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Laboratory Experiment

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Using Raman Spectroscopy and Surface-Enhanced Raman Scattering To Identify Colorants in Art: An Experiment for an Upper-Division Chemistry Laboratory

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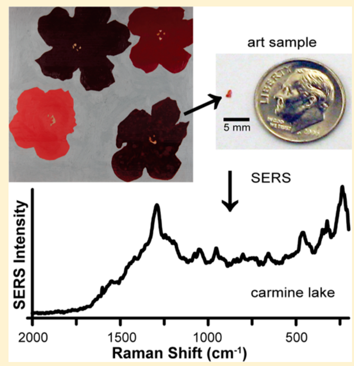
Supporting Information

ABSTRACT: Surface-enhanced Raman scattering (SERS) studies of art represent an attractive way to introduce undergraduate students to concepts in nanoscience, vibrational spectroscopy, and instrumental analysis. Here, we present an undergraduate analytical or physical chemistry laboratory wherein a combination of normal Raman and SERS spectroscopy is used to identify both inorganic and organic fluorescent colorants in an oil painting. On the basis of their experimental observations, students make procedural decisions to adjust acquisition settings and use SERS, thereby enabling the successful identification of unknowns. This laboratory engages undergraduate students by applying what they have learned about quantum mechanics, nanoscience, and spectroscopy to the real-world, problem-solving context of art conservation.



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Surface-enhanced Raman scattering (SERS) spectroscopy has become a powerful technique in analytical and physical chemistry. By integrating nanoscience with vibrational spectroscopy, SERS provides for the unambiguous and ultrasensitive detection of a wide variety of analytes.[1] For example, SERS is increasingly applied to the field of art conservation to identify colorants in minute samples from cultural heritage objects.[2−8] Indeed, SERS studies of artists' materials represent an excellent method to familiarize undergraduate students with modern applications of spectroscopy and nanoscience. Various experiments have been presented to introduce undergraduates to the field of art conservation using Raman spectroscopy,[9] UV/vis absorption,[10] NIR imaging,[11] and XRF.[12] Similarly, several laboratories have been developed to demonstrate the SERS effect and estimate enhancement factors,[13−16] but there are no existing experiments devoted to the real-world application of SERS to art conservation. The integration of SERS with art conservation enriches the learning experience by adding elements of nanoscience and advanced spectroscopy within the engaging context of art. Furthermore, this laboratory simulates a problem-solving scenario for students to detect unknown colorants in art. Students encounter problems throughout this experiment that require generating testable hypotheses and making new procedural decisions. In this problem-based learning (PBL) approach,[17] the instructor serves as a problem-solving guide by asking questions and facilitating group discussion. Ultimately, the combination of nanoparticle synthesis, normal Raman spectroscopy, and SERS in this PBL experiment enables students to learn how to identify both inorganic and natural, organic pigments in small samples from an actual oil painting.

In this experiment, students are presented with an Andy Warhol-inspired oil painting of four red flowers on a gray background. The composition for the painting is adapted from the print "Flowers" by Warhol to take advantage of the basic fields of color and to connect the experiment to an iconic element of art history. Each flower contains one of the following colorants bound in linseed oil: madder lake, lac dye, carmine lake, and vermillion (HgS).[18] The major constituents of the organic colorants are shown in Figure 1. Students are posed with the challenge of identifying two unknown paint samples. To demonstrate the differences between normal Raman spectroscopy and SERS, each group is assigned the vermillion (inorganic, nonfluorescent) paint and then chooses among the remaining unknown (organic, fluorescent) paints. Students first attempt normal Raman measurements on both unknown samples. Therefore, either Raman scattering from vermillion or molecular fluorescence from an organic paint is observed, demonstrating the utility of normal Raman spectroscopy as well as its limitations for identifying organic colorants. If an organic paint is indicated, students treat the sample with silver nanoparticles and obtain a SERS spectrum. The

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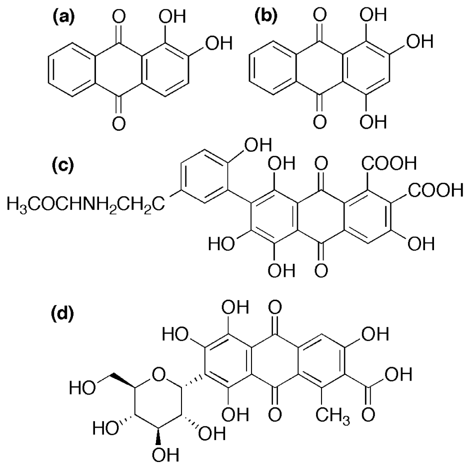

Figure 1. Chemical structures of the main chromophores in the organic colorants. Madder lake contains (a) alizarin and (b) purpurin. Lac dye is primarily comprised of (c) laccaic acid. Carmine lake is a pigment made from (d) carminic acid.

procedure is designed to be completed by groups of 2−3 students in a lit room within a 4-h laboratory period and includes: synthesis of silver nanoparticles, paint sampling, Raman measurements, and SERS measurements of treated art samples.

■ LEARNING OBJECTIVES

The learning objectives of this laboratory experiment include operations at all levels of Bloom's taxonomy:[19] knowledge, comprehension, application, analysis, synthesis, and evaluation. In particular, students apply their classroom knowledge of vibrational and electronic spectroscopy to the real-world setting of art conservation. Students use experimental observations to (1) formulate hypotheses, (2) optimize experimental conditions, and (3) propose a plan for the experiment. They interpret Raman and SERS data in order to identify unknown paints. Finally, students justify the conclusions of the experiment and evaluate the significance of their results in the laboratory report. Ultimately, this laboratory provides an engaging way to incorporate aspects of modern nanoscience and spectroscopy into the upper-level undergraduate laboratory curriculum.

■ THEORY

Raman Spectroscopy of Artists' Pigments

Raman spectroscopy provides structural information about a wide variety of analytes including artists' colorants. In order for a molecule to be Raman active, the molecular polarizability during vibration must be nonzero such that when a molecule encounters an incident electric field, a molecular dipole moment is induced.[20] Upon photoexcitation of an analyte, most incident photons will be elastically (Rayleigh) scattered at the incident frequency. However, vibrations that change the polarizability will produce inelastically (Raman) scattered photons. Scattered photons corresponding to vibrational de-excitation and excitation are termed Stokes and anti-Stokes scattering, respectively. Stokes scattering is more intense, as molecules are more likely to populate the ground vibrational level, consistent with the Boltzmann population distribution.

As a vibrational fingerprinting tool, Raman spectroscopy is frequently applied in the conservation lab setting to identify a variety of artists' colorants.[21] However, Raman is not particularly useful for the majority of natural, organic colorants due to two major issues: low concentrations and competing fluorescence. Owing to their high tinting strength, organic colorants (e.g., madder lake, carmine lake, lac) are present in low concentrations in art, which imposes a large sample requirement that is usually impossible for priceless cultural heritage objects. Furthermore, organic colorants fluoresce upon visible laser excitation, such that the relatively weak Raman scattering signal is overwhelmed by molecular fluorescence. SERS offers a solution to both of these challenges.

SERS Studies of Organic Colorants in Art

In SERS, analytes are adsorbed to the surface of noble metal nanostructures, resulting in signal enhancements that are routinely $>10^6$ relative to normal Raman scattering.[1] Because Raman intensity scales as the product of the polarizability and the incident field intensity ($|E_0|^2$), the observed signal enhancement in SERS has been explained by two mechanisms: chemical and electromagnetic (EM). The chemical mechanism is enhancement in polarizability due to chemical effects such as charge transfer and is generally considered to be modest. The EM mechanism is enhancement in the local field intensity as a result of collective excitations of the metal's conduction electrons known as surface plasmons.[22] In particular, for a small metal nanoparticle, the EM field intensity at the surface of the sphere ($|E_{out}|^2$) is given by

$$|E_{out}|^2 = 2E_0^2 \left| \frac{\varepsilon_{in} - \varepsilon_{out}}{(\varepsilon_{in} + 2\varepsilon_{out})} \right|^2 \quad (1)$$

where ε_{in} and ε_{out} are the dielectric constants of the metal and external environment, respectively. Equation 1 demonstrates that the maximum enhancement occurs when $\varepsilon_{in} = -2\varepsilon_{out}$, a resonance condition that is satisfied in the visible region for gold and silver. Because the intensity of the Raman scattering ($|E'_{out}|^2$) is also enhanced, the overall EM enhancement is expressed as an enhancement factor (EF) relative to the normal Raman scattering intensity as

$$EF = \frac{|E_{out}|^2 |E'_{out}|^2}{|E_0|^4} = \frac{I_{SERS}}{I_{NR}} \quad (2)$$

I_{SERS} and I_{NR} are the SERS and normal Raman intensity, respectively, which are normalized by the number of molecules contributing to the signal. EFs of $>10^6$ are routinely observed in SERS. For example, the substantial EM enhancements exhibited by citrate-reduced silver colloids have enabled the detection of single molecules using SERS.[23] Silver colloids are used as the SERS substrate for this laboratory due to their ease of preparation and high EM enhancement.

SERS fulfills many of the requirements of an ideal analytical technique to detect and identify organic colorants in artworks. The SERS substrate not only provides enhanced Raman signals, such that small sample sizes (i.e., ∼nanograms) are measurable, but also quenches the fluorescence generated by many organic colorants. Moreover, if the exciting laser is resonant with an electronic transition of a chromophore, further signal enhancement is observed through the resonance Raman effect. Several groups have demonstrated the applicability of SERS to the identification of colorants in artworks, the results of which are the subject of recent reviews.[5,8,24]

EXPERIMENTAL COMPONENTS

Experimental Overview

At the beginning of the lab, there is a 30 min prelab discussion. The majority of the discussion focuses on reinforcing concepts in Raman spectroscopy as well as introducing plasmonics and SERS. Each group of 2−3 students obtains two small samples from an oil painting: one from a paint containing vermillion and another organic, fluorescent pigment that is assigned by the instructor. Students in the groups split up to accomplish tasks during the first hour of lab. One student performs nanoparticle synthesis and centrifugation while the other student samples from the painting, calibrates the Raman spectrometer, and attempts normal Raman measurements of the two unknown samples. Nanoparticle synthesis and centrifugation should take less than 1 h. Each student group will have to treat one unknown sample with silver nanoparticles and measure SERS. Students compare their normal Raman and SERS results with a reference library and use spectral correlation to identify the unknown samples.

Synthesis of Colloidal Silver Nanoparticles

Glassware is cleaned with aqua regia and thoroughly rinsed with deionized water prior to the laboratory experiment. Silver colloids are formed through the reduction of $AgNO_3$ by sodium citrate trihydrate (Sigma-Aldrich).[25] To a 125 mL Erlenmeyer flask containing 50 mL ultrapure water (Fisher EasyPure, Milli-Q, 18.2 MΩ cm^{-1}) is added approximately 9 mg of $AgNO_3$ (Acros Organic, 99%+) with stirring. The solution is heated on a stirring hot plate to ~300 °C. Upon vigorous boiling of the solution, 1 mL of sodium citrate solution (1% w/v) is added initialize nanoparticle growth. The reaction proceeded for 30 min, resulting in an opaque gray-green solution. A centrifuge (Eppendorf MiniSpin) operating at 13.4 rpm for 15 min is used to concentrate 1 mL aliquots of the colloids, with ~100 μL of colloid solution remaining after supernatant removal.

Art Sample Preparation

An oil painting was created (by H.E.M. several weeks before the laboratory period) to simulate the experience of handling a cultural heritage object. All painting materials were obtained from Kremer Pigments and stored in the dark. Paints were prepared by grinding the pigments into linseed oil with a glass muller. The wood panel (see Supporting Information, Figure 4) was primed with a layer of linseed oil and the flowers were each painted with a different colorant. The gray paint is a mixture of lead white and charcoal black in linseed oil. To minimize pigment fading and dust accumulation, long-term storage of the painting is accomplished in a closed container in the dark. Students used surgical razor blades (Feather Safety Razor Company, #15) to remove ~1 mm paint samples from the wood panel, that are placed on glass coverslips (Fisher) for Raman measurements.

Raman and SERS Measurements

A DeltaNu (Intevac Photonics) benchtop Raman spectrometer equipped with a 785 nm diode laser and a right-angle attachment was used for all measurements. Laser power is <10 mW at the sample and acquisition times are ~10 s. Students identified the unknowns by comparing their spectra to a reference database compiled for the laboratory, using the spectral correlation function built into the DeltaNu NuSpec software. For SERS measurements, art samples are transferred to centrifuge tubes containing the concentrated silver colloids and mixed at 13.4 rpm for 15 min. Three 1-μL aliquots of the resulting mixture are deposited onto a coverslip.

HAZARDS

Safety goggles and gloves should be used at all times. Silver nitrate is corrosive and toxic. Vermillion and lead white paints are toxic. Paints should not be ingested. Direct eye exposure to the laser should be avoided.

RESULTS AND DISCUSSION

The normal Raman spectra of samples from vermillion, carmine lake, lac dye, and madder lake oil paints are shown in Figure 2.

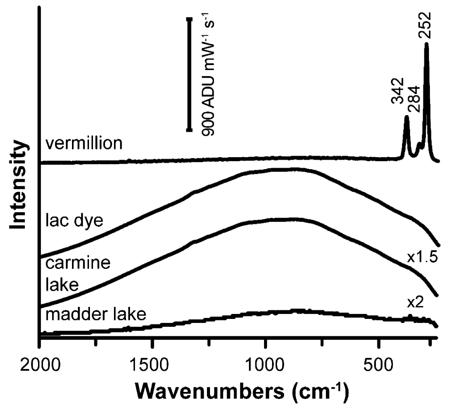

Figure 2. Student spectra for ~1 mm samples of vermillion, lac dye, carmine lake, and madder lake oil paints. The Raman spectrum of vermillion paint is clearly evident, but fluorescence precludes the measurement of Raman scattering for the organic colorants.

The normal Raman spectrum of vermillion paint exhibits characteristic peaks at 342, 284, and 252 cm^{-1}.[26,27] However, Figure 2 demonstrates that following 785 nm laser excitation, Raman scattering from the organic colorants lac dye, carmine lake, and madder lake is overwhelmed by molecular fluorescence. For fluorescent samples, students treat the paint with silver nanoparticles and perform another measurement with the Raman spectrometer. Figure 3 shows the corresponding SERS spectra of lac dye, carmine lake, and madder lake paint samples that have been treated with silver nanoparticles. Oil paint made from lac dye exhibits characteristic SERS peaks for laccaic acid at 1464, 1276, 1225, 1098, 1056, 1010, 453, and 413 cm^{-1}.[28] The SERS spectrum of carmine lake paint exhibits major peaks at 1291, 460, and 428 cm^{-1}, characteristic of carminic acid.[3] The SERS spectrum of madder lake paint contains characteristic peaks for its main constituents, alizarin and purpurin, at 1547, 1390, 1322, 1286, 1187, 1158, and 476 cm^{-1}.[7,29] Although all three organic colorants are derived from substituted anthraquinones (Figure 1), SERS measures the unique vibrational fingerprints of molecules, such that these related colorants are readily differentiated.

In total, 80 students enrolled in an undergraduate physical chemistry laboratory course have performed this experiment. Students identified the unknowns by comparing their spectra to a reference database compiled for the laboratory. Students obtained spectral correlation values of 97 ± 2%, 77 ± 10%, 70 ± 10%, and 71 ± 7%, to reference spectra for vermillion, lac dye, carmine lake, and madder lake paint, respectively, enabling the successful identification of unknowns. The student laboratory reports include experimental observations, students'

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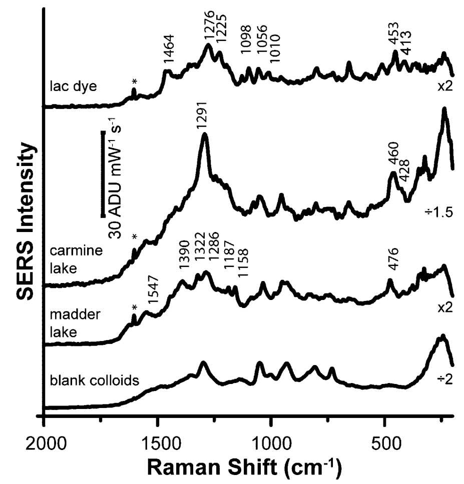

Figure 3. Corresponding SERS spectra for lac dye, carmine lake, and madder lake paint samples obtained by students after treatment with silver nanoparticles. Characteristic peaks for each colorant are labeled and SERS from blank citrate-reduced colloids is shown. Asterisks denote peaks due to the glass substrate.

rationale for using Raman or SERS, and a summary of results in the context of their significance to art conservation. The learning outcomes for this experiment are evaluated through the student laboratory reports. Successful reports explained how experimental observations are used to formulate hypotheses, optimize experimental conditions, make decisions, and identify the unknown colorants. Most students are able to successfully identify unknowns, though only ~75% of students adequately justified the conclusions of the experiment and evaluated the significance of their results in the laboratory report. Students reported that one of the strongest features of this laboratory is problem solving an investigation of art from beginning to end, which develops an understanding of the applicability of Raman and SERS spectroscopy to a real-world problem.

■ SUMMARY

Students are able to successfully synthesize nanoparticles and use the Raman instrumentation, as well as make procedural decisions and identify unknowns based on their observations. Because this laboratory involves concepts in quantum mechanics, vibrational spectroscopy, nanoscience, and instrumental analysis, the experiment is well suited for a new integrated physical and instrumental analysis laboratory course that will be introduced next year at the College of William and Mary.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The complete student laboratory manual, instructor guidelines, and a student evaluation form. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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