Forensic Dye Analysis by Direct Analysis in Real Time Mass Spectrometry and Raman Spectroscopy

by
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A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirements for graduation from the University Honors College

Term Fall 2021

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Acknowledgements

I would like to thank Dr. Zhang for his constant guidance and willingness in every aspect of this project, and for providing the necessary materials. I would also like to thank him for his help in earning MTSU's Undergraduate Research Experience and Creative Activity (URECA) gold award, as well as abstract submission to the American Academy of Forensic Science, National Conference for Undergraduate Research, and MTSU Scholars Week. Thank you to Dr. Chong for the countless hours helping me optimize techniques for extraction and Raman analysis, along with teaching me the surface enhanced Raman spectroscopy technique. I would like to acknowledge Billiquis Binitinlaiye for her collection of dyes of fibers through the HORIBA 785 nm and 532 nm wavelength.

Abstract

Differentiating similarly colored dyes is important in suspect-to-trace evidence connection. The thermal desorption/pyrolysis-direct analysis in real time-mass spectrometry (TD/Py-DART-MS) and the surface-enhanced Raman scattering (SERS) methods were developed to analyze dyes on textile fibers. Five blue dyes on different polymer matrices of fibers, including cotton, silk, and nylon, were analyzed. The mass spectra were collected from the dye standards and dyed fibers, with no prior extraction solvent step. A profile of desorption time and mass spectra was compared. The characteristic ions of dyes and polymers were identified. Dispersive Raman spectroscopy was used as a secondary method with an aqueous silver colloid to improve the signal-to-noise ratio and minimize the contribution of background polymer signal on Raman spectra. The TD/Py-DART-MS method requires a single fiber and no extraction, but it offers a multidimensional profile within 7 minutes. The method is simple and has high sample throughput potential, which may significantly contribute to the forensic identification of dyed fibers.

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CHAPTER 1

Introduction

Background

From 1979 to 1981, the fear of child abduction gripped the American South, bringing a new meaning to the popular phrase, “It’s 10 pm. Do you know where your children are?” This came after the murders of over 28 young, black children in inner-city Atlanta; their attacks and disposals were incredibly similar. Following community outcry, the FBI investigated the serial killer now dubbed: The Atlanta Monster.[1] During this time, the FBI’s Atlanta Task Force relayed photomicrographs taken of nylon fibers found on the victims’ clothes to chemists and carpet manufacturers to identify the car, or house, that would have those fibers in them. The forensic analysis led to the discovery that the nylon fibers were treated with a unique dye. The dye and fibers were linked to an already prime suspect: Wayne Williams. Through their intense focus on discriminating and differentiating unknown fibers and comparing these fibers to standards, this case became the first to highlight the importance of forensic fiber analysis in solving serious crimes. It also became the first case in American history to include fiber evidence as to the primary physical evidence at trial.[1]

Today, fibers continue to provide important trace evidence about a victim and attacker's interactions at the time of a crime.[2] This is done following the principle of exchange, established by Edmond Locard, whereas “When any two objects come into contact, there is always a transfer of material from each object on to the other”. [1] This exchange principle applies to all types of microscopic evidence that can be transferred,

yet stay in its environment to be later analyzed.[3] In the case of fibers, specifically, a transfer is seen during assaults, homicides, or other violent acts where the fibers from, particularly clothes.[3] If there is a higher intensity to the transfer, then there is a higher probability that more textile fibers will be transferred off a person.[3] However, current forensic scientists analyzing fiber samples must analyze a small quantity of transferred fiber.[3] This sample size does hold benefits because it is less readily noticed by an assailant and more likely to be recovered at the crime scene by investigators.[4]

In addition, textile fibers themselves are analyzed as two major categories: polymer backbone and dye applied to the fiber.[4] Research into the identification of these categories is quickly growing. The modern manufacturing of textiles requires dyeing techniques, which differ depending on the chemical composition of the dye itself.[5,6] These processes create individual characteristics for an examiner to compare to a possible origin.[5] The specificity gained by examining the dyes is important, especially when a large portion of textile is produced in bulk, which creates uniformity amongst dyes use.[7]

Dyes form another aspect for characterizing fiber evidence due to the many classes within one color.[8] Depending on their physical and chemical properties, the solubility and reactivity of dyes can vary significantly in different solutions and pHs, driving to various applications.[8] For example, acid dyes have deprotonated functional groups that bond with protonated polymers, but basic dyes have their positively charged functional groups that bond to negatively charged functional groups on the polymer.[8] Disperse dyes have hydrogen and weak van der Waals forces to hold them to the polymer, while reactive dyes form covalent bonds with the functional groups of the polymer.[8] Others, specifically Methyl Violet 2B are highly water-soluble textile dyes,

which is a wildly used organic pigment.[9] This study will solely focus on these dye categories in the blue/violet color. The color selection was based on the popularity of blue/violet in the textile community and how commonly it is seen on campus.

Analytical Instrumentation for Textile Fiber Analysis

To rigorously defend the fiber evidence in court, examiners rely on at least two types of analytical techniques for each of the categories: generic class, physical characteristics, and color.[2] Microscopes remain the primary instrument of analysis since they can offer characteristic information of fibers in all three categories in a nondestructive manner.[4] Some spectroscopic techniques can also be used without destroying the sample, such as Fourier-Transform infrared spectroscopy (FT-IR) and Raman spectroscopy.[2,10,11] Classical methods, such as UV-vis microspectrophotometry (MSP), liquid chromatography-mass spectrometry (LC-MS), and thin-layer chromatography (TLC), are used for the analysis of fibers and dyes at the molecular level.[2,10] While these methods are widely used in crime labs across the country, specifically in dye analysis, they do have drawbacks. The solvent extraction of dyes prior to the LC-MS analysis is critical to the success of dye analysis, but it is laborious, prone to contamination, and time-consuming (e.g., about 1 hour).[12] Due to these difficulties, research into direct analysis with no sample preparation has led to the development of methods showing a promising success in dye identification when a fiber is dyed with a light color or multiple dyes of the same color.[13]

Direct analysis in real time mass spectrometry (DART-MS) has recently emerged as an alternatively sensitive and rapid technique for the detection of various

compounds.[14] This sensitivity is derived from its excited helium metastables, in the atmosphere of Figure 1, which create a cascade of gas-phase reactions that then ionize the molecules from the sample.[14] This results in a mass spectrum of primarily molecular ions of the compounds, differentiating it from other mass spectrometric ionization methods that often show fragmentation or adduct ion patterns.[15,16] For this reason, numerous forensic labs have already utilized DART-MS in analyzing paints, drugs, and inorganic explosives.[16,17,18] Others have used DART-MS for the analysis of organic oils.[19] Some studies have coupled a thermal desorption module with DART-MS whereby a sample stage temperature increases at various heating rates over a designated amount of time to desorb the compound for the analysis. This partially preserves the volatility information of individual analytes in a complex sample.

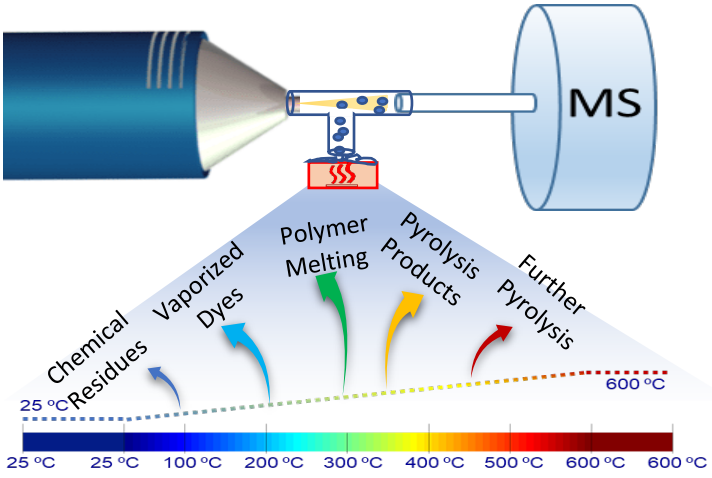


Figure 1. Schematic view of TD/Py DART-MS.

This study used DART-MS as a simple and fast method for the analysis of fibers. The method provided a multidimensional chemical profile of fiber evidence, such as the mass spectra of both the dye and polymeric material and their respective desorption temperatures. This study focused on the differentiation of various types of blue dyes on textile fibers. The research first analyzed dye standards using DART-MS and then observed the characteristic signals (i.e., characteristic ions and desorption temperature) of blue dyes on common textiles. DART-MS has demonstrated the potential of fast fiber analysis with no sample preparation, while classical methods require large amounts of fibers and lengthy sample preparation and instrumental analysis.[5] A programmable temperature gradient (from room temperature up to 600 °C in less than 7 minutes) was applied in thermal desorption/pyrolysis DART-MS (TD/Py-DART-MS, Figure 1), allowing compounds on a single fiber to be pyrolyzed and vaporized along the temperature gradient before mass spectrometric analysis.

Raman spectroscopy was also implemented in this study for fiber analysis. As a vibrational spectroscopic method, Raman spectroscopy utilizes Raman scattering whereby the laser light is absorbed by sample molecules followed by inelastic scattering, and the energy difference between scattered photons and incident photons is measured and plotted as Raman shift in reciprocal centimeters.[20] Specifically, a Raman spectrum provides fingerprint information relevant to the types and arrangement of the chemical bonds.[20] Moreover, within this study, the morphological information of each sample was used to characterize the fibers. Raman analysis provides multiple advantages in forensic analysis, such as being relatively nondestructive, a short analysis time, and possible *in situ* analysis, making it useful as a complementary technique to methods, such as light

microscopy and UV-vis microspectrophotometry.[22,23] However, there is no universal laser wavelength in the analysis of dyes, and fluorescence interference arises with impurities of dyes.[22] Typically, fluorescence interference can be attenuated using a lower laser power or longer excitation wavelength. [20] Dyes of high purity emit fluorescence interference in different wavelengths despite power settings.[20] In addition, some dyes can easily burn if a high powder is placed under a Raman laser. One method to decrease fluorescence is to photo-bleach the sample or use an excitation laser to trigger a photochemical reaction to modify the light-absorbing properties of the chromophore, thus eliminating fluorescence interference.[20] Yet, photobleaching may not be efficient for an unknown dyed fiber from a crime scene.[24] Plus, the properties of each dye category are different, and photobleaching may not be achievable for all. Therefore, fluorescence has remained a roadblock for Raman analysis of dyes.

Another option, Surface Enhanced Raman Spectroscopy (SERS), amplifies the signal with either gold or silver's nanoscale roughened surface.[25] Laser excitation changes this surface to make a localized light field, and molecules around these metals have an amplified signal.[25] To utilize this enhancement, colloids, mixtures of suspended microscopically dispersed particles in a substance, were necessary. Colloids of silver nanoparticles were formed by the reduction of silver nitrate by hydroxylamine chloride at an alkaline pH.[26] This colloid was used in the SERS analysis to allow the chemisorption of dye molecules extracted from dyed fibers via microwave irradiation. The colloidal solutions have been proven to enhance the Raman signal tremendously and overcome the fluorescence issue.[26,27]

This enhancement can be significant as previous studies have been reported to yield enhancement factors up to 10^6 on some SERS-active compounds.[27] Therefore, the analysis of the SERS enhancement, using silver colloid, with hydroxylamine hydrochloride as the reducing agent, is of forensic importance. The Raman spectra of all five dye standards used in this study were not available on Wiley's SpectraBase website. Therefore, the creation of a user-generated spectral library provided meaningful information for future studies of unknown dye and fiber combinations. The overall combination of Raman and SERS methodology with this novel TD/Py DART-MS technique has not been reported in peer-reviewed literature.

Thesis Statement

TD/Py-DART-MS method was developed to differentiate blue/violet dyes of multiple categories based on their characteristic mass spectra. The characteristic ions of dyes were identified. The DART-MS method was compared to the Raman spectroscopic method with the aid of the SERS technique. Together these methods identified and differentiated blue/violet dyes from other dyes on fibers. The results are an important addition to the analytical tools for fiber analysis in forensic laboratories.

CHAPTER 2

Experimental Procedures

Materials

Five blue dye standards in five different categories: acid, basic, disperse, methyl, and reactive dyes were tested in this study. They are Acid Blue 161, Basic Blue 9, Disperse Blue 14, Methyl Violet, and Reactive Blue 4. Warp Strip 8 Fiber Fabric strips (Testfabrics, Inc.) were the primary fabric used, including segments of Filament Acetate, Bleached Cotton, Nylon 6,6, and Spun Polyester. The primary three fiber types used in this study were cotton, nylon, and silk.

Preparation of Standards, Samples, and Silver Colloid

Each dye standard was prepared from its original powder form to a final concentration of 0.1 mg/mL with methanol. Then, 5.0 μL was transferred to the reservoir of a copper sample pot, dried for 5 min, and analyzed by TD/Py- DART-MS.

For the SERS method, silver nitrate, hydroxylamine hydrochloride, and sodium oxide were mixed into a solution and then diluted 10 times. A 1% dye solution was made from 10 mg of dye powder and 1 ml of concentrated silver colloid. The solution was labeled and vortexed in a 1.5 ml glass vial. The standard dye solution was placed into a Raman stand designed for holding liquid. Once the characteristics of each Raman spectrum were determined, the standards were saved into a personal OMNIC library. The SERS silver colloid was used to soak the target fibers (i.e., silk, cotton, and nylon). Once soaked, the strands were pulled out of the fiber strip and twisted tightly together to ensure

a diameter longer than that of the laser focus. The fiber Raman spectra were collected on the Enwave Raman instrument with a wavelength of 785 nm.

Dying Procedures for Fabric Fibers

Each fabric sheet was dyed in-house using the tie-dye technique. In brief, fabric strips were soaked in a Na_2CO_3 solution to change the pH of cellulose fibers, allowing these blank fibers to open and react with the dye permanently. The strips were placed in baths of 1.0 g/L dye aqueous solution for 10 minutes. Once they were dried for 24 hours, the excess dye was washed out until no apparent dye residues were observed in the tap water and then dried overnight. Dyed fabrics were selected based on the practical application of dyes to the corresponding fabrics and the intensity of the color. For example, if a dye is not commonly used on cotton textiles, then the dye was not analyzed on cotton fibers. The fibers were unwound from threads and cut into 5-15 mm lengths (about 30-100 μg). They were then deposited onto the reservoir of a copper sample pot with 5.0 μL of methanol before the TD-DART-MS analysis. The addition of methanol acted as an anchor for the fiber as the air suction around the T-tube may pull the fiber out of the copper pot.

Operating Conditions for DART-MS

The thermal desorption/pyrolysis temperature program was optimized in the Fall 2019 semester and was used in this project as follows: 30 °C with a 0.5-minute hold, followed by a ramp rate of 100 °C/min to 600°C, with a hold time of 0.5 minutes. The total analysis time was 6.7 min.[5]

The ion signals produced by the DART-MS were compared to the chemical structures of the standards to determine the characteristic signals derived from the dye. The temperature of desorption and time of desorption was recorded as factors that may contribute to a dye's identification. The dyed fibers were analyzed and compared. The mass spectra for solvent and the undyed fibers were collected to study the background signals and possible interference. Preliminary results showed that molecular ions were characteristic ions of some dyes, such as Disperse Blue 14, but some other dye molecules, such as Acid Blue 161, would have fragment ions dominant in the mass spectra.

Operating Conditions for Raman and SERS Analysis

Each of the five dye powders was crushed into a fine powder and placed on a hand-crafted copper slide. This slide was made copper tape with the dimensions of 0.5 × 0.5 cm^2 mounted on top of a microscope slide to reduce the Raman signal from the background. The Raman spectra of these dye powders were collected using the 785 nm Enwave Raman spectrophotometer, starting with the lowest power setting (12pm) and increasing slowly until the wide fluorescence peaks reached past the maximum absorbance. Each pm marked on the Enwave Raman instrument correlates to a percentage of laser power. Collection times for each power increase were 30, 60, and 120 integrations. The combination of power and integration for each dye was noted as unique even if there was a poor signal due to fluorescence.

Extraction Procedures for SERS

The blank spectra of both fibers and dyes were collected, analyzed, and saved. Then, dyed fiber strips were cut into approximately 5-15mm lengths and inserted into individual melting point capillaries. These capillaries were each spiked with 30 µL of SERS silver colloid to coat the entirety of fiber. The capillary tubes were capped with a silicone capillary cover and placed in a glass vial before being lowered into the test tube for microwave extraction. The parameters for microwave extraction were optimized and found that a time and temperature combination was most effective at 10 or 15 minutes while at 65°C.

After each fiber was run through the microwave extraction process, the glass capillary tube was placed on top of a clean copper slide. The Enwave Raman laser was placed on the bottom of the tube to collect spectra from the fiber. The laser was moved approximately 5 mm along the tube to the edge of the fiber, then to the center of pure colloid and dye. Raman spectra were collected at data acquisition times of 30, 60, and 90 seconds for the laser excitation at each of these locations along the capillary. The spectra containing the highest signal-to-noise ratio were selected for further processing and analysis. They were compared to the spectra from the fiber blank and standard blank.

Once the Raman analysis was complete, the fiber was taken out of the capillary and sonicated in water for 20 minutes. After sonication, the fiber was placed in a laboratory oven for approximately 1 hour. After each fiber was dried, it was weighed individually to calculate the amount of dye extracted.

CHAPTER 3

Results and Discussion

DART-MS Results

The resulting spectra of the standard dyes (Acid Blue 161, Basic Blue 9, Disperse Blue 14, Methyl Violet, and Reactive Blue 4) revealed characteristic ions that were identified through TD/Py-DART-MS. The characteristic ions of each dye were listed in Table 1. Dyes with lower molecular weights showed more intense characteristic ions ($m/z \leq 800$) in the mass spectra. The observed *m/z* signals correlated to the molecule ion or protonated/deprotonated molecule ion of each dye, for Acid Blue 161, Basic Blue 9, and Reactive Blue 4. For example, Disperse Blue 14 was run under the negatively charged mode, and *m/z* 265.3 was observed in the mass spectrum as the molecular ion of the dye (Figure 2). The dyes were analyzed by DART-MS under both positive and negative modes. It was found that 3 out of the 5 dyes (Acid Blue 161, Basic Blue 9, and Methyl Violet) were consistently observed with positive ion mode, and the characteristic signals of the other two dyes were more abundant in the negative ion mode (Disperse Blue 14, and Reactive Blue 4).

Table 1. Observed Characteristic Ions of Dye Standards

Dye Standard	Characteristic Ions (m/z)
Acid Blue 161	80, 97, 127
Basic Blue 9	257, 270, 286, 300
Disperse Blue 14	252, 266, 281, 297
Methyl Violet	360, 374
Reactive Blue 4	80, 163

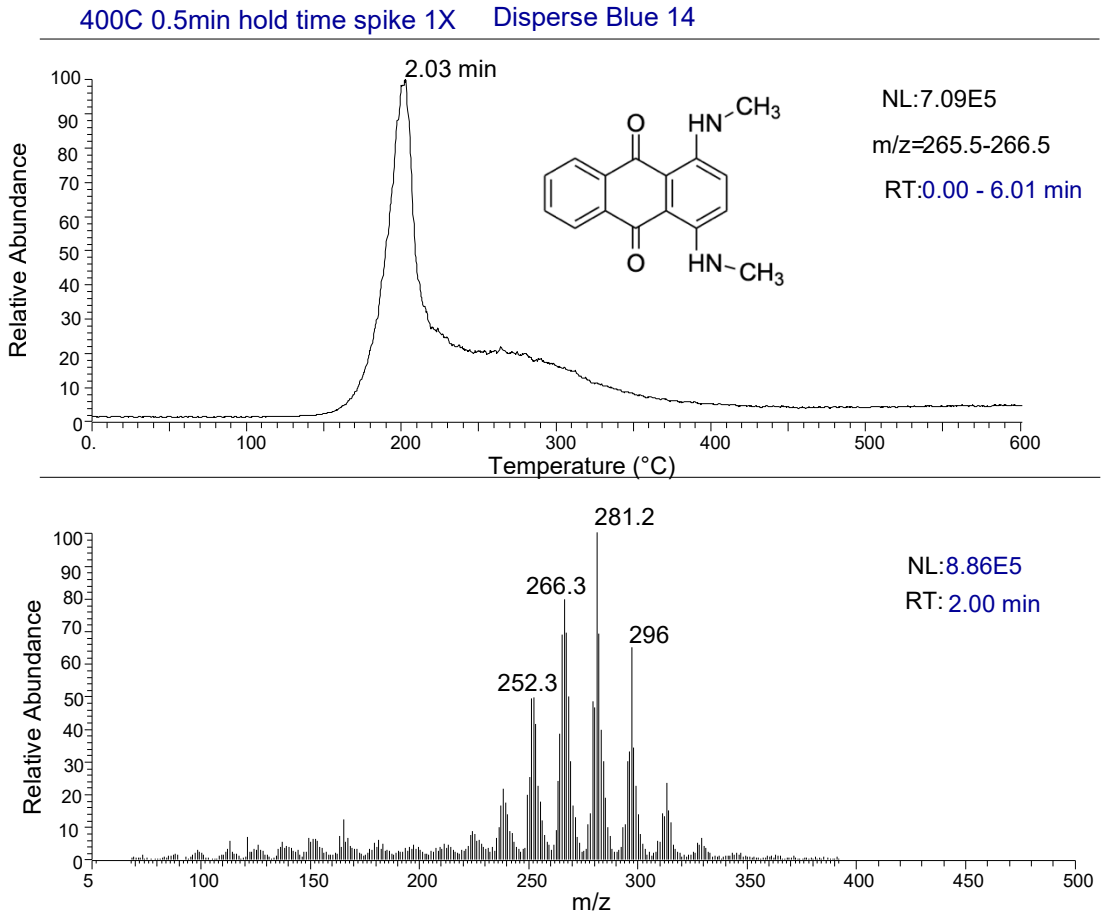


Figure 2. MS of Disperse Blue 14 dye standard. Characteristic signals are identified as well as desorption time.

However, unexpected signals of these dyes were observed. These signals were concluded to not belong to the methanol blank or as a consistent background signal. One example is *m/z* 127. This ion belonged to a mass spectrum collected from Acid Blue 161, but the structure could not be elucidated. In addition, the ions for the sulfide group (*m/z* 80) were detected in the mass spectrum of dyes containing SO_3^- and SO_2^-Na (Figure 3). Both the time and temperature at absorption are factors of distinguishability, which can be used as a foundation for further understanding each dye type on fiber.

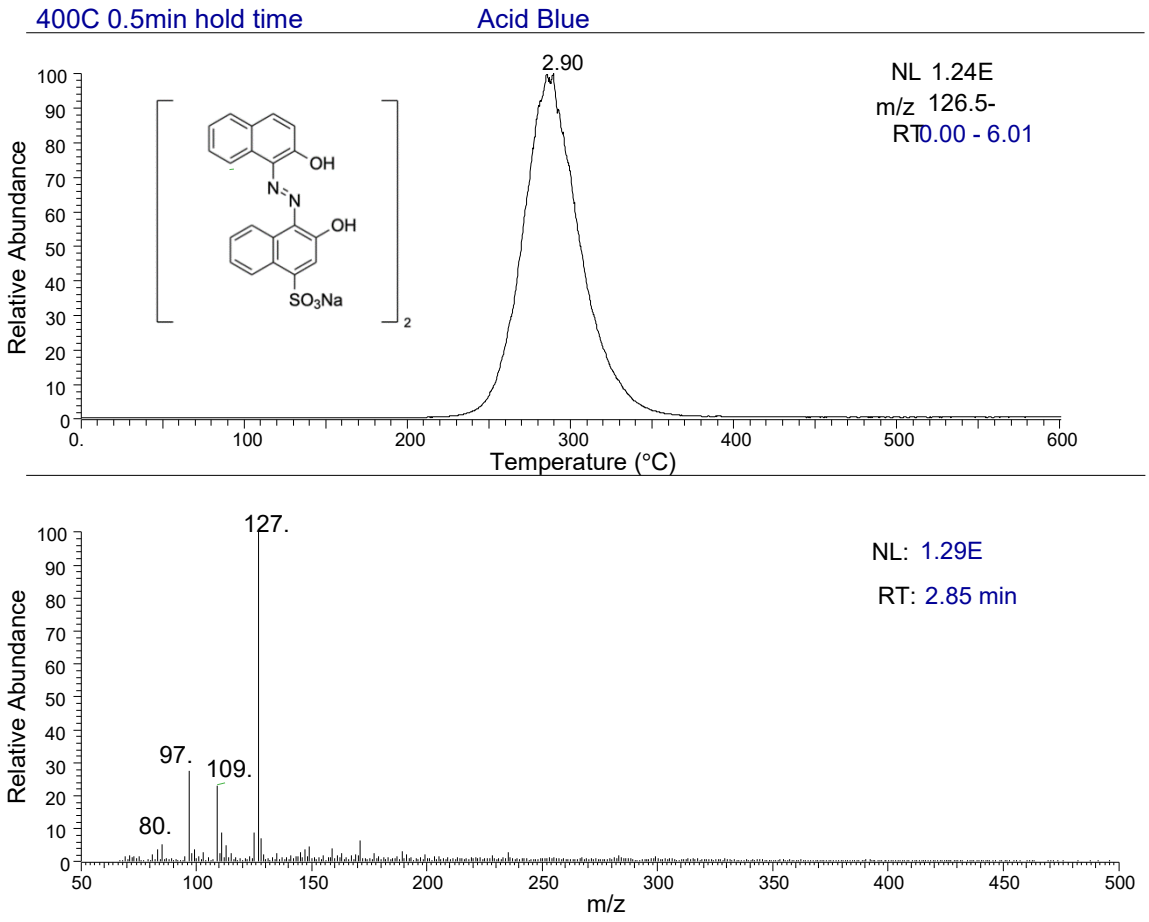


Figure 3. MS of Acid Blue 161 dye standard. Characteristic signals are identified as well as desorption time.

In some cases, the selected ions in the mass spectrum could be contributed from both dye molecules and fiber backbone materials. Then the desorption time would be critical to distinguish their signals. For example, Disperse Blue 14 had the characteristic ion of *m/z* 265.5, but the same ions were observed in the nylon blank mass spectrum. However, the signal of *m/z* 265.5 from the Disperse Blue 14 on nylon was observed at approximately 2.5 minutes, while, on the fiber blank, the absorbance was not until approximately 5.4 minutes. The desorption time of the Disperse Blue 14 (i.e., approximately 2.5 minutes) matches that of the Disperse Blue 14 standard solution. Therefore, the results indicate that both mass spectrum and desorption time/temperature could be used as characteristic signals for dye identification.

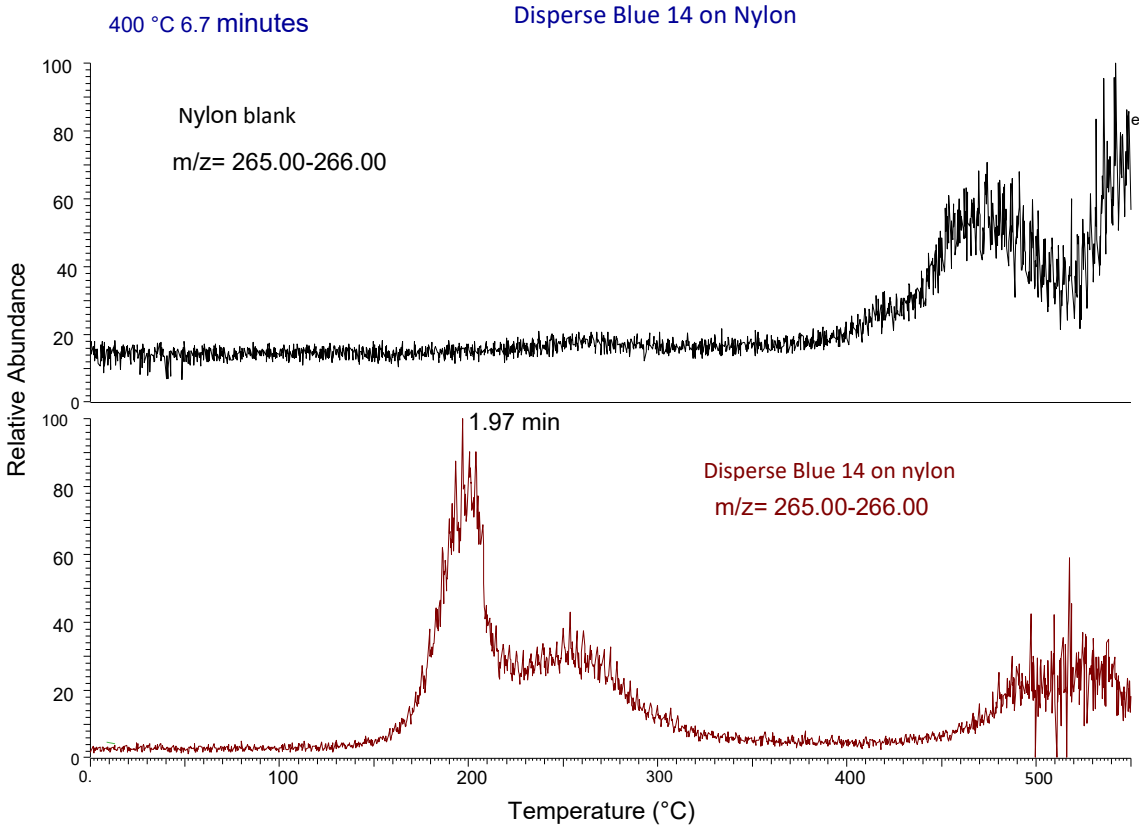


Figure 4. Comparison of spectra from nylon fiber blank and Disperse Blue 14 on nylon when a mass range *of m/z*=265-266 is inserted.

The mass range subtraction was also utilized in this project to study the relative intensity changes between different ions. This method has shown effectiveness for the observation of those characteristic ions with low intensity, such as Basic Blue 9 on silk, Acid Blue 161 on cotton, and Reactive Blue 4 on cotton, respectively. For instance, the Basic Blue 9 standard relayed a characteristic ion of *m/z* 270.2, but its intensity from the dyed fiber was immensely low which was significantly affected by the fiber background signals. In Figure 5 below, *m/z* 241.00-241.5 (a common ion in the fiber background mass spectrum) was subtracted from *m/z* 270-270.5 in the standard, fiber blank, and dyed fiber DART-MS data. There was a clear desorption peak on the dyed fiber spectra but not on the fiber, resulting in the dye identification within the fiber more feasible. It was also notable that the desorption time shift went from 2.70 minutes for Basic Blue 9 standard to 3.34 minutes for Basic Blue 9 dyed fiber. The desorption time shifts may be due to interactions of the polymer backbone and the dye molecules which require higher energy to desorb the molecules from the fibers.

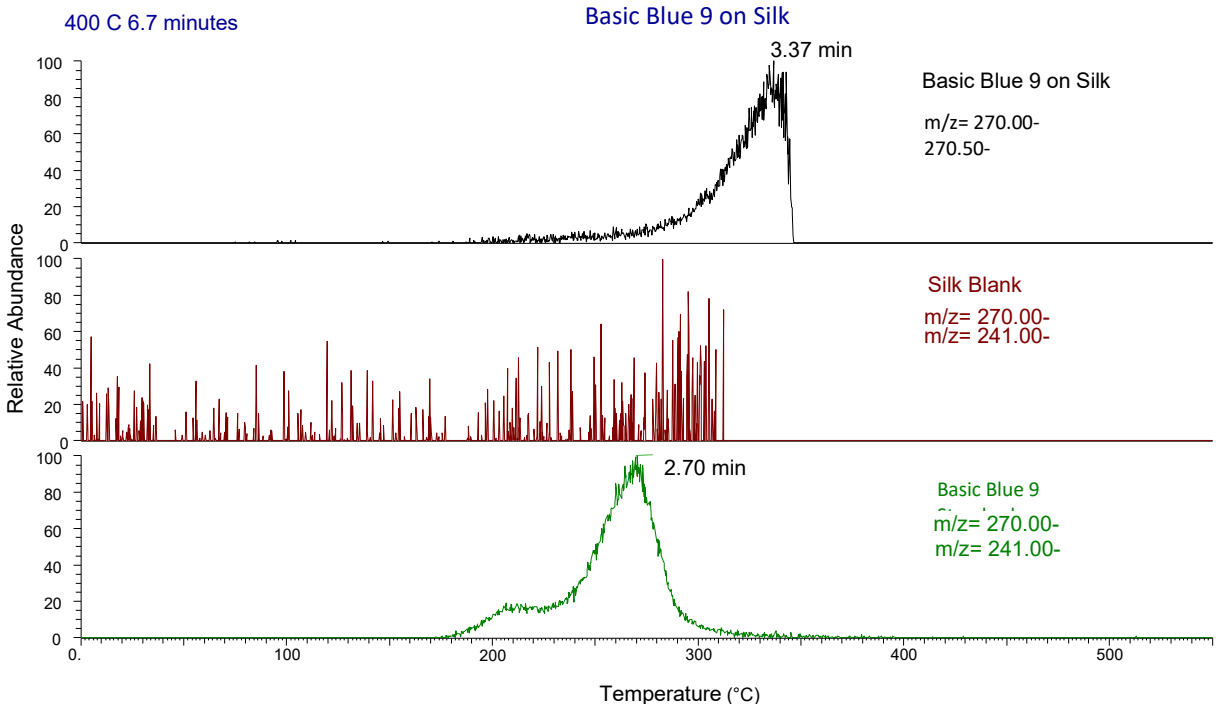


Figure 5. Comparison of spectra from Silk fiber blank and Basic Blue 9 on silk when a mass range of *m/z* 270-270.5− *m/z* 241.00-241.5 was inserted.

Raman Spectroscopy

The online spectral database named Spectrabase was searched but yielded no reference Raman spectra for any dye in the sample set. This required the collection of the spectra for dye, fiber blank, and dyed fiber through this project. However, not many commercially available dyes have sufficient purity (e.g., 97% or higher). For this reason, the SERS application, was required.

The spectral comparison of the powder sample of Methyl Violet and the SERS solution of Methyl Violet revealed stark differences (Figure 6). Each SERS silver colloid was observed with an emphasis on its bands and intensity. In the powder form, dyes were analyzed first using a low power and integration level and then were worked up, but often they began to smoke. Within a forensic setting, where the use of a minimal amount of sample is key, losing powder is not acceptable. For example, Methyl Violet's dye powder limit before burning was set at the 64% of the Raman laser's total power with only 30 integrations. However, its silver colloid mixture did not burn until it was analyzed under 75% total laser power for 60 integrations. A Rayleigh scattering event was noted in these spectra. Bands within the energy level of 100 – 400 nm were not included in distinguishing observation.

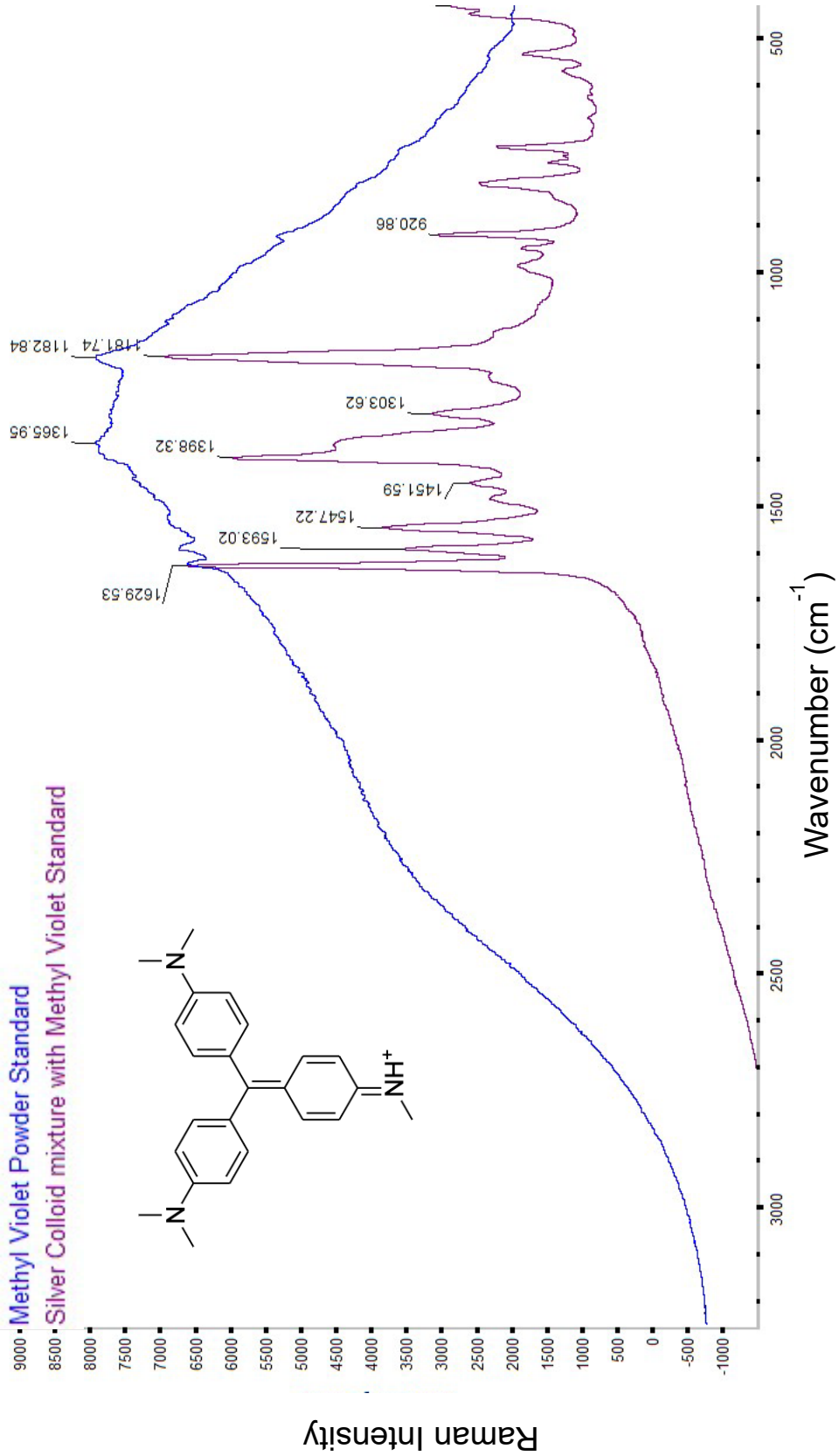


Figure 6. Overlaid spectra of Methyl Violet in powdered form and in 100 ppm solution acquired via SERS mode using silver colloid. Peaks-picking parameters that include a sensitivity of 50 and a threshold of 3717 were chosen.

The silver colloids are stable for a maximum of two days. Initially, solutions of Basic Blue 9 and colloid mix (*v/v*, 1:9) in a 1.5mL glass tube were made and analyzed. This was to ensure the colloid was able to provide observable amplification, and the enhanced sensitivity was very important for a single short fiber in the forensic setting. The blank and dyed fibers were also submerged in silver colloid and analyzed. The overall spectra collected by cotton could be compared to the overall fingerprint of cotton, in literature, that had also been collected under 785 nm laser.

Microwave extraction parameters were also evaluated, which resulted in an optimal temperature range of 65 °C for a total extraction time of 10 or 15 minutes. Compared to the DART-MS method, this extraction was more time-consuming. Table 2 provides the estimated masses of dye extracted from fiber by measuring the mass differences between dyed fiber and fibers after extraction. Table 3 shows the average weight differences at different extraction times.

Table 2. Extraction Parameters for each microwave extraction and their differences in weight before and after.

Extraction Parameters	Weight Average Difference (µg)
Acid Blue 161 on Cotton (65 °C, 10 Minutes)	28.5
Acid Blue 161 on Cotton (65 °C, 15 Minutes)	99.5
Basic Blue 9 on Silk (65 °C, 10 Minutes)	32.5
Basic Blue 9 on Silk (65 °C, 15 Minutes)	17.5
Disperse Blue 14 on Nylon (65 °C, 10 Minutes)	23.5
Disperse Blue 14 on Nylon (65 °C, 20 Minutes)	33.5
Reactive Blue 4 on Cotton (65 °C, 10 Minutes)	47.5
Reactive Blue 4 on Cotton (64 °C, 15 Minutes)	20.0
Reactive Blue 4 on Cotton (65 °C, 20 Minutes)	54.0
Methyl Violet on Cotton (65 °C, 10 Minutes)	2.0
Methyl Violet on Nylon (65 °C, 15 Minutes)	21.5
Methyl Violet on Nylon (65 °C, 20 Minutes)	31.5

Table 3. Weight difference for each time of extraction and the overall average.

10 Minute Average (µg)	15 Minute Average (µg)	20 Minute Average (µg)
26.8	39.6	42.8

The Raman spectra of Basic Blue 9 on a silk fiber, extracted for 10 minutes under 65 °C, were investigated. The overall spectra are included in Figure 7. The combination of peaks from the polymer and the dye into a wider peak, was observed. This was seen in Figure 8, at the region of approximately 1150 cm^{-1}. Here, the peak of Basic Blue 9 on silk was wider than that of the dye standard due to the signal of the silk fiber. Figure 8 is a zoomed-in spectrum from the fiber section of Basic Blue 9 on silk that was extracted at 65 °C for 10 minutes. Here the existence of dye on the fiber was evidenced by the peaks of 1187 cm^{-1} and 1188 cm^{-1} from Basic Blue 9 on silk and standard, respectively. These peaks occurred in a location where there was less fiber signal. Likewise, fiber signals were found while the laser was pointing the liquid portion of the capillary that was extracted at 65 °C for 10 minutes (Figure 9). In Figure 9, a shoulder of a peak from Basic Blue 9 on silk at the location of approximately 1160 cm^{-1} aligned with the silk blank and not the dye standard. Therefore we can conclude that minuscule amounts of fiber were released into the colloid.

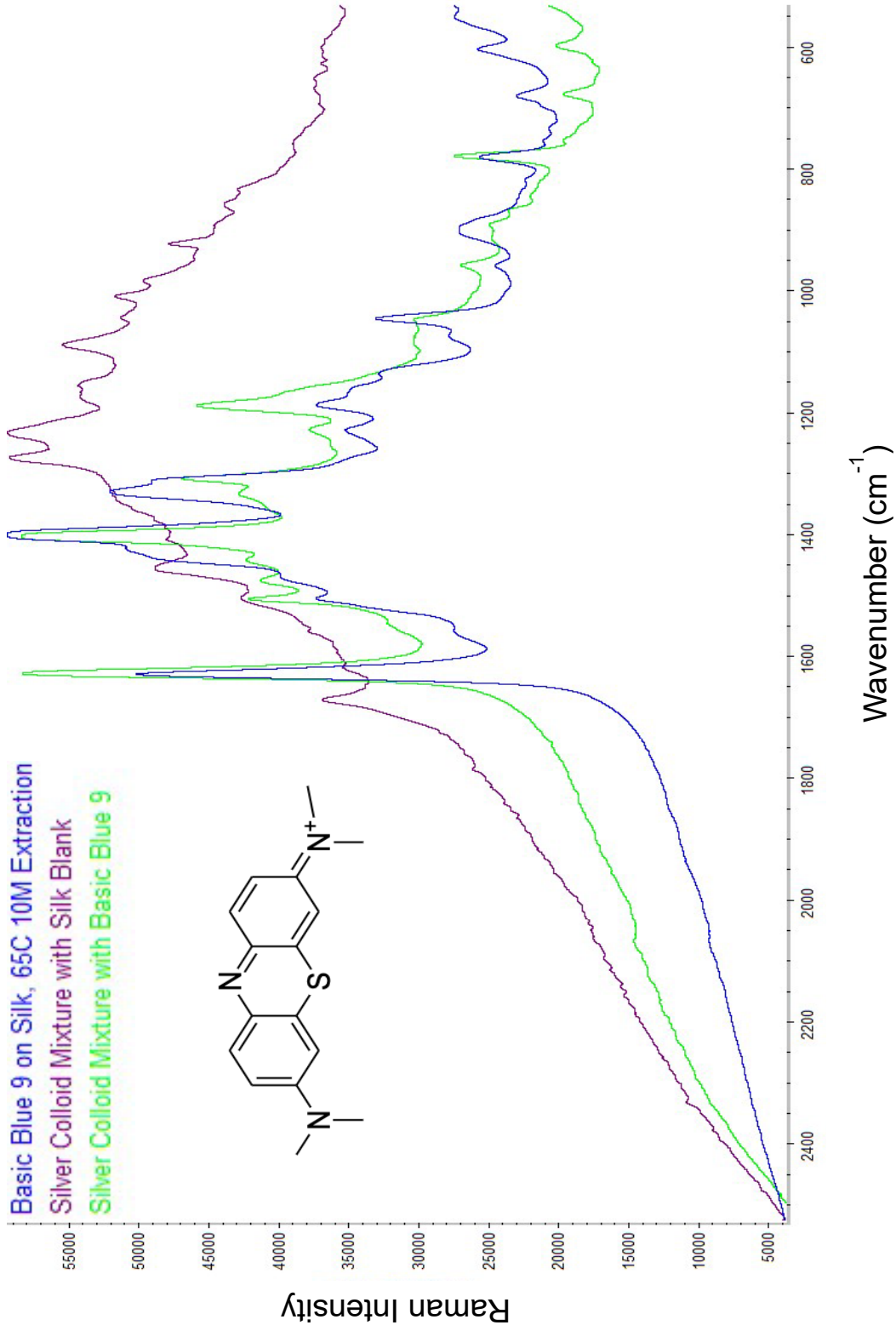


Figure 7. Overlay of Basic Blue 9 applied to silk, extracted at 65C for 10 minutes.

Laser location was on the fiber. Basic Blue dye standard in 100 ppm solution acquired via SERS mode using silver colloid. Purple spectrum: blank silk; blue spectrum: dyed silk fiber with laser location on fiber; red spectrum: dye standard.

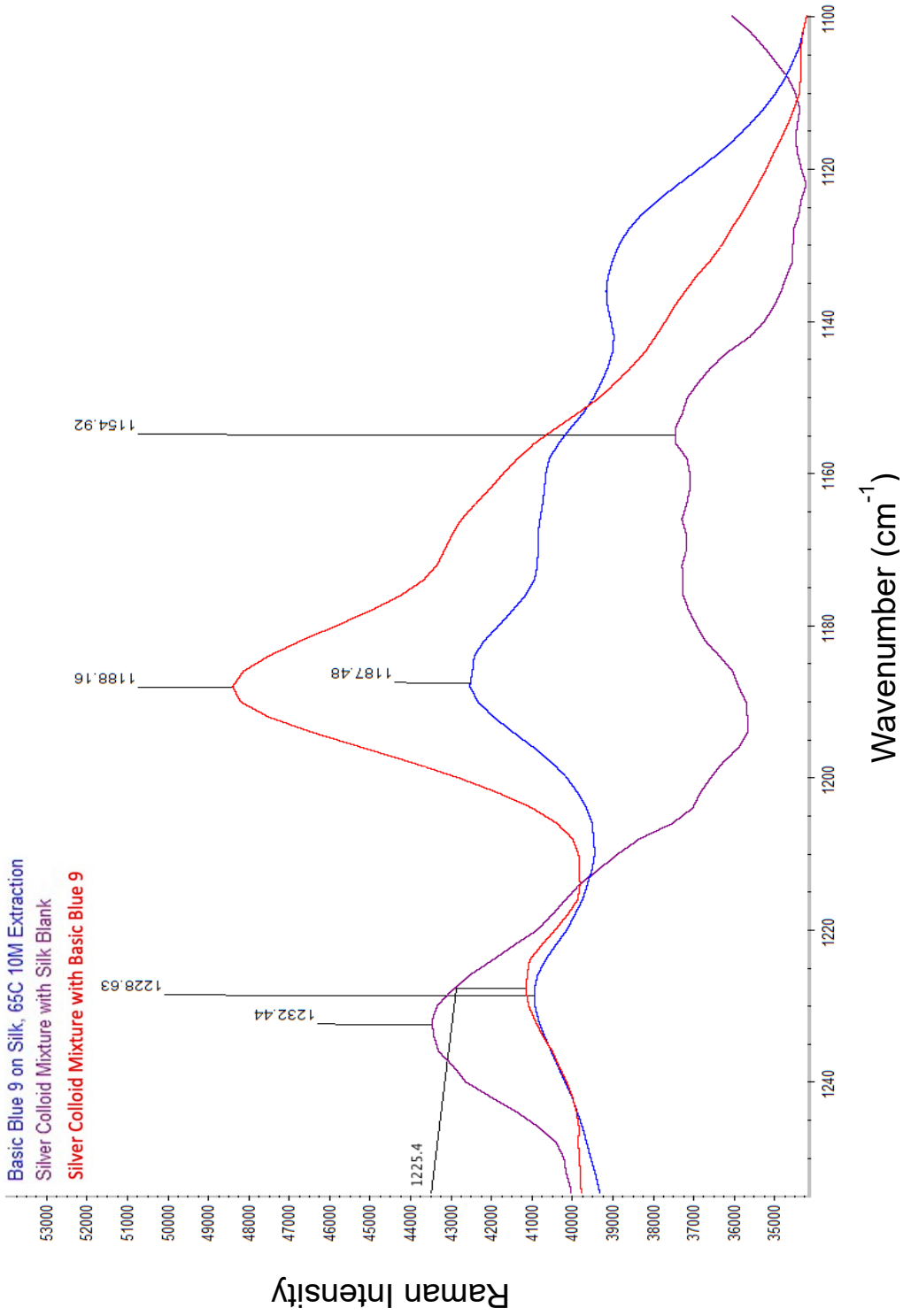


Figure 8. Basic Blue 9 on silk overlayed with SERS dye standard and SERS silk. Range: 1100cm^{-1}-1255 cm^{-1}.

Laser location was on fiber. Peaks were located with a threshold of 5496 and a sensitivity of 50. Basic Blue dye standard in 100 ppm solution acquired via SERS mode using silver colloid. Purple spectrum: blank silk; blue spectrum: dyed silk fiber with laser location on fiber; red spectrum: dye standard.

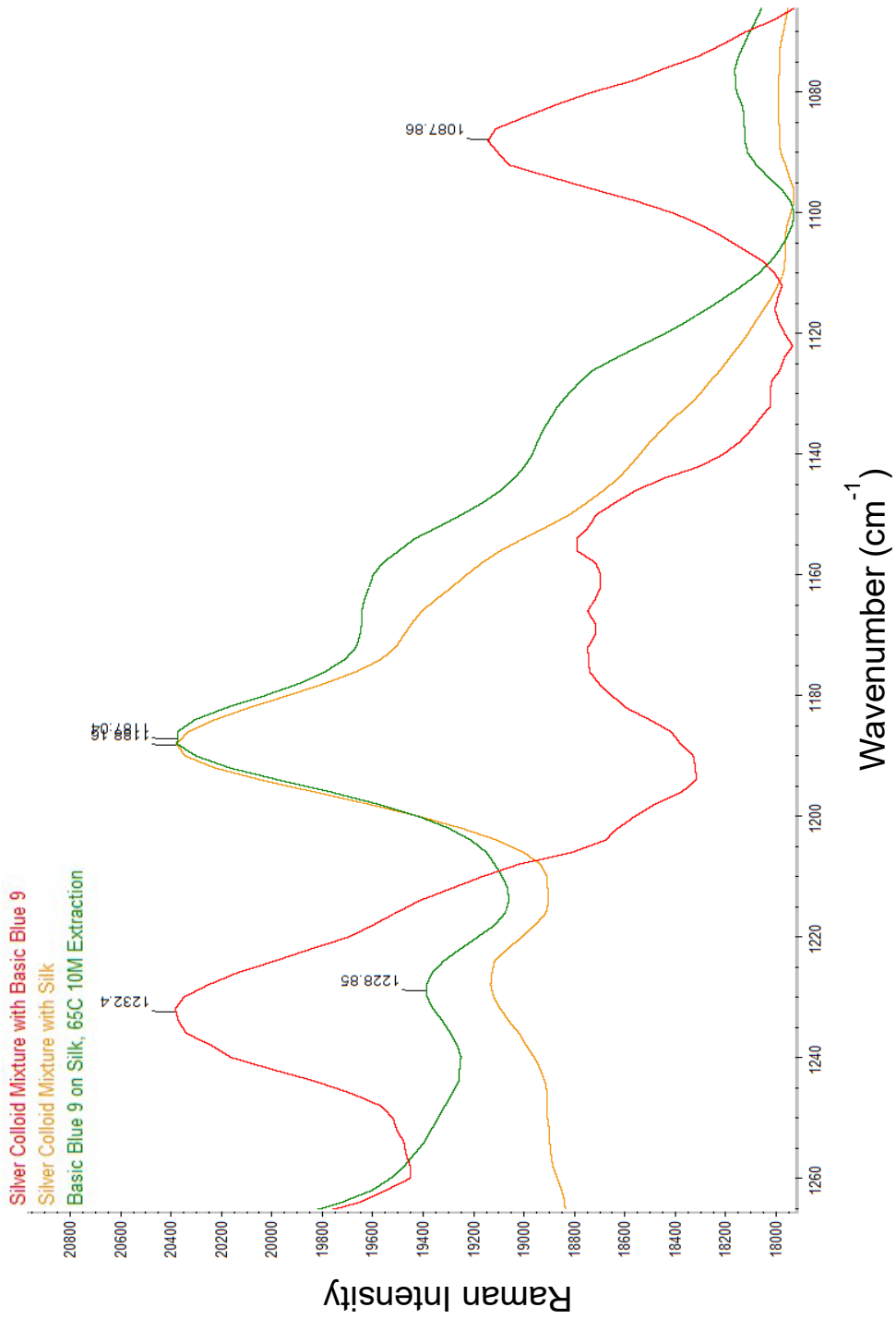


Figure 9. Basic Blue 9 on silk overlayed with SERS dye standard and SERS silk. Range: 1155cm^{-1}-1260 cm^{-1}.

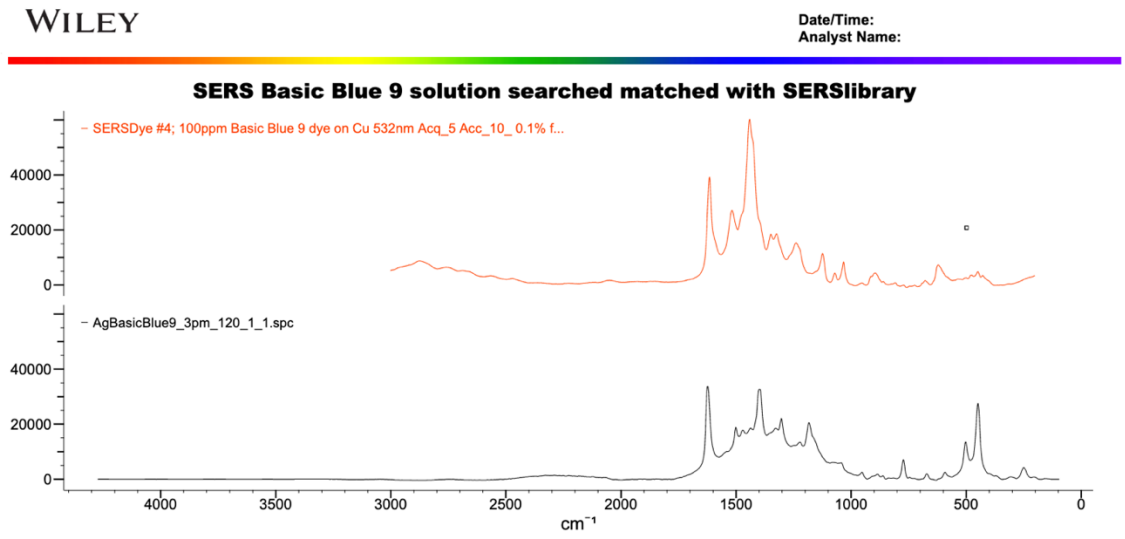
Laser location was on liquid. Peaks were located with a threshold of 5496 and a sensitivity of 50. Basic Blue dye standard in 100 ppm solution acquired via SERS mode using silver colloid. Red spectrum: blank silk; green spectrum: dyed silk fiber with laser location on liquid; yellow spectrum: dye standard.

Raman was used to identify Methyl Violet on nylon, which was difficult in DART-MS. The same phenomena for Basic Blue 9 on silk occurred when the Raman laser was focused on the fiber section of Methyl Violet with nylon (Figure 9). The peak at 1358 cm^{-1} was wider than the shoulder of Methyl Violet standard. An overlay of the nylon blank showed that its signal was slightly farther to the right of the spectra which may have influenced the dyed fiber peak to be widened. In addition, the signals of 1485 cm^{-1} and 1543 cm^{-1} on the same spectra were tied to the standard dye spectrum and therefore the Methyl Violet dye on the nylon fiber was identified.

Spectra collected from this work were uploaded to Wiley Library's KnowItAll customizable library program for comparison and confirmation. For instance, the baseline reduced Basic Blue 9 powder spectrum was matched closest to the collection of 4,4'-Ethylenedianiline, with a hit quality index (HQI) of 54.13%, when it was compared to a library of non-SERS spectra. However, when the SERS spectra library collected on a 532 nm instrument were selected along with Wiley's free sample libraries of fiber dyes, Basic Blue 9 matched a collection of Basic Blue 9 from 532 nm, with an HQI of 60.76% (Figure 10). Even higher hit quality indexes were found in cases of Methyl Violet silver colloid standard where it reached 64.53% when compared with 532 nm Methyl Violet. Results of these matches for each dye were checked for confirmation before the spectra were inserted into an OMNIC personal library for the purpose of comparing unknown dyes. The results of fibers collected on 785 nm were also placed in a personal library for fiber matching.

An HQI of 87.35% was confirmed when the spectrum of the extracted Acid Blue 161 liquid portion of the capillary was uploaded. This match was with Drimaren Navy

Blue, another name for acid navy blue textiles. The spectrum of the dye on the fiber matched Drimaren Navy Blue with an HQI of 82.25%. The consistency within this library matching also adds to a positive outlook on the ability to create forensic textile dye libraries. Once each collected spectra had been compared to spectra separately collected by a 532 nm wavelength, a personal library was made. This was for the purpose of future comparison with unknown dyes and fibers. Overall, this created an optimistic outlook on the future use of the library for unknown fiber identification.



Name	Value
Name	100ppm Basic Blue 9 dye on Cu 532nm Acq_5 Acc_10_ 0.1% fil dry
Detector Name	Synapse Plus
Instrument Name	LabRAM HR Evol
Sample Name	B

HQI	Tag	rrectio	DB	ID	Name	Spectrum
60.76			SERSDye	4	100ppm Basic Blue 9 dye on Cu 532nm Acq_5 Acc_10_ 0.1% fil dry	

Figure 10. Wiley KnowItAll library match of Basic Blue 9 on 532 nm and 785 nm.

Chapter 4

Conclusion

This project developed the TD/Py DART-MS method for high throughput forensic dye analysis without time-consuming sample preparation. Dyes, although of the same or similar colors, were effectively identified through a combination of their characteristic ions in the mass spectra and the respective desorption time/temperatures. This will be a foundation for expanding the usefulness of DART-MS for forensic dye analysis and the future inclusion of multiple dyes within one category to better understand the interactions of dyes and textile fibers. The SERS method, instead of direct Raman spectroscopic analysis of dyed fibers, can enhance the signal-to-noise ratio of the dye spectra while suppressing the fluorescence signal. This study also serves as a baseline to study more textile dyes not included in spectral databases. Based on the Raman spectroscopy and DART-MS results, there is a great deal of potential for high throughput methods to significantly contribute to the identification of unknown dyes on fibers compared to the traditional, slower, and more restrictive methods.

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