Received: 18 May 2023 | Revised: 18 July 2023 | Accepted: 19 July 2023

DOI: 10.1111/1556-4029.15347

JOURNAL OF FORENSIC SCIENCES AAFS

TECHNICAL NOTE

Criminalistics

Surface-enhanced Raman spectroscopy enables confirmatory detection of dyes on hair submerged in hypolimnion water for up to twelve weeks

Aidan P. Holman HSD[1,2] | Dmitry Kurouski PhD[2,3,4]

[1]Department of Entomology, Texas A&M University, College Station, Texas, USA

[2]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas, USA

[3]Department of Biomedical Engineering, Texas A&M University, College Station, Texas, USA

[4]Institute for Advancing Health through Agriculture, Texas A&M University, College Station, Texas, USA

Correspondence
Dmitry Kurouski, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA.
Email: dkurouski@tamu.edu

Funding information
National Institute of Justice, Grant/Award Number: 2020-90663-TX-DU

Abstract

Difficulties in the localization of bodies of homicidal or drowning victims in natural water result in their submergence for weeks if not months. Water insects and microbes drastically change the body's appearance, which significantly changes the determination of a victim's identity. DNA analysis is commonly used for identifying the decedent; however, this PCR-based approach is time-consuming and destructive of the evidence. Considering that nearly half of the people in the world dye their hair with a variety of permanent and semi-permanent dyes, one can expect that confirmatory identification of dyes on the body's hair can be used to shed light on the victim's identity. A growing body of evidence suggests that surface-enhanced Raman spectroscopy (SERS) can be used to detect and identify hair dyes. In this study, we investigated the extent to which SERS could be used to detect black and blue, permanent and semi-permanent dyes on hair submerged in hypolimnion water for up to twelve weeks. We found that SERS enabled 100% accurate identification of analyzed dyes on hair submerged in hypolimnion water for up to 8 weeks, whereas, on average, 87% accurate identification of the hair dyes could be achieved on hair exposed for 10 weeks and 50% for hair exposed 12 weeks in hypolimnion water. We also found that the aqueous environment caused progressive fading of some dyes, whereas other dyes showed substantial spectral transformations after prolonged submergence. Finally, we found that changes in the intensity of vibrational bands of dyes could be used to predict the duration of submergence of colored hair in hypolimnion water.

KEYWORDS
fading, hair dyes, hypolimnion, PLS-DA, Raman spectroscopy, SERS

Highlights

- SERS can be used to detect dyes on hair submerged in hypolimnion water for up to twelve weeks.
- Both progressive fading and chemical transformations were observed upon the prolonged submergence.
- The intensity of dye vibrational bands allows for predicting the duration of submergence.
- SERS enabled 87% accurate identification of the dyes on hair exposed for 10 weeks.

1 | INTRODUCTION

In lakes, organic matter decomposes in the lowermost region known as the hypolimnion [1]. In the hypolimnion of most lakes, the temperature ranges between 4°C and 11°C [2]. The water is stagnant, hypoxic, and is usually found greater than 3 meters below the surface [3,4]. Unless the body is weighed down by debris or other material, corpses usually remain in the hypolimnion for approximately 10–14 days before bloating causes the body to begin floating towards the surface [5]. Due to variations in the capabilities of bodies to sink when exposed to water, materials such as clothing, ropes, bags, and packs are commonly used to gravitate the body towards the bottom of the lake [5]. The constant movement of human remains further complicate their localization [5]. These obstacles prolong the duration of decedent submergence, which causes further degradation of soft tissues. As a result, only bones, teeth, and hair are available for the forensic analysis of human remains at the time of their emergence [6].

Permanent dyes and semi-permanent dyes are often used to dye hair [7]. Permanent hair dyes require developers that oxidize aromatic diamines into aromatic polymers, known as Bandrowski's bases [8]. In the hair cortex, these molecules interact with keratin when they oxidize, allowing them to remain on the hair for several months. Semi-permanent dyes are aromatic molecules with a large number of conjugated double bonds. These colorants do not require developers and do not penetrate the hair's cortex. Therefore, semi-permanent dyes easily come off upon hair washing [8]. Confirmatory analysis of hair colorants can be used to establish a connection between a decedent and their hair ritual practices, in order to help identify the decedent's identity.

Currently utilized microscopic analysis of hair can reveal the race and area of the hair origin, which is often insufficient for the identification of human remains. DNA or PCR-based analysis of hair has been shown to reveal more information about an individual through access to their genetics. However, DNA analysis is time-consuming, costly, and destructive for the analyzed samples. The majority of DNA is preserved in the bulb of a hair strand, which degrades over time [9]. This catalyzed a search for alternative techniques that can empower forensic analysis of substances of biological origin. A growing body of evidence suggests that surface-enhanced Raman spectroscopy (SERS) can be used to detect and identify artificial dyes present on hair, including the types of brands, colorants, and dye permanence [7,10-12]. SERS enhancement is based on coherent oscillations of conductive electrons on noble metal nanostructures upon their illumination with electromagnetic radiation. These coherent oscillations of conductive electrons (or localized surface plasmon resonances) provide 10^6–10^8 enhancement of Raman scattering from molecules located in proximity to metallic surfaces [13-15]. It should be noted charge transfer and molecular resonance contribute a significant portion of the total enhancement factor (EF) observed in SERS experiments [16-18]. Using SERS, Kurouski and Van Duyne found drastic differences between spectra of permanent and semi-permanent dyes present on hair [7]. These spectral differences enabled highly accurate identification of such dyes on hair. Recently, Higgins and Kurouski showed that SERS could be used to identify up to 33 different dyes with high accuracy [11]. This analytical approach also enabled confirmatory identification of dye brand and color. Furthermore, Ezparza and co-workers demonstrated that SERS could be used to reveal the dyeing history of hair [10].

To further the credibility and improve the accuracy of SERS in forensic hair analysis, we investigate the extent to which hair dye detectability remains achievable following an extensive time of hair submergence in water. In this proof-of-principle study, we acquired SERS spectra from hair colored with black and blue, permanent and semi-permanent dyes during a twelve-week period of hair submergence in hypolimnion water. We also determined whether SERS could be used to predict the duration of hair submergence.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Four commercial dyeing formulations were purchased from a local Sally Beauty supply store (College Station, TX), two permanent: Ion Jet Black (No. 305730) and Ion Tanzanite (No. 405607); and two semi-permanent: Ion Black (No. 305052) and Ion Sapphire (No. 405068). Blonde, Caucasian, virgin hair was colored with (1) black permanent (BLK^P), (2) blue permanent (BLU^P), (3) black semi-permanent (BLK^S), and (4) blue semi-permanent (BLU^S) according to the instructions provided by dye manufacturers. Dyes were applied, according to package instructions, to the undyed, virgin Caucasian hair of the same donor.

Four tubes, clean and dry, were taken to Lake Bryan, at the beginning of April, as our hypolimnion of choice and origin. A retractable tape measure was used to measure a final depth for water collection at approximately 12′5″ or 3.8 meters below the surface. All four tubes were filled and sealed with water (and soil to include microbes) from the hypolimnion in the same area. A digital thermometer was used to measure the temperature of the first group-tube water which was 6.1°C. Each group of hair was tied in rubber bands and placed into their respective lake water-filled tubes. Sampling was done every fourteen days ("week 0" being hair sampled before submergence) for twelve weeks by snipping 1–1.5 inches of 1 or 2 hair strands to be stored in clean, dry tubes. The hypolimnion is difficult to study over long periods since its microbe concentration fluctuates during seasonal and volumetric changes. In a previous study by Burns (1976), in 3 months the mean hypolimnion temperature of several northern lakes only increased by 1.83°C [19]. This created our criterion for our groups to stay between 6.1°C (temperature when it was collected) ±1.83°C (between 4.2°C and 7.9°C) which we monitored weekly using a digital thermometer (BRAND). A miniaturized fridge allowed groups to rest in temperatures between 4.4°C and 4.5°C.

2.2 | Raman spectroscopy

For each sample, 5 μL of a suspension of gold nanoparticles (AuNPs) was applied directly to hair. The AuNPs were synthesized in our laboratory following the procedure outlined by Esparza and coworkers [10]. SERS spectra were acquired from several strands of hair on the home-built confocal inverted microscope (Nikon, Model TE-2000U). Continuous wavelength 785 nm light (Necsel SLM785.0-FS-01) was focused on the sample using a 20× Nikon objective. Scattered electromagnetic radiation was collected using the same objective. Elastically scattered photons were removed using a long-pass filter. Raman photons were passed to Princeton Instruments spectrograph equipped with a 600-groove/mm grating and collected using a thermoelectrically cooled PIX-400BR CCD camera (Princeton Instruments). The laser power at the sample was ~1.8 mW. Spectral action time varied between 18 and 28 s. At least 50 spectra were collected from each class of the hair samples.

2.3 | Data analysis

Chemometric analysis of acquired spectra was done in MATLAB equipped with PLS_Toolbox 8.6.2 (Eigenvector Research, Inc., Manson, WA). Raman spectra were baseline-corrected and normalized before analysis. For PLS Discriminant Analysis (PLS-DA), Validation models partitioned in a 70:30 (Calibration v Validation) ratio were employed using the onion algorithm. Pre-processing of each model was done using MSC-mean normalization and 1st-derivative smoothing ($n = 2$, fl = 15 pt.). 100% Calibration (Cross-validation) models are included in the supporting information (SI), Tables S1 and S2. Latent Variables were selected based on the RMSE cross-validation (RMSECV1) reports, reported in SI, Figures S1 and S2.

3 | RESULTS AND DISCUSSION

In the SERS spectra acquired from BLK^P -colored hair, we detected peaks at 451, 494, 579, 734, 759, 829, 873, 950, 1003, 1135, 1210, 1269, 1319, 1436, 1517 and 1593 cm^{-1}, Figure 1, Table 1. We found a substantial decrease in the intensities of these vibrational bands in the spectra acquired from the hair submerged in hypolimnion water for 2–8 weeks. These results show that hypolimnion water causes significant degradation of BLK^P dye on hair. We also found that after week 8, no identifiable pattern of the dye could be detected in the corresponding SERS spectra. These findings suggest that BLK^P is fully degraded upon prolonged exposure of colored hair to hypolimnion water.

In the spectra acquired from BLU^P -dyed hair, we observed vibrational bands centered at 439, 513, 568, 866, 1011, 1155, 1240, 1294, 1398, 1517, and 1606 cm^{-1}, Figure 1. We found significant changes in the spectroscopic fingerprint of this dye in SERS spectra acquired from BLU^P-colored hair exposed to hypolimnion water 2–8 weeks. Specifically, we found the appearance of new vibrational bands and a drastic reduction in the intensity of BLU^P peaks. These results suggest that hypolimnion water causes changes in the chemical structure of BLU^P- dye. These chemical changes become clearly visible in the SERS spectra acquired from the BLU^P-colored hair exposed to hypolimnion water for 10 weeks. Specifically, we observed bands at 378, 430, 560, 739, and 762 cm^{-1} that were not evident in the SERS spectra acquired from the intact BLU^P -colored hair, Figure 1. Finally, only one weak band at ~430 cm^{-1} was observed in the SERS spectra acquired from hair exposed to hypolimnion water for 12 weeks. These results show that prolonged exposure of colored hair to hypolimnion water causes nearly complete degradation of BLU^P dye.

In the spectra acquired from hair colored by BLK^S at week 0, we observed vibrational bands at 466, 492, 529, 760, 1032, 1159, 1296, 1355, 1392, 1509, 1538, and 1602 cm^{-1}, Figure 1. We found that some of these bands decreased (1159, 1296, 1355, 1392, 1602 cm^{-1}), whereas others increased (466, 529, 1032, 1100, 1509, and 1538 cm^{-1}) their intensity in the SERS spectra acquired from BLK^S-colored hair at 2–8 weeks of hair exposure to hypolimnion water. These results are similar to what we found for BLU^P, hypolimnion water causes significant changes in the chemical structure of BLK^S. We also found that at weeks 10 and 12 of hair exposure to hypolimnion water, most of the vibrational bands of BLK^S could not be identified in the corresponding SERS spectra. These results demonstrate that hypolimnion water causes substantial degradation of BLK^S.

BLU^S exhibits a unique vibrational fingerprint that enables dye identification on hair [18]. Specifically, we observed vibrational bands at 314, 425, 463, 583, 760, 926, 973, 1048, 1157, 1235, 1318, 1347, 1396, 1448, 1511, and 1621 cm^{-1}, Figure 1. We found that intensities of these vibrational bands progressively decreased as the duration of hair submergence to hypolimnion water increased. Most of them were present in the spectra acquired from the hair exposed to hypolimnion water for weeks 2–12. These results demonstrate that hypolimnion water just erases the dye without causing the chemical transformation of the dye on the colored hair.

These results show that hypolimnion water can cause two distinctly different changes in the colored hair. In the first case, observed for BLK^P and BLU^S, we observed a slow dissipation of the dye from the hair without any noticeable changes in the dye composition. These conclusions can be made by the observed decrease in the intensity of vibrational bands of the dye. We found that some dyes, like BLU^S, could be detected on hair at week 12, whereas others (BLK^P) fully vanished by week 10. Thus, the resistance to dye degradation is determined by the chemical structure of the dye.

In the second case, hypolimnion water caused drastic changes in the chemical structure of the dyes, which was reflected by drastic changes in their SERS spectra. Specifically, we observed the appearance and disappearance of vibrational bands in the spectra of hair exposed to hypolimnion compared to the vibrational fingerprint of the dye observed on intact hair (week 0). We found that such changes were taken place already at week 2 of hair exposure to hypolimnion water. One can expect that confirmatory identification of such dyes would require a separate library that was partially developed in our study.

We utilized chemometrics to investigate the extent to which hypolimnion-induced changes in the vibrational bands of the dyes

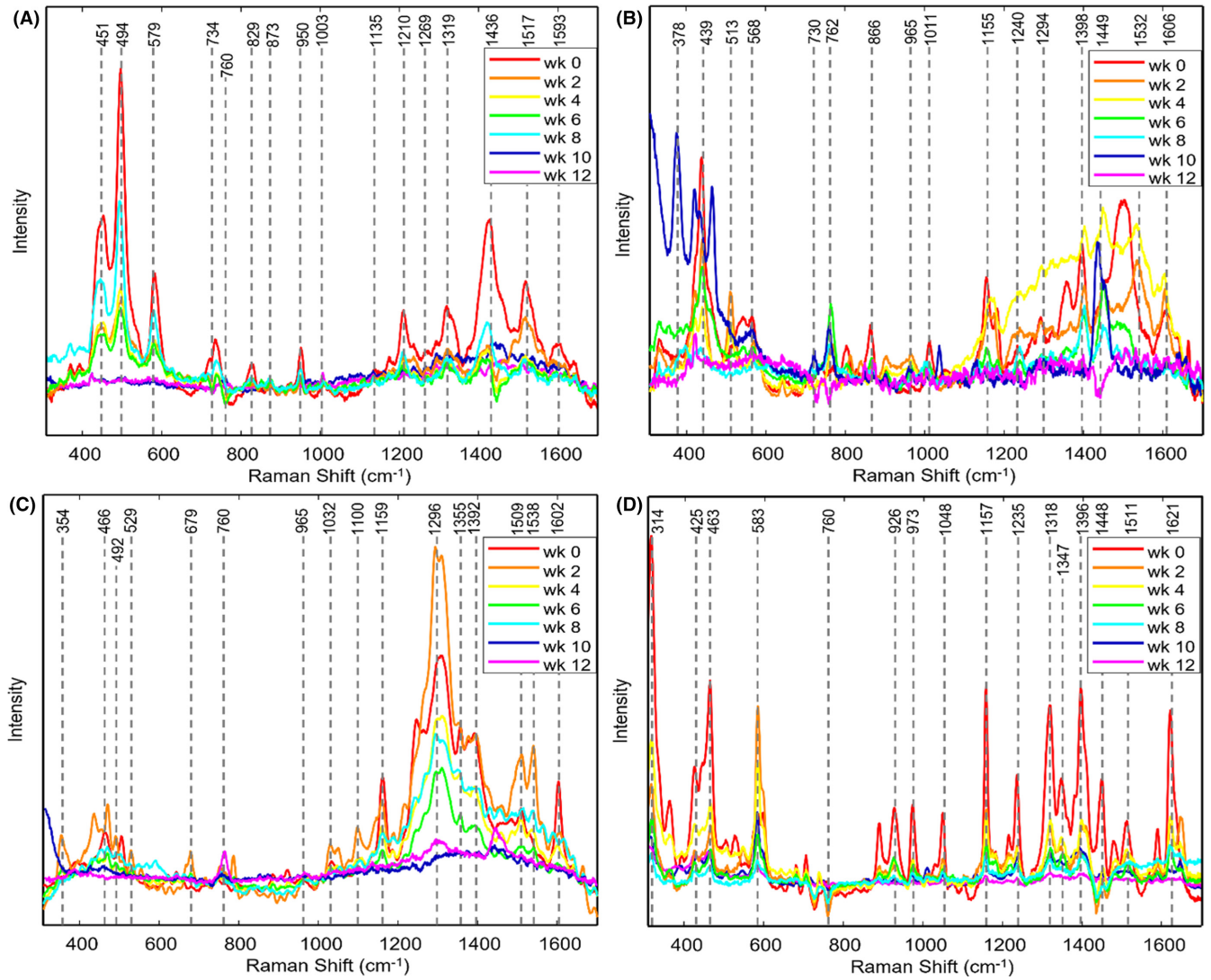

FIGURE 1 SERS spectra acquired from hair colored with BLK^P (A), BLU^P (B), BLK^S (C), and BLU^S (D) before (week 0) and after submergence to hypolimnion water for 2–12 weeks.

TABLE 1 Vibrational bands observed in SERS spectra of hair colored by BLK^P, BLU^P, BLK^S, and BLU^S dyes.

Dye	Vibrational bands, cm^{-1}
BLK^P	451, 494, 579, 734, 759, 829, 873, 950, 1003, 1135, 1210, 1269, 1319, 1436, 1517 and 1593
BLU^P	439, 513, 568, 866, 1011, 1155, 1240, 1294, 1398, 1517, and 1606
BLK^S	466, 492, 529, 760, 1032, 1159, 1296, 1355, 1392, 1509, 1538, and 1602
BLU^S	314, 425, 463, 583, 760, 926, 973, 1048, 1157, 1235, 1318, 1347, 1396, 1448, 1511, and 1621

could be used for their confirmation identification. We found that a PLS-DA model could be used to identify all dyes on hair with 100% accuracy until week 10, Table 2 and Table 3. At week 10, BLK^P and BLU^S could be identified with 100% accuracy, whereas the accuracy of identification of BLU^P and BLK^S was 91.7% and 83.3%, respectively. At week 12, however, all dyes, again, could be identified

TABLE 2 PLS-DA validation models of each sampling period's ability to correctly predict (TPR) the dye submerged in hypolimnion water.

			Dye			
			BLK^P	BLU^P	BLK^S	BLU^S
Sampling period	LV	OSR (%)	TPR (%)			
Week 0	3	100	100	100	100	100
Week 2	3	100	100	100	100	100
Week 4	3	100	100	100	100	100
Week 6	3	100	100	100	100	100
Week 8	3	100	100	100	100	100
Week 10	4	93.8	100	91.7	83.3	100
Week 12	4	100	100	100	100	100
Overall	–	99.1	100	98.8	97.6	100

OSR (%) = The overall success rate of each model, i.e., the averaged TPR for each class.

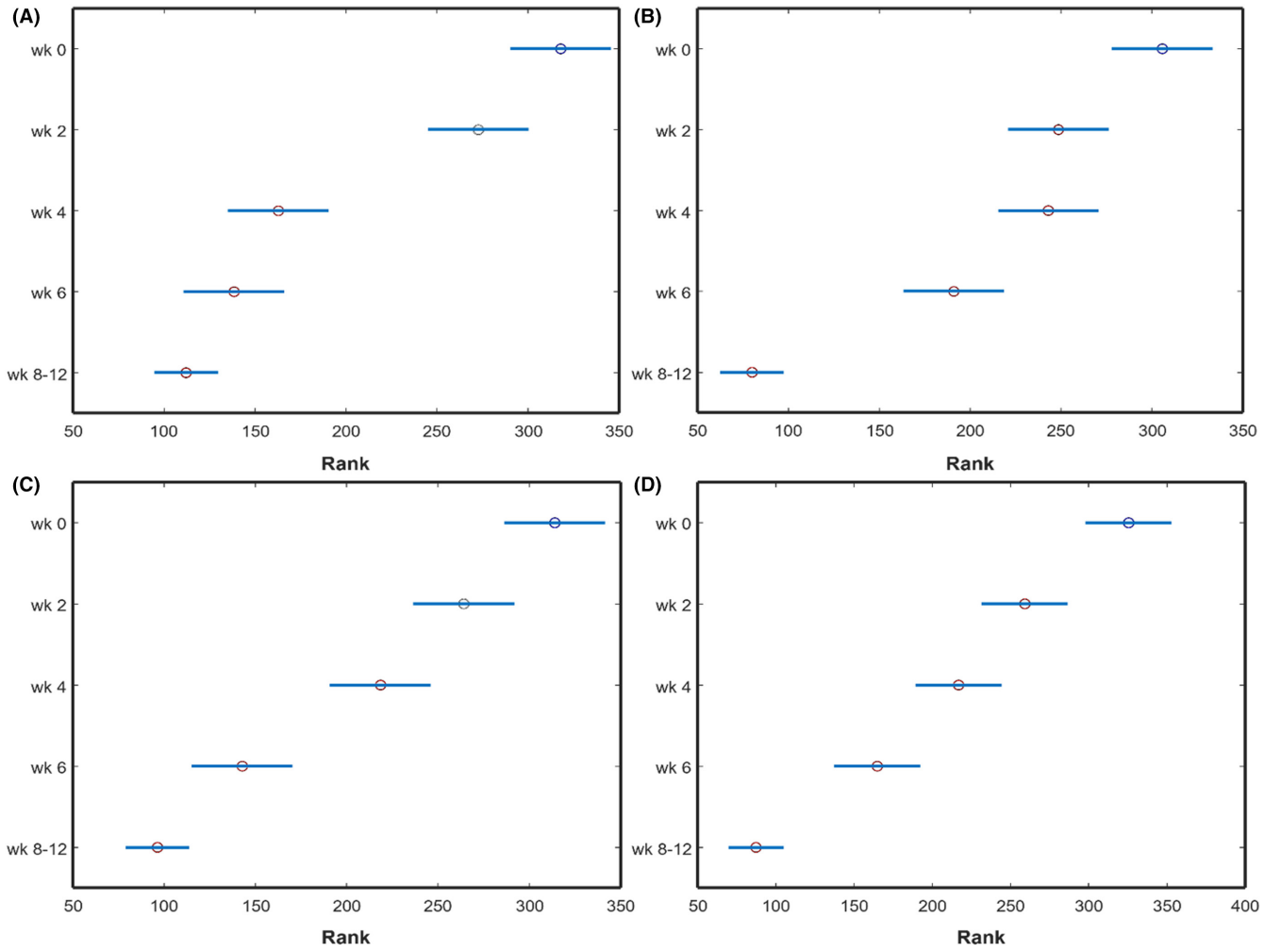

FIGURE 2 ANOVA graphs for (A) BLK^P at 1517 cm^{-1}, (B) BLU^P at 1155 cm^{-1}, (C) BLK^S at 1159 cm^{-1}, and (D) BLU^S at 1048 cm^{-1}.

TABLE 3 PLS-DA validation (prediction) models of each dye's ability to correctly predict (TPR) the time it was collected (sampling period) after submergence in hypolimnion water.

			Sampling period						
			Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Dye	LV	OSR (%)	TPR (%)						
BLK^P	11	91.1	100	93.3	50	94.1	100	100	100
BLU^P	10	96.9	100	90.9	87.5	100	100	100	100
BLK^S	11	96	100	100	87.5	84.6	100	100	100
BLU^S	11	100	100	100	100	100	100	100	100
Overall	–	96	100	96.1	81.2	94.7	100	100	100

OSR (%) = The overall success rate of each model, i.e., the averaged TPR for each class.

with 100% accuracy. These results show that although substantial changes in the intensities of vibrational bands were observed, PLS-DA enabled highly accurate identification of the dyes on hair exposed to hypolimnion water for 2–12 weeks, Table 3.

We also investigated whether any of the vibrational bands of the dyes could be used to track hypolimnion-induced dye fading. Using ANOVA, we found that 1517 cm^{-1} could be used to track fading of BLK^P, whereas changes in the intensity of 1155 cm^{-1} could be used to track BLU^P-colored hair exposure to hypolimnion water, Figure 2. Similar trends of fading were observed upon analysis of the intensity of 1159 cm^{-1} and 1048 cm^{-1} for BLK^S and BLU^S, respectively. These results demonstrate that SERS could be used to predict the duration of hair submergence to hypolimnion water (Table 3).

4 | CONCLUSIONS

Our results showed that SERS coupled with PLS-DA analysis could be used to identify permanent and semi-permanent dyes of different colors directly on hair exposed to 2–12 weeks in hypolimnion water. Detailed analysis of the acquired SERS spectra revealed that for some dyes, hypolimnion water causes only their fading, whereas for others, it triggers spectral transformations in the dyes that result in drastic changes in their SERS spectra. Nevertheless, utilization of PLS-DA enables highly accurate (100%) identification of vibrational fingerprint of analysis of dyes on hair exposed to hypolimnion water for 2–8 weeks, whereas at weeks 10–12, ~92% accurate identification could be achieved. We also found that analysis of intensity changes of some vibrational bands of the BLK^P BLU^P BLK^S and BLU^S dyes can be used to predict the duration of hair submergence in hypolimnion water. Future research concerning the effects of dye stability on hairs of different origins (e.g., races and ages), freshly dyed virgin hair versus washed, dyed virgin hair, and the ability of SERS to detect dye presence and changes in these scenarios would further improve the potential use of SERS in forensic science.

ACKNOWLEDGMENTS

The authors would like to thank Samantha Higgins, Isaac Juarez, and Mackenzi Steczkowski for their suggestions and feedback.

FUNDING INFORMATION

This project was supported by Award No. 2020-90663-TX-DU, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts to declare.

ORCID

Dmitry Kurouski https://orcid.org/0000-0002-6040-4213

REFERENCES

1. Wetzel RG, Likens GE. The inorganic carbon complex: alkalinity, acidity, CO_2, pH, total inorganic carbon, hardness. Limnological analyses. New York, NY: Springer; 1991. p. 107–28. https://doi.org/10.1007/978-1-4757-4098-1_8
2. Charlton M. Hypolimnion oxygen consumption in lakes: discussion of productivity and morphometry effects. Can J Fish Aquat Sci. 1980;37(10):1531–9. https://doi.org/10.1139/f80-198
3. Sadchikov A, Ostroumov S. Epilimnion, metalimnion, and hypolimnion of a mesotrophic aquatic ecosystem: functional role of the vertical structure of the reservoir ecosystem in terms of hydrochemical and biological parameters. Russ J Gen Chem. 2019;89(13):2860–4. https://doi.org/10.1134/S107036321913019X
4. Walker C, Lucke T. Urban lakes as a WSUD system. In: Sharma AK, Gardner T, Begbie D, editors. Approaches to water sensitive urban design: potential design, ecological health, urban greening, economics, policies, and community perceptions. Amsterdam, Netherlands: Elsevier; 2019. p. 269–85. https://doi.org/10.1016/B978-0-12-812843-5.00013-7
5. Ruffell A, Pringle J, Cassella J, Morgan R, Ferguson M, Heaton V, et al. The use of geoscience methods for aquatic forensic searches. Earth Sci Rev. 2017;171:323–37. https://doi.org/10.1016/j.earscirev.2017.04.012
6. Haglund WD, Sorg MH. Human remains in water environments. In: Haglund WD, Sorg MH, editors. Advances in forensic taphonomy: method, theory, and archaeological perspectives. Boca Raton, FL: CRC Press; 2002. p. 201–18.
7. Kurouski D, Van Duyne RP. In situ detection and identification of hair dyes using surface-enhanced Raman spectroscopy (SERS). Anal Chem. 2015;87(5):2901–6. https://doi.org/10.1021/ac504405u
8. Da França SA, Dario MF, Esteves VB, Baby AR, Velasco MVR. Types of hair dye and their mechanisms of action. Cosmetics. 2015;2(2):110–26. https://doi.org/10.3390/cosmetics2020110
9. Heywood DM, Skinner R, Cornwell PA. Analysis of DNA in hair fibers. J Cos Sci. 2003;54(1):21–8.
10. Esparza I, Wang R, Kurouski D. Surface-enhanced Raman analysis of underlaying colorants on redyed hair. Anal Chem. 2019;91(11):7313–8. https://doi.org/10.1021/acs.analchem.9b01021
11. Higgins S, Kurouski D. Surface-enhanced Raman spectroscopy enables highly accurate identification of different brands, types and colors of hair dyes. Talanta. 2022;251:123762. https://doi.org/10.1016/j.talanta.2022.123762
12. Holman A, Kurouski D. The effects of sun exposure on colorant identification of permanently and semi-permanently dyed hair. Sci Rep. 2023;13(1):2168. https://doi.org/10.1038/s41598-023-29221-8
13. Henry A-I, Sharma B, Cardinal MF, Kurouski D, Van Duyne RP. Surface-enhanced Raman spectroscopy biosensing: In vivo diagnostics and multimodal imaging. Anal Chem. 2016;88(13):6638–47. https://doi.org/10.1021/acs.analchem.6b01597
14. Kleinman SL, Sharma B, Blaber MG, Henry A-I, Valley N, Freeman RG, et al. Structure enhancement factor relationships in single gold nanoantennas by surface-enhanced Raman excitation spectroscopy. J Am Chem Soc. 2013;135(1):301–8. https://doi.org/10.1021/ja309300d
15. Wustholz KL, Henry A-I, McMahon JM, Freeman RG, Valley N, Piotti ME, et al. Structure-activity relationships in gold nanoparticle dimers and trimers for surface-enhanced Raman spectroscopy. J Am Chem Soc. 2010;132(31):10903–10. https://doi.org/10.1021/ja104174m
16. Valley N, Greeneltch N, Van Duyne RP, Schatz GC. A look at the origin and magnitude of the chemical contribution to the enhancement mechanism of surface-enhanced Raman spectroscopy (SERS): theory and experiment. J Phys Chem C. 2013;4:2599–2604. https://doi.org/10.1021/jz4012383
17. Kurouski D, Van Duyne RP, Lednev IK. Exploring the structure and formation mechanism of amyloid fibrils by Raman spectroscopy: a review. Analyst. 2015;140(15):4967–80. https://doi.org/10.1039/C5AN00342C
18. Schlucker S. Surface-enhanced Raman spectroscopy: concepts and chemical applications. Angew Chem Int Ed Engl. 2014;53(19):4756–95. https://doi.org/10.1002/anie.201205748
19. Burns N. Oxygen depletion in the central and eastern basins of Lake Erie, 1970. Can J Fish Aquat Sci. 1976;33(3):512–9. https://doi.org/10.1139/f76-069

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Holman AP, Kurouski D. Surface-enhanced Raman spectroscopy enables confirmatory detection of dyes on hair submerged in hypolimnion water for up to twelve weeks. J Forensic Sci. 2023;00:1–6. https://doi.org/10.1111/1556-4029.15347