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Excitation–emission matrix fluorescence spectroscopy combined with MCR-ALS as a tool for the forensic analysis of similar and dissimilar sets of textile fiber extracts

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Trace textile fiber evidence is found at numerous crime scenes and plays an important role in linking a suspect to the respective scene. In this work, investigations into the fluorescence of fiber dyes and fibers themselves, as well as a methodology for discriminating between fibers using room temperature fluorescence (RTF) are reported. Initial systematic analysis was conducted on dye standards and extracts taken from fibers colored with the respective dyes of interest. Absorbance, excitation and fluorescence spectra were compared between standards and extracts to determine the optimal area of the fiber to investigate dyes, fluorescent impurities or the whole fiber. High performance liquid chromatography investigations were performed to obtain detailed information on the number of dye and fluorescent components present in extracts. Three-way Excitation Emission Matrix (EEM) data were found to give the greatest amount of spectral information and provide the highest level of discrimination. Successful discrimination between non-similar and similar fibers was achieved with the aid of second order MCR-ALS chemometric analysis. The level of discrimination obtained *via* RTF-EEM spectroscopy was sufficient to differentiate among two types of visually indistinguishable fibers and among fibers obtained from two separate cloths of the same material and colored with the same dye reagent.

Introduction

Fibers are key trace evidence often found at a crime scenario. Analytical techniques that can either discriminate between similar fibers or match a known to a questioned fiber are highly valuable in forensic science and useful for forensic scientists. Cloths based on fibers usually contain additives such as dyes to impart color to a textile fiber. Microscopy, scanning electron microscopy (SEM), and optical and physical examination are often used to compare fibers with at least one distinguishable characteristic.[1,2] Differences in cross-sectional shape, type of fiber material (wool, cotton or synthetic), weave and color make it often possible to rule out a common source for two samples. The main advantage of these techniques is their non-destructive nature, which preserves the physical integrity of fibers for further court examination.

When fibers cannot be discriminated by non-destructive tests, a common approach is to solvent extract the questioned and the known fiber for further dye analysis. Established techniques for the analysis of fiber extracts include ultraviolet and visible absorption spectrometry,[1,2] thin-layer chromatography[1,2] and high-performance liquid chromatography (HPLC).[1–5] Although the discriminating power of these techniques is well suited for those cases where the optical and/or chromatographic behaviors of dyes from a questioned and a known source are different, their selectivity falls short to differentiate between two fibers that have been dyed with highly similar dyes. This is not an uncommon situation, as there are many hundreds of commercial dyes with indistinguishable colors, and minimal structural variations are encouraged by the patent process and commercial competition.

For the many hundreds of dyes used in the textile industry that appear to be the same color, that have highly similar molecular structures, virtually indistinguishable ultraviolet and visible absorption spectra and identical or highly similar chromatographic retention times, a well-suited approach is the combination of liquid chromatography and mass spectrometry (LC-MS). This technique provides high discriminating power for the

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identification of textile dyes that cannot be reliably distinguished on the bases of their ultraviolet-visible absorption profile.[4,5] Unfortunately, LC-MS analysis destroys the fiber just like all the other methods that provide chemical information based on previous dye extraction. Of the nondestructive techniques currently available for comparing dyes in textile fibers only Fourier transform infrared spectrometry and laser Raman scattering have shown some promise. However, these techniques face serious limitations in the analysis of lightly dyed fibers because of the inherently weak nature of the Raman and infrared absorption signals.[6]

This work focuses on the total fluorescence emission of fiber extracts. To the extent of our literature search, little efforts have been made to investigate the full potential of luminescence techniques for the problem at hand. Fluorescence microscopy for forensic fiber analysis has been reported,[7,8] but measurements were made with band-pass filters that take little advantage of spectral information. Recently, single fiber identification with nondestructive excitation–emission fluorescence spectroscopy combined with different chemometric techniques has been reported by our group.[9–11] This subject is of current interest, as is reported in a recent review on forensic comparison of synthetic fibers.[12]

Our approach takes room temperature fluorescence (RTF) spectroscopy to a higher level of selectivity. In addition to the contribution of the textile dye to the fluorescence spectrum of the fiber extract, we investigate the contribution of intrinsic fluorescent impurities – *i.e.* impurities imbedded into the fibers during the fabrication of the garments – as a reproducible source for fiber comparison. The accurate comparison of visually indistinguishable EEMs is best accomplished with the aid of chemometric analysis.[13] The accurate comparison of EEMs requires an algorithm to determine the number of fluorescent components that contribute to the data set of excitation and emission spectra and the emission and excitation profiles corresponding to each component. Among the algorithms that exist to compare almost identical EEMs, we chose second order multivariate curve resolution alternating least squares (MCR-ALS). This multi-way algorithm has been extensively discussed in the literature.[13–15]

Experimental

Chemicals and supplies

Fabric and dyed cloths were purchased from Testfabrics Inc., West Pittston, PA, United States. All fiber cloths were received in sealed packages. All cloths were kept as received in the dark to avoid environmental exposure. All Sigma-Aldrich dyes were purchased in reagent grade purity: Disperse Red 4, Basic Green 4, Acid Red 151, and Acid Yellow 17 and 23. All solvents used for these studies were of HPLC grade and were purchased from Fisher Scientific. Nanopure water was used throughout and obtained from a Barnstead Nanopure Infinity water purifier. Glass culture tubes were purchased from Fisher Scientific.

Solvent extraction of textile fibers

Fibers were individually pulled from cloths using tweezers. Each fiber was cut into a strand of appropriate length (4 cm, 2 cm or 5 mm) using scissors or razor blades. Tweezers, scissors and razor blades were previously cleaned with methanol and visually examined under ultraviolet light (254 nm) to prevent the presence of fluorescence contamination. Each 4 cm or 2 cm strand was cut into pieces of approximately 5 mm in length and the 5 mm strands were used as such.

The fibers were solvent extracted following the procedure recommended by the Federal Bureau of Investigations (FBI).[16] All pieces from one fiber were placed in a 6 × 50 mm glass culture tube. 200 μL of extracting solvent were added to each tube. The tubes were sealed by melting with a propane torch. Sealed tubes were placed in an oven at 100 °C for one hour. Tubes were removed from the oven, scored and broken open. The solvent was removed with a micro-pipette and placed in a plastic vial for storage.

Ultraviolet and visible absorption spectroscopy

Absorbance measurements were made with a single-beam spectrophotometer (model Cary 50, Varian) equipped with a 75 W pulsed xenon lamp, 20 nm fixed band-pass, and 24 000 nm min^{-1} maximum scan rate. Absorption measurements were made with micro-quartz cuvettes (1 cm path length × 2 mm width) that held a maximum volume of 700 μL.

Fluorescence spectroscopy

Excitation and fluorescence spectra were recorded using a commercial spectrofluorometer (FluoroMax-P from Horiba Jobin-Yvon) equipped with a continuous 100 W pulsed xenon lamp with broadband illumination from 200 to 2000 nm. Excitation and fluorescence spectra were recorded with two spectrometers holding the same reciprocal linear dispersion (4.2 nm mm^{-1}) and accuracy (±0.5 nm with 0.3 nm resolution). Both diffraction gratings had the same number of grooves per unit length (1200 grooves per mm) and were blazed at 330 nm (excitation) and 500 nm (emission). A photomultiplier tube (Hamamatsu, model R928) with spectral response from 185 to 650 nm was used for fluorescence detection operating at room temperature in the photon-counting mode. Commercial software (DataMax) was used to computer-control the instrument. Measurements were made by pouring un-degassed liquid solutions into micro-quartz cuvettes (1 cm path length × 2 mm width) that held a maximum volume of 400 μL. Fluorescence was collected at 90° from excitation using appropriate cutoff filters to reject straight-light and second order emission. EEMs from fiber extracts were collected at 5 nm excitation steps from longer to shorter wavelengths to reduce the risk of potential photo-degradation due to extensive sample excitation. The same procedures were used for blank samples to account for fluorescence background subtraction.

HPLC analysis

Dye standards and fiber extracts were analyzed using a computer-controlled HPLC system from Hitachi (San Jose, CA, USA) equipped with the following basic components: a gradient pump (L-7100), a UV (L-7400 UV) and a fluorescence (L-7485) detector, an online degasser (L-761) and a control interface

(D-7000). All HPLC operations were computer controlled with Hitachi software. Separation was carried out on an Agilent (Santa Clara, CA, USA) Zorbax EclipseXDB-C18 column with the following characteristics: 15 cm length, 2.1 mm diameter, and 5 μm average particle diameters, using ethanol as the mobile phase. All extracts and standards were injected at a volume of 20 μL using a fixed-volume injection loop. HPLC fractions were collected in 2 mL sample vials with the aid of a Gilson fraction collector (model FC 20313). A minimum of three chromatographic runs were carried out per fiber extract. Each chromatographic run lasted a maximum of 40 minutes using a 1.5 mL min^{-1} flow rate and a water/methanol mobile phase. Separation was best achieved under the following gradient mobile phase conditions: 0–15 min = 70/30 water–methanol (v/v) to 30/70 water–methanol (v/v); 15–40 min: 30/70 water–methanol (v/v) to 100% methanol. The absorption, excitation and emission wavelengths selected for HPLC detection corresponded to the maximum wavelengths of the fiber extracts.

Chemometric analysis

All chemometric calculations were done using MATLAB 7.0. Routines for MCR-ALS were available in the internet thanks to Tauler.[17] A useful MATLAB graphical interface was used for easy data manipulation and graphics presentation.[18,19] This interface provided a simple means of loading the data matrices into the MATLAB working space before running MCR-ALS.

Results and discussion

Reproducibility of spectral profiles

Previous reports on fiber analysis *via* ultraviolet-visible absorption spectrometry, thin-layer chromatography and high-performance liquid chromatography (HPLC) often recommend one of the following solvents for extracting dyes from fibers: 1 : 1 methanol–water (v/v), ethanol, 1 : 1 acetonitrile–water (v/v) and 57% pyridine–43% water (v/v).[3,4,16,20] Each type of fiber was then extracted with the four types of solvents to select an appropriate solvent for RTF spectroscopy. Consequently, based on the above mentioned previous reports and on the strong fluorescence signals we consistently observed from all its fiber extracts, a 1 : 1 acetonitrile : water (v/v) mixture or ethanol was chosen for the studies. Considering reproducible spectra as an essential characteristic for forensic fiber comparison, our next goal was to investigate the fluorescence spectral profiles of extracts obtained from single fibers belonging to the same piece of cloth. Two types of experiments were conducted to achieve the following goals: (a) spectral profiles were recorded from individual extracts belonging to adjacent fibers – *i.e.* single fibers located immediately next to each other – to investigate the reproducibility within the same area of cloth; and (b) single fibers located in four different areas of the cloth were extracted and the spectral profiles were recorded to investigate their reproducibility within the entire cloth. The four areas of cloth we arbitrarily chose were designated as top middle (TM), top corner (TC), bottom middle (BM) and bottom corner (BC).

Fig. 1 compares the excitation and fluorescence spectra of fiber extracts collected from three single fibers of Disperse Red 4 located within the same area of cloth. The extracted fibers were right next to each other so that the first fiber was lying alongside (touching) the second fiber which was alongside the third fiber. Their spectral profiles are clearly reproducible with only a slight variation in intensity. The same behavior was observed for all types of cloths. Fig. 1 illustrates the outstanding spectral reproducibility of single fiber extracts taken from any area across the cloth. Other than a slight difference in intensity, which was within the reproducibility of measurements of the instrumental response, the spectral profiles are virtually the same. For all types of investigated fibers, Disperse Red 4, Basic Green 4, Acid Red 151, and Acid Yellow 17 and 23, the spectral profiles recorded from fibers collected from different areas of cloth were also extremely reproducible.

High performance liquid chromatography and excitation–emission matrices of fiber extracts

Extracts from fibers pre-dyed with Disperse Red 4 showed strong fluorescence in both the ultraviolet and visible spectral regions. Basic Green 4 fiber extracts showed fluorescence solely in the ultraviolet region with spectral profiles virtually identical to those observed from their respective Sigma-Aldrich dye. Extracts from fibers pre-dyed with Acid Red 151 presented no fluorescence in the visible spectral region and multiple fluorescence peaks in the ultraviolet region. Acid Yellow 17 and 23 were selected to investigate the discrimination of RTF spectroscopy when handling visually indistinguishable fibers.

The investigation of the reproducibility of individual components in fiber extracts across the same piece of garment followed the same strategy as before. Adjacent fibers were collected from four different areas of the same piece of cloth, namely top corner (TC), bottom corner (BC), top middle

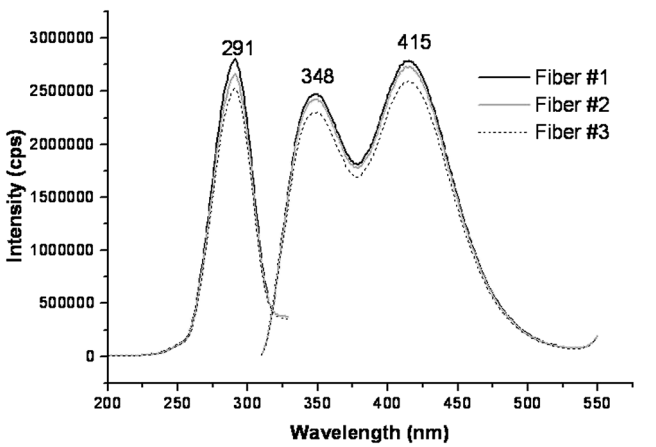

Fig. 1 Excitation and fluorescence spectra of 1 : 1 acetonitrile–water extracts taken from fibers of a polyester cloth garment pre-dyed with Disperse Red 4. Each spectrum corresponds to an extract from a single fiber. All fibers were adjacent to each other and located within the same area of cloth.

(TM) and bottom middle (BM). A minimum of three chromatographic runs were carried out per fiber extract and per dye standard solution. Fig. 2 compares the chromatograms of four fibers pre-dyed with Acid Red 151 and of the corresponding dye standard solution. The retention time of each chromatographic peak represents the average of three chromatographic runs of the same extract. The typical relative standard deviations of retention times were no larger than 5%.

The agreement among the retention times and the fluorescence intensities of the peaks in the four chromatograms in Fig. 2A demonstrates the reproducible distribution of the fluorescent impurities within fibers of the same piece of cloth. The absorption wavelength (519 nm) selected for detection in Fig. 2B was the maximum visible absorption wavelength of the Aldrich-Sigma dye. The agreement between the two retention times in the absorption chromatograms confirms the assignment of the peak in Fig. 2B to the presence of the dye in the fiber extract. Interesting to note is the less intense absorption peaks of all the extracts when compared to their respective standards. The lower intensities reveal dye concentrations in the extracts below 10 ppm, *i.e.* the concentrations of dyes in the standard solutions.

The excitation (305 nm) and emission (431 nm) wavelengths selected for the fluorescence detection in Fig. 2C correspond to the maximum excitation and emission wavelengths of the fiber extracts. The comparison of the fluorescence chromatograms of the fiber extract and of the dye in Fig. 2C clearly shows the presence of fluorescent impurities in the fiber extract and the absence of the same fluorescent impurities in the dye standard. Similar results were found for Disperse Red 4 and Basic Green 4 fiber extracts and their respective Sigma-Aldrich dye standards.

The fluorescence chromatogram of extracts from fibers pre-dyed with Acid Yellow 23 shows a number of fluorescent components and retention times that closely resemble those of Acid Red 151. It would be reasonable to assume that the similar fluorescence spectral profiles of these two extracts are due to the fluorescent components with similar elution times.

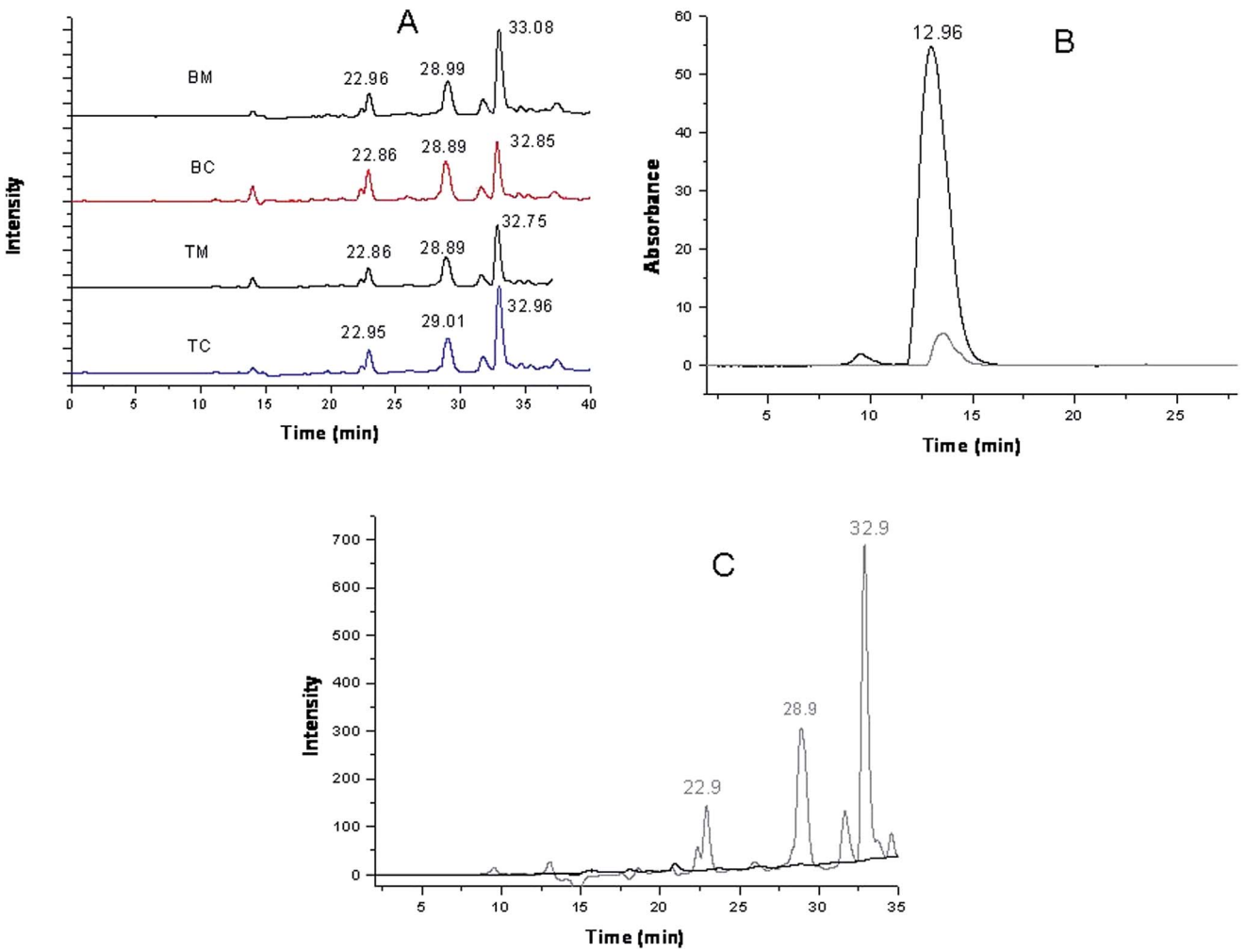

Fig. 2 HPLC chromatograms of Acid Red 151 standards and fiber extracts. (A) Fluorescence chromatograms of extracts from fibers taken from four different areas of a cloth (bottom middle, bottom corner, top middle, top corner). (B) Absorbance chromatograms of (–) standard (–) fiber extract. (C) Fluorescence chromatograms of (–) standard (–) fiber extract. Absorbance detector set = 519 nm, excitation set at 305 nm and emission set at 431 nm.

Fiber and EEM collection for MCR-ALS analysis

Large amounts of textile materials are produced each year in replicate fiber types and colors. There are cases where it is not possible to discriminate between two fibers that have been dyed with highly similar dyes. This is not an uncommon situation. Many hundreds of commercial dyes with indistinguishable colors exist as a result of minimal structural variations encouraged by the patent process and commercial competition. Acid Yellow 17 (AY17) and 23 (AY23) fall into this category. When used to color nylon 361 (N361) fibers, these dyes provide visually indistinguishable fibers. Ten single fibers of each type of material were individually extracted with ethanol and an EEM was recorded from each extract. Reproducible EEM contours were obtained for the ten fibers of each type of material. Fig. 3 provides typical EEMs recorded from the two types of fiber extracts. Although the EEMs of their extracts present noticeable contour differences, their statistical comparison within a certain confidence level should provide forensic analysis with a more robust tool for fiber discrimination. In this context, the potential of MCR-ALS for the comparison of room temperature fluorescence EEMs recorded from fiber extracts has been investigated.

All MCR-ALS comparisons were made among EEMs recorded from ethanol extracts of fibers which were visually indistinguishable. EEMs were recorded from extracts of nylon 361 fibers pre-dyed with Acid Red 151, and Acid Yellow 17 and 23.

Ten Acid Yellow 17 fibers were collected from one piece of cloth. The same was true for the ten Acid Yellow 23 fibers. Their statistical comparison allowed us to test the ability of MCR-ALS to differentiate visually indistinguishable fibers pre-dyed with two different dyes.

Acid Red 151 fibers were collected from two different pieces of cloths, with ten fibers per cloth. Their statistical comparison allowed us to test the ability of MCR-ALS to differentiate

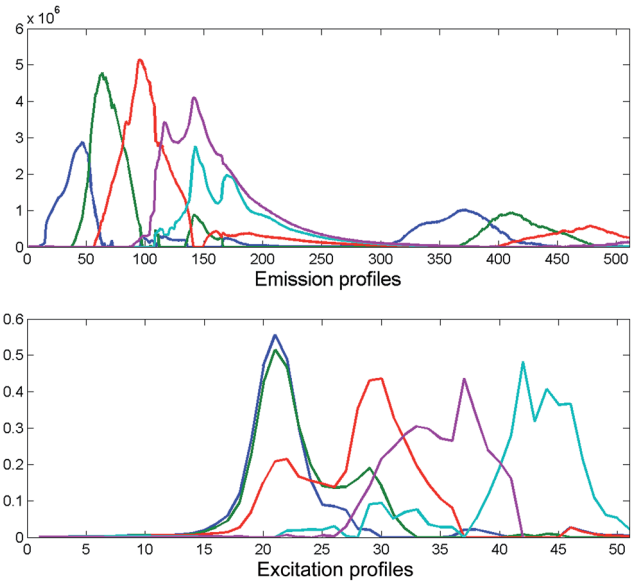

Fig. 4 Extracted emission (top) and excitation (bottom) profiles taken from the EEM of extracts from nylon fibers dyed with Acid Yellow 23.

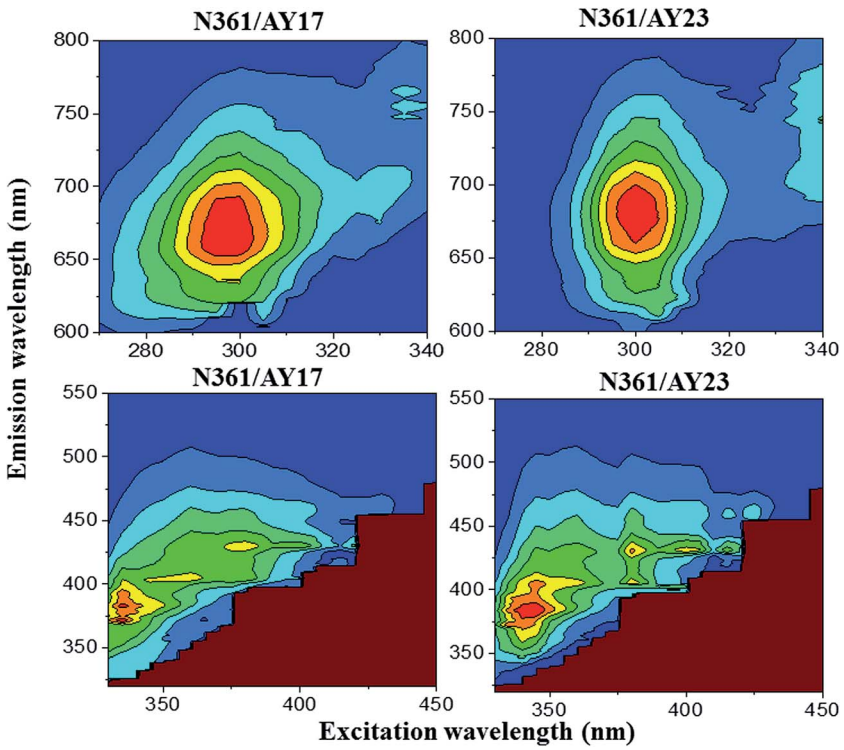

Fig. 3 EEMs of nylon 361 (N361) cloths pre-dyed with Acid Yellow 17 (AY17) and 23 (AY23) fiber extracts recorded at two different excitation/emission wavelength ranges.

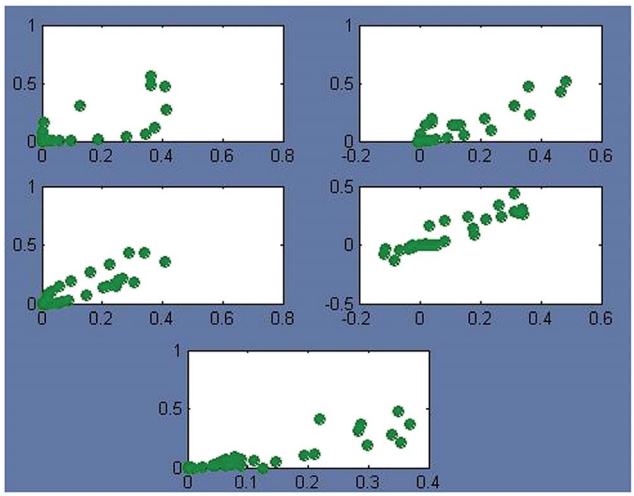

Fig. 5 Correlation of five excitation profiles extracted from the EEM of extracts collected from nylon fibers dyed with Acid Yellow 17 and 23. Correlation is performed by comparing intensities of the excitation profiles from each component *versus* wavelength. Five correlation coefficients are as follows: top left – 0.7562; top right – 0.9186; middle left – 0.8875; middle right – 0.9301, and bottom – 0.8956.

between two visually indistinguishable fibers pre-dyed with the same dye in the same textile industry but from different cloths.

MCR-ALS analysis of EEMs recorded from fiber extracts Acid Yellow 17 and 23 provided five fluorescent components per extract. The predicted excitation and fluorescence spectra of Acid Yellow 23 are shown in Fig. 4. Although the number of fluorescent components in both types of extracts is the same, the spectral profiles of the individual components of Acid Yellow 17 are considerably different to those observed for the individual components in Acid Yellow 23. Visual inspection of the excitation and fluorescence profiles confirms these correlations. Interesting to note is the good agreement between the number of fluorescent components in the EEM of Acid Yellow 23 and the number of fluorescence peaks in the chromatogram of the same type of extract.

The excitation and fluorescence spectra of the five fluorescent components in each type of fiber extract were correlated[21] (Fig. 5 and 6). The five correlations were made comparing the spectral intensities of the corresponding components in each type of extract at each excitation and fluorescence wavelength. From the calculated values of the correlation coefficients, it becomes readily apparent that only three of the five components exhibit similar spectral profiles. Close comparison of the five pairs of excitation and fluorescence spectra supports correlation coefficients close to unity for three predicted components. Based on the prediction that two components only exist in one type of fiber extract, MCR-ALS is able to discriminate among these two types of visually indistinguishable fibers.

Fig. 7 and 8 show the correlation among the intensities of the excitation and fluorescence profiles of the five components found in the EEM recorded from the two sets of Acid Red 151 fiber extracts. Close examination of correlation coefficient values in Fig. 7 reveals strong similarity of excitation profiles among four of the five fluorescent components. Three of the five correlation coefficients in Fig. 8 show strong similarity of fluorescence spectra among three of the five fluorescent components. Visual inspection of the excitation and fluorescence profiles confirms these correlations. Considering the number of similar excitation (4) and fluorescence (3) profiles it is safe to assume that three of the five fluorescent components are present in both sets of fibers. Two of the remaining four components are present in only one set of fibers. Based on the prediction that two components only exist in one type of fiber

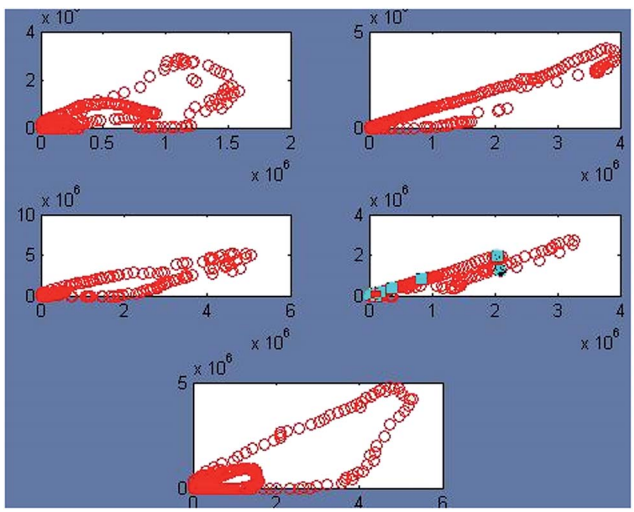

Fig. 6 Correlation of five emission profiles extracted from the EEM of extracts taken from nylon fibers dyed with Acid Yellow 17 and 23. Correlation is performed by comparing intensities of excitation profiles *versus* wavelength. Five correlation coefficients are as follows: top left – 0.7564; top right – 0.9696; middle left – 0.9480; middle right – 0.9677, and bottom – 0.8300.

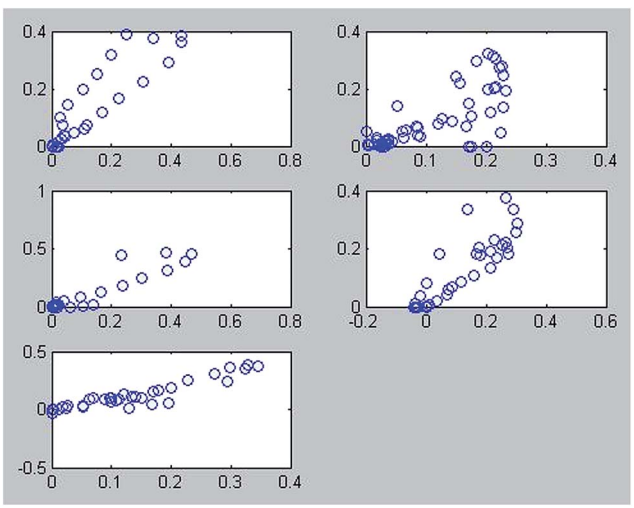

Fig. 7 Correlation of five excitation profiles extracted from the EEM of extracts taken from nylon fibers of two different cloths dyed with Acid Red 151. Correlation is performed by comparing intensities of excitation profiles *versus* wavelength. Five correlation coefficients are as follows: top left – 0.93; top right – 0.77; middle left – 0.94; middle right – 0.91, and bottom – 0.95.

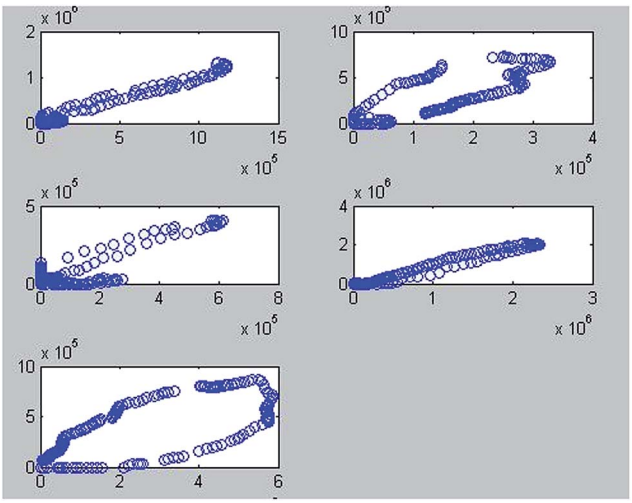

Fig. 8 Correlation of five emission profiles extracted from the EEM of extracts taken from nylon fibers of two different cloths dyed with Acid Red 151. Correlation is performed by comparing intensities of excitation profiles *versus* wavelength. Five correlation coefficients are as follows: top left – 0.99; top right – 0.85; middle left – 0.88; middle right – 0.99, and bottom – 0.77.

extract, MCR-ALS is able to discriminate among Acid Red 151 extracts of fibers collected from two different cloths.

Conclusions

The separation of fiber extracts *via* HPLC provides valuable information on the contribution of individual components to the total fluorescence of fibers. The comparison of chromatograms from the extracts of fibers collected from different areas of a cloth confirmed the reproducibility of individual fluorescent impurities within the same piece of cloth. The reproducibility of individual impurities agrees well with the fluorescence reproducibility of extracts from the fibers of the same cloth. The HPLC analysis of Aldrich-Sigma dyes provided fluorescence chromatograms with different components than those observed in their respective extracts. The observed differences can be attributed to possible variations in the chemical compositions of the standard (Sigma-Aldrich) and the dye reagent (Testfabric) and/or to the presence of fluorescent impurities in the fiber extracts adsorbed in the fabrication of Testfabric cloths. The chromatographic discrepancy among extracts and their respective dyes provided valuable information to select EEM as the best spectral format for fiber discrimination.

MCR-ALS analysis of EEM taken from the extracts of nylon fibers pre-dyed with Acid Yellow 17 and 23 predicted five fluorescent components in each type of extract. From differences in the calculated correlation coefficients of two fluorescent components, we were able to discriminate between these two types of visually indistinguishable fibers. MCR-ALS analysis of EEM taken from extracts of nylon fibers pre-dyed with Acid Red 151 also made it possible to discriminate among two visually indistinguishable fibers pre-dyed with the same dye in the same textile industry but from different cloths. Although additional studies should be made with a larger number and types of visually indistinguishable fibers, the results presented here provide the foundation to propose the combination of excitation–emission fluorescence and MCR-ALS as a promising tool for the forensic analysis of textile fibers.

Acknowledgements

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