Identification and Characterization of Artists' Red Dyes and Their Mixtures by Surface-Enhanced Raman Spectroscopy

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Silver film over nanospheres (AgFONs) were successfully employed as surface-enhanced Raman spectroscopy (SERS) substrates to characterize several artists' red dyes including: alizarin, purpurin, carminic acid, cochineal, and lac dye. Spectra were collected on sample volumes (1×10^{-6} M or 15 ng/μL) similar to those that would be found in a museum setting and were found to be higher in resolution and consistency than those collected on silver island films (AgIFs). In fact, to the best of the authors' knowledge, this work presents the highest resolution spectrum of the artists' material cochineal to date. In order to determine an optimized SERS system for dye identification, experiments were conducted in which laser excitation wavelengths were matched with correlating AgFON localized surface plasmon resonance (LSPR) maxima. Enhancements of approximately two orders of magnitude were seen when resonance SERS conditions were met in comparison to non-resonance SERS conditions. Finally, because most samples collected in a museum contain multiple dyestuffs, AgFONs were employed to simultaneously identify individual dyes within several dye mixtures. These results indicate that AgFONs have great potential to be used to identify not only real artwork samples containing a single dye but also samples containing dyes mixtures.

Index Headings: **Surface-enhanced Raman spectroscopy; SERS; Ag film over nanosphere; AgFON; Art conservation; Red dye.**

INTRODUCTION

The identification of artists' materials in works of art is important in the field of art history, as well as for conservation and restoration efforts. Furthermore, the analysis of artists' materials can often be applied to date and authenticate artifacts. Raman spectroscopy is a noninvasive method that has been successfully applied to characterize many artists' pigments and dyes.[1–5] Because every molecule scatters light uniquely, Raman spectroscopy is an excellent analytical tool to "fingerprint" unknown compounds. However, because only subnanogram levels of these dyes are needed to impart intense color to fibers or paint, normal Raman spectroscopy often lacks the sensitivity to probe these materials at such low concentrations. Furthermore, many organic dyestuffs of natural origin are extremely fluorescent, an effect that dominates the weak optical process of Raman scattering.

Recently, a number of publications have reported the use of surface-enhanced Raman spectroscopy (SERS) to successfully characterize and identify highly fluorescent red dyes and lake pigments.[6–13] It is well known that the SERS effect significantly enhances the Raman scattering signal when the Raman-active molecule is spatially confined within the electromagnetic fields generated upon excitation of the localized surface plasmon resonance (LSPR) of the nanostructured noble metal surface.[14] The SERS signals of ensemble-averaged molecules demonstrate enhancements up to eight orders of magnitude over normal Raman signals.[15,16] Furthermore, the use of a noble metal SERS substrate can quench fluorescence, therefore reducing or even removing harmful background. Additionally, resonance Raman will occur when the Raman excitation wavelength is of sufficient energy to promote an electronic transition in the molecule of interest, from the ground to some electronically excited state resulting in further enhancement of the normal Raman signal ($\sim 10^2$).[15,17] Because each of these dyes absorbs in the visible region, resonance Raman and pre-resonance Raman enhancements should occur when using 532.17 nm and 632.8 nm excitation wavelengths, respectively. Furthermore, when the LSPR of the SERS substrate is also in the proper energy region, the conditions of surface-enhanced resonance Raman spectroscopy (SERRS) are met, which will result in enhancement factors that are approximately the product of the enhancement factors for non-resonant SERS of the substrate and the resonant Raman spectrum of the adsorbate.[17] Throughout this work, the authors will use the term SERS to describe all data preceded with either "pre-resonance", "resonance", or "non-resonance" to describe the experimental conditions. Finally, another benefit of this technique is that it can be used to identify extremely small amounts (7×10^{-15} g) of material,[18] which is often all that is available for analysis when a sample is removed from priceless museum objects.

Previously, we have successfully employed silver island films (AgIFs) as SERS substrates to identify and characterize several red dyes.[11,12] However, because AgIFs are non-uniform, it can be difficult and time consuming to collect consistent spectra.[19] Alternatively, this work uses silver film over nanospheres (AgFONs) as SERS substrates to overcome these issues resulting in the acquisition of consistent spectra. The Van Duyne group has successfully used AgFONs as SERS substrates for the detection of analytes such as glucose and anthrax.[20–22] AgFONs are not only easily fabricated, economical, and stable for several weeks,[22] but are also composed of a uniform surface that provides highly consistent surface-enhanced Raman (SER) spectra. Furthermore, AgFONs have been successfully used to detect extremely small amounts of analyte, which makes them ideally suited for analyzing the small samples often available in a museum setting.[22,23] The LSPR of a AgFON can be fine tuned simply by changing the size of the underlying nanospheres, ensuring that the substrate can be excited with numerous laser wavelengths.[22] As a result, AgFONs can be utilized with any Raman system, thus effectively allowing the custom-tailoring of the substrate/laser system for each specific application.

Surface-enhanced Raman spectroscopy has been successfully applied in the past to identify samples containing a single reference dye.[6–9,11–13] However, there has been little work focusing on the identification of individual components in dye mixtures. The ability to accurately characterize dye mixtures is

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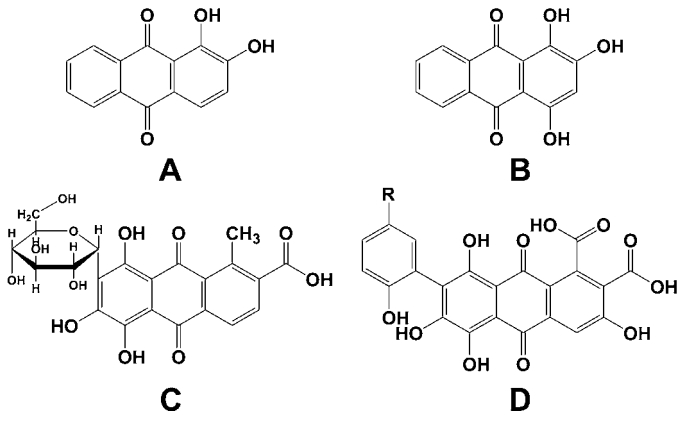

FIG. 1. Natural and organic red compounds (**A**) alizarin, (**B**) purpurin, (**C**) carminic acid, and (**D**) lac dye. Five structures of laccaic acid are present in lac dye: laccaic acid A ($R = (CH_2)_2NHCOCH_3$), laccaic acid B ($R = (CH_2)_2OH$), laccaic acid C ($R = CH_2CH(NH_2)COOH$), laccaic acid D (= flavokermesic acid), and laccaic acid E ($R = CH_2NH_2$). Laccaic acid A is the most prominent structure in lac dye.

necessary because a combination of dyes is often employed in real works of art.[24,25] Therefore, in order for SERS to be a viable technique to analyze dye samples in artwork, the method must be capable of distinguishing individual dyes in mixtures. Murcia-Mascarós and co-workers were able to successfully identify alizarin in a mixture of alizarin and carminic acid using Raman spectroscopy.[10] The authors prepared a Zr–Ormosil polymer complex that not only selectively incorporated alizarin but quenched fluorescence and induced intense Raman spectra.[10] While the Zr–Ormosil polymer can effectively be used to identify alizarin, it is materials specific. Alternatively, a materials general substrate such as an AgFON would allow for the identification of a variety of individual dyes within mixtures.

The work presented herein utilizes AgFONs as materials general SERS substrates to characterize several red dyes and organic molecules, including alizarin (Fig. 1A), purpurin (Fig. 1B), carminic acid (the chromophore in the dyestuffs cochineal) (Fig. 1C), and laccaic acid (contained in lac dye, a natural product comprising a mixture of acids) (Fig. 1D). All spectra were collected on sample volumes (1×10^{-6} M or 15 ng/μL) that were similar to real museum sample volumes The pre-resonance SER spectra collected in this work show higher resolution and improved consistency than was observed in our previous work.[11,12] The use of AgFONs has resulted in pre-resonance SER spectra that were previously not possible to collect with either normal Raman or AgIF SERS substrates. For example, to the best of the authors' knowledge, the pre-resonance SER spectra taken on AgFONs of a sample of the artists' pigment cochineal were the highest resolution to date.[11,12] Because the red dyes absorb in the visible light region, resonance and pre-resonance SERS conditions can be met by using both 532.15 nm or 632.8 nm laser excitation wavelengths (respectively) in combination with a SERS substrate with a LSPR value similar to the laser excitation wavelength.[26,27] Experiments were therefore conducted with carminic acid and lac dye to optimize the combination of AgFON LSPR maxima and laser excitation wavelength for the analysis of these dyes. Finally, experiments were carried out to demonstrate that AgFONs can be used to effectively identify dyes in dye mixtures. AgFONs were successfully applied to characterize individual dyes in mixtures of (1) alizarin and lac dye, (2) alizarin and purpurin, and (3) lac dye and purpurin.

EXPERIMENTAL

Materials. Silver (Ag) pellets (99.99%) were purchased from Kurt J. Lesker Co. (Clairton, PA). Borosilicate glass substrates, No. 2 Fisherbrand 18 mm circular cover slips were acquired from Fisher Scientific (Pittsburg, PA). Methanol, H_2SO_4, H_2O_2, and NH_4OH were obtained from Fisher Scientific (Fairlawn, VA). SiO_2 spheres with diameters of 300 nm, 390 nm, and 550 nm were received in a suspension of water from Bangs Laboratories, Inc (Fishers, IN). Millipore cartridges (Marlborough, MA) were used to purify water to a resistivity of 18 $M\Omega cm^{-1}$. Alizarin 97% (CAS # 72-48-0) and purpurin (CAS# 81-54-9) were purchased from ACROS organics and used as received, without further purification. Carminic acid was acquired from Aldrich (CAS # 1260-17-9). Lac dye, made from *laccifer lacca* secretion, was obtained from Kremer Pigments Inc (36020; indicated as extracted from *coccus lacca* on the supplier's original label). The two cochineal carmine lake samples were obtained from Penick & Co. and F. Weber. The cochineal sample manufactured by F. Weber was taken from the Forbes reference collection of pigments containing over 1600 specimens of artists' materials accumulated in the 1930s by E. W. Forbes, former director of the Fogg Art Museum at Harvard University, Boston, MA; this collection is widely used as a source of reference materials for museum research.[28]

Silver Film Over Nanospheres Fabrication and Incubation Procedure. Glass substrates were cleaned in a piranha etch solution (3:1 H_2SO_4/30% H_2O_2) for 30 min at 80 °C. After rinsing with water, the substrates were sonicated for 60 min in 5:1:1 $H_2O/H_2O_2/NH_4OH$ in order to create a hydrophilic surface on the substrate to facilitate self-assembly of the nanosphere masks. Finally, the substrates were rinsed and stored in water for future use. Approximately 2.5 μL of SiO_2 sphere solution was drop-coated onto the pretreated glass substrates and allowed to dry in ambient conditions. Then, 200 nm of Ag was deposited by electron beam (e-beam) deposition in a Kurt J. Lesker PVD 75 electron beam deposition system (Pittsburg, PA) with a base pressure of 10^{-6} torr. The mass thickness and deposition rate (2 Å/s) was monitored using a Sigma Instruments 6 MHz gold-plated quartz-crystal microbalance (QCM) (Fort Collins, CO). AgFON substrates were stored in the dark at room temperature prior to use. In anticipation of SERS analysis, AgFONs were then incubated in dye solutions of 1×10^{-6} M or 15 ng/μL concentrations overnight. All dye solutions were made in methanol.

Surface-Enhanced Raman Spectroscopy. A Jobin-Yvon Horiba Labram 300 confocal Raman microscope was used, equipped with an Andor multichannel air-cooled open electrode charge-coupled device detector (CCD: 1024 × 256), BXFM open microscope frame (Olympus), holographic notch filter, and 1800 grooves/mm dispersive grating.

The excitation lines of an air-cooled frequency doubled Nd:Yag solid state laser ($\lambda_0 = 532.15$ nm), He–Ne laser ($\lambda_0 = 632.8$ nm), and a solid-state diode laser ($\lambda_0 = 785.7$ nm) were focused through a 100× objective onto the samples and Raman scattering was back collected through the same microscope objective. Power at the samples was kept at 6 μW–0.5 mW by using appropriate neutral density filters in order to avoid any thermal and photochemical damage. The diameter of the

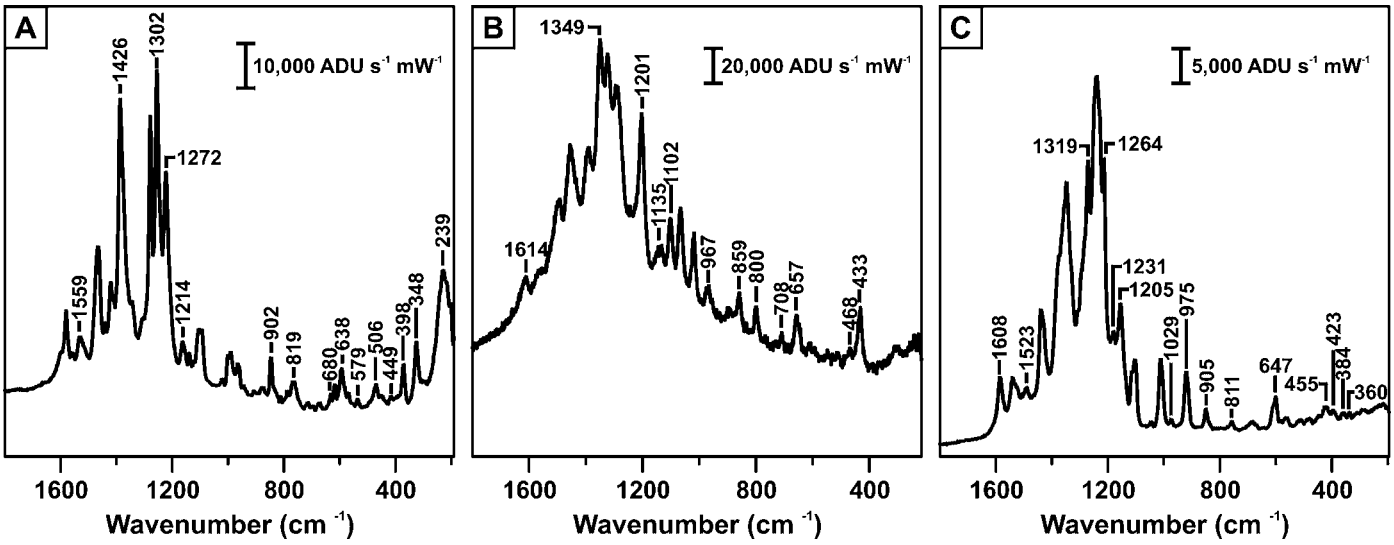

FIG. 2. Pre-resonance SER spectra of (**A**) 1×10^{-6} M alizarin (60 s acquisition), (**B**) 1×10^{-6} M purpurin (15 s acquisition), and (**C**) 15 ng/μL lac dye (15 s acquisition). All samples were prepared on AgFONs fabricated with 390 nm diameter SiO_2 spheres and spectra were collected with $\lambda_{excitation} = 632.8$ nm.

confocal hole was set at 500 μm and collection times varied in the range of 15 to 60 seconds. Some of the spectra required post-processing, involving spike removal due to cosmic rays.

Ultraviolet–Visible Extinction and Reflectance Spectroscopy. Ultraviolet–Visible absorption measurements were taken using an Ocean Optics (Dunedin, FL) SD2000 fiber-optically coupled spectrometer. All spectra of the methanolic solutions of the alizarin (1×10^{-4} M), carminic acid (1×10^{-4} M), cochineal (1500 ng/μL), lac dye (1500 ng/μL), and purpurin (1×10^{-4} M) were collected using a standard transmission geometry with unpolarized white-light excitation. AgFON spectra were collected in a reflectance geometry using a reflectance probe (Ocean Optics, Dunedin, FL) coupled to the spectrometer. AgFON spectra were acquired using a mirror-like Ag film on a glass substrate as a reference.

RESULTS AND DISCUSSION

Identification and Characterization of Red Dyes on Silver Film Over Nanospheres Substrates. Pre-resonance SER spectra were collected using 632.8 nm laser excitation for the red dyes alizarin (1×10^{-6} M) (Fig. 2A), lac dye (15 ng/μL) (Fig. 2B), and purpurin (1×10^{-6} M) (Fig. 2C). All samples were prepared on AgFONs fabricated with 390 nm diameter SiO_2 spheres. The low concentration of dyes in the sample solution was intended to mimic real museum sample sizes. The spectra were collected from each sample from five separate sample areas with extremely consistent results every time. Furthermore, all spectra obtained had higher resolution, improved consistency, and required shorter acquisition times than previously published work.[11,12] Diagnostic peaks were selected for each red dye and are labeled in Fig. 2.

Figure 3 compares pre-resonance SER spectra of cochineal from two different manufacturers (Figs. 3A and 3B) with a resonance SER spectrum of high purity carminic acid (the molecule that contributes the red color in cochineal) (Fig. 3C). The similarity of the spectra is evident, confirming the effectiveness of the methodology to correctly identify the chromaphore, in this case carminic acid, in the artists' pigment cochineal. Diagnostic peaks were selected and are labeled in the spectra in Fig. 3. Again, spectra from each sample were collected in five separate areas with consistent results each time. Additionally, to the best of the authors knowledge, the pre-resonance SER spectra of cochineal presented in this work are the highest resolution to date.[1,12]

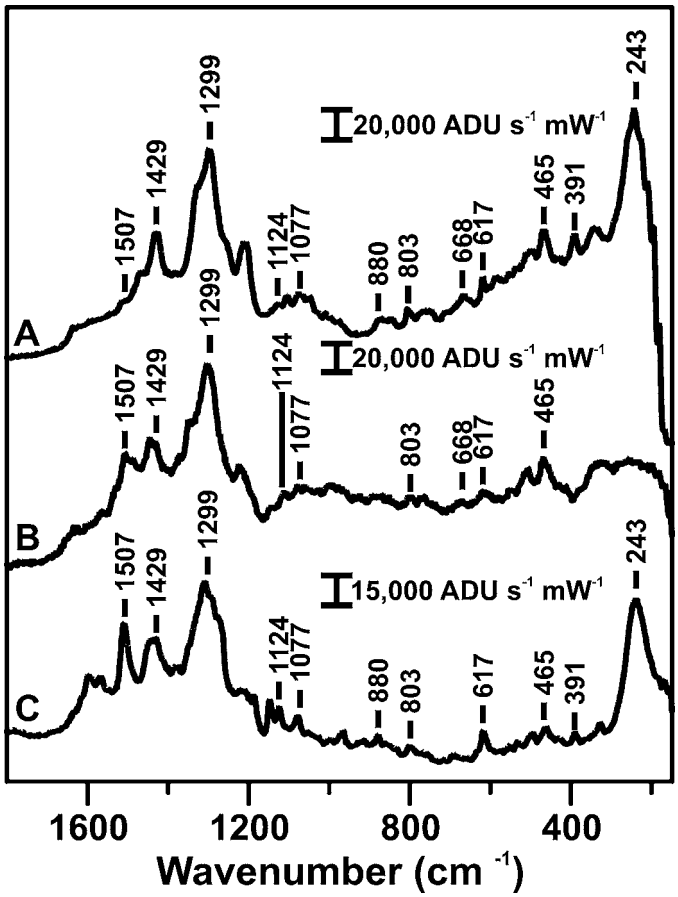

FIG. 3. Pre-resonance SERS spectra of (***A***) 15 ng/μL cochineal (Penick & Co., 1981) (30 s acquisition), (***B***) 15 ng/μL cochineal (Cochineal Carmine lake (F. Weber)) (30 s acquisition), and (***C***) 1×10^{-6} M carminic acid (45 s acquisition). All samples were prepared on AgFONs fabricated with 390 nm diameter SiO_2 spheres and spectra were collected with $\lambda_{excitation} = 632.8$ nm.

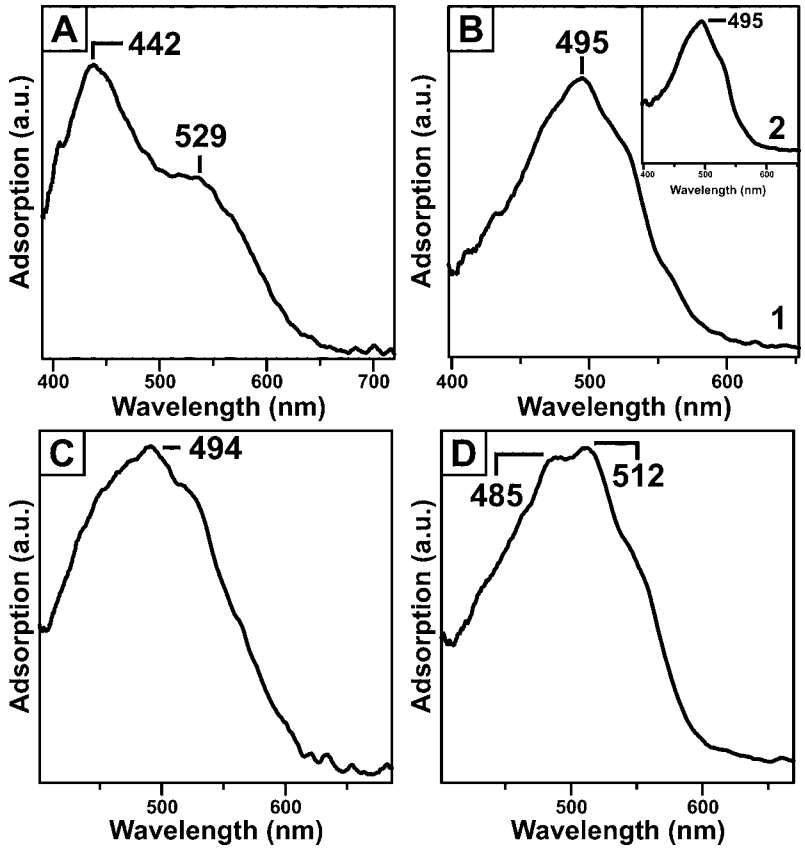

FIG. 4. UV-vis spectra of (**A**) 1×10^{-5} M alizarin, (**B**) (***1***) 150 ng/μL cochineal and (***2***) 1×10^{-5} M carminic acid, (**C**) 150 ng/μL lac dye, and (**D**) 1×10^{-5} M purpurin.

Surface-Enhanced Raman Spectroscopy Substrate Optimization. The sample size available in museums is often extremely small and therefore it is important to optimize the experimental parameters in order to find the most sensitive detection system. The work discussed here takes advantage of two Raman enhancing effects: (1) resonance Raman and (2) SERS. Experiments were conducted with two of the investigated red dyes (carminic acid and lac dye) to evaluate the contributions of both resonance Raman and SERS. Figure 4 depicts UV-Vis absorption spectra of alizarin (Fig. 4A), cochineal (Fig. 4B1), carminic acid (Fig. 4B2), lac dye (Fig. 4C), and purpurin (Fig. 4D). Because each of these dyes absorbs in the visible region, resonance Raman and pre-resonance Raman enhancements should occur when using 532.17 nm and 632.8 nm excitation wavelengths, respectively. The maximum SERS intensity is obtained from a AgFON surface when the laser excitation wavelength is slightly blue with respect to the LSPR maxiumum.[29] Therefore, by matching AgFON LSPR maxima with similar laser excitation wavelengths, optimum SERS enhancement conditions will be met. Figure 5 depicts UV-Vis reflectance spectra of AgFONs fabricated with 300 nm (Fig. 5A), 390 nm (Fig. 5B), and 550 nm (Fig. 5C) diameter SiO_2 spheres with LSPR maxima located at 561 nm, 644 nm, and 747 nm, respectively. Collecting spectra of the two dyes using 532.17 nm and 632.8 nm lasers on AgFONs with correlating LSPR maxima will result in both resonance and SER enhancements simultaneously. Alternatively, using a 785.7 nm laser with a AgFON with a similar LSPR maxima will only result in SERS. By comparing the resonance SERS spectra collected with the 532.17 nm and 632.8 nm lasers to the non-resonant SERS spectra collected with the 785.8 nm laser, the individual contributions due to resonance Raman and SERS can be determined.

Figure 6 depicts non-resonant SER (Figs. 6A1 and 6B1), pre-resonant SER (Figs. 6A2 and 6B2), and resonant (Figs. 6A3 and 6B3) SER spectra of carminic acid (Fig. 6A) and lac dye (Fig. 6B). All the spectra collected with the three laser excitation wavelengths (532.17, 632.8, and 785.7 nm) of carminic acid contained the diagnostic peaks that were previously shown (Fig. 3C). Similar results were observed for the spectra collected of lac dye. In the case of both carminic acid and lac dye, spectra collected with the 785.7 nm laser were approximately two orders of magnitude less intense than the spectra collected with the 532.15 nm and 632.8 nm lasers. These results can be attributed to the fact that resonance conditions were not met. One result of working on resonance is the amplification of specific vibrational bands. Examples of this can be seen in the 1429 cm^{-1} peak in the carminic acid spectra and the 1201 cm^{-1} peak in the lac dye spectra. Both the 1429 cm^{-1} (carminic acid) and the 1201 cm^{-1} (lac dye) peaks are weak in the spectra collected with the 785.7 nm laser, while they are significantly enhanced in the spectra collected with both the 632.8 nm and 532.15 nm lasers. These experiments show that by working in the resonance and pre-resonance regions of these red dyes, significant enhancement of SERS spectra will be achieved.

Identification of Individual Dyes in Mixtures. Artists often mix several types of pigments or dyes together; therefore,

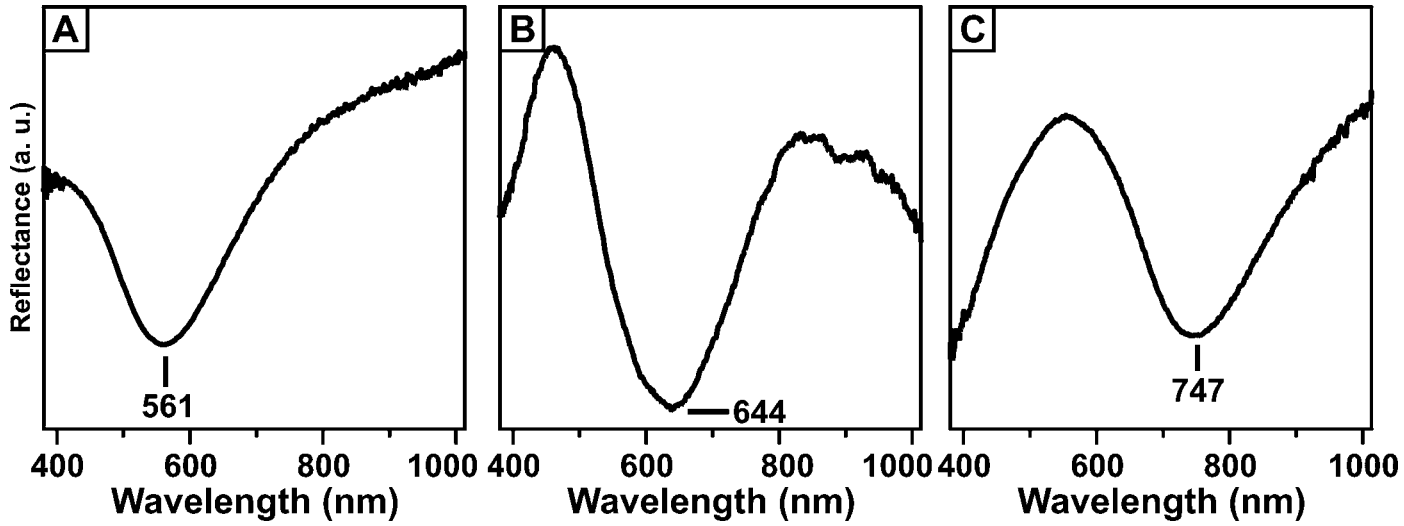

FIG. 5. UV-vis reflectance spectra of AgFONs made with (**A**) 300 nm, (**B**) 390 nm, and (**C**) 550 nm diameter SiO_2 spheres.

samples that are collected from most artwork contain a mixture of compounds. Consequently, it is important to be able to identify multiple dyes simultaneously in a single sample. Solutions were prepared containing low concentrations (1×10^{-6} M or 15 ng/μL) of two dyes, as would be found after extraction from historic textiles or artists' paint layers. Pre-resonance SER spectra were then collected with the 632.8 nm laser from SiO_2 AgFONs fabricated with 390 nm diameter spheres that had been incubated in these dye mixtures overnight. Figure 7 depicts pre-resonance SER spectra of dye mixtures containing alizarin and lac dye (Fig. 7A3), alizarin and purpurin (Fig. 7B3), and lac dye and purpurin (Fig. 7C3).

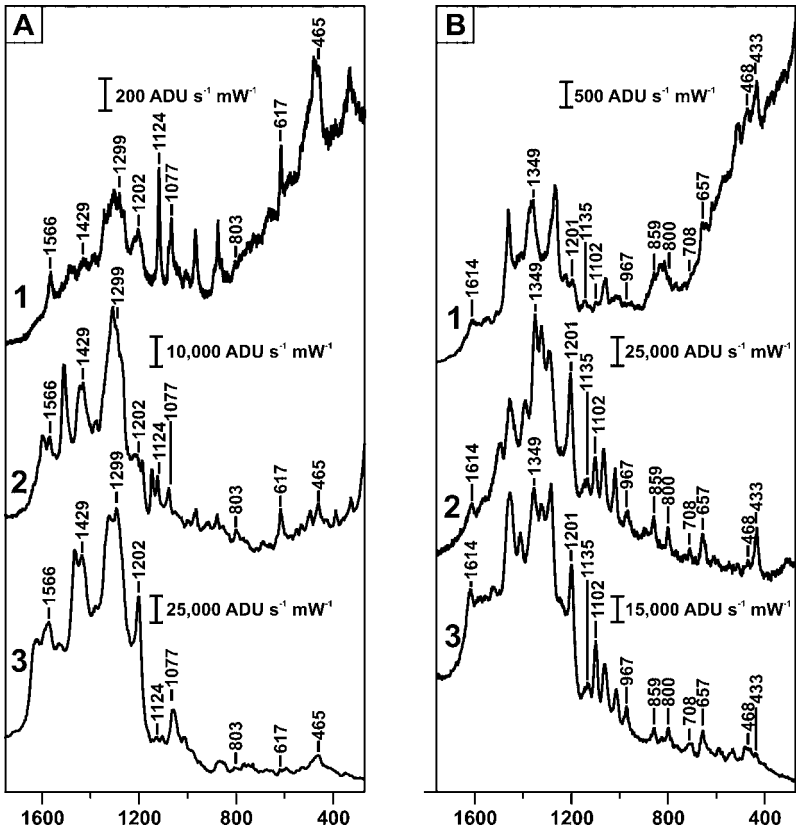

FIG. 6. Pre-resonance, resonance, and non-resonance SER spectra of (**A**) carminic acid and (**B**) lac dye collected with various excitation wavelengths paired with SiO_2 AgFONs with similar LSPR values. (***A1*** and ***B1***) $\lambda_{excitation} = 785.7$ nm, AgFONs fabricated with 550 nm diameter SiO_2 spheres, 15 s acquisition. (***A2*** and ***B2***) $\lambda_{excitation} = 632.8$ nm, AgFONs fabricated with 390 nm diameter SiO_2 spheres, 15 s acquisition. (***A3*** and ***B3***) $\lambda_{excitation} = 532.15$ nm paired with Ag FONs fabricated with 300 nm diameter SiO_2 spheres, 15 s acquisition.

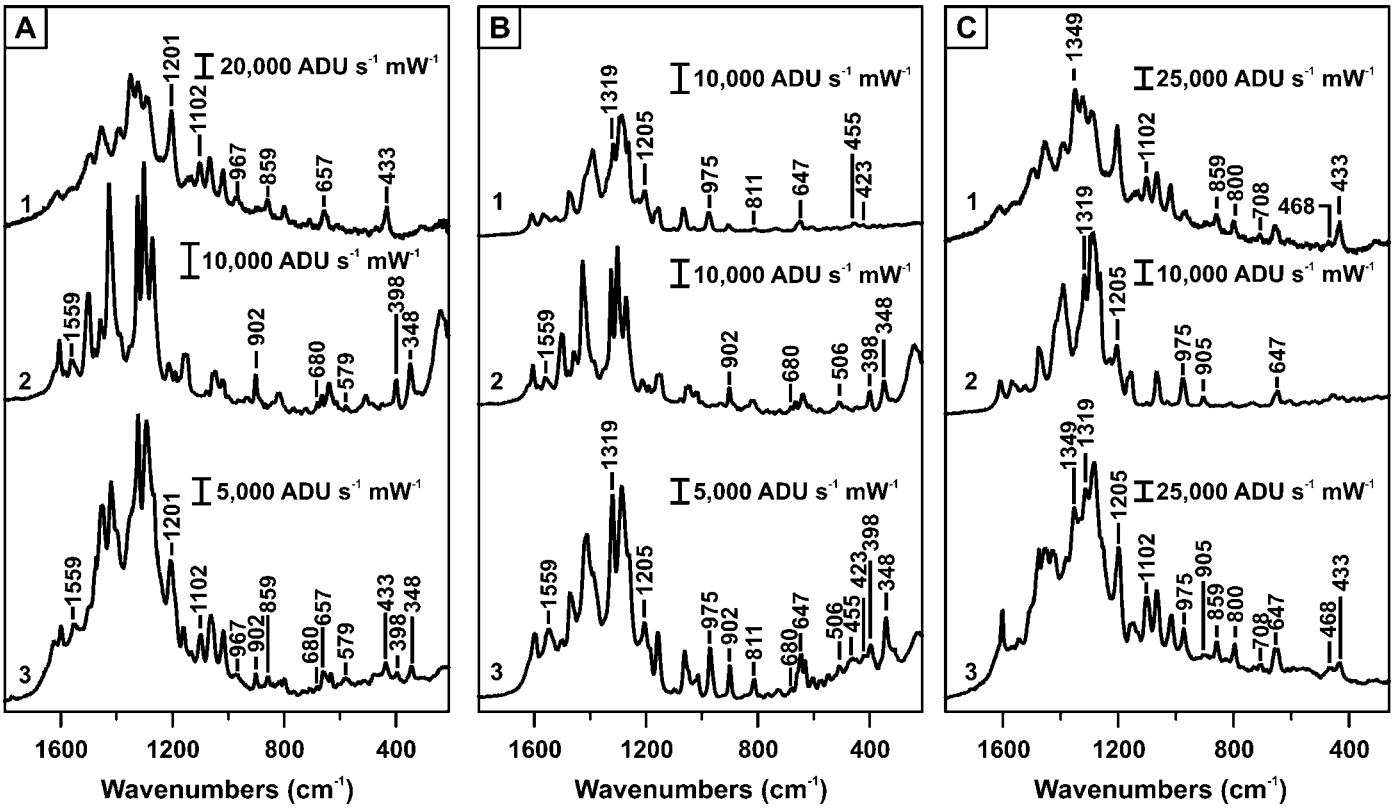

FIG. 7. Pre-resonance SER spectra of (**A**) (*1*) 15 ng/μL lac dye (15 s acquisition), (*2*) 1×10^{-6} M alizarin (60 s acquisition), and (*3*) a mixture of 15 ng/μL lac dye and 1×10^{-6} M alizarin (60 s acquisition); (**B**) (*1*) 1×10^{-6} M purpurin (15 s acquisition), (*2*) 1×10^{-6} M alizarin (60 s acquisition), and (*3*) a mixture of 1×10^{-6} M purpurin and 1×10^{-6} M alizarin (20 s acquisition); and (**C**) (*1*) 15 ng/μL lac dye (15 s acquisition), (*2*) 1×10^{-6} M purpurin (15 s acquisition), and (*3*) a mixture of 15 ng/μL lac dye and 1×10^{-6} M purpurin (20 s acquisition). (632.8 nm excitation, 390 nm diameter SiO_2 AgFONs.)

For the purpose of comparison, spectra of the mixtures are paired with reference spectra of the individual dyes: alizarin (Figs. 7A2 and 7B2), lac dye (Figs. 7A1 and 7C1), and purpurin (Figs. 7B1 and 7C2). The spectra collected from all of the dye mixtures contain diagnostic peaks from both of the individual red lake dyes and can therefore be identified as a mixture of the two dyes. The mixture containing alizarin and lac dye (Fig. 7A3) contains peaks located at 433, 657, 859, 967, 1102, and 1201 cm^{-1} that can be attributed to lac dye (Fig. 7A1) and peaks located at 348, 398, 579, 680, 902, and 1559 cm^{-1} that can be attributed to alizarin (Fig. 7A2). The mixture made from purpurin and alizarin (Fig. 7B3) contains peaks from both the individual purpurin (423, 455, 647, 811, 975, 1205, and 1319 cm^{-1}) and alizarin (348, 398, 506, 680, 902, and 1559 cm^{-1}). Finally, the mixture composed of lac dye and purpurin (Fig. 7C3) contains peaks due to both lac dye (433, 468, 708, 800, 859, 1102, and 1349 cm^{-1}) and purpurin (647, 905, 975, 1205, and 1319 cm^{-1}). These experiments show that AgFONs exhibit great potential to be employed as SERS substrates to identify and characterize artwork samples containing mixtures of pigments and dyes.

CONCLUSION

This work demonstrates that AgFONs are effective SERS substrates for the identification and characterization of artists' red dyes and their mixtures. AgFONs are easily fabricated and are capable of collecting high resolution and consistent spectra. Because museum sample sizes are often extremely small, all SERS experiments were conducted with sample sizes similar to those found in museums (1×10^{-6} M or 15 ng/μL). Detailed and reproducible pre-resonance SER spectra were collected for the dyestuffs alizarin, purpurin, lac dye, carminic acid, and cochineal. Furthermore, to the best of the authors' knowledge, the pre-resonance SER spectra collected for cochineal are the highest resolution to date. Experiments were conducted with various laser excitation wavelengths (532.15 nm, 632.8 nm, and 785.7 nm) matched with correlating AgFON LSPR maxima (561 nm, 644 nm, and 747 nm) in order to determine the most sensitive detection system to study the red dyes. Because these red dyes absorb visible light, resonance and pre-resonance Raman conditions could be met using the 532.15 nm and 632.8 nm lasers, respectively, resulting in further enhancement. Alternatively, using a 785.7 nm laser will only result in SERS. By comparing the spectra, it was determined that resonance effects will enhance SERS spectra by approximately two orders of magnitude. Finally, samples collected from artwork often contain dye mixtures. Therefore, in order for SERS to be a successful technique for the analysis of real artwork samples, the identification of individual dyes within mixtures must be possible. Consequently, experiments were successfully preformed to simultaneously identify individual dyes contained in mixtures of (1) alizarin and lac dye, (2) alizarin and purpurin, and (3) purpurin and lac dye. These initial experiments show promise towards utilizing AgFONs as SERS substrates to correctly identify individual dyes in mixed samples in real artwork.

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