USING SURFACE-ENHANCED RAMAN SPECTROSCOPY (SERS) FOR THE DETECTION OF UNDERLAYING DYES IN RE-DYED HAIR

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Using Surface-Enhanced Raman Spectroscopy (SERS) for the Detection of Underlaying Dyes on Re-Dyed Hair

Hair evidence is one of the most common types of evidence in the field of forensic sciences, yet analysis is subjective and is interpreted by an analyst, adding in bias to the outcome of the analysis. By applying Surface Enhanced Raman Spectroscopy (SERS) to the analysis of dyed hairs, forensic hair analysis can become more than just a subjective comparison between two samples and can help include or exclude individuals from being present at the scene of a crime. This alone shows the importance and need for more advanced and objective forms of hair analysis methodology in the field of forensic sciences. In order to remove the subjectivity from forensic hair analysis, the application of surface-enhanced Raman spectroscopy (SERS) is effective and nondestructive. Through this technique, we have shown that SERS can be utilized to identify hair dyes on a single strand of hair, detect underlying dyes that are present when a sample of hair has been dyed multiple times, and determine that a dye can be detected on hair that was dyed up to 9 weeks prior to analysis.

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NOMENCLATURE

BLU^{SP}	Blue (semi-permanent) dye on hair sample
BLK^{SP}	Black (semi-permanent) dye on hair sample
BLK^{P}	Black (permanent) dye on hair sample
$BLBK^{P}$	Blue-Black (permanent) dye on hair sample
(D)	Indicates the dye is isolated (not on a hair sample) on a microscope slide (e.g. BLU^{SP}(D))
→	Indicates the application of secondary dye (e.g. BLU^{SP}→BLK^{P} is black permanent dye on top of blue semi-permanent dye)

CHAPTER I

INTRODUCTION

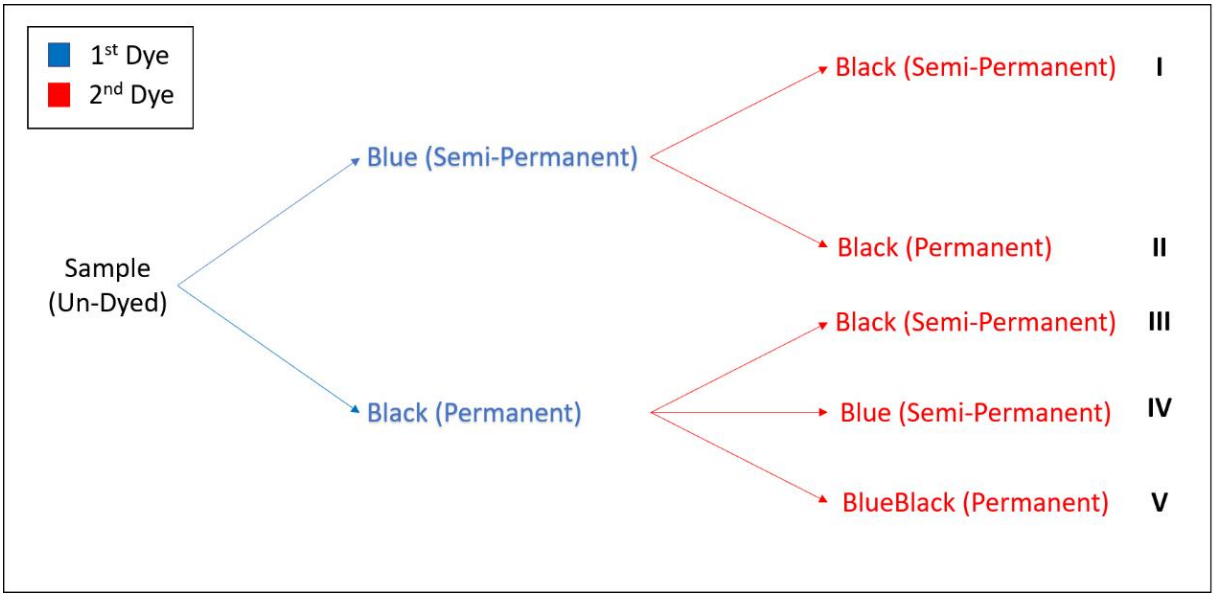
In the field of forensic sciences, hair is one of the most common pieces of evidence that can be found at the scene of a crime (1). Hair analysis is interpreted by a forensic hair analyst and relies on visual comparison techniques along with the expertise of a forensic hair analyst (2). These standards add in subjectivity to the outcome of the analysis and can cause variation amongst the same results. By applying Surface Enhanced Raman Spectroscopy (SERS) to the analysis of dyed hairs, forensic hair analysis can become more than just a subjective comparison between a sample from a scene to that of a suspect.

Due to the important nature of forensic evidence, it is highly preferable that physical evidence, such as hair remain intact during its analysis, to preserve its integrity and allow for future re-analysis. Raman spectroscopy (RS) proves to be a non-destructive technique that has proven to be effective when analyzing many types of forensic evidence including physical evidence and controlled substances (3). When analyzing a sample with RS, Raman scattering is experienced as the laser hits the sample. Because the desired signals for SERS that are produced from RS are so weak, it is necessary to amplify or enhance these signals. To accomplish this, noble metal (such as gold) nanoparticles are required. These nanoparticles, when in contact with the laser, produce localized surface plasmon resonances (LSPRs) which in turn enhance the electromagnetic field (4). This process enhances the initial Raman signal in order to detect desired signals that SERS utilizes.

Previously, it was found that SERS could be used to detect and identify hair dyes on hair samples. Using these methods, the type of hair dye (permanent or semi-permanent) could be

concluded as well as which brand was used to dye the hair (5). In order to explore SERS capabilities for the use of forensic hair evidence, we test whether an underlaying dye can be detected if a hair sample is re-dyed with another dye, and if we can detect colorant on a hair that had been dyed 9 weeks prior to analysis. Both semi-permanent and permanent dyes were utilized for this study. The difference between the two types of dyes is how they react with the hair itself. Permanent dyes undergo a chemical reaction that cause the molecules of the dye to polymerize within the shaft of the hair, allowing them to stay in the hair for longer periods of time. Semi-permanent dyes do not undergo a chemical reaction and simply stick to the hair. This explains why semi-permanent dyes do not stay on hair for extended periods of time (6).

In order to answer the research questions, two groups of experiments were conducted, testing different combinations of semi-permanent and permanent dyes as the underlaying dye. The dye procedure that was followed for each sample can be found in Scheme 1. In the first group of experiments, an undyed hair was dyed with a blue semi-permanent dye (BLU^{SP}). After this process, a black semi-permanent dye (BLK^{SP}) dye was dyed on top of the previously dyed hair (Scheme 1, I). In this same group, the initial sample that was dyed with BLU^{SP} was also re-dyed with a black permanent dye (BLK^{P}) (Scheme 1, II). These two samples test to see if an underlaying blue semi-permanent dye can be detected when re-dyed with a black semi-permanent dye and black permanent dye, respectively. To test if a permanent dye can be detected as the underlaying dye, the original hair sample was dyed first with a black permanent dye (BLK^{P}). This sample was then re-dyed with BLK^{SP} as the overlaying dye (Scheme 1, III). This was repeated with a BLU^{SP} (Scheme 1, IV) and BlueBlack permanent dye ($BLBK^{P}$) (Scheme 1, V).



Scheme 1 (I-V). Hair dyeing procedures. Blue indicates underlaying dye and red indicates secondary dye.

CHAPTER II

METHODS

Sample Collection:

Hair samples were gathered from barbershops in College Station, Texas as well as from an individual from the lab group. A preference for natural hair color or texture was not used. Samples of hair were only taken from individuals with no prior history of dyeing their hair. Samples were stored in 50 mL falcon tubes until treated with dyes.

Dyeing Procedure:

Different permanent and semi-permanent hair dyes (Table 1) were purchased from Sally Beauty Supply in College Station, Texas.

Table 1. Dye color/type, abbreviated name, and commercial name of dyes used.

Dye color/type	**Abbreviation**	**Commercial Name**
Blue (Semi-permanent)	BLU^{SP}	Ion Color Brilliance Sky Blue
Black (Semi-permanent)	BLK^{SP}	Ion Color Brilliance Blackest Black
Black (Permanent)	BLK^{P}	Ion Color Brilliance Jet Black
Blue-Black (Permanent)	$BLBK^{P}$	Ion Color Brilliance Blue Black

Note: Salon Care 20 Volume Crème Developer was added to permanent dyes to complete dye process

Semi-permanent dyes (BLU^{SP} and BLK^{SP}) were placed into respective 50 mL falcon tubes. Enough dye was placed into the tubes to successfully color the hair samples. Hair samples were placed into the falcon tubes and immersed in the dye. Samples were allowed to sit in the dye for the amount

of time recommended by the dye instructions. Following this time, the samples were washed extensively with water, until all traces of the dye in both the water and on the hair were gone. For permanent dyes ($BLBK^P$ and BLK^P), equal parts dye and developer (Salon Care 20 Creme developer) were added to falcon tubes. Hair was immersed in the dye for the recommended time. Washing was conducted following allotted time in the same methods that were used for semi-permanent hair samples. The samples were allowed to dry and were stored in respective 50 mL dry falcon tubes. Mixture samples experienced both or one process depending on the combination of dyes that were used (e.g semi-permanent on semi-permanent, semi-permanent on permanent, etc.)

Sample Preparation:

Three samples (strands of hair) were taken from each of the groups that contained more than one dye as well as from samples which were only dyed once. This resulted in 27 samples being prepared on microscope slides. These samples included each of the mixture samples which contained two dyes, and also samples which were only dyed once. Samples containing only one dye were prepared in order to determine the spectra that are gained from individual dyes. The resulting spectra help us determine what each underlaying spectra should look like when no mixture is applied. In addition to the individual dyes on hair being analyzed, the same dyes were analyzed without being dyed onto a hair. To do this, the dyes were prepared, and a drop of the dye was placed onto the microscope slide alone. These samples were prepared to verify that when dyed onto a hair, no nuances would be experienced from the dye alone. Each strand of hair was placed onto individual microscope slides and the edges of the hair were adhered to the slide with small pieces of packaging tape to ensure that the sample was flat against the slide. This also ensured that the samples did not fall from the slides. Once adhered to the slides, each sample was coated with

20 µL of gold nanorod (AuNR) solution, or enough for the hair to be completely coated with the solution. The samples were allowed to sit until the AuNR solution had dried to the surface of the hair. Each slide was placed into a 50 mL falcon tube for storage until they were analyzed.

Sample Analysis:

Samples were analyzed on an inverted confocal microscope. A laser with a wavelength of 785 nm was used to excite the sample. A spectrometer was used to collect signals that were retrieved from the samples. The resulting spectra were analyzed using GRAMS/AI 7.0. The spectra that were gathered experienced no treatment and are the original spectra that were collected.

CHAPTER III

RESULTS AND DISCUSSION

To determine if the underlaying dyes could be detected, the underlaying dye alone was dyed to find what peaks it exhibits. When analyzing the BLU^{SP} dye on a hair sample, peaks were observed at 884, 1310, 1344, 1390, 1444, 1472, 1508, 1583, 1617, and 1640 cm^{-1} (Figure 1). The same peaks were observed when analyzing the dye alone (BLU^{SP}(D)), showing that dyes could be detected when applied to hair. The BLU^{SP} sample was then dyed again with a BLK^{SP} dye (BLU^{SP}→BLK^{SP}). After analysis of the BLU^{SP}→BLK^{SP} mixture, most of the peaks that were observed corresponded with the overlaying BLK^{SP} dye. In addition to these peaks, signals that could also be attributed to the BLU^{SP} dye could also be found (Figure 1). These findings suggest that both the underlaying and overlaying dye could be detected when a sample was dyed with a semi-permanent dye on top of another semi-permanent dye.

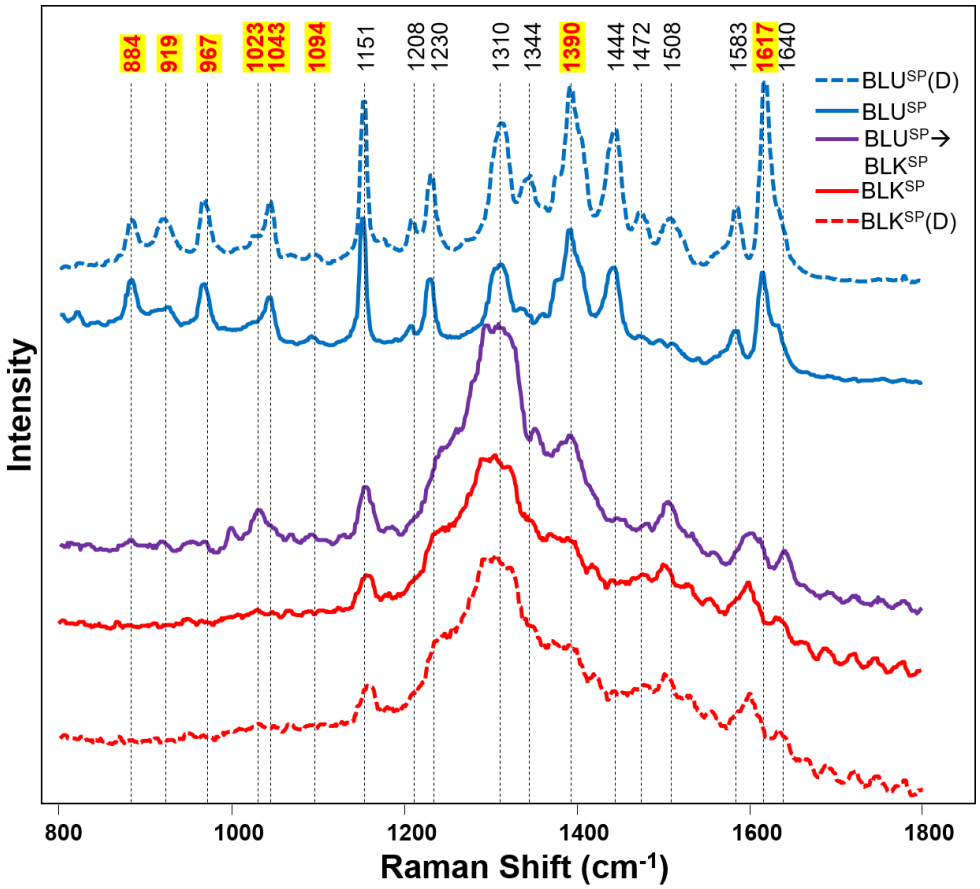


Figure1. SER spectra of hair dyed with BLU^{SP} and re-dyed with BLK^{SP} (BLU^{SP}→BLK^{SP}), BLU^{SP} dye on hair, BLU^{SP} dye alone (BLU^{SP}(D)), BLK^{SP} on hair, and BLK^{SP} dye alone (BLK^{SP}(D)).

To determine whether the same BLU^{SP} dye could be detected when a permanent dye was dyed over it, another BLU^{SP} sample was re-dyed with a BLK^{P} dye (BLU^{SP}→BLK^{P}). In this mixture, two groups of spectra were observed (Figure 2, I and II). Group I produced signals that corresponded with the initial BLU^{SP} dye (969, 1043, 1151, 1230, 1390, 1444, and 1617 cm^{-1}), while group II produced peaks that could be attributed to the secondary BLK^{P} dye (884, 949, 1208, 1310, 1344, 1414, 1472, 1505, 1583, and 1640 cm^{-1}). These findings indicate that although to the naked eye, an individual's hair seems to be uniformly dyed, some areas of the hair may in

fact be undyed by the second dye. This leaves pockets on the hair that may only contain the primary dye. These areas can be found under the microscope and expressed with the spectra that were seen.

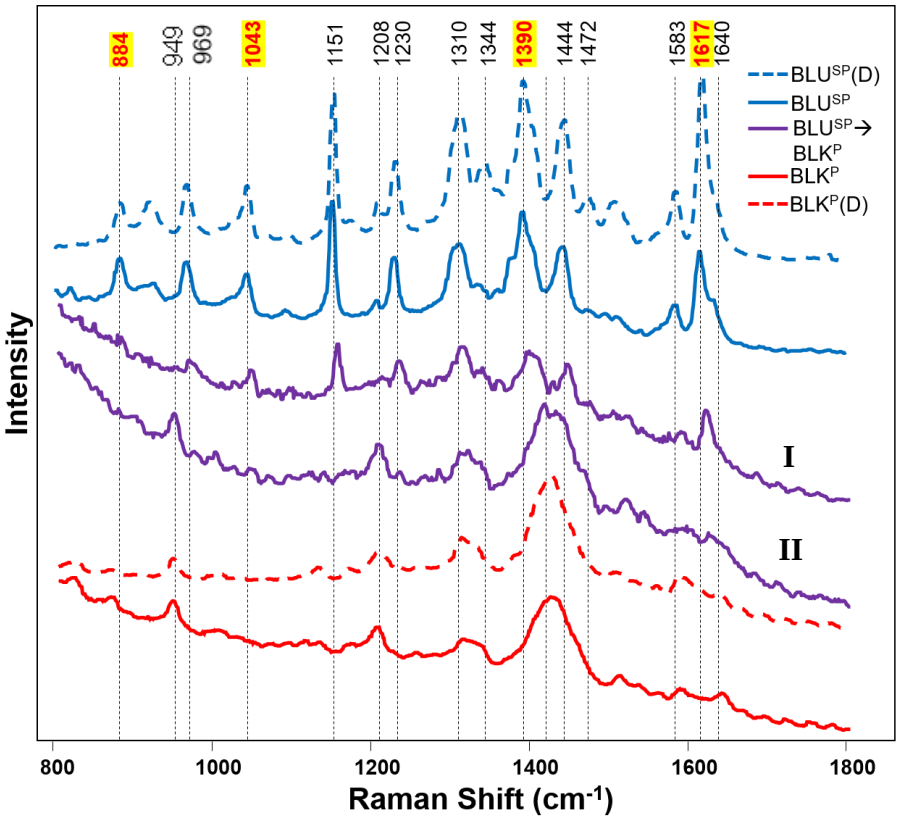


Figure 2. SER spectra of hair dyed with BLU^{SP} and re-dyed with BLK^{P} (BLU^{SP}→BLK^{P}), BLU^{SP} dye on hair, BLU^{SP} dye alone (BLU^{SP}(D)), BLK^{P} on hair, and BLK^{P} dye alone (BLK^{P}(D)).

To determine whether an underlaying permanent dye can be detected using SERS when re-dyed, a sample of hair was initially dyed with a BLK^{P} and re-dyed with a BLK^{SP} dye (BLK^{P}→BLK^{SP}). The BLK^{P}→BLK^{SP} sample was then analyzed (Figure 3). In the mixed sample, peaks that resulted from the analysis could be attributed to both the underlaying BLK^{P} dye (1151,

1208, 1310, 1390, 1508, 1583, and 1640 cm^{-1}) and the overlaying BLK^{SP} dye (946 and 1415 cm^{-1}).

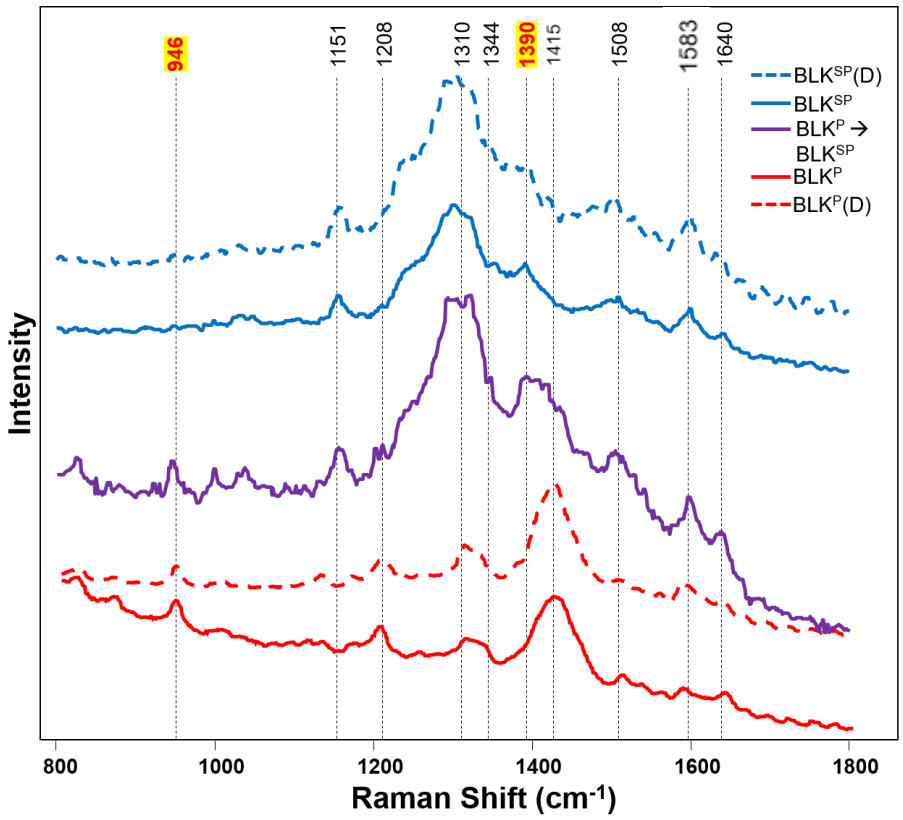


Figure 3. SER spectra of hair dyed with BLK^{P} and re-dyed with BLK^{SP} (BLK^{P}→BLK^{SP}), BLK^{P} dye on hair, BLK^{P} dye alone (BLK^{P}(D)), BLK^{SP} on hair, and BLK^{SP} dye alone (BLK^{SP}(D)).

To verify that a permanent dye could be detected under a semi-permanent dye, the BLK^{P} sample was re-dyed with the BLU^{SP} dye (BLK^{P}→BLU^{SP}) (Figure 4). Like in figure 2, this sample also resulted in the presence of two groups of spectra. Group I exhibited peaks that could be attributes to the underlaying BLU^{SP} dye (969, 1043, 1151, 1230, 1390, 1444, and 1617 cm^{-1}), while group II could be attributed to the overlaying BLK^{P} dye (930 and 1423 cm^{-1}).

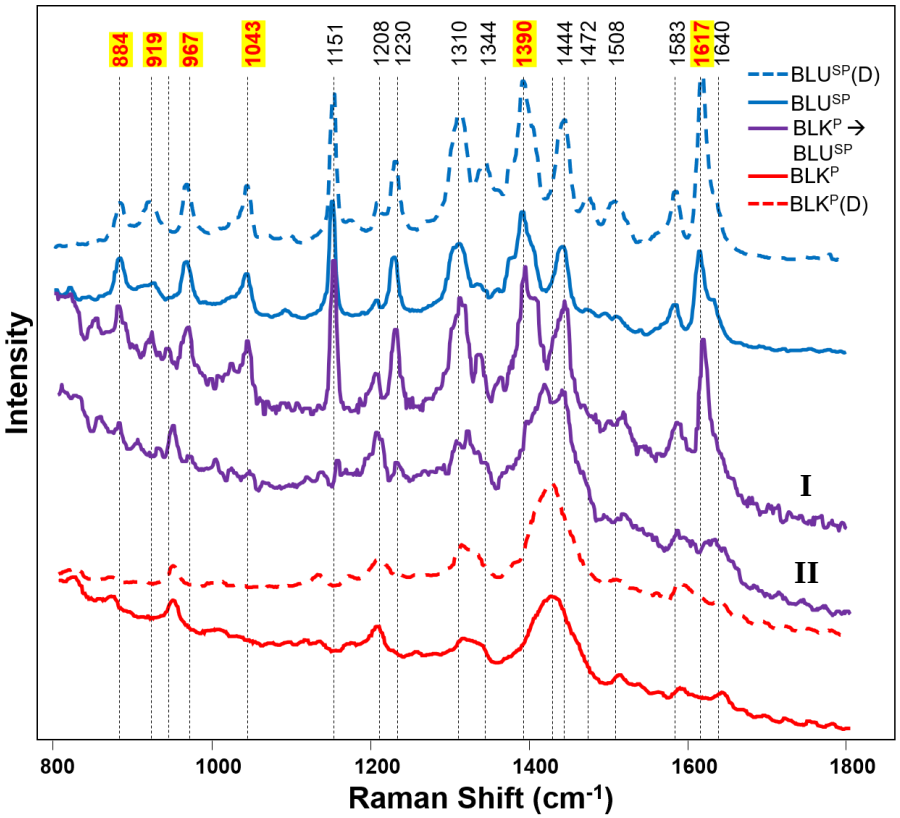


Figure 4. SER spectra of hair dyed with BLK^P and re-dyed with BLU^{SP} ($BLK^P \rightarrow BLU^{SP}$), BLK^P dye on hair, BLK^P dye alone ($BLK^P(D)$), BLU^{SP} on hair, and BLK^{SP} dye alone ($BLU^{SP}(D)$).

Lastly, in order to fully test all combinations of semi-permanent and permanent dyes, a BLK^P dye was re-dyed with a $BLBK^P$ dye ($BLK^P \rightarrow BLBK^P$). This final trial answers whether a permanent dye can be detected underneath another permanent dye. After analysis of this mixed sample, it was found that each of the component dyes (BLK^P and $BLBK^P$) individually produced such similar peaks, that they were not able to be distinguished from one another (Figure 5). This means that even when mixed, the peaks that were produced in the mixed sample could not be attributed to one of the permanent samples over the other.

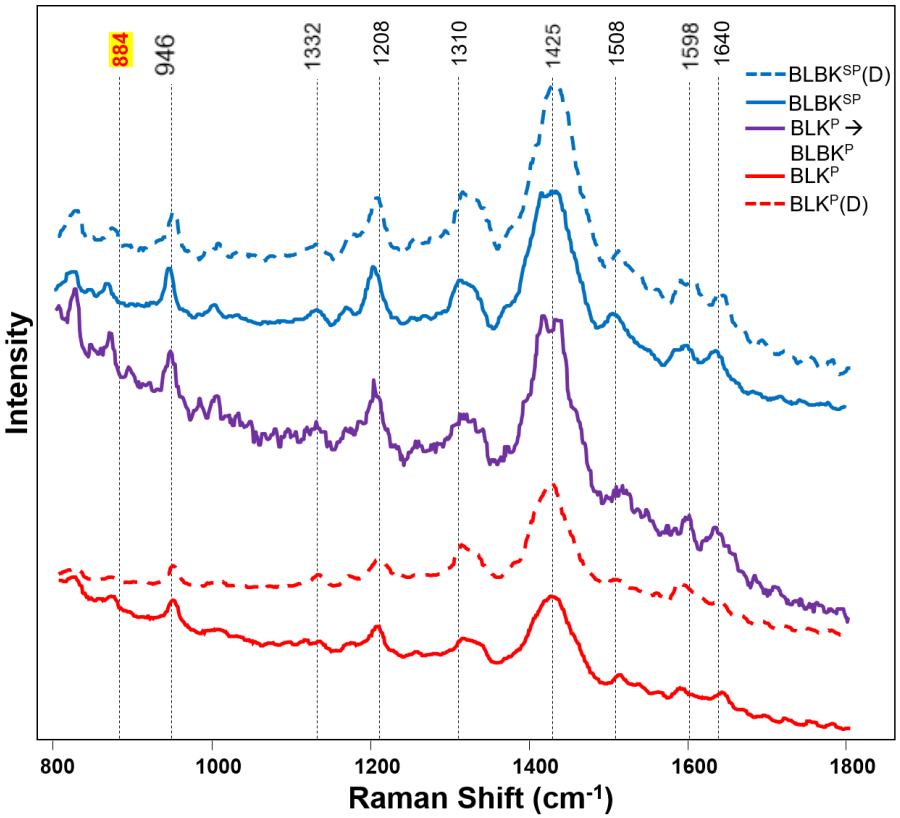


Figure 5. SER spectra of hair dyed with BLK^{P} and re-dyed with BLBK^{P} (BLK^{P}→BLBK^{P}), BLK^{P} dye on hair, BLK^{P} dye alone (BLK^{P}(D)), BLBK^{P} on hair, and BLBK^{P} dye alone (BLBK^{P}(D)).

To answer the final research question of if a dye can be detected if the hair had been dyed up to 9 weeks prior, an individual within the lab group dyed their hair with a blue semi-permanent dye. This individual proceeded with regular washing and hair care routines, and took samples of their hair once every week. These samples were prepared in the same ways and were analyzed with SERS. After analysis, it was found that in all samples, the spectra that is attributed to the blue semi-permanent dye, could be detected (Figure 6). 9 weeks (or 2 months) was chosen specifically because it proves to be the average amount of time it takes for hair to regrow and lose its colored characteristics. This is also when individuals tend to re-dye their hair. The

changes in intensity can be explained by the uneven coating of AuNRs that are deposited on the hair samples. Like dye, AuNRs also do not uniformly cover the hair. Changes in intensity that can be seen, can be explained by the higher or lower concentration of these nanorods in that certain area.

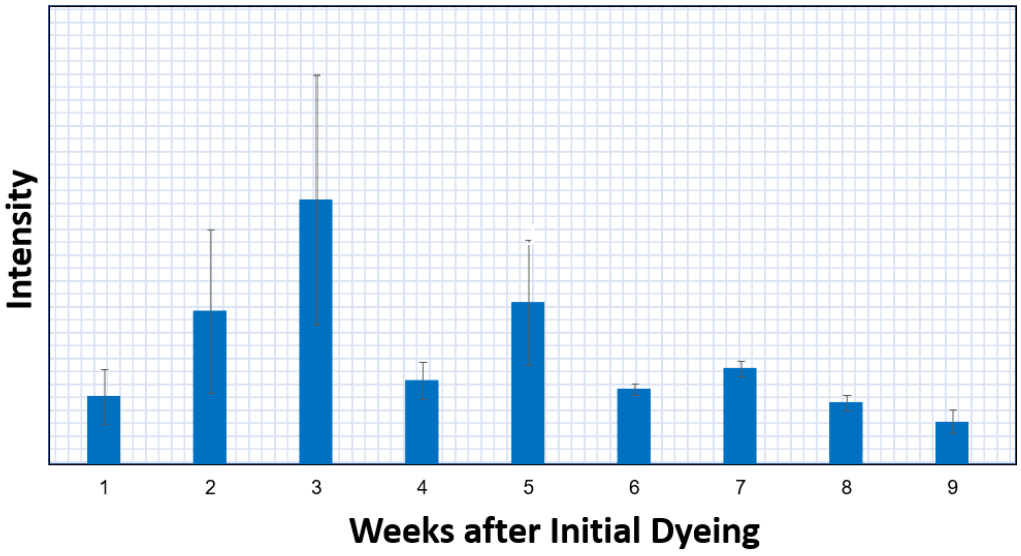


Figure 6. Intensity shifts of BLU^{SP} dye on hair sample over the duration of 9 weeks (2 months)

CHAPTER IV

CONCLUSION

After analysis of different combinations of underlaying and overlaying dye types, it can be concluded that SERS can be successfully utilized to detect a blue semi-permanent underneath both a black semi-permanent dye and a black permanent dye, respectively. It could also be concluded that utilizing these methods, a black permanent dye could be detected when re-dyed with a blue semi-permanent and black semi-permanent dye, respectively. When analyzing a hair that first contained a black permanent dye and was re-dyed with a blue/black permanent dye, it was found that SERS could not distinguish between the two component dyes.

Because in some cases, two completely different spectra could be found from the same samples, it can be inferred that although we may see one colorant, under the microscope, pockets can be found that only contain the primary dye. This shows that overall, hair is not uniformly dyed. In this study, it is also shown that even with normal use, hygiene, and care routine, a blue semi-permanent dye can still be detected when the hair was dyed up to 9 weeks (if not more) prior to analysis.

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