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PAPER

CRIMINALISTICS

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Principal Component Analysis and Analysis of Variance on the Effects of Entellan New on the Raman Spectra of Fibers

ABSTRACT: During the forensic examination of textile fibers, fibers are usually mounted on glass slides for visual inspection and identification under the microscope. One method that has the capability to accurately identify single textile fibers without subsequent demounting is Raman microspectroscopy. The effect of the mountant Entellan New on the Raman spectra of fibers was investigated to determine if it is suitable for fiber analysis. Raman spectra of synthetic fibers mounted in three different ways were collected and subjected to multivariate analysis. Principal component analysis score plots revealed that while spectra from different fiber classes formed distinct groups, fibers of the same class formed a single group regardless of the mounting method. The spectra of bare fibers and those mounted in Entellan New were found to be statistically indistinguishable by analysis of variance calculations. These results demonstrate that fibers mounted in Entellan New may be identified directly by Raman microspectroscopy without further sample preparation.

KEYWORDS: forensic science, Raman microspectroscopy, fibers, principal component analysis, analysis of variance, Entellan New, PCA, ANOVA

One of the first steps in the forensic analysis of recovered textile fibers is the visual inspection of fiber tapings under a stereomicroscope. A questioned fiber is selected from the tapings and mounted on a glass slide in a semi-permanent mounting medium for examination using comparison microscopy and to determine the generic class of the fiber from its optical properties. Synthetic fibers that are found to be indistinguishable from the target fiber are then further characterized by infrared (IR) spectroscopy to unambiguously identify the polymer type (1–3). However, there are three significant disadvantages to IR analysis: (i) the fibers must be demounted from the microscope slide because the glass slide and cover slip are not IR transparent, (ii) the fiber must be flattened to a thin film, either under an attenuated total reflectance prism or in a diamond anvil cell, which will destroy the morphology of the fiber, and (iii) a single textile fiber may not be large enough to be analyzed by IR spectroscopy. While IR spectroscopy is a useful method, an alternative method that provides the same information without the need for additional sample preparation would be preferred.

Raman microspectroscopy combines the resolving power of a microscope with the ability of molecular spectroscopy to provide information about the molecular structure of small samples such as single, synthetic textile fibers. Moreover, the information obtained by Raman spectroscopy complements that obtained by IR analysis (4–8). Raman spectroscopy has been used for a variety of forensic examinations, including paint chips (9,10), inks (11,12), drugs (13,14), and natural and synthetic textile fibers (4,5,8,15–23). Raman spectroscopy can be used to identify the generic polymer class, and in some cases the subclass, of unknown synthetic textile fibers (5). This technique is characterized by its nondestructive nature, ease of sample preparation, and a weak interfering signal from glass. Confocal Raman microspectroscopy has further advantages, such as relatively high microscope magnifications which allow for the analysis of single textile fibers (4), and the ability to collect spatially resolved chemical information on selected regions of the sample.

Statistical and multivariate data analyses can be useful tools in the evaluation of spectroscopic data. Analysis of variance (ANOVA), a statistical technique used to determine if the means of various groups differ, has been applied to the forensic analysis of glass (24–26), soil (27,28), and DNA (29,30). Alternatively, principal component analysis (PCA), one of the widely used types of multivariate data analyses, has been used for pattern recognition within sets of Raman spectra (1–3,5). The main advantage of multivariate data analysis is that it uses all of the information contained in the entire spectrum rather than relying on the information contained in only a few characteristic peaks or bands.

Until recently, XAM neutral medium-improved white mounting medium was commonly used in many laboratories to mount textile fibers for forensic examination. However, as a result of the European Directive 98/79/EC (31), this product is no longer available. Recently, Wiggins and Drummond (32) identified Entellan New as a suitable mounting medium for textile fibers that had many characteristics similar to XAM. Here, the effect of mounting medium Entellan New on the Raman spectra of textile fibers is investigated.

Materials and Methods

Materials

Acrylic, nylon, and polyester fiber samples were obtained from the 2001 Forensic Fiber Reference Collection (Microtrace, Elgin,

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IL). The fibers were mounted in three different ways: (i) taped down on a glass microscope slide with no cover slip, (ii) taped down on a glass microscope slide and covered with a cover slip, and (iii) mounted in Entellan New (Electron Microscopy Sciences, Hatfield, PA) on a glass microscope slide and covered with a cover slip.

Raman Spectroscopy

Raman spectra were obtained using a Raman microscope (DXR; Thermo Scientific, Waltham, MA) equipped with a 532 nm frequency-doubled Nd:YVO_4 laser source and a charge-coupled device detector. A 60× objective (Olympus, Orangeburg, NY) was used to focus the laser onto a 2 μm spot on the sample, which was irradiated with 10 mW of laser power. Each sample was analyzed by collecting five exposures (30 sec each) over the range of 100–3380 cm^{-1} with the resulting signals averaged into a single spectrum.

Raman Spectra Processing and Data Analysis

Each spectrum was collected, and the baseline manually corrected, using the Omnic for Dispersive Raman software (version 8.1.42; Thermo). The spectrum was then normalized to the tallest peak and mean-centered prior to PCA. PCA was performed using Minitab Statistical Software (Release 14.1; Minitab Inc., State College, PA) using the covariance matrix over the spectral range of 600–1500 cm^{-1}, where most of the peaks unique to the textile fiber were located. One-way ANOVA was performed in an Excel spreadsheet (Microsoft Corporation, Redmond, WA) from user-created functions and the results were confirmed using the built-in ANOVA functions in Minitab. A 95% confidence level (alpha value of 0.05) was used to determine the $F_{critical}$ value. Smoothing functions were not applied to any of the spectra.

Results and Discussion

Ideally, a sample should be in its natural state (e.g., bare fiber) when acquiring its Raman spectrum so that there is no interference from the surrounding matrix (i.e., mounting medium, cover slip, etc). However, it has been shown that mounted textile fibers can be analyzed and identified effectively by Raman microspectroscopy. For example, Miller and Bartick (4) showed that the spectrum of nylon can be obtained by performing a spectral subtraction. However, the nylon peaks in the raw spectrum of the Permount-mounted nylon (on a glass slide with a cover slip) were heavily masked by a broad glass fluorescence band and peaks from the mounting medium. Spectral subtractions can also result in a low signal-to-noise ratio when compared to a spectrum acquired from a bare fiber. Therefore, it is desirable to use a mounting medium that does not contribute to, or significantly mask, the Raman spectrum of a textile fiber.

In a previous report, Wiggins and Drummond identified Entellan New as the best mounting medium for textile fiber examination compared to a "home-made" Phytohistol (33), Clarion, Eukitt, Euparal, and Practamount (32). In their study, several characteristics of the mounting media were taken into consideration—the refractive index, bubble or crystal formation, color, shrinkage, toxicity, and ease of handling of the medium—when identifying which mounting medium performed similar to or exceeded the properties of XAM. Here, we used multivariate data analysis and statistical analysis to evaluate the suitability of Entellan New as a mounting medium for the collection of Raman spectra from single textile fibers.

Principal Component Analysis

Prior to analyzing the effect of the mounting medium on the Raman spectrum of a textile fiber, PCA was first performed on three different fiber classes to illustrate what would be observed when there is a significant difference between fiber spectra. Acrylic, nylon, and polyester fibers were taped to a microscope slide, without a cover slip, and their Raman spectra collected. A representative Raman spectrum from each fiber is shown in Fig. 1 where it can be seen that the spectra of the three fibers are considerably different from one another. The differences between the fiber types are clearly shown as three distinct groups in the PCA score plot (Fig. 2), where the first two principal components (PCs) represent 96.8% of the variation in the data (PC1 = 0.864 and PC2 = 0.104).

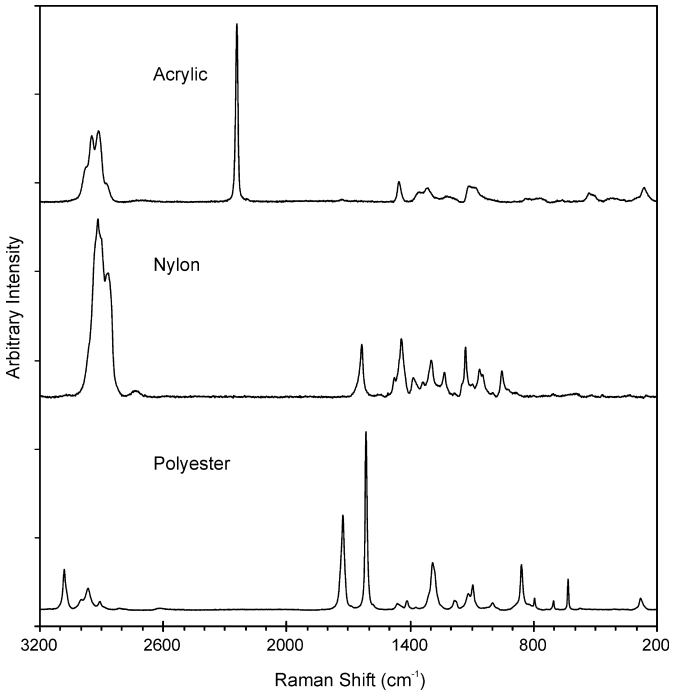

FIG. 1—*Raman spectra of bare (top) acrylic, (middle) nylon, and (bottom) polyester fibers. The spectra have been normalized to the tallest peak.*

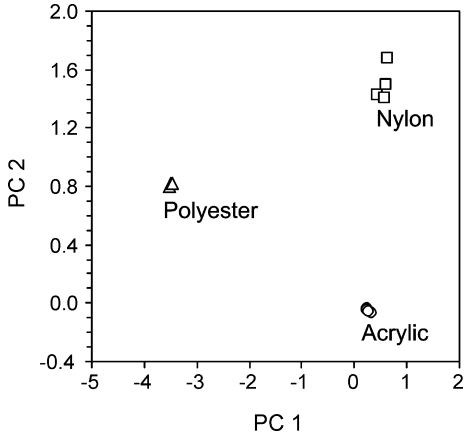

FIG. 2—*A principal component analysis score plot obtained using a covariance matrix for the spectra of various bare acrylic (○), nylon (□), and polyester (△) fibers.*

Keeping in mind that fibers having different Raman spectra form distinct groups in the PCA score plot, a single textile fiber was chosen to determine the effect of the mounting medium on its Raman spectrum. The Raman spectra of a single polyester fiber were acquired at five different locations along the fiber for each of the three different mounting methods (Fig. 3). Although visual inspection of the spectra did not show any major differences between the three mounting methods, very weak peaks belonging to the mountant were observed in the spectra of a polyester fiber mounted in Entellan New. However, these weak peaks did not contribute significantly to the spectrum because the PCA score plot showed that all the data points overlap each other without forming distinct groups (Fig. 4). The first two PCs shown in Fig. 4 represent 69.7% of the variation in the data (PC1 = 0.489 and PC2 = 0.208).

Analysis of Variance

ANOVA was performed on the polyester fiber spectra to compare the similarity between spectra of a bare fiber ($n = 5$) and a fiber mounted in Entellan New ($n = 5$). Eleven peaks that were unique to the polyester spectrum were chosen for the calculations. Two separate ANOVA calculations, one based on peak position and the other based on peak height, were performed on the data set. It can be seen in Table 1 that the ANOVA calculations

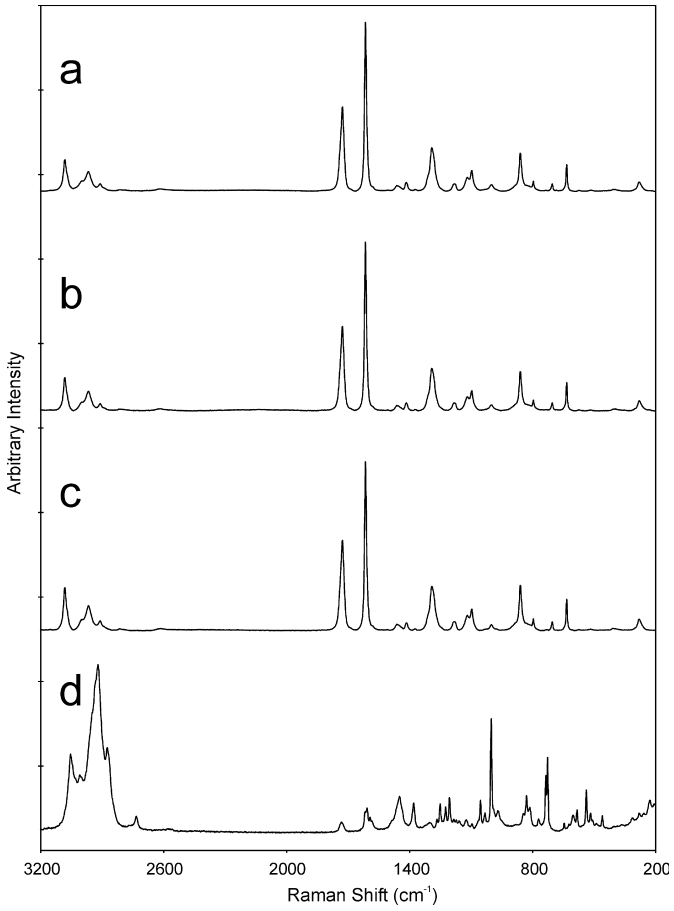

FIG. 3—*Raman spectra of the polyester fibers (a) taped onto a microscope slide with no cover slip or mounting medium, (b) taped onto a microscope slide and covered with a cover slip, (c) mounted in Entellan New, and (d) Raman spectrum of Entellan New on a microscope slide. The spectra have been normalized to the tallest peak.*

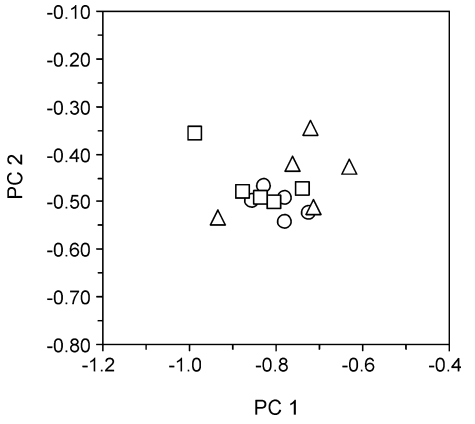

FIG. 4—*A principal component analysis score plot obtained using a covariance matrix for the spectra of polyester fibers taped onto a microscope slide with no cover slip or mounting medium (○), taped onto a microscope slide and covered with a cover slip (□), and mounted in Entellan New (△).*

TABLE 1—*Calculated ANOVA table comparing the peak positions from a bare polyester fiber and the same fiber mounted in Entellan New.*

Source of Variation, peak (cm^{-1})	SS	MS	*F*-value*	*p*-Value
3081	0.014, 0.061	0.014, 0.008	1.894	0.206
1726	0.037, 0.134	0.037, 0.017	2.223	0.174
1614	0.052, 0.063	0.052, 0.008	6.612	0.033
1415	0.128, 2.841	0.128, 0.355	0.360	0.565
1290	0.161, 0.868	0.161, 0.108	1.487	0.257
1095	0.144, 0.639	0.144, 0.080	1.802	0.216
858	0.077, 0.139	0.077, 0.017	4.452	0.068
795	0.008, 0.161	0.008, 0.020	0.417	0.536
702	0.003, 0.032	0.003, 0.004	0.644	0.445
632	0.013, 0.066	0.013, 0.008	1.561	0.247
278	0.088, 0.425	0.088, 0.053	1.662	0.233

Data reported in the following order: between group, within group.

ANOVA, analysis of variance; SS, sum of the squares; MS, mean square; d.f., degrees of freedom.

*$F_{critical} = 5.32$ where $\alpha = 0.05$, $d.f._{between\ group} = 1$, $d.f._{within\ group} = 8$.

comparing the small variations in each peak position of the unmounted bare fiber with the fiber mounted in Entellan New resulted in one peak being statistically different. Mehrens et al. (34) stated that "if the ANOVA analysis returns one peak in a form that is statistically different, then the whole data set is considered different" (p. 1359), which implies that there is a statistically significant difference between the spectra of the bare fiber and that mounted in Entellan New. However, these authors found that the ANOVA on individual peaks from the pharmaceutical compound furosemide showed the data sets to be statistically different when, in fact, they were of the same form (34). Therefore, ANOVA was applied to all of the selected peaks at the same time. To use ANOVA to compare several peaks, the data from each peak had to be normalized by subtracting the mean peak position ($n = 10$) for a given peak (e.g., 3081 cm^{-1}) from the corresponding peak position in each of the 10 spectra (five spectra of bare fibers and five spectra of fibers mounted in Entellan New). Peak normalization thus reduced the data to two larger data sets, one for bare fiber peaks ($n = 55$) and one for fibers mounted in Entellan New ($n = 55$). The ANOVA results for all 11 peaks combined showed that the spectra of the bare fibers and those embedded in Entellan New were statistically indistinguishable (Table 2). Similar calculations were performed to

TABLE 2—*Calculated ANOVA table comparing the 11 peak positions (Table 1) from a bare polyester fiber and the same fiber mounted in Entellan New.*

Source of Variation	SS	MS	*F*-value*	*p*-Value
11 peaks	0.130, 6.03	0.130, 0.056	2.328	0.130

Data reported in the following order: between group, within group.
ANOVA, analysis of variance; SS, sum of the squares; MS, mean square; d.f., degrees of freedom.
*$F_{critical}$ = 3.93 where α = 0.05, d.f.$_{between\ group}$ = 1, d.f.$_{within\ group}$ = 108.

determine whether there were any significant differences between the peak heights of the fiber spectra using the two mounting methods. Spectra (exported as XY data files) were first normalized to the tallest peak height (1614 cm^{-1}), which left 10 peaks whose height could be compared using ANOVA. First, the height data for each individual peak was compared using ANOVA and each were found to be statistically indistinguishable (Table 3). The heights of the 10 peaks were then normalized by subtracting the mean peak height for a given peak (e.g., 3081 cm^{-1}) from the corresponding peak height in each of the 10 spectra (five spectra of bare fibers and five spectra of fibers mounted in Entellan New). This procedure reduced the data into two larger data sets, one for bare fiber peaks (n = 50) and one for fibers mounted in Entellan New (n = 50). An ANOVA calculation was performed on the reduced data and the heights of the 10 selected peaks in the spectra of the bare fibers were found to be statistically indistinguishable from the spectra of those fibers mounted in Entellan New (Table 4).

TABLE 3—*Calculated ANOVA table comparing the normalized peak heights from a bare polyester fiber and the same fiber mounted in Entellan New.*

Source of Variation, peak (cm^{-1})	SS ($\times 10^{-4}$)	MS ($\times 10^{-4}$)	*F*-value*	*p*-Value
3081	0.015, 74	0.015, 9.3	0.002	0.969
1726	0.013, 18	0.013, 2.2	0.006	0.941
1415	0.045, 1.0	0.045, 0.13	0.341	0.575
1290	0.41, 1.2	0.41, 0.15	2.779	0.134
1095	0.88, 2.3	0.88, 0.28	3.129	0.115
858	1.1, 30	1.1, 3.8	0.297	0.601
795	0.052, 2.7	0.052, 0.34	0.152	0.707
702	0.053, 1.1	0.053, 0.14	0.384	0.553
632	0.43, 25	0.43, 3.1	0.139	0.719
278	0.14, 3.5	0.14, 0.44	0.317	0.589

Data reported in the following order: between group, within group.
ANOVA, analysis of variance; SS, sum of the squares; MS, mean square; d.f., degrees of freedom.
*$F_{critical}$ = 5.32 where α = 0.05, d.f.$_{between\ group}$ = 1, d.f.$_{within\ group}$ = 8.

TABLE 4—*Calculated total ANOVA table comparing the 10 normalized peak heights (Table 3) from a bare polyester fiber and the same fiber mounted in Entellan New.*

Source of Variation	SS ($\times 10^{-4}$)	MS ($\times 10^{-4}$)	*F*-value*	*p*-Value
10 Peaks	2.2, 160	2.2, 1.6	1.315	0.254

Data reported in the following order: between group, within group.
ANOVA, analysis of variance; SS, sum of the squares; MS, mean square; d.f., degrees of freedom.
*$F_{critical}$ = 3.94 where α = 0.05, d.f.$_{between\ group}$ = 1, d.f.$_{within\ group}$ = 98.

Conclusion

The main goal of this study was to determine whether or not the presence of a mounting medium would affect the Raman spectrum obtained from a single synthetic textile fiber. When comparing fibers of different polymer types, the PCA score plots showed that each fiber type could be classified into a distinct group. When PCA was used to compare the spectra of polyester fibers mounted as bare fibers (with and without a cover slip) with those mounted in Entellan New, the results showed that the weak Raman peaks arising from Entellan New did not interfere with the Raman spectra of the fiber under examination. This result was confirmed by ANOVA, which showed that the spectra of the bare fiber and that mounted in Entellan New were statistically indistinguishable. These results show that textile fibers mounted in Entellan New can be analyzed *in situ* by Raman spectroscopy with no further sample preparation.

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