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FINAL SUMMARY OVERVIEW

THE EFFECT OF ULTRAVIOLET RADIATION ON THE MICROSPECTROPHOTOMETRY (MSP) OF DYED FIBERS

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1.0 Purpose

Dyed or pigmented fibers are known to change or lose color as the colorant (dye or pigment) undergoes photodegradation after exposure to ultraviolet (UV) radiation. This is problematic for forensic scientists performing fiber comparisons, especially if the fibers were exposed to sunlight or UV radiation for any extended length of time. For example, two fibers may have originated from the same source, but their color (and spectra) may be different if one has changed color after sunlight exposure.

This research aimed to assess the effect of UV radiation over time on the color of different types of dyed fibers and determine if any observed change was predictable for a given colorant using UV-Vis microspectrophotometry (MSP). MSP is an instrumental analysis technique utilized in most forensic science laboratories to objectively compare the color of microscopic particles, including fibers, viewed through a microscope. The microspectrophotometer generates a spectral graph plotting the absorbance (or percentage of transmittance) of ultraviolet and visible wavelengths of radiation transmitted through the fiber, and is the most appropriate instrument for measuring color and any color change. Quantifiable, statistically relevant, objective color comparison using MSP provides more discriminating results than ordinary, visual color comparison of fibers.

Without the proper research to understand the degradation process and effect of UV radiation on colored fibers, forensic scientists and the criminal justice system are at a disadvantage. Prior to this research, there were no forensic science studies that examined the effect of extended exposure of colored fibers to UV radiation using MSP.

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This project addressed this need and examined the nature and degree of the photodegradation effect on a large variety of dyed fibers after they were exposed to ultraviolet radiation. MSP data collected in this research were used for comparison of control (unexposed), artificial UV-exposed, and sunlight-exposed fibers and fabrics.

2.0 Project Design and Methods

This project included the analysis of a total of 53 swatches from a variety of fiber types and dyes of commercial and custom-dyed manufactured fibers that were exposed to UV radiation (artificial UV radiation in the laboratory in Chicago, and sunlight exposure in Arizona) for a duration of 18 months, using MSP to measure the fading effect of UV radiation on color. The fiber samples were all manufactured and included common types, such as polyester, nylon, acrylic and rayons with dyes typical for each fiber type. McCrone Research Institute (McCrone) obtained fiber and fabric samples from two sources: the reference-traceable fiber collection from Microtrace LLC (Elgin, IL) and custom-dyed fabrics from Testfabrics, Inc. (West Pittston, PA).

The fibers from Microtrace represented a wide variety of color and included only those that are colored with single dyes. From the Microtrace fiber collection, 43 fibertype and dye-type combinations were chosen based on their suitability for this research. The fibers obtained from Microtrace include information about manufacturer, dye process, dye applied (if available), and year of manufacture.

McCrone obtained an additional set of 10 custom-dyed fabric swatches from Testfabrics that included information about dye type, dye class, and Colour Index numbers (when available), as well as information on how the fabrics were dyed in the

Testfabrics laboratory. All Testfabrics samples were dyed at 1.50% on weight of fiber (OWF). A detailed spreadsheet that contains basic information for the samples in this study including fiber type, dye, colour index number (when available), chromophore and optical properties can be found in the Appendix. These samples were divided in two subsets. Subset A1-A10 was sent to Q-Lab Test Services (Buckeye, AZ) for sunlight exposure: the textile swatches were placed 45° south-facing following ASTM G-7 Standard Practice for Atmospheric Environmental Exposure Testing of Nonmetallic Materials. Subset B1 – B10 was placed into the artificial UV radiation light box in the laboratory in Chicago. Temperature and percent relative humidity were recorded for both indoor and outdoor sunlight exposure environments. The ranges recorded in the indoor artificial UV-radiation light box were $23.2 - 44.1^\circ$ C and $5.4 - 46.9\%$ relative humidity. Outdoors in Arizona, the recorded temperature range was 2 – 46° C and the relative humidity was 15 – 82%.

2.1 Preparation of Fabric Swatches

The original 10 Testfabrics swatches were approximately 8 x 8 in. square, as received. For sunlight exposure to the Arizona site, they were cut into approximately 2 x 2 in. sections and mounted onto 10 half-inch thick untreated plywood boards and secured using four stainless steel staples, Figure 1. These sample boards were shipped to Arizona for sunlight exposure, Figure 2.

Each of the 53 fabric swatches were prepared for the UV-radiation light box in the laboratory in Chicago: 43 from the Microtrace collection plus 10 from Testfabrics. The 43 Microtrace samples were all approximately 1 cm square and used as received. The Testfabrics samples were cut into strips measuring approximately 1 x 2 cm. A small piece of adhesive tape was folded over one end of each swatch, creating an extended grip surface for the small binder clips, which were used to hold the swatches. The handles of the binder clips were folded upwards, and fishing line was used to suspend the binder clips in between the UV lamps inside the light box. Each piece of tape attached to a swatch was labeled in pencil, each binder clip was labeled, and a detailed label was also attached to the fishing line, Figure 3.

above, and a small portion of these subsamples was extracted for analysis using MSP.
At the Arizona site, one board was removed from exposure, packaged and returned to Prior to both the indoor and outdoor exposure experiments, a control for each sample (T0) was retained. Every 8 weeks, swatches were subsampled using a stereomicroscope and new razor blades. All subsamples were prepared as described Chicago every 8 weeks.

Both indoor and outdoor exposures spanned over a period of 80 weeks and in addition to a control sample at T0, samples were collected after exposure times of 8, 16, 24, 32, 48 weeks (T8 through T48), and 56, 64, 72, and 80 weeks, when necessary (T56, T64, T72, and T80, respectively).

2.2 Instrumentation

 can be set between 80 x 80 µm and 2.5 x 2.5 µm with a 36x reflecting objective. A CRAIC FLEX/508 PV UV-Visible-NIR microspectrophotometer (CRAIC Technologies, San Dimas, CA) was used for this study. The spectrometer is coupled to a Zeiss AXIO research microscope (Carl Zeiss AG, Oberkochen, Germany). The excitation source was a Xenon short arc lamp (Ushio, Inc., Japan). The sampling area

LambdaFire Integrated Spectral Acquisition and Imaging software, version 1.2.75.2 was used for the capture and management of MSP spectra.

2.3 Data Acquisition

Using a stereomicroscope, individual fibers from subsampled swatches were dispersed on quartz slides and covered with a quartz coverslip; distilled water was added as a mounting medium.

The microscope of the microspectrophotometer was first set up in Köhler illumination, then the field diaphragm was opened slightly beyond the edge of the field of view (as seen through the eyepieces), and the condenser numerical aperture (NA) was set at just less than 0.2 NA Spectra and photomicrographs were collected from 10 individual fibers, five spectra were collected from each fiber for a total of 50 spectra and photomicrographs per sample. Spectra were collected within a spectral range of 200 – 875 nm and a 3 nm path. A UV blocking filter was used between all spectra collection. A new dark and reference spectra were collected for each fiber. All spectra were collected in absorbance, using a 36x reflecting objective and an instrument aperture of "4" (7.8 x 7.8 µm). The software was used to set the integration time to 15 ms and use a resolution factor of "0".

Photomicrographs were stored in JPG file format. Spectral data were stored in MSP, SPC, and CSV file formats; while MSP is specific to CRAIC, SPC is a universal format that can be read on any spectroscopy software. The CSV file format is in the form of a spreadsheet and was used for data analysis in this study. High-dimensional data frames were created for each week of exposure, and then a master data frame for each sample was assembled: the columns consisted of the variables that is 1146

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absorbance values for a spectral range set of 200 – 700 nm and a path of about 0.4 nm. The rows consisted of the observations, that is, the 50 spectra collected from the fibers for each sample, for a given week.

3.0 Data Analysis and Results

3.1 Data Analysis

Statistical spectral comparisons were carried out to detect sets of spectra at a given exposure time that did not fit in the range of variation for spectra sets collected without previous UV exposure and collected at previous exposure times. Visual spectral comparisons were initially conducted to identify features of spectral alterations as a function of UV exposure.

The methods of data reduction and visualization of principal component analysis (PCA) and t-distributed stochastic neighbor embedding (t-SNE) were used to visualize the spectral data. These two methods are complementary in the sense that while PCA is a linear technique that aims to keep the low-dimensional representations of *dissimilar* data points far apart, t-SNE aims to maintain the low-dimensional representations of *very similar* data points close together. Data inspection was also carried out using K-Medoids partitional clustering in the classic form of Partitioning Around Medoids (PAM). The K-Medoids is preferred to the K-Means because it is more robust to outliers. Analyses were carried out using R software, version 3.5.1 (R Core Team, 2018). The MASS package, Rtsne and Cluster packages were used. Standardized protocols in the forms of R codes have been created to analyze data from different samples consistently.

3.2 Results

3.2.1 Recommended practices

Although MSP is considered a non-destructive technique, caution should be exercised to avoid the risk to unnecessarily expose the fiber samples to the xenon lamp source. Samples should be pre-screened to determine their susceptibility to photofading prior to analyzing them using the MSP. The following guidelines are recommended:

1) Perform an instrument-induced photobleaching assessment that spans the anticipated time it will take to collect all of the necessary spectra from the sample. In this study, it was found that a few samples displayed marked photobleaching in just a few minutes; therefore, these were excluded from the study.

2) Samples should be well dispersed throughout the slide; this will help avoid extended light exposure to samples that are nearby during spectrum collection. Keep in mind that the area being exposed to the light source extends beyond the field of view (as seen through the eyepieces).

3) A UV blocking filter should be used between all sessions of spectra collection.

4) Fibers selected for spectral collection should be chosen primarily on their suitability of orientation on the prepared slide, meaning that the fibers should be lying flat surrounded completely by mounting media and be reasonably free of noticeable defects or damage in the collection area, an example is shown in Figure 4.

3.2.2 Visual changes in fiber appearance

Over time, it was observed that many fibers not only faded in color but also became physically degraded. This physical degradation, seen visually through the microscope, had an effect on the MSP spectra. It was also noted that, in most cases, alterations within spectral curves started before visually observing the color fading in the fiber samples.

3.2.3 Photobleaching effects

 but one sample. Spectral alterations occurred at different times, ranging from as little as Photofading of fiber dyes can be expected when fiber samples are continuously exposed to an intense light source, such as the xenon light source, over time. Therefore, preceding the detection of spectral alteration, the potential effect of instrument-induced photobleaching over time was evaluated by continuously exposing each of the dyed fibers to the MSP light source from 0 to about 3,700 seconds, with a spectrum collected every 60 seconds. This was controlled with the use of CRAIC TimePro software. In the photobleaching study, spectral alterations were observed in all 20 seconds to as much as 1,730 seconds. Spectral changes were observed to occur in various forms: band shifts in the x-axis (wavenumber units) were commonly observed. There were cases where spectral shapes were preserved, but the overall intensity of all the bands was out of the range of the intra-source variation of spectra collected, without further exposing the fibers to the excitation radiations. Changes in intensity values of one or a few bands within a spectrum were also observed. Effects of band flattening, bump formation, bands fusion, slope change, and band disappearance were also noted. Combinations of the previously described spectral alteration modes were also recorded. In 28 instances, spectral alterations were observed in the visible range of the spectrum; while in 13 samples, such changes were observed in the short UV range. In nine cases, spectral changes were observed in both the UV and visible ranges, where they did occur at different times.

This assessment emphasized the importance to use a UV blocking filter between all spectra collection and when no active data acquisition is being carried out.

3.2.4 Spectral differences as a function of time

also noted as: visible $(\sim]380 - 700$ nm), UV (200 – $\sim]380$ nm) or both. Changes at T8 Differences in spectral curves obtained at different exposure times were observed for all the samples. The week where spectral alterations were first observed (Tn) was recorded. Also, the spectral region where these changes were observed was were noted for 22 samples (12 in the visible range and 10 in both the visible and the UV spectral ranges); changes at T16 were noted for 14 samples (six in the visible range, one in the UV range, and seven in both); changes at T24 were noted for nine samples (eight in the visible range and one in the UV range); changes at T32 were noted for six samples (three in the visible range and three in both); changes at T64 were noted for one sample in the visible range and changes at T80 were noted for both UV and visible ranges.

About 40% of the samples underwent spectral alterations between 0 and 8 weeks. An important consideration of this study is that it was not possible to correlate the properties of the fibers (i.e., type of polymer) or the properties of the dyes (i.e., color, chemical class, or application mode) to their observed spectral alterations because every selected period included a variety of combinations of these properties. The fading process is complex and may depend on the interaction between the fiber substrate and the impregnated dye content and was not part of the study of this research.

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3.3 Planned Scholarly Products and Past Presentations

The final result of this research includes at least one scientific paper suitable for publication in an appropriate, peer-reviewed journal such as *Journal of Forensic Sciences*, *Forensic Science International*, *The Microscope* journal, etc. It is anticipated that there will be a total of three forthcoming publications. The first will focus on the technical aspects of this project including methods, materials, and suggested best practices (MSP Methodology for Dyed Fibers, P. Buzzini, M. King, S. Sparenga, G. Laughlin, in preparation). The second publication will address photobleaching effects and the classification of different types of spectral alterations (MSP Photobleaching Effects and Spectral Alterations of Dyed Fibers, P. Buzzini, M. King, S. Sparenga, G. Laughlin, in preparation). The third publication will encompass the overall project, examining spectral differences as a function of time (The Effect of Ultraviolet Radiation on the MSP of Dyed Fibers, P. Buzzini, M. King, S. Sparenga, G. Laughlin, in preparation).

The following oral presentation was delivered at the Inter/Micro 2017 international microscopy symposium June 12-14, 2017 in Chicago, Illinois:

The Effect of Ultraviolet Radiation on the Microspectrophotometry (MSP) of Dyed Fibers — Phase 1 Meggan B. King, McCrone Research Institute, Inc.

The following oral presentations were delivered at the Inter/Micro 2018 international microscopy symposium, hosted by McCrone Research Institute, 11 – 15

June 2018 in Chicago:

The Effect of Ultraviolet Radiation on the Microspectrophotometry (MSP) of Dyed Fibers, Part 1: Photobleaching Effect

Meggan B. King — McCrone Research Institute Patrick Buzzini and Carrie Polston — Sam Houston State University

The Effect of Ultraviolet Radiation on the Microspectrophotometry (MSP) of Dyed Fibers, Part 2: Spectral Differences as a Function of Time Patrick Buzzini and Carrie Polston — Sam Houston State University Meggan B. King — McCrone Research Institute

The following oral presentation by Dr. Patrick Buzzini was delivered at the 71st

Annual American Academy of Forensic Sciences meeting on February 22, 2019 in

Baltimore, Maryland:

The Effect of Ultraviolet Radiation on the Microspectrophotometry (MSP) of Dyed Textile Fibers: Types of Spectral Alterations

Meggan B. King, B.S.¹; Sebastian B. Sparenga, M.S.¹; Gary J. Laughlin, Ph.D.¹; and Patrick Buzzini, Ph.D.²

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²Department of Forensic Science, Sam Houston State University, 1003 Bowers Blvd., Huntsville, TX 77340

The following presentations were given on June 12, 2019 at the Inter/Micro

International Microscopy Conference in Chicago, Illinois.

A Look at How Visual Aspects of Fiber Appearance Affect MSP Spectra by Meggan King — McCrone Research Institute, Inc.

 Fibers as a Function of Time Using UV-Vis Microspectrophotometry An Update on the Effect of Ultraviolet Radiation on the Degradation of Dyed Patrick Buzzini — Department of Forensic Science, Sam Houston State University

4.0 Implications for Criminal Justice Policy and Practice in the U.S.

One of the most important aspects of forensic fiber examination is the ability to address the question of whether a fiber, through its observed characteristics (optical properties, color, diameter, etc.), could have come from a particular source. The forensic scientist must be able to give opinions to the courts or triers of fact based on reliable data. A scientific basis for reporting non-differentiations (or exclusions) of sets of questioned and reference fibers is needed. Currently, with little published research on the effects of photofading of fiber dyes, the forensic scientist may be unable to fulfill this obligation. The consequences may be small (e.g., the degraded fiber is deemed unworthy for comparison and no analyses are done) or large (e.g., the degraded fiber is erroneously associated or excluded from a source). This project provides an understanding of the occurrence of changes that dyed manufactured fibers undergo during exposure to artificial UV radiation and outdoor sunlight and their effect on MSP results to assist trace evidence examiners with the interpretation of their fiber cases.

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Figure 1. Sample board with Testfabrics swatches, samples A1-A10.

Figure 2. Sample boards with Testfabrics swatches in position at Q-Lab Test Services in Arizona, October 2017.

Figure 3. Microtrace fabric swatches inside the UV-radiation light box at McCrone Research Institute, Chicago, October 2017.

Figure 4. The photomicrographs (sample A19, acrylic basic red 14) outlined in green (left and right) show areas of fibers that are considered suitable for spectra collection because they are lying flat. Spectra are collected from the area marked by a black square. The photomicrograph outlined in red (center) shows a fiber positioned in an orientation that is not suitable for spectrum collection because it is positioned on its edge.

SAMPLE INFORMATION ‐ GROUP A

SAMPLE INFORMATION - GROUP A

APPENDIX

APPENDIX

SAMPLE INFORMATION ‐ GROUP B

SAMPLE INFORMATION - GROUP B

APPENDIX

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