Room Temperature Fluorescence Spectroscopy As A Tool For The Forensic Trace Analysis Of Textile Fibers

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ROOM TEMPERATURE FLUORESCENCE SPECTROSCOPY AS A TOOL FOR THE FORENSIC TRACE ANALYSIS OF TEXTILE FIBERS

by

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B.S. Bemidji State University, 2001

A dissertation submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in the Department of Chemistry in the College of Sciences at the University of Central Florida Orlando, Florida

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ABSTRACT

Trace textile fiber evidence is found at numerous crime scenes and plays an important role in linking a suspect to the respective scene. Several methods currently exist for the analysis of trace fiber evidence. Microscopy provides information regarding the fibers material, color and weave. For more detailed chemical analysis chromatographic methods are employed and for discrimination between dyes, liquid chromatography coupled with mass spectrometry (LC-MS) is currently the method providing the most discrimination. These methods have primarily focused on the dyes used to color the fibers and have not investigated other components that can potentially discriminate among fibers.

This dissertation deals with investigations into the fluorescence of the fiber dyes, (contaminants?) and the fibers themselves, as well as methodology for discriminating between fibers using fluorescence. 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 give detailed information on the number of dye and fluorescent components present in extracts.

Our investigations then focused on the best room-temperature fluorescence (RTF) data format for analysis and discrimination of fiber samples. An excitation emission matrix (EEM) was 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 Chemometric analysis. The level of discrimination obtained via RTF-EEM
spectroscopy was sufficient to differentiate among fibers obtained from two separate cloths of the same material and colored with the same dye reagent.

Final studies deal with examining exposure of the fiber to various environmental contaminants. Clothing fibers are typically exposed to myriad numbers of contaminants, from food stains to cigarette smoke. The challenge then becomes detecting fluorescence signals from trace amounts of these environmental contaminants. We demonstrate the detection and classification of polycyclic aromatic hydrocarbons (PAH) present on fibers after exposure to cigarette smoke. This dissertation also investigates the change in fluorescence emission after laundering fibers numerous times.

The main drawback of chemical analysis of fibers is the destructive nature of the methods. To extract a dye or contaminant from a fiber essentially destroys the evidence. This leaves the investigator without their original sample in the courtroom. This also provides a finite amount of sample for testing and analysis. This is true of chromatographic methods and for the method detailed in this dissertation which makes use of extracts taken from fiber samples. Lastly, we propose an instrumental setup coupling a microscope to a spectrofluorimeter for the purpose of taking EEM directly from a fiber sample. This setup makes use of the superior optics of the microscope for focusing excitation light onto the fiber sample. Initial studies have been performed on extracts from a single textile fiber and EEM collected from said fiber.
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CHAPTER 1: GENERAL INTRODUCTION

1.1 Chemical Analysis for Forensic Examination of Textile Fibers

“The wherever he steps, whatever he touches, whatever he leaves, even unconsciously, will serve as a silent witness against him. Not only his fingerprints or his footprints, but his hair, the fibers from his clothes, the glass he breaks, the tool mark he leaves, the paint he scratches, the blood or semen he deposits or collects. All of these and more, bear mute witness against him. This is evidence that does not forget. It is not confused by the excitement of the moment. It is not absent because human witnesses are. It is factual evidence. Physical evidence cannot be wrong, it cannot perjure itself, it cannot be wholly absent. Only human failure to find it, study and understand it, can diminish its value.”

The above quote is from Edmond Locard, and details the Locard exchange principle. Simply put the Locard exchange principle states that every contact leaves a trace, which is the basis of forensic trace evidence investigation. While some transfer does take place at a crime scene, it may not be detected. The amount of material transferred may in fact be too small to detect or analyze. The goal of the forensic scientist then is to develop the ability to detect and analyze smaller and smaller trace samples of evidence.

When dealing with textile fibers the number of fibers transferred is shown to depend on several factors, including: area of the surfaces in contact, number of contacts, time of contact, pressure applied during the contact, type of fiber and weave of the donor and recipient garment. There are several methods of recovering fibers from a crime scene. The first step is to consider all fiber items relating to the victim and the suspect. These are considered donor items from known sources. After initial inspection of the scene all extraneous fibers large enough to be seen by the naked eye are collected as evidence. After collecting all of these extraneous fibers, the
locations where a transfer could occur are considered and a series of tape lifts are executed to pull up fibers too small to be noticed. Fibers are removed from the tape lift by cutting a window on top of the tape lift and placing a drop of xylene on the fiber. This fiber can then be mounted under a small circular cover glass. The advantage of storing the fiber this way is safe handling, numbering and the easy removal for further testing.

Analytical techniques that can either discriminate between similar fibers or match a known to a questioned fiber are highly valuable in forensic science. Microscopy, scanning electron microscopy (SEM), 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 often possible to rule out a common source for the two samples. The main advantage of these techniques is their non-destructive nature, which preserves the physical integrity of the 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 hundred 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 identification of textile dyes that cannot be reliably distinguished on the basis 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. \[6\]

1.2 Our Proposition

This dissertation tackles a different aspect of fiber analysis as it focuses on the total fluorescence emission of fiber extracts. To the extent of our literature search, no 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 on spectral information. Our proposition takes 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 fluorescence impurities – i.e. impurities imbedded into the fibers during the fabrication of the garments - as a reproducible source for fiber comparison.

The experimental approach we followed attempted to answering the following questions:
• Should we concentrate on the fluorescence of textile dyes, the fluorescence of intrinsic impurities or the total fluorescence of the fiber?
• Are intrinsic impurities reproducible sources of fluorescence?
• What is the best fluorescence data format for fiber discrimination?
• What instrumental approach one should use for fiber discrimination?
• How do we deal with fluorescence from environmental impurities?

1.3 Fundamental Concepts of Fluorescence and Phosphorescence Phenomena

Figure 1.1 illustrates a partial Jablonski diagram, detailing the various activation and deactivation processes for an aromatic molecule [9]. Under normal conditions, the orbitals of lowest energy of a molecule are occupied by pairs of electrons with spin in opposite directions. The resulting electron spin is zero, since most organic molecules have an even number of valence electrons. Such a state, with no net spin is called the singlet state. The ground state is the singlet state of the lowest energy and is shown in the Jablonski diagram, figure 1.1, as S0. Each electronic state has several vibrational levels, which under different conditions can represent the energy state of a molecule.

Through the absorption of electromagnetic radiation, also known as excitation, a molecule can pass from the ground state to a higher energy excited state. This process is shown in the Jablonski diagram by arrows moving upward from the S0, singlet ground state, to the S1 and S2, first and second singlet excited states, respectively. The absorption or excitation process occurs rapidly, on the order of $10^{-15}$ seconds and entails the promotion of an electron from the highest occupied orbital to a previously unoccupied one. If the transition occurs with no change in the spin of the promoted electron, the excited state will have two unpaired electrons with anti-
parallel spins and, therefore, no net spin. An electronic state with these characteristics is known as a singlet excited state. If the transition involves a change in electronic spin, the excited state will be characterized by two unpaired electrons with parallel spins. In this case, the net spin is one, and this excited state is known as the triplet state. In the Jablonski diagram, the triplet state of lowest energy is shown as T₁, while higher triplet states would be shown as Tₙ.

After excitation to S₂, a molecule typically releases excess of vibrational energy to reach the lowest vibrational level of the excited state. This radiationless deactivation process, which is known as vibrational relaxation (vr), results from thermal energy transfer to the surrounding medium by the excited molecule. The process of vibrational relaxation (vr) involves vibrational levels of the same electronic state and takes on the order of 10⁻¹³ seconds or less [10-11].

After reaching the lowest vibrational level of the S₂ excited state, the excited molecule might go into the highest vibrational level of the S₁ excited state via internal conversion (ic). The efficiency of this process is determined by the similarity in energy of the ground vibrational level of the S₂ state and the upper vibrational level of the S₁ state. This process is the result of the transformation of exciting energy into vibrational-rotational energy and occurs between electronic states of the same multiplicity. Internal conversion (ic) occurs on the order of 10⁻¹² seconds. The lowest vibrational level of S₁ is then reached by vibrational relaxation. Once in the S₁ state the molecule may deactivate through several processes. One possibility is external conversion (ec), a non-radiative deactivation process in which the excited molecule transfers its excess electronic energy to surrounding molecules or solvent molecules through collisions. Since this deactivation process is collision based, both, cooling or isolating the sample will reduce contributions from external conversion (ec) to the deactivation of S₁. From the lowest vibrational level of S₁, the molecule has two ways of directly returning to the ground state,
through internal conversion, without the emission of radiation, or by the emission of a photon with no change in the electronic spin. The latter process is responsible for the emission of fluorescence and occurs in a period to time between $\sim 10^{-6}$ to $\sim 10^{-8}$ seconds. The energy of the emitted photon corresponds to the energy gap between the lowest vibrational level of $S_1$ and the ground state ($S_0$). Fluorescence from the $S_2$ state will only occur if the energy gap between the $S_1$ and $S_2$ states is too large to favor internal conversion ($ic$). When the lowest vibrational level of the $S_1$ state overlaps with $S_0$, the excited state is deactivated by non-radiative relaxation and the emission of fluorescence from $S_1$ does not occur.

A process termed resonance fluorescence is observed when the emitted photon has the same energy as the one initially absorbed. More often, however, the energy loss in vibrational relaxation and internal conversion results in the emission of fluorescence at wavelengths longer than the excitation wavelength, and resonance fluorescence is not observed.

![Figure 1.1. Jablonski Diagram](image)

Figure 1.1. Jablonski Diagram
The remaining possibility for returning to the S\(_0\) state from S\(_1\) begins with a process called intersystem crossing (isc). Intersystem crossing is a radiationless mechanism involving systems of different multiplicity which requires a change in the electronic spin. Although this kind of transition has a much lower probability to occur than spin allowed transitions, the time scale of intersystem crossing is similar to the one for fluorescence (10\(^{-8}\) to 10\(^{-7}\) seconds) and, therefore, it competes with fluorescence for the deactivation of the S\(_1\) excited state. From S\(_1\), the molecule can then pass to the excited triplet state (Tn) and - by a series of \(vr\) and \(ic\) processes - will reach the lowest vibrational level of T\(_1\). From T\(_1\), and through \(isc\), the molecule can revert back to the excited singlet state. Since the triplet states have lower energy than the corresponding singlet states, the transition from T\(_1\) to S\(_1\) requires some additional activation energy. This activation energy can be obtained either by a thermal process or by the interaction of two molecules in the triplet state to produce one molecule in the excited singlet state. When the molecule returns to S\(_0\) by the emission of a photon, an identical spectrum to the one of conventional fluorescence is obtained. This process, which is not represented in the Jablonski diagram, is known as delayed fluorescence and has a lifetime longer than 10\(^{-8}\) seconds [9-11].

If reverse \(isc\) does not occur, the molecule has two other possibilities to return from T\(_1\) to S\(_0\). Through \(isc\) followed by \(vr\), or through the emission of radiation in a process known as phosphorescence (P). The emission of phosphorescence involves states of different multiplicity and, as a consequence, has a longer lifetime than fluorescence (between 10\(^{-3}\) to 10 seconds). Since the energy gap between T\(_1\) and S\(_0\) is usually smaller than the one between S\(_1\) and S\(_0\), phosphorescence occurs in a region of lower energy than fluorescence.

Radiative processes, such as fluorescence, are always in competition with the various non-radiative deactivation processes. The fluorescence intensity will depend upon the relative
efficiencies of all competing processes. The efficiency of fluorescence is most often expressed in terms of quantum yield ($\phi_F$). The ratio of the rate of fluorescence (photons emitted/second) with the rate of absorption (photons absorbed/second) defines fluorescence quantum yield, $\frac{\Phi_F}{\Phi_A}$, where $\Phi_F = k_F n_{S1} V$ and $\Phi_A = k_A n_{S0} V$. $V$ is the volume of the sample illuminated; $n_{Sx}$ is the number of molecules occupying the given electronic state $x$; and $k_F$ and $k_A$ in emitting photons per seconds and photons absorbed per second, are the rate of fluorescence and absorption, respectively. Under steady state conditions, we can assume that $n_{S1} = n_{S0} k_A / (k_F + k_{nr})$, where $k_{nr}$ is the sum of rates for the non-radiative processes (external conversion, $k_{ec}$; internal conversion, $k_{ic}$; and inter system crossing, $k_{isc}$). Using these relationships, the fluorescence quantum yield in terms of rates of the various activation and deactivation processes is given by equation (1.1):

$$\phi_F = \frac{k_F}{k_F + k_{nr}}$$

(1.1)

which shows that, in order to improve the fluorescence quantum yield, and directly the fluorescence intensity, one needs to minimize the rate contributions from non-radiative processes.

**1.4 Excitation-Emission Matrixes**

Among the numerous photoluminescence techniques in the analytical field, RTF spectroscopy is the most popular. RTF offers the advantages of relatively inexpensive instrumentation, calibration curves with linear dynamic ranges extending over 2 to 3 orders of magnitude and limits of detection that often allow to reaching trace concentration levels. In most cases de-oxygenation of the sample is not critical and fluorescence measurements are rapidly performed by placing a quartz cell in the sample compartment of a spectrofluorimeter.
Conventional fluorimetric methods - in which either the excitation or the emission wavelength is set at its maximum position while the other is scanned - present limited selectivity for the analysis of single fluorescent components in fluorescence mixtures. The spectral overlapping among broad room-temperature excitation and fluorescence bands makes difficult to characterize single components on the basis of wavelength assignments. Several strategies exist to improve the selectivity of RTF measurements. These include temporal (lifetime) resolution, EEM and synchronous excitation. Although EEM have been extensively applied to environmental [12] and drug analysis [13] our literature search revealed no applications to the forensic analysis of textile fibers or their extracts.

Figure 1.2 illustrates the process involved in the collection of RTF-EEM. The spectrofluorimeter to record steady-state EEM typically consists of a continuous wave excitation source, excitation and an emission monochromators and a photomultiplier tube. The resulting I by J data matrix (EEM) is collected from an array of two-dimensional fluorescence spectra.
(intensity versus wavelength) while the excitation wavelength is increased incrementally between each scan [14,15]. Each I row in the EEM corresponds to the emission spectrum at the \(i\)th excitation wavelength. Each J column in the EEM corresponds to the excitation spectrum at the \(j\)th emission wavelength [15]. For a single emitting species in a sample, the elements of the EEM are given by:

\[
M_{ij} = 2.303 \Phi F I_0(\lambda_i) \varepsilon(\lambda_i) bc \gamma(\lambda_j) \kappa(\lambda_j) \tag{1.2}
\]

where \(I_0(\lambda_i)\) is the intensity of the incident light exciting the sample in units of quanta/s; \(2.303 \varepsilon(\lambda_i) bc\) represents the optical density of the sample, which results from the product of the analyte’s molar extinction coefficient \(\varepsilon(\lambda_i)\), the optical path-length \(b\), and the concentration of the emitting species \(c\); \(\Phi F\) is the quantum yield of fluorescence; \(\gamma(\lambda_j)\) is the fraction of fluorescence photons emitted at wavelength \(\lambda_j\); and \(\kappa(\lambda_j)\) shows the wavelength dependence of the sensitivity of the analyzing system of the analyzing system, i.e. quantum efficiency of the detector, monochromator’s grating, etc [15]. Equation 1.2 is based on the assumption that the optical densities of the analytes \(i\) are low enough so that the condition \(2.303(i) bc \ll 1\) is satisfied for all \(\lambda_i\). The condensed versions of equation 1.2 may be expressed as:

\[
M_{ij} = a x_i y_j \tag{1.3}
\]

where \(a = 2.303 \Phi F bc\) is a wavelength independent factor containing all of the concentration dependence, \(x_i = I_0(\lambda_i) \varepsilon(\lambda_i)\) and \(y_i = \gamma(\lambda_j) \kappa(\lambda_j)\).

The observed relative fluorescence excitation spectrum may be represented by \{\(x_i\}\}, the wavelength sequenced set, and thought of as a column vector, \(x\) in \(\lambda_i\) space. The wavelength sequenced set, \{\(y_i\)\}, may be thought of as a row vector \(y\) in \(\lambda_i\) space, representing the observed fluorescence emission spectrum. Therefore, for a single component, \(M\) is simply represented as:

\[
M = axy \tag{1.4}
\]
Where $M$ is the product of the vectors $x$ and $y$ multiplied by the compound specific parameter $\alpha$. Equations 1.2 thru 1.4 require that the fluorescence spectrum is independent of the excitation wavelength, and that the excitation spectrum is independent of the emission wavelength. Dilute solutions of molecules containing only one fluorescent chromophore have almost universally displayed these conditions.

When data is taken from a sample containing multiple, $r$, different species, $M$ is given by the following expression:

$$M = \sum_{k=1}^{r} \alpha_k x^k y^k$$

(1.5)

where $k$ is used to detail the species. For the observed $M$ the analysis of the data then relies on finding $r$, $\alpha_k$, $x^k$, and $y^k$.

1.5. Chemometrics

The accurate comparison of visually indistinguishable EEM is best accomplished with the aid of Chemometric analysis. [16] The accurate comparison of EEM requires the algorithm to determine the number of fluorescence components that contribute to the data set of excitation and emission spectra and the emission and excitation profile corresponding to each component. Among the algorithms that exist to compare almost identical EEM, we chose parallel factor analysis (PARAFAC) and multivariate curve resolution alternating least squares (MCR-ALS). These two algorithms have been extensively discussed in the literature [16-19]. The main difference between the two resides on the procedures to determine the number of fluorescent components (factors) and the algorithms that extract the emission and excitation profile of each fluorescent component.
When using PARAFAC to analyze multiple EEM taken from several samples, the EEM are stacked to create an EEM cube. This EEM cube is created with the three dimensions being excitation and emission wavelengths and the number of samples, see figure 1.3. Before PARAFAC algorithms can extract fluorescence spectra and concentrations from this cube, the number of fluorescent components must be determined. The number of components is determined by running a core consistency diagnostic. Two different algorithms are used upon the PARAFAC core and compared; the agreement between these two algorithms is then checked and reported as a percentage. Each time the algorithms are run, the number of estimated fluorescent components must be selected. This process starts the number of components at one and increases sequentially by one for each subsequent comparison, i.e. one component, two components, and so on. One additional component to the maximum correct number of components will cause the agreement to fall dramatically below 100%. An example would be a core consistency score of 100% for a number of components varying from 1 to 5, but a score of 65% for six components [20]. The correct number of components to use is always the maximum number. In the previous example, the correct number would then be five.

PARAFAC is a tri-linear model of component (factor) analysis, meaning it analyzes the number of factors present and uses algorithms to extract information from a three dimensional matrix or array, in this case: the excitation wavelengths, emission wavelengths and concentrations. Equation 1.6 is used for PARAFAC analysis and is described below.

\[ X_{ijk} = \sum_{r=1}^{R} a_{ir} b_{jr} c_{kr} \]  

(1.6)

Where \( X_{ijk} \) represents the EEM cube and is a three way array with dimensions \( i \) (# of samples), \( j \) (emission wavelengths), and \( k \) (excitation wavelengths). The values of \( a_{ir} \) are the relative
concentration (intensity) of analyte r in sample i. The J vector \( b_r \) with elements \( b_{jr} \) is the estimated emission spectrum of the analyte and \( c_{kr} \) is the estimated excitation spectrum [18,21,22]. Once the number of fluorescent components is determined, equation 1.6 is used to decompose the EEM cube. This decomposition extracts the excitation \( (c_{kr}) \) and emission spectra \( (b_{jr}) \) for each factor as well as the concentration \( (a_{ir}) \).

Figure 1.3 Diagram of EEM corresponding to the number of samples and tri-linear PARAFAC model. Each matrix represents one dimension of the cube containing R factors.

After determining the number of fluorescent components present in an EEM and estimating the emission and excitation spectra for each component, individual spectra from one EEM may now be compared to those of a different EEM. The shape and intensity (concentration) of each emission and excitation spectra may be correlated between data sets to determine the statistical similarity between EEM.

Figure 1.4 shows a schematic diagram of the steps involved in MCR-ALS. The first step is to organize each data set of excitation and emission spectra into an EEM (bilinear matrix).
The number of factors (or components) present in the mixture is then determined through principle component analysis (PCA) or a similar method such as singular value decomposition (SVD) [16,17,22]. After determining the number of factors, MCR-ALS decomposes a bilinear matrix into excitation and emission profiles for all fluorescent components in the matrix. In the matrix form, this bilinear data matrix of excitation and emission profiles is expressed as:

\[ D = C S^T + E \]  

Where \( D(I,J) \) is the matrix of experimental data of dimensions I samples by J wavelengths. \( C(I,K) \) is the matrix of emission profiles of the different K analytes; \( S^T(K,J) \) is the excitation profiles of K analytes at J wavelengths, \( E(I,J) \) is the matrix associated to the experimental error [22,23].

Several programs exist which make use of MCR-ALS algorithms to decompose these data matrices. The program developed by R. Tauler on the MCR-ALS homepage [22] is the one we will use for our analyses. With this program one may select the number of factors and the number of EEM. The number of EEM could be one or higher. Whereas PARAFAC is able to decompose a tri-linear matrix (utilizing not only excitation and emission, but also number of samples) MCR-ALS is only able to decompose a bilinear matrix. If more than one EEM (number of samples) is used, an augmented bilinear data matrix is created to accommodate the third dimension of data. An augmented matrix is used to represent the coefficients and the solution vector of each equation set (see figure 1.5). In this instance the augmented data matrix would take into account the number of EEM that were recorded. The augmented matrix can then be decomposed either by rows (excitation) or by columns (emission). If decomposed by columns MCR-ALS would yield sets of emission profiles for each factor (one set for each EEM) and one set of excitation profiles for each factor (or component). If the matrix is decomposed by rows,
MCR-ALS would yield one set of emission profiles for each factor and sets of excitation profiles (one set for each EEM) for each factor. A single set of emission or excitation profiles may then be selected for comparison or all recorded sets may be averaged to create one set of emission or excitation profiles. The intensity at each wavelength is then related to the concentration of each component in the mixture.

**Figure 1.4 Multivariate Curve Resolution-Alternating Least Squares theory.** Extracting pure component information from matrix.

\[
A = \begin{bmatrix}
1 & 3 & 2 \\
5 & 2 & 2 \\
2 & 0 & 1 
\end{bmatrix}, \quad B = \begin{bmatrix}
4 & 3 & 2 \\
3 & 0 & 1 \\
1 & 2 & 2 
\end{bmatrix}
\]

Then \((A|B) = \)

\[
\begin{bmatrix}
1 & 3 & 2 & 4 & 3 & 2 \\
5 & 2 & 2 & 3 & 0 & 1 \\
2 & 0 & 1 & 1 & 2 & 2 
\end{bmatrix}
\]

**Figure 1.5 Augmented Matrix.** Details how an augmented matrix is created from two separate matrices.
CHAPTER 2: ABSORBANCE AND FLUORESCENCE INVESTIGATIONS COMPARING TEXTILE DYES AND FIBER EXTRACTS

2.1 Introduction

Careful examination of the open literature provided insufficient information for the direct application of RTF spectroscopy to the analysis of fibers. A systematic study was undertaken in our lab to better understand the fluorescence characteristics of textile fibers. The investigated fibers were selected to cover a wide range of cloth materials (acetate, polyester, cotton, and nylon) and reagent dyes commonly manufactured in the textile industry. Table 2.1 summarizes the fourteen types of fibers investigated in this study, which were purchased in the form of fiber cloths of different shapes and sizes. Appendix A shows the molecular structures of their respective dyes, which fit into one of the following four categories: direct, disperse, basic, and acid dyes. Direct dyes are those that have high affinity for cellulose materials such as cotton, rayon, etc. Disperse dyes are slightly soluble in water and typically used for synthetic fibers made of nylon, polyester, etc. Acid dyes are anionic dyes often applied from an acid dye-bath and basic dyes are cationic dyes characterized by their affinity for acrylic fiber and occasionally silk, wool, or cotton.
<table>
<thead>
<tr>
<th>Type of Fiber</th>
<th>Respective Textile Dye</th>
<th>Cloth Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate 154</td>
<td>Disperse Red 1</td>
<td>1meter x 1meter</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Disperse Red 4</td>
<td>1meter x 1meter</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Disperse Red 13</td>
<td>30cm x 30cm</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Disperse Blue 56</td>
<td>10cm x 10cm</td>
</tr>
<tr>
<td>Poly-acrylic 864</td>
<td>Basic Green 4</td>
<td>1meter x 1meter</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Basic Red 9</td>
<td>30cm x 30cm</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Basic Violet 14</td>
<td>30cm x 30cm</td>
</tr>
<tr>
<td>Cotton 400</td>
<td>Direct Blue 1</td>
<td>11cm x 15cm (100 pieces)</td>
</tr>
<tr>
<td>Cotton 460</td>
<td>Direct Blue 71</td>
<td>10cm x 10cm</td>
</tr>
<tr>
<td>Cotton 400</td>
<td>Direct Blue 90</td>
<td>11cm x 15cm (100 pieces)</td>
</tr>
<tr>
<td>Nylon 361</td>
<td>Acid Red 151</td>
<td>11cm x 15cm (100 pieces)</td>
</tr>
<tr>
<td>Nylon 361</td>
<td>Acid Yellow 17</td>
<td>30cm x 30cm</td>
</tr>
<tr>
<td>Nylon 361</td>
<td>Acid Yellow 23</td>
<td>30cm x 30cm</td>
</tr>
<tr>
<td>Nylon 361</td>
<td>Acid Green 27</td>
<td>30cm x 30cm</td>
</tr>
</tbody>
</table>

The main goals of this chapter were the following: (a) to select an appropriate extracting solvent for the RTF spectroscopy of each type of fiber; (b) to select a general extracting solvent for the RTF spectroscopy of all studied fibers; (c) to investigate the minimum fiber length that still provides an adequate fluorescence signal for RTF spectroscopy; (d) to investigate the reproducibility of spectral profiles recorded from fluorescence extracts of fibers belonging to the same piece of cloth; and (e) to investigate the reproducibility of fluorescence spectral profiles recorded from fluorescence extracts of the same types of fibers belonging to different pieces of the same type of cloth but from the same commercial batch.

2.2 Experimental

2.2.1. Chemicals and Supplies

Fabric and dyed cloths were purchased from Testfabrics Inc.; West Pittston, PA. 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 at reagent grade purity (see Table 2.2). No additional information was provided by Sigma-Aldrich on the complete chemical composition of the dye reagents.

<table>
<thead>
<tr>
<th>Dye Dye Content (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Red 1 95</td>
</tr>
<tr>
<td>Disperse Red 4 NA b</td>
</tr>
<tr>
<td>Disperse Red 13 95</td>
</tr>
<tr>
<td>Disperse Blue 56 NA b</td>
</tr>
<tr>
<td>Basic Green 4 80</td>
</tr>
<tr>
<td>Basic Red 9 85</td>
</tr>
<tr>
<td>Basic Violet 14 NA b</td>
</tr>
<tr>
<td>Direct Blue 1 NA b</td>
</tr>
<tr>
<td>Direct Blue 71 50</td>
</tr>
<tr>
<td>Direct Blue 90 NA b</td>
</tr>
<tr>
<td>Acid Red 151 40</td>
</tr>
<tr>
<td>Acid Yellow 17 60</td>
</tr>
<tr>
<td>Acid Yellow 23 90</td>
</tr>
<tr>
<td>Acid Green 27 65</td>
</tr>
</tbody>
</table>

a (%) = mass/mass x 100; b NA = not available.

All solvents used for these studies were 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.

2.2.2. Solvent Extraction of Textile Fibers

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

Fibers were solvent extracted following the procedure depicted in Figure 2.1, which is recommended by the Federal Bureau of Investigations (FBI). [24] All pieces from one fiber were
placed in a 6x50mm glass culture tube. 200μL 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.

![Figure 2.1: Solvent Extractions of Textile Fibers](image)

2.2.3. 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 (1cm path length x 2mm width) that held a maximum volume of 700μL.

2.2.4. RTF 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·mm⁻¹) and were blazed at 330nm (excitation) and 500nm (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 (1cm path length x 2mm width) that held a maximum volume of 400μL. Fluorescence was collected at 90° from excitation using appropriate cutoff filters to reject straight-light and second order emission.

2.3. Results and Discussion

2.3.1. Selecting the Best Solvent for Fiber Extraction

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). [3,4,24,25] Each type of fiber in Table 2.1 was then extracted with the four types of solvents to select an appropriate solvent for RTF spectroscopy. All measurements were performed with fibers collected from the top left corner of sample cloths, i.e. a cloth area arbitrarily selected with the sole purpose of consistency.

Appendix B compiles the excitation and fluorescence spectra of the extracts from fibers in Table 2.1 with the four types of solvent systems. All spectra represent an average of nine spectral runs recorded from three aliquots of three independent fiber extractions. The slit widths of the excitation and emission monochromators were adjusted to obtain a satisfactory
compromise between signal intensity and spectral resolution. For comparison purposes, all spectra in Appendix B were recorded with the same excitation (4nm) and emission (2nm) band-pass. Under these conditions, most of the studied fibers showed strong fluorescence in the four types of extracting solvents. For the few cases where relatively weak fluorescence was observed, further adjustment of excitation and emission band-pass was unsuccessfully attempted as weak fluorescence signals – i.e. barely distinguishable from the blanks - were still observed under 10nm excitation and emission band-passes. Under these conditions, the signal intensities of the strongly fluorescent fibers fall beyond the upper linear limit (2 x 10^6 counts) of our instrument detection unit.

The visual comparison of fluorescence spectra in Appendix B reveals two main types of spectral profiles, i.e. emission spectra with only one and with more than one fluorescent peaks. Assuming the absence of spectral overlapping and synergistic effects among fluorescence components in the fiber extract, fluorescence spectra with a single fluorescence peak suggest the emission of one fluorescence component at the recorded excitation wavelength. Still under the same assumptions but disregarding the possibility of vibronic structural resolution at room temperature, the presence of multiple emission peaks can then be attributed to the emission of multiple fluorescence components at the recorded excitation wavelength. Under this prospective, spectral comparison within the same type of fiber reveals some cases with strong dependence on the chemical nature of the extracting solvent. The differences observed in Figures 2.2 – 2.6, which compare intensity-normalized spectra recorded from the same types of fibers but extracted with different solvents, could then be attributed to the chemical affinities of the extracting solvents for fluorescence components in the textile fibers.
Figure 2.2 Fluorescence Spectra of Extracts in Two Different Solvents from Fibers Pre-Dyed with Disperse Red 4. (—) Extract in 1:1 acetonitrile water (v:v) and (—) extract in ethanol. Maximum excitation wavelength used for each extract, 291 nm and 298 nm respectively. Excitation and emission slits set for 4 nm and 2 nm band-pass, respectively.
Figure 2.3 Fluorescence Spectra of Extracts in Two Different Solvents from Fibers Pre-Dyed with Disperse Red 13. (---) Extract in 1:1 methanol/water (v:v) and (—) extract in ethanol. Maximum excitation wavelength used for each extract, 290nm and 302nm respectively. Excitation and emission slits set for 4nm and 2nm band-pass, respectively.
Figure 2.4 Fluorescence Spectra of Extracts in Two Different Solvents from Fibers Pre-Dyed with Basic Red 9. (—) Extract in ethanol and (—) extract in 1:1 acetonitrile/water (v:v). Maximum excitation wavelength used for each extract, 295nm and 298nm respectively. Excitation and emission slits set for 4nm and 2nm band-pass, respectively.
Figure 2.5 Fluorescence Spectra of Extracts in Two Different Solvents from Fibers Pre-Dyed with Direct Blue 1. (—) Extract in 1:1 methanol/water (v:v) and (—) extract in ethanol. Maximum excitation wavelength used for each extract, 303nm and 333nm respectively. Excitation and emission slits set for 4nm and 2nm band-pass, respectively.
Figure 2.6 Fluorescence Spectra of Extracts in Two Different Solvents from Fibers Pre-Dyed with Direct Blue 71. (—) Extract in 1:1 methanol/water (v:v) and (——) extract in ethanol. Maximum excitation wavelength used for each extract, 297nm and 271nm respectively. Excitation and emission slits set for 4nm and 2nm band-pass, respectively.

2.3.2. Minimum Fiber Length

Extensive work in 1974 dealing with forensic fiber comparisons [26] had shown that the average length fiber transferred between clothing materials was about 5mm. Subsequent work in 1991 reemphasized the limited length and size distribution of retained transferred fibers in typical casework, with the result that the average recovered transferred fiber had a length of approximately 2mm, when removed eight hours after the transfer took place. [27] The rather short lengths of available fibers often encountered in crime scenes stress the importance of an extracting solvent that provides strong fluorescence emission from the extracted fiber. Table 2.3
summarizes the extracting solvent with the highest fluorescence intensity for each type of studied fiber. The comparison of the maximum excitation and fluorescence wavelengths shows the possibility to discriminate the studied fibers on the basis of their spectral profiles.

Table 2.3: Maximum Excitation and Fluorescence Wavelengths of Fiber Extracts with the Highest Fluorescence Intensity

<table>
<thead>
<tr>
<th>Fiber Dye</th>
<th>Excitation Peaks (nm)(^a)</th>
<th>Emission Peaks (nm)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Red 1</td>
<td>256, 347</td>
<td>386</td>
</tr>
<tr>
<td>Disperse Red 4</td>
<td>291</td>
<td>348, 415, 571, 607</td>
</tr>
<tr>
<td>Disperse Red 13</td>
<td>303</td>
<td>357, 404, 430</td>
</tr>
<tr>
<td>Disperse Blue 56</td>
<td>319</td>
<td>385</td>
</tr>
<tr>
<td>Basic Green 4</td>
<td>271, 303</td>
<td>366</td>
</tr>
<tr>
<td>Basic Red 9</td>
<td>295</td>
<td>350, 404, 430</td>
</tr>
<tr>
<td>Basic Violet 14</td>
<td>284</td>
<td>330</td>
</tr>
<tr>
<td>Direct Blue 1</td>
<td>317, 333</td>
<td>372, 430</td>
</tr>
<tr>
<td>Direct Blue 71</td>
<td>300, 355</td>
<td>417</td>
</tr>
<tr>
<td>Direct Blue 90</td>
<td>335</td>
<td>406</td>
</tr>
<tr>
<td>Acid Red 151</td>
<td>305</td>
<td>358, 387, 406, 431, 459</td>
</tr>
<tr>
<td>Acid Yellow 17</td>
<td>287</td>
<td>338, 403, 430</td>
</tr>
<tr>
<td>Acid Yellow 23</td>
<td>289</td>
<td>345, 430</td>
</tr>
<tr>
<td>Acid Green 27</td>
<td>245, 303</td>
<td>412</td>
</tr>
</tbody>
</table>

\(^a\)In the case of multiple peaks, most intense is underlined.

2.3.3. Selecting a General Solvent for Fiber Extraction

Table 2.4 summaries the extracting solvent with the highest fluorescence intensity for each type of studied fiber. The comparison of the maximum excitation and fluorescence wavelengths shows the possibility to discriminate the studied fibers on the basis of their maximum wavelengths.

Having to select an optimum solvent for fiber extraction prior to fiber analysis might not a feasible approach for forensic comparisons, mainly if one considers that each loose fiber in a forensic investigation represents – and must be handled as – a unique item of evidence in the case. Under this prospective, we thought it would be valuable to propose a common extracting
solvent for all the studied fibers. Based on the strong fluorescence signals we consistently observed from all its fibers extracts, a 1:1 acetonitrile:water (v:v) mixture appears to be the best solvent choice.

**Table 2.4 Type of Fiber with Dye Used to Pre-dye Fabric and the Best Extracting Solvent for Fluorescence**

<table>
<thead>
<tr>
<th>Type of Fiber</th>
<th>Respective Textile Dye</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate 154</td>
<td>Disperse Red 1</td>
<td>1:1 acetonitrile/water (v:v)</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Disperse Red 4</td>
<td>1:1 acetonitrile/water (v:v)</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Disperse Red 13</td>
<td>ethanol</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Disperse Blue 56</td>
<td>4:3 pyridine/water (v:v)</td>
</tr>
<tr>
<td>Poly-acrylic 864</td>
<td>Basic Green 4</td>
<td>1:1 acetonitrile/water (v:v)</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Basic Red 9</td>
<td>ethanol</td>
</tr>
<tr>
<td>Polyester 777</td>
<td>Basic Violet 14</td>
<td>1:1 acetonitrile/water (v:v)</td>
</tr>
<tr>
<td>Cotton 400</td>
<td>Direct Blue 1</td>
<td>ethanol</td>
</tr>
<tr>
<td>Cotton 400</td>
<td>Direct Blue 71</td>
<td>1:1 acetonitrile/water (v:v)</td>
</tr>
<tr>
<td>Cotton 400</td>
<td>Direct Blue 90</td>
<td>4:3 pyridine/water (v:v)</td>
</tr>
<tr>
<td>Nylon 361</td>
<td>Acid Red 151</td>
<td>ethanol</td>
</tr>
<tr>
<td>Nylon 361</td>
<td>Acid Yellow 17</td>
<td>ethanol</td>
</tr>
<tr>
<td>Nylon 361</td>
<td>Acid Yellow 23</td>
<td>ethanol</td>
</tr>
<tr>
<td>Nylon 361</td>
<td>Acid Green 27</td>
<td>1:1 methanol/water (v:v)</td>
</tr>
</tbody>
</table>

Figures 2.11-2.13 compare the excitation and fluorescence profiles of the fourteen types of fibers extracted with this solvent. As shown in figure 2.11, fibers pre-dyed with Disperse Red 1, Direct Blue 1, Disperse Red 13 and Acid Green 27 showed extracts with similar excitation and fluorescence profiles. The same is true for fibers pre-dyed with Basic Red 9, Basic Violet 14, Disperse Blue 56 and Acid Yellow 17 (see figure 2.12). On the other end, fibers pre-dyed with Basic Green 4, Disperse Red 4, Direct Blue 71, Direct Blue 90, Acid Yellow 23 and Acid Red 151 showed distinct excitation and fluorescence spectra. Although the comparison of fibers in figure 2.11 and figure 2.12 would certainly benefit from additional parameters of selectivity, the different excitation and fluorescence maxima still makes possible their visual discrimination on the basis of spectral profiles. The strong fluorescence intensities of fibers in 1:1 acetonitrile/water (v:v) extracts make also possible their analyses with 2mm lengths.
The best extracting solvent for each fiber was then used to investigate the possibility to analyze fibers with 2mm lengths. Figures 2.7-2.10 compare fluorescence spectra of extracts obtained from the same type of fiber with different lengths. Although the overall intensities of fluorescence spectra decreased with the lengths of the extracted fibers, 2mm fiber lengths still provided sufficient fluorescence to characterize the fibers on the basis of their spectral profiles.

**Figure 2.7 Excitation and Emission Spectra of Disperse Red 4 Extracts from Different Lengths of Fibers.** Extracts in 1:1 acetonitrile/water (v:v) taken from fibers pre-dyed with Disperse Red 4 of lengths (---) 2cm (—) 1cm and (—) 2mm. Extracts excited at 291nm and emission set at 415nm to collect excitation spectra. Slits set at 4 and 2 nm bandpass for excitation and emission, respectively.
Figure 2.8 Excitation and Emission Spectra of Acid Red 151 Extracts from Different Lengths of Fibers. Extracts in ethanol taken from fibers pre-dyed with Acid Red 151 of lengths (---) 2cm (—) 1cm and (——) 2mm. Extracts excited at 305nm and emission set at 430nm to collect excitation spectra. Slits set at 4 and 2 nm bandpass for excitation and emission, respectively.
Figure 2.9 Excitation and Emission Spectra of Basic Violet 14 Extracts from Different Lengths of Fibers. Extracts in 1:1 acetonitrile/water (v:v) taken from fibers pre-dyed with Basic Violet 14 of lengths (---) 2 cm (—) 1 cm and (—) 2 mm. Extracts excited at 285 nm and emission set at 330 nm to collect excitation spectra. Slits set at 4 and 2 nm bandpass for excitation and emission, respectively.
Figure 2.10 Excitation and Emission Spectra of Disperse Blue 56 Extracts from Different Lengths of Fibers. Extracts in 4:3 pyridine/water (v:v) taken from fibers pre-dyed with Acid Disperse Blue 56 of lengths (---) 2cm (—) 1cm and (—) 2mm. Extracts excited at 320nm and emission set at 383nm to collect excitation spectra. Slits set at 4 and 2 nm bandpass for excitation and emission, respectively.
Figure 2.11 Comparison of Four Different Fiber Extracts in Same Solvent, 1:1 Acetonitrile/Water (v:v). A- Disperse Red 1  B-Direct Blue 1  C-Disperse Red 13  D-Acid Green 27. Excitation set at maximum wavelength for each extract and excitation and emission slits set at 4 and 2 nm band-pass, respectively.
Figure 2.12 Comparison of Four Different Fiber Extracts in Same Solvent, 1:1 Acetonitrile/Water (v:v). A- Basic Red 9 B-Basic Violet 14 C-Disperse Blue 56 D-Acid Yellow 17. Excitation set at maximum wavelength for each extract and excitation and emission slits set at 4 and 2 nm band-pass, respectively.
Figure 2.13 Comparison of Six Different Fiber Extracts in Same Solvent, 1:1
Acetonitrile/Water (v:v). A- Basic Green 4 B-Disperse Red 4 C-Direct Blue 71
D-Direct Blue 90 E-Acid Yellow 23 F-Acid Red 151. Excitation set at maximum wavelength for
each extract and excitation and emission slits set at 4 and 2 nm band-pass, respectively.
2.3.4. Reproducibility of Spectral Profiles

Considering reproducible spectra 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 recorded to investigate their reproducibility within the entire cloth. Figure 2.14 illustrates the four areas of cloth we arbitrarily chose and designated as top middle (TM), top corner (TC), bottom middle (BM) and bottom corner (BC).

Figure 2.14. Fiber collection to investigate the reproducibility of fluorescence spectral profiles within fibers of the same piece of cloth. Abbreviations represent four areas of cloth as follows: TC = top corner, TM = top middle, BC = bottom corner, BM = bottom middle.

Figures 2.15 compares the excitation and fluorescence spectra of fiber extracts collected from three single fibers 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 in Table 2.1. Figures 2.16 – 2.17 show additional examples of the excellent reproducibility among the spectral profiles of extracts from adjacent fibers.

Figure 2.15 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.
Figure 2.16 Excitation and fluorescence spectra of 1:1 acetonitrile-water extracts taken from fibers of a polyester cloth garment pre-dyed Basic Violet 14. 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.
Figure 2.17 Excitation and fluorescence spectra of 1:1 acetonitrile-water extracts taken from fibers of a cotton cloth garment pre-dyed with Direct Blue 71. 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.

Figure 2.18 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, the spectral profiles recorded from fibers collected from different areas of cloth were extremely reproducible. Additional examples of the observed reproducibility are shown in figures 2.19 to 2.20.
Figure 2.18. 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 located at different areas within the same cloth.
Figure 2.19 Excitation and Fluorescence Spectra of 1:1 Acetonitrile/Water Extracts Taken from Fibers of a Polyester Cloth Garment Pre-Dyed with Basic Violet 14. Each spectrum corresponds to an extract from a single fiber. All fibers were located at different areas within the same cloth.
2.3.5. Comparison among the Same Type of Fibers belonging to Different Pieces of the Same Type of Cloth

This type of comparison was made with fiber cloths pre-dyed with Direct Blue 90, Direct Blue 1 and Acid Red 151. All the remaining fibers were acquired as one piece of cloth. Direct Blue 90, Direct Blue 1 and Acid Red 151 were purchased as a set of 100 pieces of cloths. To the extent of our knowledge (verbal communication), each set of 100 pieces of cloths belongs to the same commercial batch. Our studies, therefore, do not provide information on batch-to-batch reproducibility. Four pieces of each type of cloth were randomly selected to collect one fiber
from the top left corner of each garment (see TC area in figure 2.13). Each fiber was cut to a 1cm length and extracted with its best extracting solvent for RTF spectroscopy.

Among the three types of fibers, the largest spectral differences were observed with Direct Blue 1 fibers. Figure 2.21 compares the excitation and fluorescence profiles of the four Direct Blue 1 fiber extracts in ethanol. With the exception of fiber extracts from cloths 2 and 4, which showed almost identical spectra, the remaining spectral profiles allow one to visually differentiate among the four fiber extracts. One should notice that all fluorescence spectra were recorded at the same excitation wavelength (maximum excitation wavelength = 333nm). As such, the possibility to enhance fluorescence spectral differences upon selective excitation was not explored. The same is true for all excitation spectra, which were recorded at the same fluorescence wavelength (maximum fluorescence wavelength = 373nm). Although the differentiation of the same type of fiber from different pieces of cloth appears possible on the basis of two-dimensional excitation and fluorescence spectra, we feel that the forensic examination of textile fibers would certainly benefit from additional selectivity.
Figure 2.21: Excitation and Fluorescence Profiles Comparing Four Direct Blue 1 Fiber Extracts in Ethanol from Different Cloths.  A-Cloth 1 vs. Cloth 2  B-Cloth 1 vs. Cloth 3  C-Cloth 1 vs. Cloth 4  D-Cloth 2 vs. Cloth 3  E-Cloth 2 vs. Cloth 4  F-Cloth 3 vs. Cloth 4.
2.4. Conclusion

The visual comparison of fluorescence spectra in Appendix B reveals two main types of spectral profiles, i.e. emission spectra with only one and with more than one fluorescent peaks. Spectral comparison within the same type of fiber and the same extracting solvent reveals some cases with strong dependence on the chemical nature of the extracting solvent. The differences observed can be attributed to the chemical affinities of the extracting solvents for fluorescence components in the textile fibers. When fiber extraction was carried out with the best extracting solvent for RTF spectroscopy, all fiber extracts showed excitation and fluorescence profiles different from each other. As shown in Table 2.3, the comparison of their maximum excitation and fluorescence wavelengths shows the possibility to discriminate the studied fibers on the basis of their spectral profiles.

Considering the disadvantages associated to selecting the best solvent for fiber extraction prior to RTF spectroscopy in forensic comparisons, we propose a 1:1 acetonitrile:water (v/v) mixture as the common extracting solvent for all the studied fibers. Similar to our observations with the best extracting solvents, the strong fluorescence intensities of fibers in 1:1 acetonitrile:water (v/v) extracts made also possible their analyses with 2mm lengths. The comparison of excitation and fluorescence profiles of the fourteen types of fibers extracted with this solvent showed three distinct groups. Fibers pre-dyed with Disperse Red 1, Direct Blue 1, Disperse Red 13 and Acid Green 27 showed extracts with similar excitation and fluorescence profiles. The same is true for fibers pre-dyed with Basic Red 9, Basic Violet 14, Disperse Blue 56 and Acid Yellow 17. On the other end, fibers pre-dyed with Basic Green 4, Disperse Red 4, Direct Blue 71, Direct Blue 90, Acid Yellow 23 and Acid Red 151 showed distinct excitation and fluorescence spectra. Although the comparison of fibers within the first two groups would
certainly benefit from additional parameters of selectivity, the different excitation and fluorescence maxima still makes possible their visual discrimination on the basis of spectral profiles.

The reproducibility of excitation and fluorescence spectral profiles was demonstrated with two types of experiments. Spectral profiles were recorded from individual extracts belonging to adjacent fibers – i.e. single fibers located immediately next to each other – within the same area of cloth – and from single fibers located in four different areas of the cloth. Both types of experiments provided outstanding reproducibility of spectral profiles with minimum variations in fluorescence intensities. The comparison among the same type of fibers belonging to different pieces of the same type of cloth was only made with three types of fibers, namely Direct Blue 90, Direct Blue 1 and Acid Red 151. All the remaining fibers were acquired as one piece of cloth. Although the differentiation of the same type of fiber from different pieces of cloth appeared to be possible on the basis of two-dimensional excitation and fluorescence spectra, we feel that the forensic examination of textile fibers would certainly benefit from additional selectivity.
CHAPTER 3: COMPARISON OF FIBER EXTRACTS WITH DYE STANDARDS

3.1. Introduction

A head-to-head comparison of the fluorescence characteristics of dye reagents with fiber extracts should take into consideration the possible variations that often exist in the chemical composition of dye reagents from different commercial sources and/or variations that may also exist within dye reagent batches from the same source. Bearing this possibility in mind, we made several attempts to acquire the dye reagents that were actually used to pre-dye the studied fibers. If fluorescence impurities are incorporated into the fiber during the fabrication of the cloth, subtraction of the spectral profile of the dye from that of the extract should provide a spectral signature of the impurities and – as such - an additional level of fiber discrimination for forensic comparisons.

Unfortunately, Testfabrics did not provide us with matching reagent dyes for the studied fibers. The unsuccessful outcome of our attempts led us to an investigation of the absorption and fluorescence properties of Sigma-Aldrich dye standards, a popular source of reagent dyes for the textile industry. Not much is published of the RTF characteristics of textile dyes. A comprehensive literature search on fluorescence of reagent dyes from Sigma-Aldrich revealed only two reports. [28,29] The fluorescence characteristics of Direct Blue 1 and Direct Blue 71 were recorded in water, methanol and aqueous triton-X. [28] The fluorescence features of Disperse Red 1 were investigated in methanol, ethylene glycol, glycerol, and phenol. [29] Both reports use the reagent dyes as received with no attempts of further purification. In the case of Disperse Red 1, [29] the highest fluorescence signal was observed upon sample excitation at
532nm, i.e. an excitation wavelength nominally selected to attempt the exclusive excitation of the dye in the presence of unknown reagent impurities.

The main goal of this chapter then became to estimate the contribution of dyes and impurities to the overall fluorescence of their respective fiber extracts. Keeping in mind that we are dealing with un-exposed fibers to environmental contamination, possible spectral differences among dye and fiber extracts could originate from the following sources: (a) existing impurities in the reagent dye used to pre-dye the fiber and/or (b) from physical contact with other chemical(s) during the their fabrication process. The ideal approach should only provide a spectral matching for the dye reagent that was used to pre-dye the investigated fiber. If fluorescence impurities are incorporated into the fiber during the fabrication of the cloth, the spectral profile of the Aldrich-Sigma dye should provide a different spectral signature to the one recorded from the “respective” Testfabrics fiber extract.

3.2 Experimental

See section 2.2 for experimental details.

3.3. Results and Discussion

3.3.1. Absorbance of Dye Standards and Fiber Extracts

The absorption spectra of several dyes in Table 2.1 are well documented in the Sigma-Aldrich Handbook of Stains, Dyes and Indicators. [25] Among the four solvent systems we investigate here for fiber extraction, the most common solvent used to record absorption spectra in the Aldrich-Sigma Handbook was methanol/water. “A” spectra in appendix C compiles the absorption profiles of the fourteen dye standards recorded in 1:1 methanol/water (v:v) solutions.
All spectra showed strong absorption peaks in the ultraviolet and visible wavelength regions. Based on the visible color of dye standards solutions, the absorption peaks in the visible most likely reflect the presence of the dye in the commercial standard. Because the concentration of the dyes in the commercial standards are always lower than 100% (see Table 2.2), the absorption peaks in the ultraviolet range of the spectra could be attributed to the presence of impurities in the commercial mixtures of unknown composition.

The comparison of “A” spectra in appendix C to the absorption spectra of dyes in the best fiber extracting solvent for fluorescence (see “B” spectra in Appendix C) shows the effect of solvent composition and dye solubility in the spectral features of the studied dyes. With the exception of Direct Blue 1, all the other dyes showed very similar spectra in both types of solvents. The absence of the visible peak in the absorption spectrum of Direct Blue 1 is due to the poor solubility of the dye in ethanol, i.e. the best fiber extracting solvent for fluorescence. The presence of ultraviolet peaks in both spectra probably reflects the presence of soluble impurities in both types of solvents.

Comparison of “B” spectra to the absorption spectra of fiber extracts in the best fluorescence extracting solvent (see “C” spectra in Appendix C) should take into consideration possible variations in the composition of the Aldrich-Sigma standards and the dye reagents used to pre-dye Testfabrics fibers. If the visible portion of the spectra results from the presence of the dyes in the fibers, the comparison among “B” and “C” spectra should then provide qualitative information on the ability of the best fluorescence solvents to extract the dyes from the fibers.

Figures C-6 to C-8 and C-12 show the absence of the absorption peak in the visible spectrum of the extract. The lack of visible peak in the absorption spectrum of Direct Blue 1 (figure C-8) can be attributed to the poor solubility of the dye in the best extracting solvent for
fluorescence. The lack of visible absorption peaks in Basic Red 9 (figure C-6), Basic Violet 14 (C-7) and Acid Yellow 17 (figure C-12) can be attributed to two possible reasons, namely the best fluorescence solvent was unable to extract the dye from the fiber and/or the concentration of the dye in the extract was too low to be measured via absorption spectrometry. All the remaining “C” spectra show visible peaks in the fiber extracts. When comparing the relative intensities of the visible peaks to those in “B” spectra, one should consider the unknown amount of dye in the fiber because their relative intensities do not provide quantitative information on the extraction efficiency of the best fluorescence solvent. The comparison only demonstrates the ability of the best fluorescence solvent to extract the dye from the fiber. Table 3.1 summaries the maximum absorption wavelengths for the dyes and their respective fiber extracts.

**Table 3.1: Absorbance Peaks a of Dye Standards and Extracts in Best Extracting Solvent**

<table>
<thead>
<tr>
<th>Dye Standard</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Red 1</td>
<td>263, 318, 483</td>
</tr>
<tr>
<td>Disperse Red 4</td>
<td>257, 512, 547</td>
</tr>
<tr>
<td>Disperse Red 13</td>
<td>211, 284, 503</td>
</tr>
<tr>
<td>Disperse Blue 56</td>
<td>302, 592, 632</td>
</tr>
<tr>
<td>Basic Green 4</td>
<td>256, 359, 621</td>
</tr>
<tr>
<td>Basic Red 9</td>
<td>211, 289, 546</td>
</tr>
<tr>
<td>Basic Violet 14</td>
<td>240, 291, 546</td>
</tr>
<tr>
<td>Direct Blue 1</td>
<td>214, 274</td>
</tr>
<tr>
<td>Direct Blue 71</td>
<td>299, 591</td>
</tr>
<tr>
<td>Direct Blue 90</td>
<td>631</td>
</tr>
<tr>
<td>Acid Red 151</td>
<td>230, 351, 511</td>
</tr>
<tr>
<td>Acid Yellow 17</td>
<td>224, 403</td>
</tr>
<tr>
<td>Acid Yellow 23</td>
<td>260, 430</td>
</tr>
<tr>
<td>Acid Green 27</td>
<td>254, 286, 410, 603, 645</td>
</tr>
</tbody>
</table>

*aIf more than one peak was observed, the peak of maximum intensity is underlined.*

**3.3.2. Fluorescence of Dye Standards**

With the exception of Disperse Red 13 and Acid Red 151, all the dye standards in Table 2.2 showed fluorescence when dissolved in the best extracting solvent for their corresponding
fibers (see Table 2.3). One should notice that the lack of fluorescence from Disperse Red 13 and Acid Red 151 does not reflect the solubility of the dyes in the extracting solvent as both dyes were soluble in ethanol, i.e. the best fluorescence solvent for their corresponding fibers. Appendix D compiles the excitation and fluorescence spectra of the remaining dyes recorded with the same excitation and emission band-passes used to record spectra from fibers extracts in Appendix B. Initial wavelength selection for fluorescence excitation was based on absorption spectra. For those dyes that showed absorption peaks in the ultraviolet and visible region, fluorescence excitation was attempted in both spectral regions. Dispersed Red 4 (see spectra D-2) was the only dye to show different fluorescence peaks upon excitation in the ultraviolet (272nm) and the visible (512nm) regions of the spectrum. Upon excitation at 253nm, Basic Red 9 showed two fluorescence peaks with maximum wavelengths at 350nm and 689nm (see spectra D-6). The remaining dyes showed one fluorescence peak either in the ultraviolet or the in visible spectral regions. In some cases, their excitation spectra recorded at the maximum fluorescence wavelength showed more than one prominent peak. Although the unknown and heterogeneous composition of commercial standards in Table 2.2 make difficult the assignment of peaks to impurities and/or dyes, the comparison of spectra in Appendix D clearly shows the possibility to discriminate among commercial dyes on the basis of excitation and fluorescence profiles.

Figures 3-1 to 3-4 provide a head-to-head comparison of the spectral profiles of the commercial dyes and their corresponding fiber extracts in the best solvent for fluorescence intensity. With the exception of Basic Green 4 and its corresponding fiber extract, all the remaining pairs showed different spectral profiles. The observed differences provide compelling evidence of the important contribution that fluorescence impurities make on the total fluorescence spectra of fiber extracts. The presence of impurities on the extracts of fibers pre-
dyed with Disperse Red 13 and Acid Red 151 make possible their discrimination on the basis of fluorescence spectroscopy. The maximum excitation and fluorescence wavelengths of dyes standards are summarized in Table 3.2.

*Table 3.2 Maximum Excitation and Fluorescence Wavelengths* of Dye Standards

<table>
<thead>
<tr>
<th>Dye Standard</th>
<th>Excitation</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Red 1</td>
<td>258</td>
<td>307</td>
</tr>
<tr>
<td>Disperse Red 4</td>
<td>272, 512</td>
<td>300, 570</td>
</tr>
<tr>
<td>Disperse Red 13</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Disperse Blue 56</td>
<td>326, 586</td>
<td>380, 661</td>
</tr>
<tr>
<td>Basic Green 4</td>
<td>272</td>
<td>368</td>
</tr>
<tr>
<td>Basic Red 9</td>
<td>252, 290</td>
<td>350, 689</td>
</tr>
<tr>
<td>Basic Violet 14</td>
<td>257, 299</td>
<td>363</td>
</tr>
<tr>
<td>Direct Blue 1</td>
<td>267, 340</td>
<td>397</td>
</tr>
<tr>
<td>Direct Blue 71</td>
<td>250, 296, 350</td>
<td>431</td>
</tr>
<tr>
<td>Direct Blue 90</td>
<td>250</td>
<td>306</td>
</tr>
<tr>
<td>Acid Red 151</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Acid Yellow 17</td>
<td>278</td>
<td>302</td>
</tr>
<tr>
<td>Acid Yellow 23</td>
<td>275, 423</td>
<td>299, 547</td>
</tr>
<tr>
<td>Acid Green 27</td>
<td>600</td>
<td>689</td>
</tr>
</tbody>
</table>

*a*In the case of multiple peaks, most intense is underlined.
Figure 3.1 Excitation and Fluorescence Comparison of Disperse Red 4 Standards and Extracts in 1:1 Acetonitrile/Water (v:v). (—) Fiber extract and (—) Standard. Maximum excitation wavelength for each sample was used, excitation and emission slits were set at 4 and 2nm band-pass, respectively.
Figure 3.2 Excitation and Fluorescence Comparison of Basic Green 4 Standards and Extracts in 1:1 Acetonitrile/Water (v:v). (—) Fiber extract and (—) Standard. Maximum excitation wavelength for each sample was used, excitation and emission slits were set at 4 and 2nm band-pass, respectively.
Figure 3.3 Excitation and Fluorescence Comparison of Direct Blue 71 Standards and Extracts in 1:1 Acetonitrile/Water (v:v). (—) Fiber extract and (—) Standard. Maximum excitation wavelength for each sample was used, excitation and emission slits were set at 4 and 2nm band-pass, respectively.
3.4. Conclusions

Absorbance spectra of standards gave spectra closely matching that of dyes from the Sigma Aldrich Handbook of Stains, Dyes and Indicators. As expected, all dye standards had strong peaks in the visible range most likely attributed to the presence of the dyes. The fiber extracts showed similar visible peaks when the dye was extracted, but also showed stronger peaks in the UV region. It is believed that these peaks confirm the presence of unknown impurities in the fiber. Considering the hypothesis of the dyes to absorb exclusively in the visible spectral region and the concomitants to absorb exclusively in the ultraviolet spectral
region, our results indicate that all the studied dyes are fluorescent with the exception of Disperse Red 13 and Acid Red 151.

The majority of dye standards showed relatively weaker fluorescence than their corresponding fiber extracts. Fiber extracts showed the contribution of unknown fluorescence impurities. Several possibilities exist for the origin of these impurities. They could be imparted upon clothes during processing, but before dyeing. They could come from impurities present in the impure dyes used to color the fabrics or impurities present in the equipment and reagents used for dyeing the cloths. Since the fluorescence given by the fiber extracts appears to be irrespective of the dyes these fluorescence signals could provide another level of discrimination for forensic fiber analysis beyond examining dyes. The potential exists for differentiating visually indistinguishable fibers, i.e. of the same material, dyed with the same dye but with the presence of different impurities. Based on the possible fluorescence sources, textile fibers could then fit into one of six possible categories (see Figure 3.5). If no synergistic effects exist among the dye and the unknown concomitants, the fluorescent outcome of fiber analysis type A and B should be the same. With the exception of fibers type E and F, Room-Temperature Fluorescence (RTF) Spectroscopy should be able to provide useful information in all cases.
Type A
Fibers pre-dyed with 100% pure fluorescent reagent dye

Type B
Fibers pre-dyed with a fluorescent reagent dye containing non-fluorescent comitants

Type C
Fibers pre-dyed with a fluorescent reagent dye containing fluorescent comitants

Type D
Fibers pre-dyed with a non-fluorescent reagent dye containing fluorescent

Type E
Fibers pre-dyed with a 100% pure non-fluorescent reagent dye

Type F
Fibers pre-dyed with a non-fluorescence reagent dye containing non-fluorescent

Figure 3.5: Classification of Fibers According to Potential Fluorescent Sources.
4.1. Introduction

The broad nature of room-temperature excitation and fluorescence spectra and the possible spectral overlapping that might result from the presence of several fluorescence concomitants makes difficult to track down the individual components of fluorescence fiber extracts. Figure 4.1 shows the experimental design we followed in this chapter to obtain a better insight on the fluorescence of textile fibers and Sigma-Aldrich dyes. The separation of fiber extracts via high-performance liquid chromatography (HPLC) should provide valuable information on the contribution of individual components to the total fluorescence of fibers. The HPLC analysis of Aldrich-Sigma dyes should make possible to observe the fluorescence characteristics of pure dyes without the contribution of impurities. Separation of extracts via HPLC should also give information on the number of individual components that contribute to the total fluorescence of each EEM contour and their reproducibility for forensic fiber comparison. Further analysis of HPLC fractions via RTF spectroscopy will make possible the comparison of the spectral characteristics of each concomitant. This information should be valuable to assess the potential advantage of lowering the temperature-temperature to minimize spectral overlapping for better fiber discrimination.
Based on the spectral characteristics of fibers extracts in Chapters 2 and 3 we selected five types of fibers as representative cases of the fourteen fibers in Table 2.1. Extracts from fibers pre-dyed with Dispersed 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. Their spectral profiles were different from those observed from their respective Sigma-Aldrich dye. The main reason for selecting fibers pre-dyed with Acid Yellow 17 and Acid Yellow 23 was the opportunity to
investigate the discrimination of RTF spectroscopy when handling visually indistinguishable fibers.

4.2. Experimental

4.2.1. Chemicals and Supplies

Fabric and dyed cloths were the same as those reported in chapter 2. The same is true for Sigma-Aldrich dye standards. All solvents used for these studies were 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.

4.2.2. Solvent Extraction of Textile Fibers

See procedure previously described in chapter 2.

4.2.3. Ultraviolet and Visible Absorption Spectroscopy

The same procedure and instrumentation described in chapter 2 were used to record absorption spectra from HPLC fractions.

4.2.4. RTF Spectroscopy

The same procedure and instrumentation described in chapter 2 was used to investigate the spectral characteristics HPLC fractions. Two-dimensional (2D) excitation and 2D-fluorescence spectra were recorded via the classic method of setting one monochromator to the maximum wavelength while scanning the other. EEM from fiber extracts were collected at 5nm 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.

4.2.5. 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 operation was 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. 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).

4.3. Results and Discussion

4.3.1. HPLC Analysis

The investigation of the reproducibility of individual components in fiber extracts across the same piece of garment followed the same strategy as the one described in chapter 2. Adjacent fibers were collected from four different areas of the same piece of cloth, namely top corner (TC), bottom corner (BC), top middle (TM) and bottom middle (BM). A minimum of three chromatographic runs were carried out per fiber extract and per dye standard solution. Figure 4.2 compares the chromatograms of four fibers pre-dyed with Dispersed Red 4. 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
absorption wavelength (512nm) selected for detection in figure 4.2-B was the maximum visible absorption wavelength of the Aldrich-Sigma dye (see appendix C). The agreement among the retention times and the absorption intensities of the four chromatograms demonstrates the reproducible distribution of the dye within fibers of the same piece of cloth. The excitation (291nm) and emission (345nm) wavelengths selected for the fluorescence detection in figure 4.2-C correspond to the maximum excitation and emission wavelengths of the fiber extracts (see appendix B). The retention times of the fluorescent peaks are statistically different from those in figure 4.2-A. This fact demonstrates the presence of fluorescence impurities in the fiber extract. The skewed peaks at 12.9 min are due to the strong fluorescence of the main impurity that almost reached the upper linearity limit of the HPLC detector. The agreement among the retention times and the fluorescence intensities of the peaks in the four chromatograms demonstrates the reproducible distribution of the fluorescence impurities within fibers of the same piece of cloth.
Figure 4.2. HPLC Chromatograms of Disperse Red 4 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 = 512nm, Excitation set at 291nm and emission set at 415nm.

Figure 4.2-A and B compares the absorption and fluorescence chromatograms of the fiber extracts to the Sigma-Aldrich dye standard. The agreement between the two retention times in the absorption chromatograms confirms the previous assignment of the peak in figure 4.2-B to the presence of the dye in the fiber extract. The comparison of the fluorescence chromatograms in figure 4.2-C clearly shows the absence of the same fluorescence impurities in the dye standard, i.e. concomitants that show the emission of fluorescence at 345nm upon excitation at 291nm.
Figure 4.3. HPLC Chromatograms of Basic Green 4 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 = 621nm, Excitation set at 272nm and emission set at 366nm.

Similar results are shown in figures 4.3 and 4.4 for Basic Green 4 and Acid Red 151 fiber extracts and their respective Sigma-Aldrich dye standards. 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 10ppm, i.e. the concentrations of dyes in the standard solutions. A direct correlation between the concentration of the dye in the extract
and its mass on the extracted fiber requires the extraction efficiency of the solvent, which was not investigated in these studies.

Figures 4.4 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 = 519nm, Excitation set at 305nm and emission set at 431nm.

Figure 4.5 shows the fluorescence chromatogram of extracts from fibers pre-dyed with Acid Yellow 23. The number of fluorescent components and retention times closely resemble that of Acid Red 151. It would be reasonable to assume that the similar fluorescence spectral profiles of these two extracts are due to the fluorescence components with similar elution times.
Figure 4.5 Fluorescence Chromatogram of Extracts from Fibers Pre-Dyed with Acid Yellow 23. Excitation set at 290nm and emission detector set at 345nm.

Figure 4.6-A compares the absorption spectrum of a 10ppm Disperse Red 4 dye standard solution in 50% methanol-water (volume/volume) to the absorption spectrum of the HPLC fraction collected from the extract at 14.9 min (see figure 4.2-A for reference). The agreement between the two spectra can be attributed to the similarity of the spectral features of the commercial dye reagent and the purified dye via HPLC analysis or to the unsuccessful separation of impurities from the dye via HPLC analysis of the extract. Considering the strong probability of the chemical composition of the commercial dye reagent to be different from the chemical composition of the extract, the latter appears to be unlikely. Figure 4.6-B shows the absorption spectrum of the HPLC fluorescence fraction collected from the extract at 12.9 min (see figure 4.2-B for reference). The lack of extract absorbance with similar characteristics to those from the
dye confirms the assignment of fluorescence peaks in figure 4.2-B to the presence of impurities and not to the presence of the dye. Similar results are shown for fibers pre-dyed with Acid Red 151 in figure 4.7.

Figure 4.6 Absorbance Spectra of HPLC Fractions from Extracts of Fibers Pre-Dyed with Disperse Red 4. A-Absorbance spectra of (—) 10ppm Disperse Red 4 standard in 1:1 methanol/water (v:v) and (—) fraction collected at 14.9 minutes from HPLC chromatogram of Disperse Red 4 extract. B-Absorbance spectra of fraction collected at 12.9 minutes from HPLC chromatogram of Disperse Red 4 extract.

Figure 4.7 Absorbance Spectra of HPLC Fractions from Extracts of Fibers Pre-Dyed with Acid Red 151. A-Absorbance spectra of (—) 10ppm Acid Red 151 standard in 1:1 methanol/water (v:v) and (—) fraction collected at 12.96 minutes from HPLC chromatogram of Acid Red 151 extract. B-Absorbance spectra of fraction collected at 32.9 minutes from HPLC chromatogram of Acid Red 151 extract.
Figure 4.8-A compares the excitation and fluorescence spectra of a 10ppm Disperse Red 4 dye standard solution in 50% methanol-water (volume/volume) to the fluorescence spectrum of the HPLC fraction collected from the extract at 14.9 min (see figure 4.2-A for reference). Figure 4.8-B shows the excitation and emission spectra of the HPLC fraction collected from the extract at 12.9 min (see figure 4.2-B for reference). These spectra were recorded at the same excitation (512nm) and emission (570nm) wavelengths as the spectra in figure 4.8-A. The similarity of the spectral features in figure 4.8-A and the lack of fluorescence in Figure 4.8-B confirms the assignment of the absorption peak in figure 4.2-B to the presence of the dye and not to the presence of impurities.

Figure 4.9-A compares the excitation and fluorescence spectra of the extract from fibers pre-dyed with Disperse Red 4 to those recorded from the HPLC fluorescence fraction of the
same extract collected at 12.9min (see figure 4.2-C for reference). Figure 4.9-B shows the excitation and emission spectra recorded from the HPLC absorption fraction of the same extract collected at 14.9min (see figure 4.2-B for reference). The similarity of the spectral features in figure 4.9-A and the lack of fluorescence in figure 4.9-B confirms that the fluorescence of the extract in the ultraviolet spectral region is due to the presence of fluorescence impurities and not to the presence of the dye. One should notice that similar differences in the relative intensities of the two fluorescence peaks with maximum wavelengths at 348 and 415nm were also observed in the spectra of extracts from fibers with different lengths. Spectra from extracts of fibers with 2cm and 1cm lengths showed the highest peak at 415nm. Extracts from 2 mm fibers showed the highest fluorescence peak at 348nm. These facts indicate a correlation between the spectral features of the extract and the concentration of its fluorescence impurities. Further studies are needed to understand the role of the concentration of fluorescence impurities on synergistic and inner filter effects.
Figure 4.9 UV Excitation and Emission Spectra of HPLC Fractions from Extracts of Fibers Pre-Dyed with Disperse Red 4. A-Fluorescence spectra of (—) extract from fibers pre-dyed with Disperse Red 4 in 1:1 methanol/water (v:v) and (—) fraction collected at 12.9 minutes from HPLC chromatogram of Disperse Red 4 extract. B-Fluorescence spectra of fraction collected at 14.9 minutes from HPLC chromatogram of Disperse Red 4 extract. Exciting at maximum excitation (291nm) and collecting excitation with emission set at (348nm). Excitation slits set at 4 and emission slits set at 2 nm bandpass respectively.

Figure 4.10 overlays the excitation and fluorescence spectra of the extract from fibers pre-dyed with Acid Red 151 to those recorded from the HPLC fluorescence fraction of the same extract collected at 22.95 min (4.10-A) and 29.01 min (4.10-B). The reader should refer to chromatogram 4.4 for guidance. Their comparison clearly demonstrates the main contribution of two different fluorescence impurities to the total fluorescence spectrum of the extract.
Figure 4.10 UV Excitation and Emission Spectra of HPLC Fractions from Extracts of Fibers Pre-Dyed with Acid Red 151. A-Fluorescence spectra of (—) extract from fibers pre-dyed with Acid Red 151 in ethanol and (—) fraction collected at 22.9 minutes from HPLC chromatogram of Acid Red 151 extract. Exciting at maximum excitation (295nm) and collecting excitation with emission set at (405nm). B-Absorbance spectra of fraction collected at 28.9 minutes from HPLC chromatogram of Acid Red 151 extract. Exciting at maximum excitation (303nm) and collecting excitation with emission set at (430nm) Excitation slits set at 4 and emission slits set at 2 nm bandpass respectively.

4.3.2. RTF-EEM Spectroscopy

Nine types of textiles fibers were investigated via RTF – EEM spectroscopy. The studied fibers were randomly selected among the fourteen types of fibers in Table 2.1. For the purpose of comparison, EEM were also recorded from their respective Sigma-Aldrich standards. Appendix E compares the EEM of the fiber extracts to those recorded from the dye standards. Clearly, all the EEM from the extracts are different from the EEM of their respective dyes. Table 4.1 summarizes the comparison of 2D-spectral profiles and EEM recorded from the seven types of fibers and their respective dye standards.
Table 4.1 Qualitative Comparison of 2D RT-Excitation Spectra, Fluorescence Spectra and EEM Recorded from Dye Reagents and Fiber Extracts

<table>
<thead>
<tr>
<th>Type of Fiber</th>
<th>2D-Excitation</th>
<th>2D-Fluorescence</th>
<th>EEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Red 4</td>
<td>different</td>
<td>different</td>
<td>different</td>
</tr>
<tr>
<td>Disperse Blue 56</td>
<td>similar</td>
<td>similar</td>
<td>different</td>
</tr>
<tr>
<td>Basic Green 4</td>
<td>same</td>
<td>same</td>
<td>different</td>
</tr>
<tr>
<td>Basic Red 9</td>
<td>different</td>
<td>different</td>
<td>different</td>
</tr>
<tr>
<td>Acid Red 151</td>
<td>different</td>
<td>different</td>
<td>different</td>
</tr>
<tr>
<td>Acid Yellow 17</td>
<td>different</td>
<td>different</td>
<td>different</td>
</tr>
<tr>
<td>Acid Yellow 23</td>
<td>different</td>
<td>different</td>
<td>different</td>
</tr>
<tr>
<td>Acid Green 27</td>
<td>different</td>
<td>different</td>
<td>different</td>
</tr>
</tbody>
</table>

Figure 3.2 compares the 2D-excitation and 2D-fluorescence spectra of the fiber extracts pre-dyed with Basic Green 4 to those recorded from their respective Sigma-Aldrich dye standard. The spectral profiles of the extracts and the standard are virtually the same, which makes visual discrimination within these two not possible.

Figure 4.11 compares the HPLC chromatograms of the extract and the standard for Disperse Blue 56. The chromatographic profiles within each pair reflect the difference on the composition of fluorescence impurities of the extract and the standard. The inability of 2D-excitation and fluorescence spectra to discriminate between these two pairs reflects the partial excitation of the same fraction of fluorescence components in the extracts and the standards.
Figure 4.11 HPLC Fluorescence Chromatogram of Disperse Blue 56 Standard and Fiber Extracts. (—) Disperse Blue 56 10 ppm, excitation set at 583nm with emission detector set to 660nm. (—) Extract from fibers pre-dyed with Disperse Blue 56, excitation set at 320, emission detector set at 385nm.

Figure 4.12 shows the EEM of Disperse Blue 56 standard and its HPLC chromatograms recorded upon absorption (590nm) and fluorescence (583/660nm) detection within the excitation and emission wavelength range of one of the EEM contours. Both chromatograms indicate the presence of seven components that absorb and fluorescence within this wavelength region. Figure 4.13 compares the fluorescence chromatograms of the same standard and the fiber extract recorded with two sets of excitation and emission wavelengths. Considering the chromatographic retention times of 20.5 min (583/660nm) and 20.88 (320/385nm) statistically similar (P = 95%, N₁ = N₂ = 3), [30], the several peaks with different retention times indicate the presence of several fluorescence concomitants in the extract. The main reason EEM were able to differentiate
between these two pairs of extracts and standards is their ability to convolute the contribution of all fluorescence components within the ultraviolet and visible spectral regions. Close examination of all EEM in appendix E reveal the possibility to discriminate among the extracts of all the studied fibers. Interesting to note is the possibility to spectrally resolve all the fluorescence components via high-resolution luminescence spectroscopy. The optimization of Fluorescence Line-Narrowing Spectroscopy for the analysis of Disperse Blue 56 extract could provide a spectral pattern with at least seven peaks for fiber discrimination.

Figure 4.12. EEM of Disperse Blue 56 fiber extract and its HPLC chromatograms. Absorption (590nm) and fluorescence (583/660nm) detection within the excitation and emission wavelength range of one of the EEM contours.
Figure 4.13 EEM and HPLC Chromatograms of Disperse Blue 56 Standard and Extract from Fibers Pre-Dyed with Disperse Blue 56. Top- EEM and fluorescence chromatograms of Disperse Blue 56 10ppm with excitation set at 583nm and the emission detector set at 660nm. Bottom- EEM and fluorescence chromatograms of Disperse Blue 56 fiber extract with excitation set at 320nm and the emission detector set at 385nm.

4.4 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 extracts of fibers collected from different areas of a cloth confirmed the reproducibility of individual fluorescence impurities within the same piece of cloth. The reproducibility of individual impurities agrees well with the fluorescence reproducibility of
extracts from 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 fluorescence 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.
CHAPTER 5: PARAFAC ANALYSIS OF RTF-EEM RECORDED FROM SIMILAR AND DISSIMILAR SETS OF TEXTILE FIBER EXTRACTS

5.1 Introduction

Figure 5.1 compares the RTF-EEM of extracts from Acid Yellow 17 and Acid Yellow 23. These two types of fibers are visually indistinguishable. Although the EEM of their extracts present noticeable contour differences, their statistical comparison within a certain confidence level should provide the forensic analyst with a more robust tool for fiber discrimination. In this chapter, we investigate the potential of PARAFAC and MCR-ALS for the comparison of RTF-EEM recorded from fiber extracts.

Figure 5.1 EEM Recorded from Extracts of Fibers Pre-Dyed with Acid Yellow 17 (left) and Acid Yellow 23 (right).

5.2 Experimental

5.2.1. Chemicals and Supplies

See section 4.2.1 for experimental details.
5.2.2. Solvent Extraction of Textile Fibers

See procedure previously described in chapter 2.

5.2.3. RTF Spectroscopy

The same procedure and instrumentation described in chapter 4 was used to record EEM from fiber extracts.

5.2.4. Chemometric Analysis

These studies were carried out in collaboration with Dr. Hector C. Goicoechea, Associate Professor, Catedra de Quimica Analitica I, Facultad de Bioquimica y ciencias Biologicas, Universidad Nacional del Litortal, Ciudad Universitaria, CC 242-S30001 Santa Fe, Argentina. All chemometric calculations were done using MATLAB 6.0. Routines for PARAFAC and MCR-ALS were available in the Internet thanks to Bro [31] and Tauler [22], respectively. A useful MATLAB graphical interface was available in Dr. Goicoechea’s lab for easy data manipulation and graphics presentation. [32] This interface provided a simple means of loading the data matrices into the MATLAB working space before running PARAFAC. MCR-ALS analysis was carried out with the aid of appropriate software currently available in the MCR-ALS home page. [22]

5.3. Results and Discussion

5.3.1. Fiber and EEM Collection for PARAFAC Analysis

The investigated fibers were separated in three different sets (see figure 5.2). Set #1 included ten nylon fibers pre-dyed with Acid Red 151 and collected from different areas of the same piece of cloth. Set #2 included ten nylon fibers pre-dyed with Acid Red 151 but collected from a different piece of cloth than the fibers in set #1. Set #3 included ten cotton fibers pre-dyed with
Direct Blue 1 and collected from the same piece of cloth. Each fiber from each set was individually extracted with ethanol and one EEM per fiber extract was recorded. Because no previous application of PARAFAC for the purpose has been published, the main idea behind fiber selection was to confirm – or not – the ability of PARAFAC to separate the three sets of fibers into appropriate statistical groups.

Figure 5.2. Detail of three sets of 10 EEM taken from three different cloths. Fiber sets #1 and #2 were collected from two separate nylon cloths pre-dyed with the same dye (Acid Red 151). Fiber set #3 was collected from a cotton cloth pre-dyed with Direct Blue 1.
Figures 5.3 and 5.4 compare eight of the twenty EEM recorded from fiber sets #1 and #2, respectively. The visual comparison of EEM contours shows strong spectral similarity among the four fiber extracts of set #1. The same is true for the four EEM recorded from the four fiber extracts in set #2. The comparison of EEM recorded from fibers of different sets show noticeable contour differences. Under the prospective of forensic fiber comparison, this is an extremely positive result because it shows the ability of RTF-EEM to differentiate between two visually indistinguishable fibers pre-dyed with the same dye in the same textile industry but from different cloths.

Figure 5.3. Four EEM from Ten Fiber Samples of set #1, Nylon Cloth Pre-Dyed with Acid Red 151. Samples were excited from 500 to 200nm while collecting emission from 290 to 800nm. Excitation slits were at 4 nm bandpass and emission slits set at 2 nm bandpass.
Figure 5.4. Four EEM from Ten Fiber Samples of Set #2, Second Nylon Cloth Pre-Dyed with Acid Red 151. Samples were excited from 500 to 200nm while collecting emission from 290 to 800 nm. Excitation slits were at 4 nm bandpass and emission slits set at 2 nm bandpass.
Figure 5.5 details the strong similarities that exist among the four EEM contours recorded from the extracts of fibers in set #3. As expected, the comparison of these EEM to those in figures 5.3 and 5.4 show remarkably different contours.

**Figure 5.5.** Four EEM from Ten Fiber Samples of Set #3, Cotton Cloth Pre-Dyed with Direct Blue 1. Samples were excited from 200 to 600 nm while collecting emission from 290 to 800 nm. Excitation slits were at 4 nm band-pass and emission slits set at 2 nm band-pass.
The spectral de-convolution of EEM via PARAFAC provided the best fit for a five fluorescence component mixture in the three cases. Figure 5.6 shows the emission and excitation loadings of the five-component PARAFAC model for extracts from fibers in set #1.

Figure 5.6. Profiles of five components extracted from EEM of Set #1 with PARAFAC. Top-emission loadings of a five component PARAFAC model, bottom- excitation loadings of five component PARAFAC model.

Figure 5.7 depicts the statistical grouping of the 30 EEM recorded from the individual extracts of the 30 fibers. The intercepts of the of the three PC values – which correspond to the maximum intensities of the first three spectral components of each EEM – place each EEM within a statistical (elliptical) domain. The statistical domain is defined using the bivariate method to achieve a confidence level of 95%. Although PARAFAC groups EEM from set #1 and
#2 within the same statistical group, fiber extracts from set #1 show EEM with similar PC values while EEM from set #2 show PC values scattered over the entire elliptical domain. Under this prospective, PARAFAC provides some sort of discrimination between fiber extracts from set #1 and #2.

Figure 5.7. Statistical Grouping of 30 EEM from the 30 Fibers in Sets #1, #2 and #3. Set#1 is represented by blue circles, Set #2 by red and Set #3 by green circles. Each point is the intersect of the intensities from the first three fluorescent spectral components extracted from the EEM.

5.3.2. Fiber and EEM Collection for MCR-ALS Analysis

All MCR-ALS comparisons were made among EEM recorded from extracts of fibers visually indistinguishable. EEM were recorded from extracts of nylon fibers pre-dyed with Acid Red 151,
Acid Yellow 17 and Acid Yellow 23. Acid Red 151 fibers were collected from two different pieces of cloths, ten fibers per cloth. Ten Acid Yellow 17 fibers were collected from one piece of cloth. The same was true for the ten Acid Yellow 23 fibers. Each fiber was extracted with ethanol and an EEM was recorded from each extract. Examples of EEM recorded from the two sets of fiber extracts Acid Red 151 are shown in figure 5.3 and 5.4. Their statistical comparison allowed us to test the ability of MCR-ALS to differentiate between two visually indistinguishable fibers pre-dyed with the same dye in the same textile industry but from different cloths. Figure 5.1 provides examples of the EEM recorded from fiber extracts Acid Yellow 17 and Acid Yellow 23. Their statistical comparison allowed us to test the ability of MCR-ALS to differentiate between two visually indistinguishable fibers pre-dyed with two different dyes. The molecular structures of Acid Yellow 17 and Acid Yellow 23 can be compared in Appendix A.

MCR-ALS analysis of EEM recorded from fiber extracts Acid Yellow 17 and Acid Yellow 23 provided five fluorescence components per extract. Their predicted excitation and fluorescence spectra are shown in figure 5.8 (Acid Yellow 17) and figure 5.9 (Acid Yellow 23). Although the number of fluorescence 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 the individual components in Acid Yellow 23. Their excitation and emission spectra are overlaid in figures 5.10 and 5.11, respectively. Interesting to note is the good agreement between the number of fluorescence components in the EEM of Acid Yellow 23 and the number of fluorescence peaks in the chromatogram of the same type of extract (see figure 4.5).
Figure 5.8. Extracted emission (top) and excitation (bottom) profiles taken from EEM of extracts from nylon fibers dyed with Acid Yellow 17.
Figure 5.9. Extracted emission (top) and excitation (bottom) profiles taken from EEM of extracts from nylon fibers dyed with Acid Yellow 23.
Figure 5.10. Overlay comparison of the five extracted excitation profiles taken from EEM of nylon fibers dyed with Acid Yellow 17 and 23. Stars represent profiles of Acid Yellow 17 and Spectra represented by circles are profiles from Acid Yellow 23. Components two (red spectra), four (cyan spectra) and five (green spectra) show the strongest correlation.
Figure 5.11. Overlay comparison of the five emission profiles extracted from EEM of Extracts from Nylon Fibers Pre-Dyed with Acid Yellow 17 and 23. Solid lines represent profiles of Acid Yellow 17 and spectra represented by dotted lines are profiles from Acid Yellow 23. Components two (purple spectra), three (red spectra) and four (green spectra) show the strongest correlation.

Figures 5.12 and 5.13 correlate the excitation and fluorescence spectra of the five fluorescence components in each type of fiber extract. 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 support 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.
Figure 5.12 Shows Correlation of the Five Excitation Profiles Extracted from EEM of Extracts Collected from Nylon Fibers Pre-Dyed with Acid Yellow 17 and 23. Correlation is done 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, bottom-0.8956.
Figure 5.13. Shows Correlation of Five emission profiles extracted from EEM of extracts taken from nylon fibers dyed with Acid Yellow 17 and 23. Correlation is done by comparing intensity of excitation profile versus wavelength. Five correlation coefficients are as follows top left-0.7564; top right-0.9696; middle left-0.9480; middle right-0.9677, bottom-0.8300.

Figures 5.14 and 5.15 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 figure 5.14 predict strong similarity of excitation profiles among four of the five fluorescence components. Three of the five correlation coefficients in figure 5.15 predict strong similarity of fluorescence spectra among three of the five fluorescence components. Visual inspection of the excitation and fluorescence profiles overlaid in figure 5.16 and 5.17 confirm these correlations. Considering the number of similar excitation (4) and fluorescence (3) profiles it is safe to assume that three of
the five fluorescence components are present in both sets of fibers. Two of the remaining four components are present in only one set of fibers. The differences among the spectra of the two pairs of different components explain the EEM contour differences observed in figures 5.3 and 5.4. Based on the prediction that two components only exist in one type of fiber extract, MCR-ALS is able to discriminate among Acid Red 151 extracts of fibers collected from two different cloths.

Figure 5.14. Shows correlation of five excitation profiles extracted from EEM of extracts taken from nylon fibers of two different cloths dyed with Acid Red 151. Correlation is done by comparing intensity of excitation profile versus wavelength. Five correlation coefficients are as follows top left-0.93; top right-0.77; middle left-0.94; middle right-0.91, bottom-0.95.
Figure 5.15. Shows correlation of five emission profiles extracted from EEM of extracts taken from nylon fibers of two different cloths dyed with Acid Red 151. Correlation is done by comparing intensity of excitation profile versus wavelength. Five correlation coefficients are as follows top left-0.99; top right-0.85; middle left-0.88; middle right-0.99, bottom-0.77.
Figure 5.16. Comparison of the five excitation profiles extracted from EEM of nylon fibers collected from two different clothes dyed with Acid Red 151. Stars represent profiles of Acid Red 151 cloth #1 and Spectra represented by circles are profiles from Acid Red 151 circles. Components one (red spectra), three (blue), four (cyan spectra) and five (purple spectra) show the strongest correlation.
5.4. Conclusions

MCR-ALS analysis of EEM taken from 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 among 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 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 RTF-EEM and MCR-ALS as a promising tool for the forensic analysis of textile fibers.
CHAPTER 6: EFFECTS OF ENVIRONMENTAL FACTORS AND CONTAMINANTS ON FLUORESCENCE OF TEXTILE FIBERS

6.1 Introduction

From the time of manufacture to the time they are found at the crime scene, textile fibers will most likely be exposed to a wide variety of environmental factors and contaminants. These include, but are not limited to cigarette smoke, food stains, weathering and laundering. Their exposure to these uncontrollable variables might play a role on fiber discrimination via RTF spectroscopy. This chapter examines three possible environmental factors that could alter the fluorescence characteristics of textile fibers. Cigarette smoke is one of the possible contaminants that clothing may be exposed to in a real world environment. Contained within cigarette smoke are a number of strongly fluorescing compounds known as polycyclic aromatic hydrocarbons (PAH). Our investigations in this chapter expose fibers to cigarette smoke and examine the resulting extracts to detect the possible presence of PAH. Clothing in a real world environment will be exposed to laundering at some point. It is not unlikely that fibers discovered at a crime scene may have been exposed to multiple launderings previous to becoming evidence. Fibers from suspect clothing may have been laundered after a crime was committed. It becomes then essential to understand the effect that multiple washings will have on the fluorescence characteristics of fibers over time. Repetitive laundering of fibers might be able to remove fluorescence concomitants originally present in the fibers. The repetitive exposure to detergents and fabric softeners might add additional fluorescence spectral components to the total fluorescence of the fiber. In this chapter, we investigate the effect of repetitive washing on the intrinsic fluorescence of fibers, the fluorescence characteristics of detergents and fabric softeners and their effects upon repetitive washing of textile fibers.
6.2. Experimental

6.2.1. Chemicals and Supplies

Naphthalene, phenanthrene, anthracene, pyrene and benzo(a)pyrene were purchased from Sigma-Aldrich at their highest available purity. A Supelco 16 PAH mixture (EPA 610) in methanol:methyl chloride (1:1 v/v) from Supelco was used as the reference standard for HPL analysis. Marlboro cigarettes were purchased locally and tested in the lab. The following laundry detergents were purchased locally and tested as is, in the lab: All, Snuggle, Tide, Woolite, and X-TRA. For all remaining chemicals and supplies see section 2.2.1.

6.2.2. Exposure of Textile fibers to Cigarette Smoke

Disperse Red 1 fibers were exposed to cigarette smoke as detailed in figure 6.1. Two holes were cut into a 591mL plastic bottle. The first hole was made on the top of the cap lid and the second at the bottom of the bottle. The bottom hole was made to partially introduce a lit cigarette into the bottle. A metal screw with a textile fiber wound around it was introduced into the bottle via the top hole of the screw cap lid. Prior to introducing the cigarette into the bottle, the cigarette was lit with the aid of a rubber pipet bulb. Once the lit cigarette was partially placed into the bottle, the bulb was slowly depressed to simulate “smoking” of the cigarette. Following verbal communications from several smokers, the simulation of cigarette smoking was carried out over an average time of seven minutes to expose textile fibers to cigarette smoke. The cigarette was removed from the bottle after it burned down to the filter. The textile fiber was then removed from the bottle with the aid of the metal screw. Clean tweezers were used to place the exposed fiber inside a plastic micro-centrifuge tube. All steps involving cigarette smoking simulations were performed under an exhaust hood. Solvent extraction of PAH from exposed fibers was carried out with n-octane according to the procedure previously described (see section
2.2.2). Blanks of un-exposed fibers were submitted to the same solvent extraction procedure to investigate the possible octane co-extraction of inherent fluorescence components.

![Diagram](image)

**Figure 6.1 Diagram illustrating the procedure of exposing fibers to cigarette smoke.**

6.2.3. Washing of Textile Fibers

Five square samples (5 cm x 5 cm) of cloth were cut from a 1 m x 1 m piece of cloth pre-dyed with Disperse Red 4. Each sample cloth was placed into a separate plastic bag and set aside in the dark for storage. Four of the five sample cloths were individually washed in the presence of full load of cloths. The remaining cloth sample was kept unwashed for reference purposes. Each washing was carried out using the normal wash cycle setting of the same washing machine located at the Laundromat of a local apartment complex. Each piece of cloth was subsequently washed five times. The only difference among washings of the same sample was
the load of cloths in the washing machine, which had previous exposure to unknown potential contaminants. All washing were done in cold water with ALL detergent. After each washing, the sample was dried in the same commercial drier with the entire load of cloths. After each drying, one fiber of 1 cm length was removed from the sample cloth and set aside for further experiments in a plastic micro-centrifuge tube. All collected fibers were storage in the dark at room temperature. Fiber extractions were carried out with 1:1 acetonitrile-water (v/v) according to experimental procedure described in section 2.2.2.

6.2.4. Absorption Spectroscopy Measurements

See section 2.2.3 for experimental details.

6.2.5. RTF Spectroscopy Measurements

See section 2.2.4 for experimental details.

6.2.6. HPLC Analysis

The separation of PAH was accomplished using a mixture of methanol/water as the mobile phase. Column conditions include a 1.5mL/min flow rate, isocratic elution with 40/60 water/methanol for 5 min, and then linear gradient to 99% methanol over 20 min. The total separation time of the 16 EPA-PAH was approximately 40 min. For all remaining details on HPLC analysis refer to section 4.2.5.

6.2.7. High-Resolution Luminescence Spectroscopy

6.2.7.1. Instrumentation: Samples were excited with the output of a Northern Lights tunable dye laser (Dakota Technologies, Inc.) through a KDP frequency-doubling crystal. The dye laser was operated Rhodamine 590 and DCM laser dye (Exciton), and was pumped with the
second harmonic of a 10-Hz Nd:YAG Q-switched solid state laser (Big Sky Brilliant Laser). Fluorescence was detected with a multi-channel detector consisting of a front illuminated ICCD (Andor Technology). The CCD has the following specifications: active area, 690 x 256 pixels (26 mm² pixel size photocathode); dark current, 0.002 electrons/pixel/s; readout noise, 4 electrons at 20 kHz. The ICCD was mounted at the focal plane of a spectrograph (SPEX 270M) equipped with a 1200 grooves/mm grating blazed at 500 nm. The gating parameters were controlled with a digital delay generator (DG535, Stanford Research Systems, Inc) via a GPIB interface. Custom Labview software, developed in house, was used for instrument control and data collection.

6.2.7.2. Cryogenic Probe: the probe assembly consisted of one excitation and six collection fibers fed into a 1.25 m long section of copper tubing that provided mechanical support for lowering the probe into the liquid cryogen. All the fibers were 3 m long and 500 μm core diameter silica-clad silica with polyimide buffer coating (Polymicro Technologies, Inc.). At the analysis end, the excitation and emission fibers were arranged in a conventional six-around-one configuration, bundled with vacuum epoxy (Torr-Seal®, Varian) and fed into a metal sleeve for mechanical support. The copper tubing was flared stopping a swage nut tapped to allow for the threading of a 0.75 ml polypropylene sample vial. At the instrument end, the emission fibers were bundled with vacuum epoxy in a slit configuration, fed into a metal sleeve and aligned with the entrance slit of the spectrometer.

6.7.2.3. Lifetime Analysis: fluorescence lifetimes were determined via a three-step procedure: (1) full sample and background WTM were collected; (2) the background decay curve was subtracted from the fluorescence decay curve at the wavelength of maximum fluorescence for each PAH; (3) the background corrected data were fit to single exponentials. In cases where the exact sample composition was unknown and the formulation of a correct blank
for lifetime background subtraction was impossible, the fluorescence decay at the base of the target peak was used for background subtraction at the target wavelength. The accuracy of this procedure was investigated previously (18). Origin software (version 5, Microcal Software, Inc.) was used for curve fitting of fluorescence lifetimes. The decay curve data were collected with a minimum 10 ns interval between opening of the ICCD gate and the rising edge of the laser pulse, which is sufficient to avoid the need to consider convolution of the laser pulse with the analyte signal. Fitted decay curves \( y = y_0 + A_1 \exp^{-(x-x_0)t} \) were obtained by fixing \( y_0 \) and \( x_0 \) at a value of zero.

6.2.7.4. Experimental Procedure for 77K Measurements: Samples were frozen with the following procedure. After 0.2 ml of undegassed sample solution was pipetted into the sample vial, the tip of the fiber optic probe was positioned and held constant with the screw cap below the solution surface. Sample freezing was accomplished by lowering the copper tubing into the liquid cryogen. The liquid nitrogen was held in a Dewar with 5L storage capacity. Complete sample freezing took less than 90 s. The approximately one-minute probe clean up procedure involved removing the sample vial from the cryogen container, melting the frozen matrix and warming the resulting solution to approximately room temperature with a heat gun, rinsing the probe with n-alkane, and drying it with warm air from the heat gun. The entire freeze, thaw, and cleanup cycle took no longer than 5 minutes.

6.3 Results and Discussion

6.3.1 Investigation of PAH After Cigarette Smoke Exposure

Solvent extraction of fibers unexposed to cigarette smoke provided n-octane extracts with no fluorescence. This fact agrees well with previous results from fiber extractions with polar
solvents. Both cases support the presence of polar fluorescence impurities in Disperse Red 1 fibers. Solvent extraction of fibers exposed to cigarette smoked provided n-octane extracts with strong fluorescence. Attributing the observed fluorescence to the adsorption of PAH from cigarette smoke, n-octane fiber extracts were submitted to PAH analysis via HPLC [ref] and Laser-Excited Time-Resolved Shpol’skii Spectroscopy (LETRSS) [ref].

Figure 6.2 compares the absorption chromatogram of the EPA-PAH standard mixture to a chromatogram recorded from one fiber extract. It can be noted that several peaks in the extract chromatogram match the retention times of EPA-PAH. The unknown peaks in the fiber extract can be attributed to the presence of naphthalene, phenanthrene, anthracene, pyrene and benzo(a)pyrene. Their presence was then confirmed via LETRSS analysis of the fiber extract.

Figure 6.3 shows an example of the type of data obtained for the investigated PAH. Analysis of fiber extracts under selective parameters for pyrene determination – excitation wavelength = 313nm, delay time = 100 ns and gate time = 2000 ns - provided fluorescence profiles almost identical to those obtained from pure pyrene solutions. Figure 6.4 shows similar results for benzo(a)pyrene. Both spectra were recorded under selective conditions for benzo(a)pyrene, i.e. excitation wavelength = 297.5 nm, delay time = 10 ns and gate time = 200 ns. Because site-selective excitation was not attempted and fluorescence was not recorded with optimum band-pass for best spectral resolution, all fluorescence peaks correspond to the emission of multi-sites in the Shpol’skii matrixes.
Figure 6.2 HPLC absorbance chromatogram of (—) 16 EPA PAH 1-Naphthalene*, 2-Acenaphthylene, 3-Acenaphthene, 4-Fluorene, 5-Phenanthrene*, 6-Anthracene*, 7-Fluoranthene, 8-Pyrene*, 9-Benzo(a)anthracene, 10-Chrysene, 11-Benzo(b)fluoranthene, 12-Benz(k)fluoranthene, 13-Benzo(a)pyrene*, 14-Dibenzo(a,h)anthracene, 15-Benzo(g,h,i)perylene, 16-Indeno(1,2,3-cd)pyrene (—) Octane extract taken from 1cm of acetate 154 fiber dyed with Disperse Red 1 that was exposed to cigarette smoke.
Figure 6.3 Fluorescence emission of pyrene and fiber extract at 77K. (—) Fiber extract and (—) 10ppm Standard. Exciting at 313 nm a delay of 100ns and gate width of 2000 ns.

Figure 6.4. Fluorescence emission of benzo(a)pyrene and fiber extract at 77K. (—) Fiber extract and (—) 10ppm Standard. Exciting at 297.5 nm a delay of 10ns and gate width of 200 ns.
Lifetime measurements made at the maximum wavelengths of fluorescence peaks with highest intensities confirmed the spectral assignments to the sole emission of the investigated PAH. Figures 6.5 and 6.6 compare the fluorescence decays of pure standards of pyrene and benzo(a)pyrene to those recorded from fiber extracts. The single exponential decays observed from fiber extracts demonstrate the emission of single components at the excitation and fluorescence wavelengths of measurements. The comparison of the lifetime values to those recorded from pure standards confirm the peaks assignments to pyrene and benzo(a)pyrene.

Figure 6.5 Fluorescence decay of pyrene and extract at 77K. (—) Fiber extract and (—) 10ppm Standard. Exciting at 313 nm and collecting decay at 371 nm. Decay collected with a delay of 100ns, gate width of 2000 ns and 40 steps of 50 ns. Lifetime for standard = 480.5 ns; lifetime for extract = 506.0 ns.
Figure 6.6. Fluorescence decay of benzo(a)pyrene and fiber extract at 77K. (—) Fiber extract and (—) 10ppm Standard. Exciting at 297.5 nm and collecting decay at 402 nm. Decay collected with a delay of 10ns, gate width of 200 ns and 40 steps of 5 ns. Lifetime for standard = 40.7 ns; lifetime for extract = 39.6 ns.

6.3.2 Spectral Characteristics of Detergents and Fabric Softeners

The absorption and fluorescence characteristics of commercial detergents and fabric softeners were investigated using solutions with typical laundering concentrations. Their concentrations were calculated taking into consideration the average volume (40 L) of a common washing machine and the quantity of detergents and fabric softeners recommended for use by the manufacturer. Of the five detergents and fabric softeners that were investigated four of them showed strong absorbance in the ultra-violet spectral region. Figures 6-7 overlays their spectral profiles and Table 6.1 lists their maximum absorption wavelengths.
Figure 6.7. Absorbance Spectra of Laundry Detergents and Fabric Softeners Overaid for Comparison.

Table 6.1 Absorbance of Laundry Detergents

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>223</td>
</tr>
<tr>
<td>Snuggle</td>
<td>N/P</td>
</tr>
<tr>
<td>Tide</td>
<td>225, 253</td>
</tr>
<tr>
<td>Woolite</td>
<td>224</td>
</tr>
<tr>
<td>XTRA</td>
<td>223</td>
</tr>
</tbody>
</table>

All the investigated detergents and softeners showed strong fluorescence upon excitation in the ultraviolet spectral region. Their excitation and fluorescence spectra are shown in figures 6.8 – 6.12. In all cases, fluorescence extends from the ultraviolet to the visible region.
Figure 6.8 XTRA laundry detergent Excited at 278nm. Excitation and emission slits set at 4 and 2nm band-pass, respectively.
Figure 6.9 Woolite laundry detergent excited at 262nm. Excitation and emission slits set at 4 and 2nm band-pass, respectively.
Figure 6.10 Tide Laundry Detergent Excited at 288nm. Excitation and emission slits set at 4 and 2nm band-pass, respectively.
Figure 6.11 Snuggle fabric softener Excited at 230nm. Excitation and emission slits set at 4 and 2nm band-pass, respectively.
Figure 6.12. All laundry detergent Excited at 263nm. Excitation and emission slits set at 4 and 2nm band-pass, respectively.

6.3.3. Fluorescence Characteristics of Disperse Red 4 Fiber Extracts after Laundering

Figure 6.13 shows the change in fluorescence spectra of Disperse Red 4 fiber extracts as a function of fiber laundering with ALL detergent. Table 6.2 lists the signal intensities measured at the maximum wavelengths of the two fluorescence peaks. All measurements were made upon sample excitation at 291nm, i.e. the maximum excitation wavelength of the fiber extract in the absence of detergent. The comparison of signal intensities in Table 6.2 shows no significant change at 344nm. On the other end, a significant intensity increase is observed at 414nm. Comparison of the spectral features of ALL in figure 6.12 to those in figure 6.13 shows that the main fluorescence peak of the extract (maximum wavelength at 344nm) falls within the wavelength valley (~320 nm to ~400nm) at which the detergent emits low fluorescence. The
second fluorescence peak of the extract (maximum wavelength at 414nm) strongly overlaps with the fluorescence peak of the detergent at 431nm. The intensity enhancement observed at 414nm could then be attributed to the residual amount detergent on the fiber, which apparently increases with the number of washings.

**Table 6.2 Intensity of Fluorescence From Disperse Red 4 Extracts After Washing**

<table>
<thead>
<tr>
<th># of Washings</th>
<th>Intensity (cps)a</th>
<th>Intensity (cps)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.40 x 10^6</td>
<td>2.73 x 10^6</td>
</tr>
<tr>
<td>1</td>
<td>5.29x10^6</td>
<td>2.67x10^6</td>
</tr>
<tr>
<td>2</td>
<td>5.23x10^6</td>
<td>3.52 x 10^6</td>
</tr>
<tr>
<td>5</td>
<td>5.27x10^6</td>
<td>3.42 x 10^6</td>
</tr>
</tbody>
</table>

a Intensity measured from peak at 344nm  
b Intensity measured from peak at 414nm
Figure 6.13 Fluorescence emission of extracts taken from fibers dyed with Disperse Red 4 dye after a variable number of launderings in a washing machine. Samples excited at 291 nm with excitation and emission slits set at 4 and 2 nm band-pass, respectively.

6.4 Conclusions

Many researchers believe that environmental contaminants increase the individualization of the fiber for forensic analysis [33-37]. The research we conducted in this chapter does not intend to provide a definite answer to all the questions related to environmental factors. Our objective was relatively modest as we only wanted to initiate an investigation on the potential usefulness of environmental contaminants as an additional source of RTF discrimination. Although we are not convinced one way or the other, we feel that the fluorescence of environmental contaminants could prove useful for fiber comparison. For instance, the spectral features of PAH adsorbed on the fiber could be used to discriminate between fibers on the basis
of their exposure history. The same is true with the spectral changes observed upon washing. In the case of non-polar contaminants such as PAH, one possibility is to remove them from the fiber prior to its extraction. Because of the polar nature of intrinsic fluorescence components, fiber rinsing (or even extraction) with a non-polar solvent should be harmless to the inherent fluorescence of the fiber. Because detergents and softeners are soluble in water, the choice of an appropriate solvent for their selective extraction requires further studies. Fiber discrimination via RTF spectroscopy should benefit from a comprehensive spectral database of commercial detergents and softeners. Comparison of this database to the spectral characteristics of fiber extracts should aid the analyst to track down the exposure of fibers to laundering contaminants.
CHAPTER 7: OVERALL CONCLUSIONS AND FUTURE WORK

The work presented in this dissertation provides an optimistic foundation to pursue the full potential of RTF Spectroscopy for forensic fiber discrimination. Dye analysis has long been recognized as an important feature for forensic fiber comparisons due to the diversity of visually and microscopically indistinguishable dye materials. Numerous methods have been reported for isolating dyestuffs from fibers and for comparing dyes and dye mixtures with thin-layer chromatography (TLC) techniques. These include TLC systems for disperse, acid and basic dyes extracted from polyester, polyamide and acrylic fibers [38], for direct and reactive dyes extracted from cellulose fibers [39], for acid and metallised acid dyes from wool [40] and for disperse, acid and metallised dyes from polypropylene [41]. Some of these methods were compiled and presented as an analytical protocol in 1983 by the Trace Evidence Study Group of the California Association of Criminalists. [42] This work was later extended to cotton and rayon fibers by Laing et al. [43] Typically, the protocol described by Wiersema involves three major steps: (a) identification of the fiber type (usually involving microscopy); (b) selection and application of increasingly powerful extracting solvents to identify dye types; and (c) selection of appropriate TLC systems to discriminate extracted dyes. Although these procedures and their limitations pose few if any problems when large samples of fiber or fabric are available for comparison, they represent substantial difficulties in typical forensic fiber comparisons. These difficulties center on the fact that each loose fiber in a forensic investigation represents, and must be handled as, a unique item of evidence in the case. Extensive work in 1974 [44] had shown that the average length fiber transferred between clothing materials was about 5mm. Subsequent work in 1991 reemphasized the limited length and size distribution of retained transferred fibers in
typical casework, with the result that the average recovered transferred fiber had a length of less than 2mm, when removed eight hours after the transfer took place. [45] This fiber length poses a significant challenge to current forensic protocols, [38-45] which require a minimum fiber length between 5 and 20mm. Extracting solvents are not universally applicable and because dye extraction is a destructive technique evidence fibers are rendered useless for future comparisons or reanalysis.

Our prospective to fiber analysis was fundamentally different as we focused on the total fluorescence of fiber extracts. We demonstrated that fluorescence impurities inherent to the chemical composition of textile fibers are reproducible sources for fiber comparison. A common solvent (1:1 acetonitrile-water v/v) to all investigated fibers provided characteristic fluorescence profiles for discrimination with 2mm lengths. MCR-ALS analysis of EEM taken from fiber extracts made possible to discriminate among visually indistinguishable fibers. With this approach, we were able to discriminate among two visually indistinguishable fibers pre-dyed with the same dye in the same textile industry but from different cloths.

The role of environmental contaminants on the RTF Spectroscopy of textile fibers remains an open question. Their presence in the fibers could benefit the individualization of the fiber for forensic analysis. The spectral features of PAH adsorbed on textile fibers exposed to cigarette smoke could be used to discriminate between fibers on the basis of their exposure history. The same is true with the fluorescence spectral changes upon washing. In the case of non-polar contaminants such as PAH, one possibility is to remove them from the fiber prior to its extraction. Because of the polar nature of intrinsic fluorescence components, fiber rinsing (or even extraction) with a non-polar solvent should be harmless to the inherent fluorescence of the fiber. Because detergents and softeners are soluble in water, the choice of an appropriate solvent
for their selective extraction requires further studies. One way or the other, fiber discrimination via RTF spectroscopy should benefit from a comprehensive spectral database of PAH and their resulting spectral profiles in the smoke from commercial cigarette brands, detergents and softeners. Comparison of these databases to the spectral characteristics of fiber extracts should aid the analyst to track down the history of fibers to environmental exposure.

Current work in our lab seeks to develop RTF spectroscopy into a non-destructive technique for forensic fiber discrimination. With the purpose of making this approach an appealing tool to the forensic community, we had to envision an instrument that could be easily assembled from commercially available equipment at a cost that fit the budget of Forensic Science labs. Many tests were made in our lab with the available instrumentation and fiber samples were shipped to instrumentation companies to test additional options. Our final choice consists of an Olympus BX-51 fluorescence microscope connected to a FluoroMax 3 spectrofluorimeter (Horiba Jobin-Yvon) via fiber optic probes. The spectrofluorimeter uses a continuous wave Xenon lamp for sample excitation from 200 to 1100nm. The excitation and emission monochromators are equipped with 1,200 grooves/mm gratings blazed at 330 and 500nm, respectively. Fluorescence is detected with a photomultiplier tube (R928P) with spectral response from 180 and 850nm. Figure 7.1 shows a schematic diagram detailing the microscope unit of the instrumental set up. Excitation and emission ranges for measurements with the microscope are selected via one of the following: dichroic mirrors, filters or beam splitters. The choice of the optical component is facilitated with a turret placed immediately above the microscope objective and aligned with both the excitation and the emission optical paths. The turret can accommodate up to six optical components. The best approach to collect EEM is to use two 50/50 beam splitters, one for the ultraviolet and the other for the visible range.
The fluorescence area probe with the microscope objective can be controlled through a pinhole turret with variable sizes. This pinhole improves signal-to-noise ration minimizing the detection of excitation scatter and background emission. Fluorescence is directly collected with the emission bundle of the fiber optic probe for EEM collection with the spectrofluorimeter. With the aid of a flipper mirror, fluorescence can be directed into the camera CCD of the microscope for imaging recording and/or real time viewing of the sample on the stage.

Figure 7.1  Schematic diagram of Olympus BX-51 microscope coupling to Fluormax-P.
Figure 7.2 and 7.3 show three dimensional EEM recorded from a polyester fiber pre-dyed with Disperse Red 4. Upon excitation at 500nm, the main fluorescence peak of the resulting EEM appears at 612nm. These maximum wavelengths are similar to those observed from disperse Red 4 fiber extracts. Upon excitation in the ultraviolet, the resulting EEM shows a single fluorescence peak at 405nm. The maximum wavelength of this peak appears at a similar wavelength to the maximum observed from fiber extracts under ultraviolet excitation. Future work in our lab will extend EEM collection to the remaining fibers in Table 2.1.

Figure 7.2 Visible fluorescence EEM taken directly from Disperse Red 4 fibers
Figure 7.3 UV fluorescence EEM taken directly from Disperse Red 4 fibers.
APPENDIX A: MOLECULAR STRUCTURES OF 14 INVESTIGATED DYES
Figure A.1 Dye Structures

Disperse Red 1

Disperse Red 4

Disperse Red 13

Disperse Blue 56

Basic Green 4

Basic Red 9

Basic Violet 14

Direct Blue 1
Direct Blue 71

Direct Blue 90

Acid Red 151

Acid Yellow 17
Acid Yellow 23

Acid Green 27
APPENDIX B: ROOM TEMPERATURE FLUORESCENCE SPECTRA OF FIBER EXTRACTS IN FOUR DIFFERENT EXTRACTING SOLVENTS
Figure B-1. Fluorescence spectra of extracts taken from acetate fibers dyed with Disperse Red 1 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-2. Fluorescence spectra of extracts taken from polyester fibers dyed with Disperse Red 4 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-3. Fluorescence spectra of extracts taken from polyester fibers dyed with Disperse Red 13 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-4. Fluorescence spectra of extracts taken from polyester fibers dyed with Disperse Blue 56 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-5. Fluorescence spectra of extracts taken from poly-acrylic fibers dyed with Basic Green 4 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-6. Fluorescence spectra of extracts taken from polyester fibers dyed with Basic Red 9 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-7. Fluorescence spectra of extracts taken from polyester fibers dyed with Basic Violet 14 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-8. Fluorescence spectra of extracts taken from cotton fibers dyed with Direct Blue 1 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-9. Fluorescence spectra of extracts taken from cotton fibers dyed with Direct Blue 71 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-10. Fluorescence spectra of extracts taken from cotton fibers dyed with Direct Blue 90 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-11. Fluorescence spectra of extracts taken from nylon fibers dyed with Acid Red 151 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-12. Fluorescence spectra of extracts taken from nylon fibers dyed with Acid Yellow 17 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-13. Fluorescence spectra of extracts taken from nylon fibers dyed with Acid Yellow 23 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
Figure B-14. Fluorescence spectra of extracts taken from nylon fibers dyed with Acid Green 27 dye. Extracts are in A-1:1 methanol/water (v:v) B-ethanol C-1:1 acetonitrile/water (v:v) D-4:3 pyridine/water (v:v). Excitation and emission set at maximum wavelength.
APPENDIX C: ULTRAVIOLET/VISIBLE SPECTRA OF DYE STANDARDS AND EXTRACTS AT ROOM TEMPERATURE
Figure C-1. Absorbance spectra of Disperse Red 1 A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in ethanol C-extract taken from acetate fibers dyed with Disperse Red 1 in ethanol.
Figure C-2 Absorbance spectra of Disperse Red 4 A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in 1:1 acetonitrile/water (v:v) C-extract taken from polyester fibers dyed with Disperse Red 4 in 1:1 acetonitrile/water (v:v).
Figure C-3. Absorbance spectra of Disperse Red 13 A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in ethanol C-extract taken from polyester fibers dyed with Disperse Red 13 in ethanol.
Figure C-4. Absorbance spectra of Disperse Blue 56 A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in 4:3 pyridine/water (v:v) C-extract taken from polyester fibers dyed with Disperse Blue 56 in 4:3 pyridine/water (v:v).
Figure C-5. Absorbance spectra of Basic Green 4 A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in 1:1 acetonitrile/water (v:v) C-extract taken from poly-acryllic fibers dyed with Basic Green 4 in 1:1 acetonitrile/water (v:v).
Figure C-6. Absorbance spectra of Basic Red 9 A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in ethanol C-extract taken from polyester fibers dyed with Basic Red 9 in ethanol.
Figure C-7 Absorbance spectra of Basic Violet 14  A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in 1:1 acetonitrile/water (v:v) C-extract taken from polyester fibers dyed with Basic Violet 14 in 1:1 acetonitrile/water (v:v).
Figure C-8. Absorbance spectra of Direct Blue 1 A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in ethanol C-extract taken from cotton fibers dyed with Direct Blue 1 in ethanol.
Figure C-9. Absorbance spectra of Direct Blue 71. A-10 ppm standard in 1:1 methanol/water (v:v) B-10 ppm standard in 1:1 acetonitrile/water (v:v) C-extract taken from cotton fibers dyed with Direct Blue 71 in 1:1 acetonitrile/water (v:v).
Figure C-10. Absorbance spectra of Direct Blue 90 A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in 4:3 pyridine/water (v:v) C-extract taken from cotton fibers dyed with Direct Blue 90 in 4:3 pyridine/water (v:v).
Figure C-11. Absorbance spectra of Acid Red 151  A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in ethanol C-extract taken from nylon fibers dyed with Acid Red 151 in ethanol.
Figure C-12. Absorbance spectra of Acid Yellow 17 A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in ethanol C-extract taken from nylon fibers dyed with Acid Yellow 17 in ethanol.
Figure C-13. Absorbance spectra of Acid Yellow 23. A-10ppm standard in 1:1 methanol/water (v:v) B-10ppm standard in ethanol C-extract taken from nylon fibers dyed with Acid Yellow 23 in ethanol.
Figure C-14. Absorbance spectra of Acid Green 27 A-10ppm standard in 1:1 methanol/water (v:v) B-extract taken from nylon fibers dyed with Acid Green 27 in 1:1 methanol/water (v:v)
APPENDIX D: EXCITATION AND FLUORESCENCE OF DYE STANDARDS IN BEST EXTRACTING SOLVENT
Figure D.1 Excitation Emission Spectrum of Disperse Red 1 Standard in 1:1 Acetonitrile/Water (v:v). $\lambda_{\text{ex}} = 258\text{nm}$ and $\lambda_{\text{em}} = 307\text{nm}$
Figure D.2 Excitation Emission Spectrum of Disperse Red 4 Standard in 1:1 Acetonitrile/Water (v:v). A-Ultra-violet region $\lambda_{ex} = 272\text{nm}$ and $\lambda_{em} = 300\text{nm}$  
B-Visible region $\lambda_{ex} = 512\text{nm}$ and $\lambda_{em} = 570\text{nm}$.
Figure D.3 Excitation Emission Spectrum of Disperse Blue 56 Standard in 4:3 Pyridine/Water (v:v). A-Ultra-violet region $\lambda_{\text{ex}} = 326\text{nm}$ and $\lambda_{\text{em}} = 380\text{nm}$ B-Visible region $\lambda_{\text{ex}} = 586\text{nm}$ and $\lambda_{\text{em}} = 661\text{nm}$. 
Figure D.4 Excitation Emission Spectrum of Basic Green 4 Standard in 1:1 Acetonitrile/Water (v:v). $\lambda_{ex} = 272\text{nm}$ and $\lambda_{em} = 368\text{nm}$
Figure D.5 Excitation Emission Spectrum of Basic Red 9 Standard in Ethanol. $\lambda_{\text{ex}} = 252\text{nm}$ and $\lambda_{\text{em}} = 350\text{nm}$
Figure D.6 Excitation Emission Spectrum of Basic Violet 14 Standard in 1:1 Acetonitrile/Water (v:v). $\lambda_{ex} = 299$nm and $\lambda_{em} = 363$nm
Figure D.7 Excitation Emission Spectrum of Direct Blue 1 Standard in Ethanol.

$\lambda_{ex} = 340\text{nm}$ and $\lambda_{em} = 397\text{nm}$
Figure D.8 Excitation and Emission Spectrum of Direct Blue 71 Standard in 1:1 Acetonitrile/Water (v:v). $\lambda_{ex} = 296$ nm and $\lambda_{em} = 431$ nm
Figure D.9 Excitation Emission Spectrum of Direct Blue 90 Standard in 4:3 Pyridine/Water (v:v). $\lambda_{ex} = 250\text{nm}$ and $\lambda_{em} = 306\text{nm}$
Figure D.10 Excitation and Emission Spectrum of Acid Yellow 17 Standard in Ethanol. 
$\lambda_{ex} = 278\text{nm}$ and $\lambda_{em} = 302\text{nm}$
Figure D.11 Excitation Emission Spectrum of Acid Yellow 23 Standard in Ethanol.
A-Ultraviolet region $\lambda_{\text{ex}} = 275\text{nm}$ and $\lambda_{\text{em}} = 299\text{nm}$  
B-Visible region $\lambda_{\text{ex}} = 451\text{nm}$ and $\lambda_{\text{em}} = 550\text{nm}$. 
Figure D.12 Excitation and Emission Spectrum of Acid Green 27 Standard in 1:1 Methanol/Water. $\lambda_{ex} = 598\text{nm}$ and $\lambda_{em} = 689\text{nm}$
Figure E.1 EEM of Standards and Extracts from Fibers Pre-Dyed with Disperse Red 4 in 1:1 Acetonitrile/Water (v:v)
A-10ppm standard B-Fiber extract

Figure E.2 EEM of Standards and Extracts from Fibers Pre-Dyed with Disperse Blue 56 in 4:3 Pyridine/Water (v:v)
A-10ppm standard B-Fiber extract
Figure E.3 EEM of Standards and Extracts from Fibers Pre-Dyed with Basic Green 4 in 1:1 Acetonitrile/Water (v:v)
A-10ppm standard B-Fiber extract

Figure E.4 EEM of Standards and Extracts from Fibers Pre-Dyed with Basic Red 9 in Ethanol
A-10ppm standard B-Fiber extract
Figure E.5 EEM of Standards and Extracts from Fibers Pre-Dyed with Acid Red 151 in Ethanol
A-10ppm standard B-Fiber extract

Figure E.6 EEM of Standards and Extracts from Fibers Pre-Dyed with Acid Yellow 17 in Ethanol
A-10ppm standard B-Fiber extract
Figure E.7 EEM of Standards and Extracts from Fibers Pre-Dyed with Acid Yellow 23 in Ethanol
A-10ppm standard B-Fiber extract

Figure E.8 EEM of Standards and Extracts from Fibers Pre-Dyed with Acid Green 27 in 1:1 Methanol/Water (v:v)
A-10ppm standard B-Fiber extract
REFERENCES CITED


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