Surface-Enhanced Raman Scattering and X-ray Fluorescence Analyses of a Single Hair Colored with a Hair Dye Product

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Abstract

This paper describes a method for obtaining information that can contribute to individual identification from a single hair colored with a hair dye product using a combination of surface-enhanced Raman scattering (SERS) and X-ray fluorescence (XRF) analyses. SERS and XRF spectra of single hog hairs colored with several commercially available hair dye products were measured. SERS spectral patterns tended to be different depending on hair dye products used for hair coloring. However, SERS spectral patterns of single hog hairs colored with different hair dye products that produced or contained similar types of dyes were similar. By performing XRF analysis, characteristic metallic elements originating from some hair dye products were detected. Therefore, XRF can contribute to identifying the difference among colored hairs that cannot be identified only by SERS. SERS and XRF analyses of a single shed hair can contribute to individual identification used in forensic science.

Keywords: hair dye, individual identification, single hair, surface-enhanced Raman scattering, X-ray fluorescence
Introduction

Various types of information obtained from hair are useful for individual identification.\(^1\) Therefore, hair gathered at the crime scene can become an important clue in criminal investigation. In forensic science, information for individual identification is needed to be obtained from a trace amount of substance found at the crime scene, and a method enabling acquiring information for specifying whose hair it is from a single hair is needed. Short tandem repeat (STR) analysis of nuclear DNA is a method having a high ability to identify individuals. However, it is difficult to perform nuclear DNA analysis of hair to which hair root sheath is not adhered. Hair root sheath will not usually be adhered to hair found at the crime scene. Mitochondrial DNA (mtDNA) analysis of hair shaft\(^2\)\(^-\)\(^4\) can be used for individual identification. However, the discrimination power of mtDNA analysis is lower than that of nuclear DNA analysis.\(^5\) Therefore, when hair gathered at the crime scene does not have hair root sheath, it is desirable to acquire various types of information from the hair using several non-destructive analytical methods before performing mtDNA analysis in order to obtain various evidences for individual identification. If on-site analysis of a single hair is performed at the crime scene, either biological or chemical information can be rapidly obtained. Therefore, non-destructive on-site analytical method can contribute to rapid criminal investigation.

Kurouski et al.\(^6\) reported results of surface-enhanced Raman scattering (SERS) analysis of single hairs colored with several semipermanent hair dye products containing organic dyes and a permanent hair dye product that dyes hairs using chemical reaction involving 2-methyl-\(p\)-phenylenediaminesulfate. In this report, SERS was effective for distinguishing hair samples colored with the semipermanent hair dye products from that colored with the permanent hair dye product, and it was also useful for distinguishing among hair samples colored with several semipermanent hair dye products whose compositions of dyes were
different. They have also performed SERS measurement of a single colored hair using a portable Raman spectrometer. Recently, the number of people having hairs colored with hair dyes has increased, and analysis of hair dyes used for hair coloring will become important to specifying the criminal. SERS was also applied to analysis of dyes on textile fibers.\textsuperscript{7, 8} Although there is a possibility that noble metallic particles used for SERS measurements adhere to the analyte, almost non-destructive analysis can be performed by using SERS. The difference among hair dye products used for hair coloring can be identified by SERS. However, SERS spectral patterns of single hairs colored with different hair dye products that produces or contains similar dyes will be similar. Therefore, a hair dye product used for hair coloring cannot be identified only by SERS. Commercially available hair dye products contain, in addition to hair dyes, various types of ingredients such emulsifiers and fragrances. Metallic compounds are included as ingredients for producing dyes and other ingredients in some hair dye products, and metallic elements contained in these products will adhere to or penetrate into hairs after using these products. Therefore, acquiring information about metallic elements will contribute to identifying the difference among hairs colored with different hair dye products. X-ray fluorescence (XRF) spectrometry is a method for element analysis, and it was applied to hair analysis.\textsuperscript{9, 10} Non-destructive on-site analysis is performed by a portable XRF spectrometer. Recently, element analysis of human hairs and bleached hairs were performed using XRF analysis and laser-induced breakdown spectroscopy (LIBS).\textsuperscript{11} An analytical method for single hairs using portable Raman and XRF spectrometers will become a powerful method for rapidly obtaining information that can contribute to specifying whose hair it is.

In this study, a combination of SERS and XRF using two portable spectrometers was applied to analysis of single hog hairs colored with several hair dye products. We show that this combination is beneficial for identifying the difference among hairs colored with different
hair dye products. Because hair dyes are usually inhomogeneously distributed in or on dyed hairs, intensities of fluorescent X-rays and SERS signals originating from the hair dyes would vary depending on the region irradiated with the incident laser beam or incident X-ray beam. Therefore, quantitative SERS and XRF analyses were not performed in this study. In this study, we examined whether identifying the difference of SERS spectral patterns and acquiring the information about constituent metallic elements using XRF analysis can be effective for identifying the difference among single hairs colored with different hair dye products.

**Experimental**

White hog hairs, which were clipped from a commercially available hog hair brush, were used instead of human hairs. The following commercially available hair dye products were used for dyeing hog hairs:

1) A hair dye product containing polyphenol and iron salt (product A)
2) A hair dye product containing a plant dye called as Henna (product B)
3) A permanent hair dye product containing \( p \)-phenylenediamine and \( p \)-aminophenol as dye precursors and \( m \)-aminophenol, 5-(2-hydroxyethyl)amino-2-methylphenol, and \( p \)-amino-o-cresol, and resorcinol as couplers (product C)
4) Another permanent hair dye product containing \( p \)-aminophenol and \( p \)-phenylenediamine as dye precursors and \( \alpha \)-naphthol, \( m \)-aminophenol, and resorcinol as couplers (product D)
5) Semipermanent hair dye product containing red 33, yellow 6, CI 20470, orange 4, and ext. violet 2 as organic dyes (product E)

When using product A, hair is colored with a dye produced by a chemical reaction between polyphenol in a 1st agent and iron salt in a 2nd agent. Permanent hair dye products such as...
products C and D consist of a 1st agent containing dye precursors and couplers and a 2nd agent containing H$_2$O$_2$ as an oxidant, and a mixture of these two agents is used when coloring hairs. In the case that a permanent hair dye product is used, hair is colored with dyes produced by chemical reactions involving dye precursors, couplers, and H$_2$O$_2$. Product D also contains yellow 10 as an organic dye. In this study, hog hairs were dyed by products A-E for 1 day. After hair coloring using products A-E, these colored hog hairs were washed with distilled water and wiped in order to remove surpass dyes attached on them. Hog hairs after dyeing using products A, B, C, and D had brownish colors, and a hog hair after hair coloring using product E had a grayish color.

A portable Raman spectrometer C12710 (Hamamatsu Photonics K. K.) was used for measuring SERS spectra of single hog hair samples colored with products A-E, and suspensions of products A-E. A normal Raman spectrum of a single hog hair sample colored with product A was also measured. In this study, a procedure reported by Zaffino et al. was used as a reference when SERS measurements were performed. A single dyed hog hair sample and a 2μL droplet of distilled water, which were sandwiched between a quartz glass substrate and a silver evaporated film formed on another quartz glass substrate, were irradiated with the laser beam with a power of 62.5 mW and a wavelength of 785 nm. The procedure of the measurement of a normal Raman spectrum of the single hair sample dyed by product A was similar to that of SERS measurements, but the dyed hair sample was sandwiched between two quartz glass substrates. Lengths of single dyed hog hair samples for SERS analysis and normal Raman analysis were about 1 cm. In the case that a SERS measurement of a suspension of a hair dye product was performed, a 1 μL droplet of a suspension prepared by mixing 0.1 g of the product with 10 mL of distilled water was sandwiched between a quartz glass substrate and a silver evaporated film. In the case that a hair dye product consisted of a 1st agent and a 2nd agent, a 1 μL droplet of a suspension prepared by mixing a mixture of 0.1 g
each of 1st and 2nd agents with 10 mL of distilled water was measured. The irradiation
direction of the laser beam was from the back side of the silver-evaporated film to the single
hair sample as shown in Fig. 1a. Thicknesses of silver evaporated films used for SERS
measurements were set to 7 nm. A SERS spectrum of an analyte was obtained by averaging 5
spectra that measuring time of each spectrum was 10 s. A portable total reflection X-ray
fluorescence spectrometer\textsuperscript{12} whose setup was the same as that reported in a previous paper\textsuperscript{13} was
used for XRF measurements of single hog hair samples. X-rays emitted from an X-ray tube 50
kV Magnum (Moxtek Inc., Orem, UT, USA) with a tantalum anode were collimated by an
X-ray waveguide acting as a collimator. The X-ray tube was operated at a tube voltage of 25
kV and a tube current of 0.2 mA. A single hog hair sample was attached on an acrylic resin
plate with a hole at the center when XRF analysis was performed. Lengths of single dyed hog
hair samples for XRF analysis were from about 2 cm to about 3 cm. XRF spectra were
measured by using a silicon drift detector VITUS-SDD (Ketek GmbH, Munich, Germany) that
was placed directly above the analyte, and almost all measurements were performed in air for
600 s. However, a hog hair sample colored with product C was measured in air for 1800 s.
During XRF measurements, the acrylic resin plate holding the hair sample was tilted at 0.04° to
the horizontal, and an angle between the hair sample attached on the acrylic resin plate with a
hole and the irradiation direction of the incident X-ray beam was set to 0.04° as shown in Fig.
1b.

Results and Discussion

Fig. 2 shows representative SERS spectra of single hog hair samples colored with
products A-E, and droplets of suspensions of products A-E. A representative normal Raman
spectrum of a single hog hair dyed by product A was also shown in Fig. 2a. Strong Raman
peaks were detected in Fig. 2a when the analyte sandwiched between the silver evaporated film
and the quartz glass substrate was measured, but noticeable peaks were not detected when the analyte sandwiched between the two quartz glass substrates was measured. This result shows that intensities of Raman peaks originating from hair dye product can be enhanced by performing SERS. As shown in Figs. 2a-2e, SERS spectral patterns of single hog hair samples colored with products A-E showed qualitatively good agreement with those of droplets of suspensions of products A-E, respectively. This result indicated that ingredients in hair dye products adhered to or penetrated into hog hairs. Especially, the difference in the SERS spectral patterns of the dyed hog hair samples would show the difference in the types of hair dyes. Although wavenumbers of Raman peaks in Fig. 2c were different from those in Fig. 2d, these SERS spectral patterns were similar to each other. This would be because dye precursors and couplers in product C partially coincided with those in product D as described in Experimental and dyes produced by product C were similar to those produced by product D. In this study, SERS signals were obtained when both of a single dyed hog hair sample and a droplet of distilled water were contacted to a silver-evaporated film. However, there was a possibility that adhesion of silver nanoparticles peeled off from the silver-evaporated film to a single dyed hog hair could contribute to acquisition of SERS signals.

Fig. 3 shows representative XRF spectra of a single hog hair sample that was not colored with any hair dye products and single hog hair samples colored with products A-E. As shown in Fig. 3a, S, Ar, Ca, Fe, and Ta peaks were detected. The S peak originated from keratin in hog hair. The Ca peak was also attributed to hog hair because calcium is usually contained in hair. The Ar and Ta peaks were attributed to air containing 0.9% of argon and the anode material of the X-ray tube, respectively. Iron is also usually contained in hair. However, a weak Fe Kα line is sometimes detected even when a sample holder for TXRF analysis on which a sample is not deposited is analyzed using this spectrometer. Therefore, all or a part of the Fe Kα X-ray photons may originate from a component of this spectrometer. As shown in 3b,
strong Fe K lines originating from iron salt in product A were detected. The K and Mn Kα lines observed in Fig. 3c would be attributed to plant components in product B. The Ti Kα line in Fig. 3d originated from product C because TiO$_2$ is contained in this product. The Cl peak in Fig. 3d would also originate from product C because chlorine was not detected from the hog hair that was not colored with any hair dyes. XRF analysis was beneficial for detecting characteristic metallic elements originating from hair dye products. Characteristic metallic elements detected from single hog hair samples dyed by products A-C were as below:

Hog hair colored by product A: Fe
Hog hair colored by product B: K and Mn
Hog hair colored by product C: Ti

Intensities of the Ca K lines in Fig. 3c were higher than those in Fig. 3a, and a part of Ca K X-ray photons would originate from product B. However, Ca was not selected as characteristic metallic element attributed to product B in this study because this element is usually contained in hair. On the other hand, Fe was selected as characteristic metallic element originating from product A because there was a marked difference of the intensity of the Fe Kα line between Figs. 3a and 3b. Although the SERS spectral patterns of single hog hair samples dyed by products C and D were similar to each other as shown in Figs. 2c and 2d, elements detected from these samples were different as shown in Fig. 3d and 3e. This result shows that XRF can play a supporting role in identifying the difference among hairs dyed by different products. Characteristic metallic elements originating from products D and E were not detected in Figs. 3e and 3f. The Ca Kα line, which was observed in Fig. 3a, was not detected or scarcely observed in Figs. 3d-3f, but this reason was unclear.

As presented above, SERS is effective for identifying the difference among single hairs
colored with different hair dye products. However, SERS spectral patterns of single hairs dyed by different hair dye products that produces or contains similar types of dyes will be similar. Characteristic metallic elements originating from ingredients for producing dyes or other ingredients in hair dye products can be detected by using XRF analysis, and XRF analysis can contribute to identifying the difference among dyed hairs that cannot be identified only by SERS. SERS and XRF analyses of a single shed hair can contribute to specifying whose hair it is.

**Conclusions**

In this study, by using a combination of SERS and XRF analyses, chemical information originating from hair dye products were obtained from single hog hairs. This combination makes it possible to identify the difference among hairs dyed by different hair dye products. Using a combination of portable Raman and XRF spectrometers, on-site SERS and XRF analyses can be performed. Therefore, analysis of hair gathered at the crime scene using this combination can contribute to rapid acquisition of information about the criminal and specifying the criminal.

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Figure Captions

Fig. 1  Schematic views of experimental setups around samples for (a) SERS and (b) XRF analyses. In Fig. 1b, a schematic view of the experimental setup around hair sample viewed from the side and that viewed from directly above are shown.

Fig. 2 (a) Representative normal Raman and SERS spectra of single hog hair samples colored with product A and representative SERS spectra of single hog hair samples colored with products (b) B, (c) C, (d) D, and (e) E. These SERS spectra are displayed as black solid lines, and the normal Raman spectrum is displayed as a light grey solid line. Representative SERS spectra of 1 μL droplets of suspensions of products A-E are also shown in Figs. 2a-2e, respectively, and they are displayed as grey solid lines. Raman peaks in the SERS spectra of
products A-E whose wavenumbers are almost consistent with those of Raman peaks in the SERS spectra of single hog hair samples colored with products A-E are denoted by black dashed lines and these wavenumbers are described near the dashed lines.

Fig. 3  Representative XRF spectra of (a) a single hog hair sample that was not colored with any hair dye products and single hog hair samples colored with products (b) A, (c) B, (d) C, (e) D, and (f) E. The insets in Figs. 3c and 3d show enlarged spectra in the X-ray energy range from 3 to 7 keV. The spectra in Figs. 3a-c, 3e, and 3f were measured for 600 s. The spectrum in Fig. 3d was measured for 1800 s.

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Fig. 3 Representative XRF spectra of (a) a single hog hair sample that was not colored with any hair dye products and single hog hair samples colored with products (b) A, (c) B, (d) C, (e)
D, and (f) E. The insets in Figs. 3c and 3d show enlarged spectra in the X-ray energy range from 3 to 7 keV. The spectra in Figs. 3a-c, 3e, and 3f were measured for 600 s. The spectrum in Fig. 3d was measured for 1800 s.

**Graphical Index**

- Laser beam
- SERS signals & fluorescent X-rays
- X-ray beam
- Single colored hair
- Chemical information attributed to a hair dye product is obtained.
- This information can contribute to specifying whose hair it is.