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FORENSIC EXAMINATION OF TEXTILE FIBERS USING UV-VIS SPECTROSCOPY COMBINED WITH MULTIVARIATE ANALYSIS

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The current study aims at discriminating cotton and woolen textile fibers from dye using UV-Vis spectroscopy and chemometrics methods. For extraction of the dye from fibers, seven solvent systems have been used, and different extraction conditions have also been tested. Two different approaches, i.e., a visual comparison of peaks and chemometric analysis, have been proposed to differentiate between the textile fibers. The comparison of peaks obtained through UV-Vis spectra provides the discrimination power of 83.6% for cotton fibers and 94.3% for the woolen fibers. However, the incorporation of chemometrics has further improved the discrimination power to 100% for cotton and 98.1% for woolen fibers. The discrimination of 100% is achieved with nonsignificant values of the Welch's t-test, which illustrates that all samples are discriminated.

Keywords: *textile fiber, chemometrics, UV-Vis spectroscopy, forensic, Welch's t-test.*

КРИМИНАЛИСТИЧЕСКАЯ ЭКСПЕРТИЗА ТЕКСТИЛЬНЫХ ВОЛОКОН МЕТОДАМИ СПЕКТРОСКОПИИ УФ–ВИДИМОГО ДИАПАЗОНА С ИСПОЛЬЗОВАНИЕМ МНОГОМЕРНОГО АНАЛИЗА

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С использованием методов УФ-спектроскопии и хемометрии проведено распознавание хлопковых и шерстяных текстильных волокон по красителям. Для извлечения красителя из волокон использованы семь систем растворителей, а также испытаны различные условия экстракции. Для различения текстильных волокон предложены два различных подхода – визуальное сравнение максимумов и хемометрический анализ. Сравнение максимумов в спектрах УФ и видимого диапазонов обеспечивает степень дискриминации 83.6% для хлопковых и 94.3% для шерстяных волокон. Применение хемометрии позволило повысить степень дискриминации до 100% для хлопковых и до 98.1% для шерстяных волокон. Дискриминация в 100% достигается при незначительных величинах t-критерия Уэлша, показывающих, что все образцы различаются.

Ключевые слова: *текстильное волокно, хемометрия, спектроскопия УФ–видимого диапазонов, судебная экспертиза, t-критерий Уэлша.*

Introduction. Physical trace evidences are defined as "minute generally microscopic fragments of various types of material including fibers, human/animal hairs, paint chips, cement/soil, pollen grains, etc. that transfer between people, places, and objects and persist there for a long time" [1]. In forensic criminal investigation, fibers can be collected from many crime scenes such as hit and run, burglary, theft, rape, etc., and they play an important role in linking the suspects/victims to the crime. The primary technique of fiber examination is microscopy [2]. For example, a stereo zoom microscope can reveal the size and shape of the fiber, and infrared microscopy is routinely used for comparing the single strand of fiber in the forensic science laboratories [3]. For the discrimination of man-made fibers, microscopic methods are not sufficient. Other methods include examination by using chromatography and spectrophotometry. The most common technique of chromatography is thin-layer chromatography (TLC) [4–6]. However, this method is time-consuming. Some hyphenated techniques like mass spectroscopy coupled with its ionization method [7, 8] and capillary electrophoresis [9] have also been used.

In the spectroscopic method, the spectra of extracted dye from the fibers are recorded and compared [10–12]. For determination of chemical composition of cotton and woolen fibers, Fourier transforms infrared spectroscopy (FTIR) [3, 13] and Raman spectroscopy [14, 15] have been widely used. However, experts rely only on the visual inspection of complex multidimensional spectra/chromatograms obtained from analytical methods, which may lead to misleading results. In this regard, multivariate statistical approaches have been used nowadays for differentiation of forensic exhibits [16–18].

Among all the mentioned techniques, UV-Vis spectroscopy is cost-effective, reliable, accurate, and easily available in forensic science laboratories. Along with these valuable facts, the chemometric methods can be easily applied to the spectral dataset obtained through this technique, and the outcome provides reliable quantitative results that are highly acceptable in the forensic context.

Materials and methods. Dyed textile fiber samples are collected from Raymond Textile shops in and around the northwestern part of India. Eleven black colored cottons and fifteen dyed woolen fibers are collected to investigate the discrimination in the visually same type of fibers.

Extraction of dyes. Different solvent systems like pyridine/water, formic acid/water, aqueous oxalic acid, acetic acid, NaOH, dimethylformamide, and chlorobenzene are used to extract dyes from fibers. The best solvent for the extraction of the dye from the fibers has been selected by measuring the maximum absorbance value given by the dye after extracting with the listed solvent.

The extraction of the dye from textile fibers is carried out under different conditions in order to choose the best suitable method for the proper extraction of the dye. For this purpose, five different conditions, i.e., sonication, oven, hot water bath, vortex, and hotplate are examined for the dye extraction. This protocol has been performed for 15 min for each condition.

UV-Vis spectroscopy. All spectra of the extracted dye samples are obtained through a standard UV-2550 series (Shimadzu, Japan), UV-Vis spectrophotometer utilizing quartz cuvettes. All spectra are analyzed in the wavelength range of 200–800 nm. The instrument calibration is performed before analyzing the samples.

Principal component analysis (PCA) is the most common mathematical technique that is used in chemometrics. It is a method for identifying the data pattern. It converts multivariate data into orthogonal components (uncorrelated components) called principal components (PCs). The first component describes the maximum amount of the total variance in the observed variables. The second component explains the lower variance compared to that of the 1st PC and the variance amount reduced with the extraction of each new component [19, 20]. Before performing the multivariate statistical analysis, the spectral raw data must be preprocessed. Variations due to high and low concentrations of the dye in the sample, the amount of samples, and instrumental errors are all eliminated by normalizing the raw data. SPSS 16.0 (IBM) has been used to perform the principal component analysis of the fiber samples.

Welch's t-test is a statistical method usually applied to the samples when the variance is not equal. This allows sample discrimination on the basis of variance inequality. The significant value ≥ 0.05 indicates that the two samples are not the same; as a result, different samples are discriminated [21].

Results and discussion. After extraction of the dyes from the fibers, UV-Vis spectra of each sample were taken in the wavelength range from 400 to 700 nm (visible region), and absorbance maxima have been noted. It was concluded that the NaOH solution extracts the maximum amount of dye from the cotton samples. However, in the case of the dyed woolen fibers, Formic Acid/Water provides better results in comparison to other solvent systems.

After selecting the best solvent for extraction, the conditions for the extraction were also evaluated. The best dye extraction is obtained by heating the fiber sample in the oven for 15 min and the minimum extraction is shown by vortexing the fiber sample. The result of the UV-spectra shows the order of extraction as

Oven >Hot Plate > Water Bath > Sonication > Vortex.

Discrimination of cotton fiber samples by using UV-Vis spectroscopy. The discrimination power as given by Smalldon and Moffat [22] is calculated by comparing the visual features of the individual dye spectrum after analysis by a UV-Vis spectrophotometer. Eleven black cotton samples were analyzed to determine the chemical variation in the black dye. The result of the UV-Vis spectroscopy shows that different peaks are observed for different samples as shown in Table 1.

TABLE 1. Cotton Fiber Samples Showing the Presence of Absorbance Band at Particular Wavelength Ranges. The check (✓) marks signify the presence of a peak at the mentioned wavelength ranges

Sample No	Peaks showed by sample at different wavelengths			
	240–260 nm	480–500 nm	590–610 nm	Extra Peaks, nm
1	✓			496, 324
2			✓	293
3	✓	✓		
4	✓	✓		
5	✓	✓		
6	✓			
7	✓		✓	317
8	✓		✓	317
9	✓		✓	317
10	✓		✓	317
11	✓	✓		311

The discrimination power for these samples is calculated as follows

DP = No. of Sample Pair Discriminated × 100/ Total No. of Sample Pair

In the present case, the number of sample pairs not discriminated is 9 (samples 3, 4, and 5 having two peaks in the ranges of 240–260 and 480–500 nm, respectively, with no extra peak, and samples 7, 8, 9, and 10 having peaks at 240–260, 590–610 nm and with an extra characteristic peak at 317 nm). Here, the number of discriminated sample pairs is 46, and the total number of sample pairs is 55. Therefore, the discrimination power =46×100/55 = 83.63% is achieved.

Multivariate discrimination. The discrimination power obtained through visual analysis is although significant, but it can be enhanced by using chemometrics of the normalized spectral dataset. In chemometrics, PCA is applied to see the variance present in the data, and it is also used to see the differentiation among the samples under study.

The sample adequacy test gives a significant value, i.e., 0.65 and a $p = 0.00$ for the Kaiser-Meyer-Olkin (KMO) and Bartlett's tests, respectively. At each step, new components are extracted and the valuable components are selected with the help of the Kaiser criterion [23]. In the present study of cotton samples, the first six components show an eigenvalue greater than unity in the rotation matrix solution, and hence, follow the Kaiser criterion.

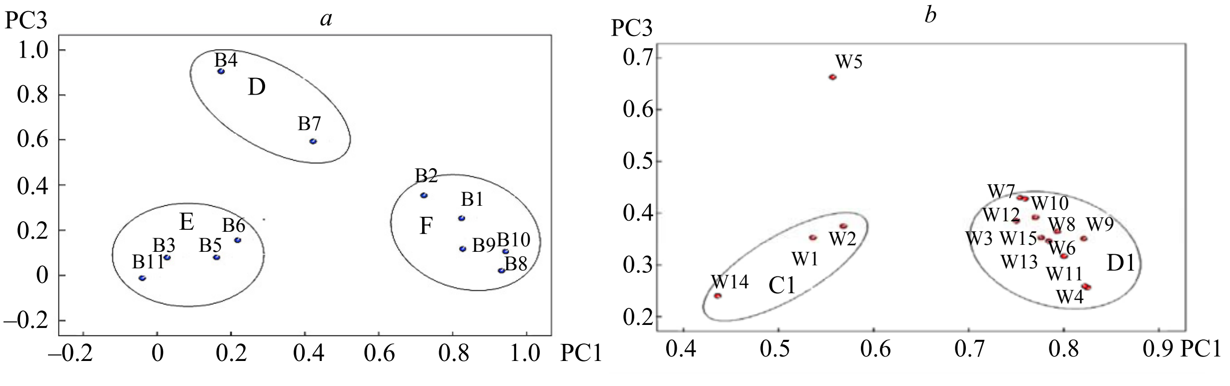

Fig. 1. The scatter plot for the component loadings values of PC1 vs. PC3 for cotton (a) and woolen (b) fiber samples.

The result of the analysis of the spectra of cotton samples (black) shows that the first six components describe 93.5% of the total variance in spectral features. To observe discrimination, a scatter plot has been developed between important PCs. Since all the components extracted from the PCA analysis are uncorrelated, the scatter plot of PC1 versus PC3 is enough to explain the pairwise discrimination of fiber samples. The plot shows clearly distinguished fiber samples in all three different groups, i.e., D, E, and F as shown in Fig. 1a. Therefore, 100% pairwise discrimination is obtained by using chemometrics.

Discrimination of woolen fiber samples by visual inspection. UV-Vis spectral analysis of the woolen fiber samples has been performed, which shows clear differences in the spectral features of different fiber samples as shown in Table 2. Here, the total number of sample pairs is 105 and the number of sample pairs discriminated is 99. The number of sample pairs that show similar types of visual characteristics and hence are not discriminated is 6 (sample W5, W8, W9, and W10). Therefore, the discrimination power for the woolen fiber samples is 99/105×100 = 94.28%.

TABLE 2. Absorption Wavelength Shown by Woolen Samples along with Some Extra Peaks. The check (✓) mark shows the presence of a peak at particular wavelength ranges

Sample No.	215–225 nm	245–255 nm	Extra peak, nm
W1		✓	
W2		✓	214
W3	✓	✓	572
W4		✓	367, 517
W5	✓		590
W6	✓	✓	365
W7	✓		
W8	✓		494
W9	✓		492, 370
W10	✓		576
W11	✓	✓	544, 488
W12	✓		370, 568
W13	✓		392
W14	✓	✓	
W15	✓	✓	451

Multivariate discrimination. The value of the Kaiser-Meyer-Olkin test comes out significant, and hence PCA is applied to all spectral datasets. These spectra analysis shows that the first three components in the rotation solution show 98.89% variance among the spectral features of the woolen fiber samples as shown in Table 3. After the first three components, the eigenvalue is dropped to unity, and hence the rest of the components are not included for further analysis [24].

The discrimination is calculated by plotting the scatter plot between PC1 versus PC2. All the samples are divided into two groups labeled as A1 (samples W1, W2, and W14) and B1 (samples W3–W13 and W15). Group A1 shows samples clearly distinguished from each other. However, group B1 shows the discrimination of all samples except for samples W4, W7, W11, and W12. These four samples show pairwise overlapping, i.e., W4/W11 and W7/W12. Therefore, these samples are not distinguished by chemometrics, as shown in Fig. 1b. Thus, the pairwise comparison gives the discrimination power of 98.10%, since only two pairs of samples are not discriminated by PCA.

TABLE 3. Result Showing Total Variance Explained by the First Three Important Principal Components for Woolen Fiber Samples

Component	Initial Eigen values			Rotation Sums of Squared Loadings		
	Total	Variance, %	Cumulative, %	Total	Variance, %	Cumulative, %
1	14.27	95.16	95.16	7.93	52.86	52.86
2	0.41	2.72	97.88	4.74	31.59	84.45
3	0.17	1.12	99.00	2.17	14.45	98.90
4	0.051	0.341	99.34	0.06	0.40	99.30

Discrimination by using Welch's t-test. The scatter plot of the woolen fiber samples showed that samples W4/W11 and W7/W12 overlapped. Now, to verify that these samples are similar or not similar, the Welch's *t*-test is used for a pair of W4/W11 samples, and the outputs are shown in Table 4. The Welch's *t*-test value signifies that both pairs of the sample are not the same. If these samples are similar, then the value of the Welch's *t*-test must be ≤0.05. This value comes out to be greater than 0.05.

TABLE 4. Results Obtained through Welch's *t*-Test

Parameter	Welch	Brown-Forsythe
Statistic	0.00	0.00
degree of freedom 1	1	1
degree of freedom 2	1199.37	1.00
Significat *p*-value	1199.37	1.00

Similar results are obtained for the sample pair of W7/W12. All the woolen fiber samples are separated out by using a combined approach to the PCA analysis and Welch's *t*-test. Therefore, 100% pairwise discrimination is achieved by using this method. This approach provides a better validation method to check the similarity/dissimilarity between the fiber samples.

Conclusion. The current work describes a cost-effective and reliable quantitative method for discrimination of cotton and woolen fibers by using UV-Vis spectroscopy in combination with principal component analysis. In this research, a sequence of examination methods is chosen in order to obtain the best possible discrimination. NaOH and formic acid/water provide the best extraction of dyes from cotton and woolen fibers, respectively. The extraction efficiency is increased under controlled heating temperature (oven at 100°C). The incorporation of chemometrics has provided enhanced discrimination (100% for cotton and 98.10% for woolen) compared to manual discrimination. The result of the Welch's *t*-test rejects the null hypothesis, which illustrates that all woolen samples are differentiated giving 100% discrimination.

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