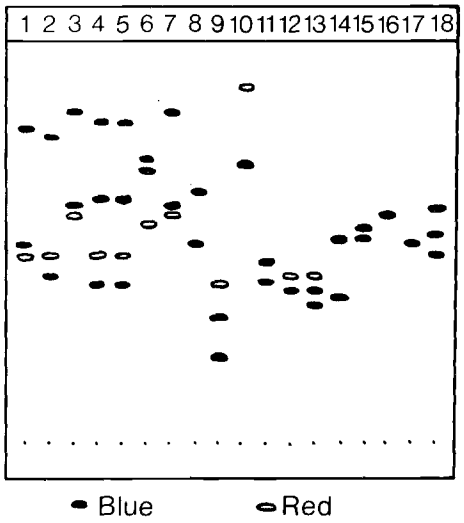


FIG. 6—*Thin-layer chromatogram obtained for the 18 blue wools.*

described failed to discriminate any further samples of blue or black wools. However, three additional pairs of red wools were discriminated and hence by using three systems the discriminating power of thin-layer chromatography, for this color group, was increased to 0.99.

## General Discussion

The discriminating powers obtained for the red, blue, and black wool samples with microscopy, microspectrophotometry, solution spectrophotometry, and thin-layer chromatography are compared in Table 6. It is quite clear that the various analytical techniques have discriminated many pairs of fibers that appeared similar by microscopy in white and UV light. However, since microscopic comparisons will remain the basic technique for fiber examinations in forensic science, it is the merits of the other three methods which require discussion.

Table 6 shows that the discriminating powers of the three analytical techniques are very similar and therefore the approach chosen for case material will depend on other factors. Before proceeding further it is useful to discuss whether or not the methods are complementary (do they discriminate different pairs of fibers?). Comparing the pairs of fibers not discriminated by each of the three techniques shows that, generally, microspectrophotometry and solution spectrophotometry yield the same combinations of indistinguish-

TABLE 6—*The discriminating powers of various techniques for red, blue, and black wools.*

| Technique | Red Wools[a] | Blue Wools[b] | Black Wools[a] |
|---|---|---|---|
| Comparison microscopy, white light | 0.53 | 0.47 | 0.17 |
| Comparison microscopy, white + UV light | 0.80 | 0.68 | 0.21 |
| Microspectrophotometry | 0.94 | 0.99 | ...[c] |
| Thin-layer chromatography | 0.94[d] | 0.99 | 0.94 |
| Solution spectrophotometry | 0.97 | 0.98 | 0.93 |

[a] 66 paired comparisons examined.
[b] 153 paired comparisons examined.
[c] Samples too intense to obtain spectra.
[d] Increased to 0.99 by two additional systems (see text).

able fibers, and hence these methods, as might be expected, are not complementary. However, in rare instances different pairs are discriminated by these methods, probably because of solvent effects on the dyestuffs after extraction. Thin-layer chromatography was found, in a number of instances, to discriminate pairs of fibers not differentiated by spectroscopic techniques and vice versa. Thus it would appear that either form of spectrophotometry and thin-layer chromatography are to some extent complementary.

Microspectrophotometry is in some ways a natural partner to comparison microscopy, since the same mounted fibers used in microscopy can be examined without any further manipulative effort. The examination can be performed on lengths of fiber no longer than the fiber's diameter (in this work 20 $\mu$m). However, in practice, there might be some advantage in using a slit aperture instead of a circular diaphragm both to increase light levels and to average out any local variations along the fiber. A permanent record of the fiber color is provided nondestructively in a few minutes. The data can be stored and processed and a much-needed data bank could be built up for the assessment of the evidential value of fiber comparisons.

The microspectrophotometer chosen for this type of work should be carefully selected so that it can record spectra at high absorbances and deal with highly scattering samples. The problems with deeply dyed samples, such as black fibers, are apparent from the work reported here. The collection of light over a wide angle will be necessary to deal with heavily delustered and irregularly cross-sectioned synthetic fibers. Microspectrophotometers are expensive, whereas the other types of examinations can be conducted fairly cheaply.

It is appropriate to consider solution spectrophotometry and thin-layer chromatography jointly since both methods can be used on the same dye extract. In the present work considerable effort was made to optimize both methods so that the results represent the best currently obtainable. It is clear that, if sufficient fiber is available and the dyes can be efficiently extracted, these two methods offer excellent discrimination. However, it is inevitable that in some case material applications insufficient sample will be available and in others it may not be possible to extract the dyes in sufficient quantity. The fiber examiner may also be reluctant in some circumstances to destroy his limited specimens. Solution spectra can be stored and processed in the same way as microspectra, but standardization and storage of thin-layer chromatograms is difficult.

Although further work remains to be done, particularly on heavily dyed and light-scattering samples, it appears to the authors that microspectrophotometry could become an effective and routine technique that would greatly enhance the value of fiber comparisons. On suitable occasions solution spectrophotometry and thin-layer chromatography on dye extracts could be used to deal with problem samples or to provide additional discrimination.

**Summary**

The dyestuffs from three groups of visually similar wool samples (12 reds, 18 blues, and 12 blacks) have been characterized by five different methods. Mounted fibers were examined nondestructively with white light microscopy, UV fluorescence microscopy, and microspectrophotometry; after dye extraction, solution spectrophotometry and thin-layer chromatography were used. Considerable increases in discriminating power were obtained by using techniques in addition to microscopy. For the red and blue wools microspectrophotometry, on 20-$\mu$m lengths of single fibers, produced discriminating powers similar to those obtained for the two destructive methods on samples 500 times larger (1 cm). However, the black fibers absorbed so strongly that useful spectra could not be recorded on the available microspectrophotometer. An objective method for the comparison of absorption spectra is described that could also be used to assess the evidential value of fiber comparisons, in conjunction with a suitable data bank of reference spectra. The considerable advantages of microspectrophotometry and some of its possible limitations are discussed in relation to the other methods.

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