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Ad-hoc Surface-Enhanced Raman Spectroscopy Methodologies for the Detection of Artist Dyestuffs: Thin Layer Chromatography-Surface Enhanced Raman Spectroscopy and in Situ On the Fiber Analysis

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Tailored ad-hoc methods must be developed for successful identification of minute amounts of natural dyes on works of art using Surface-Enhanced Raman Spectroscopy (SERS). This article details two of these successful approaches using silver film over nanosphere (AgFON) substrates and silica gel coupled with citrate-reduced Ag colloids. The latter substrate functions as the test system for the coupling of thin-layer chromatography and SERS (TLC-SERS), which has been used in the current research to separate and characterize a mixture of several artists' dyes. The poor limit of detection of TLC is overcome by coupling with SERS, and dyes which co-elute to nearly the same spot can be distinguished from each other. In addition, in situ extractionless non-hydrolysis SERS was used to analyze dyed reference fibers, as well as historical textile fibers. Colorants such as alizarin, purpurin, carminic acid, lac dye, crocin, and Cape jasmine were thus successfully identified.

Elucidation of organic dyes and pigments contained in historical artworks has important relevance in the fields of art history and art conservation. In addition, such an analysis can be important for dating and authenticating artworks. To date, several analytical techniques have found use in the detection of natural and early synthetic organic dyes, including HPLC,[1−3] UV−vis spectroscopy,[4] FTIR,[5,6] NIR,[7] and Raman spectroscopy.[8,9] Currently HPLC has demonstrated the ability to identify the widest spectrum of colorants, provided enough sample (∼5 mm of fiber) is available. Surface-Enhanced Raman Spectroscopy (SERS) can be advantageous over these techniques because the noble metal substrate effectively quenches fluorescence and provides a greatly enhanced Raman signal, thus offering highly specific, molecular-level identification of extremely small samples.[10−13] Indeed, in recent years, several papers have successfully reported on the use of SERS to identify organic, highly fluorescent dyes that are significant for artistic production.[14−16] However, very few papers have focused on the identification of chromophores in a sample which contains two or more dye components. Whitney et al. successfully described a proof of concept experiment that showed promising results as to the ability of SERS to identify components in solutions containing two dye components;[17] however, this analysis was not applied to an actual historical sample of relevance to the art conservation community. It is well-known that materials present in samples from real works of art may be affected by aging or contamination by previous treatments. Additionally, the dye is invariably embedded in a complex matrix, thus rendering the transfer of a method found to be successful on pure or standard materials to actual artwork samples very challenging, a roadblock that is often encountered in biomedical applications of SERS.[18]

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The objective of this paper is to develop and test two ad-hoc SERS methodologies to successfully detect dye components in reference samples, as well as samples from works of art. A 3-fold methodology was adopted: first to examine the use of citrate-reduced colloids on a silica gel matrix as a SERS-active substrate, comparing the SERS activity to silver film over nanosphere substrates; second, to use these citrate-reduced colloids as the SERS-active mechanism for TLC-SERS of mixed dye components; and third, to investigate the possibility of in situ *on the fiber* analysis of dyed textiles, without the necessity of extraction/hydrolysis.

Coupling of TLC and SERS was first reported by Henzel,[19] and since then has been applied successfully to the separation of various analytes,[20−22] including pharmaceuticals.[23] However, although TLC has been used as a separation technique for the identification of dye components,[24,25] it has not, to date, been coupled to a molecule-specific detection method to identify dye components of art-historical interest. In the current study, the usefulness of combined TLC-SERS for analyzing mixtures of red dyes was probed. Often times the preparatory work required to extract a dye from a sample, such as a dyed textile, can be laborious and can result in degradation of the sample components. Typically the size of sample taken from a precious artwork is miniscule, precluding the use of analytical methods which require large amounts of sample. In light of this, in situ non-destructive/microdestructive analysis techniques are required. The first example of in situ *on the fiber* non-extractive SERS was reported by Jurasekova et al.,[26] who used photoreduced silver nanoparticles on the fiber to generate the SERS signal from a reference fiber dyed with luteolin and apigenin. The current study seeks to extend this work to the hydroxyanthroquinone dyes using citrate reduced colloids, measuring SERS directly on both dyed reference fibers and actual historical textile samples.

EXPERIMENTAL SECTION

Materials. Silver (Ag) wire (99%) was purchased from D.F. Goldsmith (Evanston, IL). Borosilicate glass substrates (No. 2, 18 mm) were acquired from Fisher Scientific (Pittsburgh, PA). SiO_2 spheres (350 and 390 nm diameter, 10% by weight, in water) were purchased from Bangs Laboratories Inc. (Fishers, IN). Silver nitrate (99+%), sodium citrate (98+%), potassium aluminum sulfate dodecahydrate (>99%), alizarin (97%), carminic acid (98+%), and crocin (98+%) were all purchased from Sigma Aldrich (St. Louis, MO). Purpurin (98+%) was purchased from ACROS (Morris Plains, NJ). Madder root was obtained from Kremer pigments (New York, NY). Silica gel thin layer chromatography (TLC) plates on aluminum foil and glass developing chambers were purchased from Sigma Aldrich. Clean (degreased) undyed wool was obtained from Esther's Place Fibers (Big Rock, IL). In addition, dyed fiber samples of *Gardenia augusta* L. (Cape jasmine; main component crocin) were generously provided by the Getty Conservation Institute.

Silver Film Over Nanosphere Fabrication and Incubation Procedure. Glass substrates (18 mm circular, No. 2, Fisher Scientific, Pittsburgh, PA) were cleaned in a freshly prepared piranha etch solution (3:1 H_2SO_4:H_2O_2) for 30 min. After rinsing with Milli-Q water (18.2 MΩ cm, Millipore, Billerica, MA), the glass substrates were sonicated for 60 min in 5:1:1 $H_2O/H_2O_2/NH_4OH$, followed by copious rinsing, to create a hydrophilic surface on the glass to facilitate sphere packing. Approximately 2.5 μL of the SiO_2 sphere solution was drop-coated onto the surface of the glass and was allowed to dry under ambient conditions. Next, 200 nm of silver was deposited onto the surface of the sphere mask using a modified Consolidated Vacuum Corporation (Rochester, NY) thermal deposition chamber with a base pressure of 10^{-6} Torr. The deposition rate (∼2Å/s) of the silver was monitored using a Leybold Inficon XTM/2 quartz crystal microbalance (QCM) (East Syracuse, NY). AgFON substrates were incubated in methanolic dye solutions (1.0×10^{-3} M) or the dyed fiber extracts overnight, and were rinsed with methanol prior to SERS measurements.

Silica Gel with Silver Colloid Substrates. Citrate reduced silver colloids were prepared using the standard Lee and Miesel preparation,[27] and were centrifuged 10 times (relative centrifugal force = 36000, 15 min per cycle) to concentrate the colloid. The colloids prepared this way were stable at room temperature (stored in the dark) for up to 3 weeks. Silica gel TLC plates were activated in an oven at 130 °C for 60 min, prior to being cut up into 2.5 × 2.5 cm squares. Five microliters of the sample (1.0×10^{-3} M methanolic dye solution or dyed fiber extract) were micropipetted onto the TLC plate, and 5 μL of the centrifuged citrate reduced colloid was then spotted on top of the spot. SER spectra were recorded soon after application of the colloids; however, SERS activity from such samples was observable for up to 3 weeks.

SERS. All SER spectra were collected on a custom-built macro setup. 632.8 nm excitation was obtained using a HeNe laser (12 mW output power, 9 mW at the sample, 0.8 mm beam diameter) (Research Electro-Optics, Boulder, CO). The SERS measurements employ 1″ interference and notch filters (Semrock, Rochester, NY), a single-grating monochromator with the entrance slit set to 100 μm (model VM-505, Acton Research Corporation, Acton, MA), a liquid N_2 cooled CCD detector (model Spec10:400B, Roper Scientific, Trenton, NJ), and a data acquisition system (Photometrics, Tucson, AZ). The spectral positions of the CCD pixels were calibrated using a neon lamp. The spectral resolution was 4 cm^{-1}. While excitations of 532 and 785 nm were also evaluated for SERS of these dyes, 633 nm excitation was found to give the most intense SERS signal, with a minimum of interfering fluorescence.

Wool Mordanting and Dyeing. Clean (degreased), undyed wool was subjected to a mordanting procedure according to literature.[28] Briefly, 10 g of the wool was rinsed with Milli-Q water,

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and was then placed in a mordanting bath containing 2.5 g of potassium aluminum sulfate dodecahydrate (therefore 25% w/w of mordant) in 400 mL of Milli-Q water. The mordanting bath was then heated to a temperature of 90 °C for 60 min. After cooling to room temperature, the mordanted wool was rinsed repeatedly with Milli-Q water. To dye the wool, a small amount of the wool (~0.5 g) was added to a methanolic stock solution of red dye(s) (methanol was used to increase the solubility of the dye component), and left overnight. The next day, the dyed wool was removed from the dye bath and was rinsed repeatedly with methanol to remove any unbound dye component. The dyed wool was stored in closed containers in the dark until needed.

Extraction of Dye Components from Dyed Wool. To remove the pigment from the mordanted, dyed wool, various extraction protocols were tested. Traditionally used strong acid extractions using HCl[1] or H_2SO_4 resulted in excellent color removal but caused extreme degradation of the wool. Such degradation was found to interfere with spectroscopic measurements on the extracts, and therefore extractions focused on formic acid/methanol mixtures to achieve a gentler, however incomplete, removal of the colorant.[29,30] Several volume ratios were tested, ranging from 5 to 20 volume % of formic acid, and the best extraction solvent was found to be an 85:15 v/v% mixture of methanol: formic acid. At this ratio of methanol to formic acid, very good color removal was observed with minimal sample deterioration. A small sample of the dyed wool was heated in this extraction solvent for 60 min at 50 °C in a hot water bath. The wool was then removed, and the extract was evaporated to dryness under a gentle nitrogen flow, and then reconstituted in a small (several microliters) volume of methanol.

TLC-SERS. Conventional TLC of the red dye components was done using the activated silica gel TLC plates and glass developing chambers. The optimal developing solvent was found to be 9:2:2 v/v% toluene/acetic acid/methanol, a modification of the eluent reported by Grygar et al.[31] Typical developing times ranged from 20 to 30 min. After development and drying of the TLC plate, 5 μL of the concentrated silver colloid was deposited onto each spot, and the SERS of each spot was recorded. Initial experiments aimed at making the entire plate SERS-active were evaluated, which would provide a surface that could be probed by scanning the laser across it. This was attempted by both pre and post treatment of the entire TLC plate with silver nanoparticles, using methods that have been reported in the literature.[32,33] Unfortunately, pre treatment of the plate with silver resulted in very poor separation of the dyes, and difficult spot identification. Post treatment of the plate was laborious and resulted in a poor distribution of the silver nanoparticles. As a result, this concept was abandoned in favor of deposition of silver colloids directly on the spot of interest.

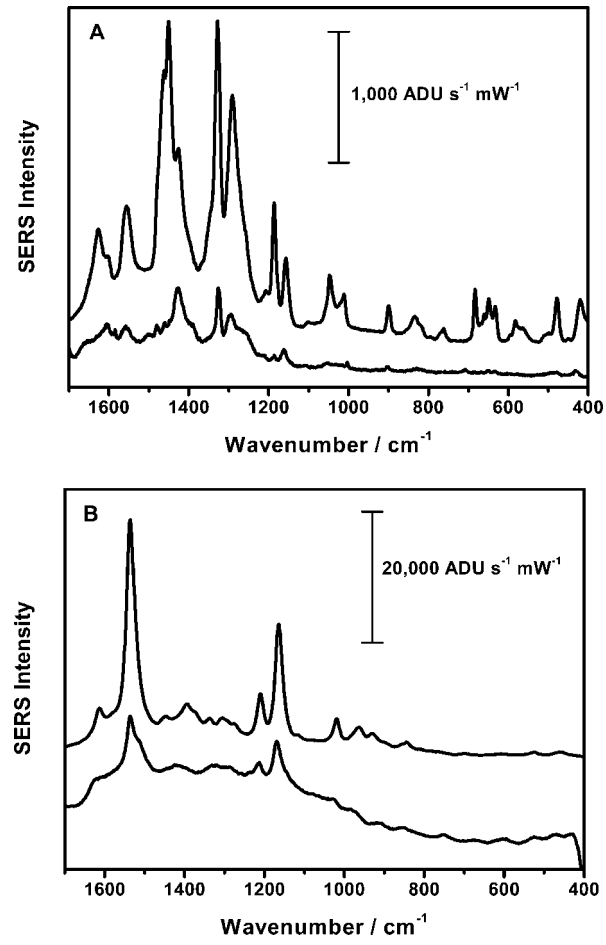

Figure 1. Comparison of SER spectra obtained from AgFON surfaces (bottom curves) and silica gel with silver colloid surfaces (top curves) for (A) 1.0×10^{-3} M alizarin (B) 1.0×10^{-3} M crocin.

RESULTS AND DISCUSSION

Comparison of AgFON and Silica gel with Ag Colloid Substrates Using Reference Dye Component Solutions. Preresonance surface-enhanced Raman spectra were recorded using 632.8 nm excitation for reference stock solutions (1.0×10^{-3} M) of the three red dye components (alizarin, purpurin, and carminic acid) and one yellow dye component (crocin) investigated in this project. AgFONs used with the red dyes were fabricated using 390 nm SiO_2 spheres, while AgFONs used with the yellow dye were fabricated using 350 nm SiO_2 spheres. These differing sphere sizes give rise to maximum localized surface plasmon resonances at 644 and 572 nm, respectively (data not shown). An example comparison of SER spectra for the red dye alizarin and the yellow dye crocin on the AgFON and silica gel plus silver colloid substrates is shown in Figure 1. (Interested readers can find tabulated peak positions for each dye investigated in this work in the Supporting Information, Table S-5.) It is obvious that the AgFON spectra have larger backgrounds, and poorer resolution than the silica gel plus silver colloid spectra. While the AgFON in theory should provide better spectra as it is tuned to optimize both the laser excitation and the absorption maxima of the dye molecule, it suffers from carbon contamination issues, especially during the silver deposition phase. The binding affinity of the organic dyes is apparently weaker than the binding affinity of the carbon contamination, and as a result, the organic dyes do not displace the contamination on the

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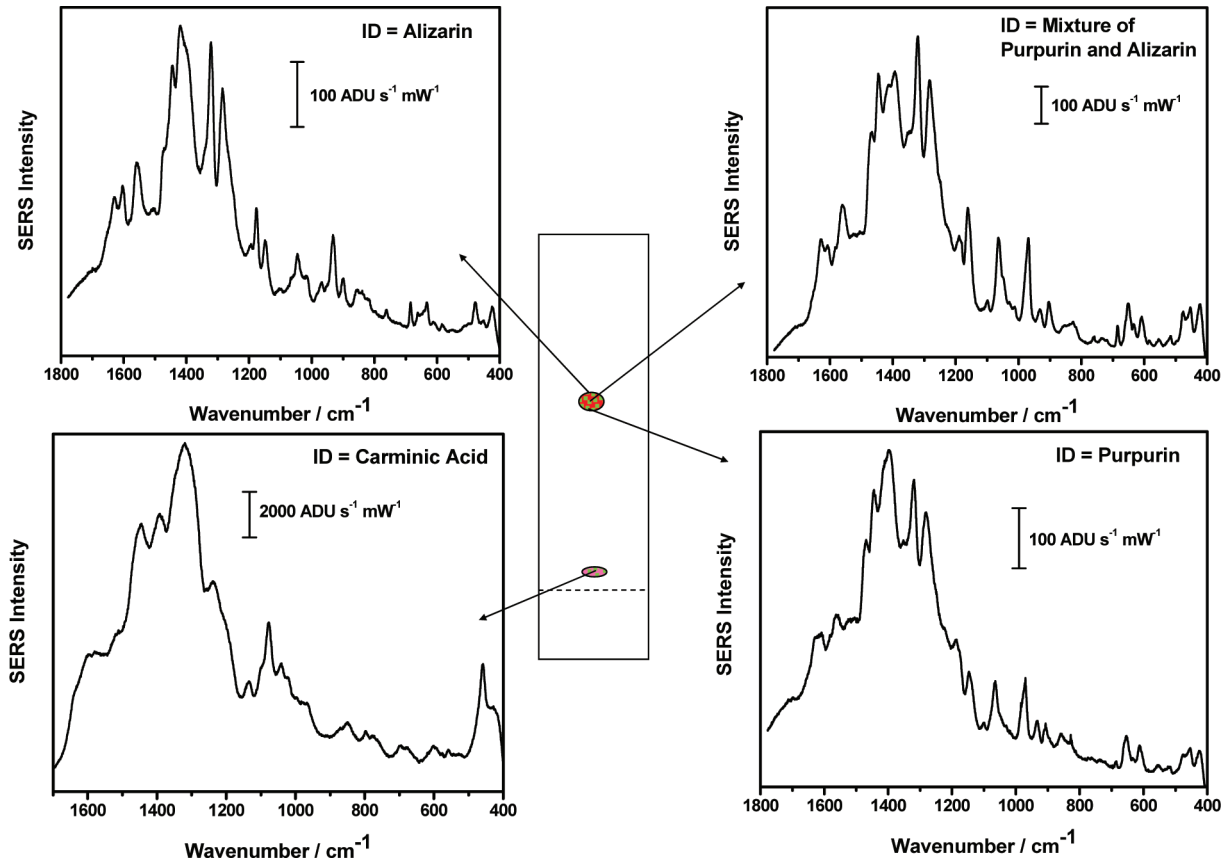

Figure 2. TLC-SERS of the separation of three red organic dye components, carminic acid, purpurin, and alizarin. Purpurin and alizarin are both present in the spot which moved further along the TLC plate.

surface, as is the case for analytes such as alkanethiols. The enhanced SERS signal for the silica gel with silver colloid substrates over the AgFON substrates may be explained by three factors: (i) the heterogeneous sizes of the colloidal particles provide a large range of LSPRs, of which some will be exactly tuned to the dye absorption; (ii) the citrate capping ligand is more readily displaced by the dye than is the carbon contamination on the AgFON; and (iii) there are small nanoparticle aggregates formed on the silica gel that provide "hot spots" for SERS.

Coupling of TLC and SERS for Dye Analysis. Results from the previously described experiments indicated that the silica gel with silver colloid system works as an excellent substrate for SERS. The next step was to use the silica gel for its intended application, as the stationary phase in TLC. Initially, a plate was spotted with 5 μL each of the red dyes, and various eluents were tested which would give good separation of the components along the TLC plate with minimal trailing. The best eluent was found to be 9:2:2 v/v% toluene/acetic acid/methanol. Next, the three red dyes were mixed together in equal aliquots, and 5 μL of the mixture was micropipetted on the TLC plate, and the chromatograms were developed. After development and drying of the TLC plate, the spots corresponding to the dyes were each treated with 5 μL of the silver colloids. Within a period of several hours, the SER spectra were recorded for each developed spot.

It is interesting to note that for the TLC of the red dye mixture only two spots were visible by eye, even though there are three dyes in the mixture. This is because alizarin and purpurin have nearly the same chemical structure (Supporting Information, Figure S-1), and hence nearly identical polarities, and as a result they migrate to the same position on the TLC plate for this elution system. Typically polyamide or acetylated cellulose plates are used to separate anthraquinone dyes by TLC.[34,35] Unfortunately this stationary phase would be expected to have a large SERS background, and so silica gel plates were used instead, even though the latter substrate gives a less than optimal separation of the dyes. However, even though the two components contained within this spot cannot be distinguished visually, SER spectra recorded at three positions within this spot clearly indicate that alizarin is on the leading edge of the spot, purpurin is on the trailing edge, and there is a mixture of both components in the middle of the spot. Figure 2 presents the SER spectra for both spots, including the identification of the dye resulting from that spot. This is an illustration of the powerful combination of SERS with a simple separation technique, which on its own has poor resolution of similar molecules.

Having demonstrated that TLC-SERS is a viable method for the separation of minute quantities of organic dyes, the next step was to test it on a fiber which was dyed with more than one dye component. A small wool sample (~0.001 g) which was dyed with both alizarin and carminic acid was treated using the formic acid/methanol extraction protocol listed above, and 5 μL of the reconstituted extract was micropipetted on the TLC plate, devel-

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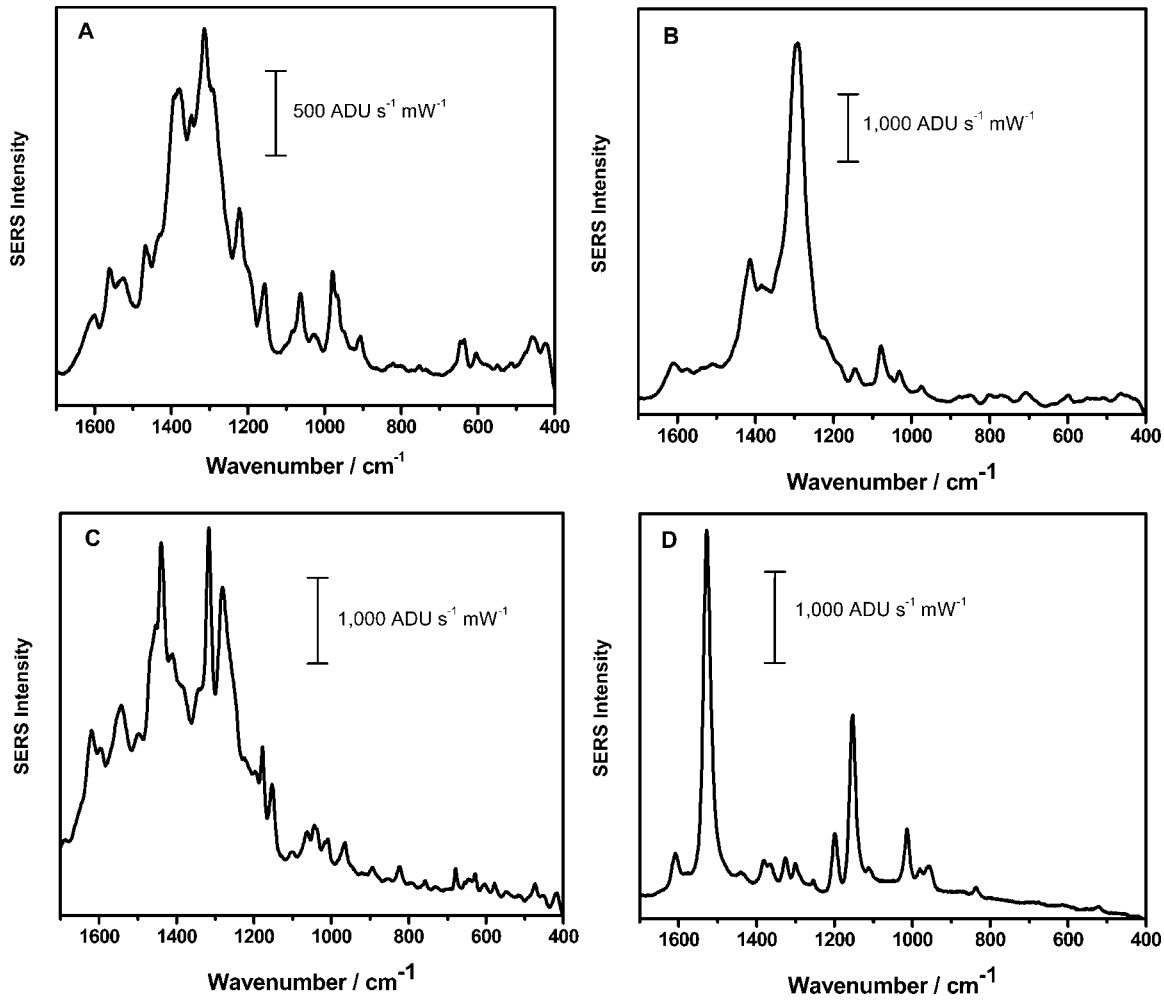

Figure 3. In-situ *on the fiber* extractionless non-hydrolysis SERS of reference fibers dyed with (A) Purpurin, (B) Carminic acid, (C) Madder, and (D) Cape jasmine.

oped, and treated with silver colloid. SERS measurements were done on the developed spots, and show that both dye components are clearly identifiable (Supporting Information, Figure S-2).

In-Situ on the Fiber Extractionless Non-Hydrolysis SERS. In cases where only one colorant was used in an artwork, or where sample preparation is difficult and the sample is extremely small, in situ methodologies which can be done directly on the fiber or art sample are desirable. Given this motivation, in situ *on the fiber* extractionless non-hydrolysis SERS using citrate-reduced silver colloids was developed as part of this project and is being demonstrated here for the first time on actual historical textiles. Reference fibers dyed with purpurin, carminic acid, madder, and Cape jasmine were treated with the concentrated silver colloids, and the SER spectra for these fibers are shown in Figure 3. It is evident that the SER spectra are excellent and demonstrate that removal of the dye from the mordanted fiber is not necessary to obtain spectra. As described in the experimental section, the dyed fibers were repeatedly rinsed with methanol prior to analysis; thus, the spectra presented here represent the actual dye−metal ion−biofiber complex. Figure 4 shows the comparison between the in situ SERS for Cape jasmine, and the SERS recorded for an extract (dye components not separated) of Cape jasmine taken from a sample of dyed fiber, measured using the silica gel with

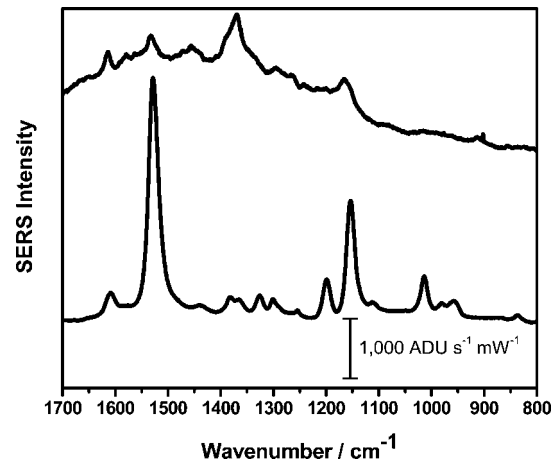

Figure 4. Comparison of SER spectra obtained for in situ extractionless non-hydrolysis SERS on a Cape jasmine dyed fiber (bottom curve), and for an extract from a Cape jasmine dyed fiber recorded on a silica gel with silver colloids substrate (top curve).

citrate reduced colloids. It is obvious that the in situ *on the fiber* SER spectra exhibit greater intensities, and less background interference.

One important aspect to consider when doing *on the fiber* SERS of dyes on actual historical samples that may not have been

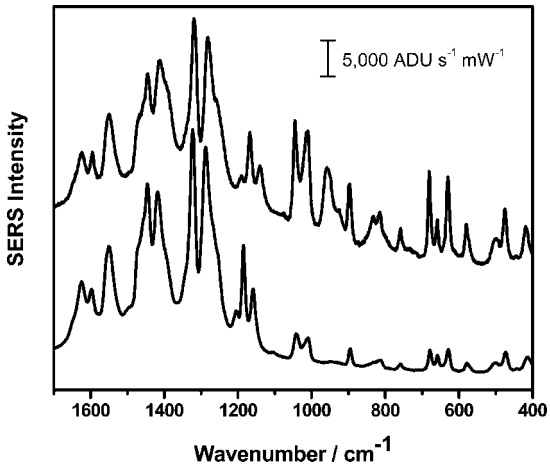

Figure 5. Comparison of the SER spectra for free alizarin dye (bottom curve) and mordanted alizarin (top curve), complexed with aluminum to form a lake pigment.

thoroughly rinsed, is the extent to which one is actually probing the dye complexed with mordant, or free dye which is unbound to the fiber. To investigate the spectral differences in a red dye when complexed to a mordant and when not complexed, stock solutions of 1.0×10^{-3} M alizarin were prepared, and to one of the alizarin samples, 1 mL of a concentrated aluminum potassium sulfate solution was added. An immediate color change from yellow to orange-red was observed, indicating formation of the dye-mordant complex (or lake pigment). Figure 5 compares the SER spectra of the free alizarin and the alizarin-mordant complex (measured using the silica gel with silver colloids substrate). Comparing the free dye spectrum to the complexed dye spectrum, some differences are noted. First, the 1160 cm^{-1}, 1186 cm^{-1}, 1205 cm^{-1}, 1287 cm^{-1}, 1323 cm^{-1} and 1418 cm^{-1} bands, assigned to mainly C−C stretching and C−H bending vibrations (ring breathing modes),[36,37] shift to lower frequencies in the case of the dye complex, to 1141, 1169, 1191, 1281, 1318, and 1412 cm^{-1}, respectively. In addition, in the case of the mordanted dye, a band appears at 960 cm^{-1}, which is consistent with the Al−O bending vibration,[38] an indication that the dye-mordant complex is intact.

Finally, once the best methodology to test several different systems was determined, the in situ *on the fiber* extractionless non-hydrolysis procedure was applied to three historical textile samples. The first one, shown in Supporting Information, Figure S-3 is a cover from Italy, which dates to the early 17th century. The size of the sample silk fiber used in the SERS analysis (~100 μm in length), coated in silver colloid, is shown as an inset in Supporting Information, Figure S-3. Figure 6 shows the SER spectrum taken from the aforementioned textile fiber, which appears to most closely resemble the red organic dye component purpurin, which would point to the use of madder (*Rubia tinctorum* L.). Figure 7 shows an image of the second historical textile tested, which is a portion of a carpet from Turkey, Istanbul or Bursa, dating to the late 16th/early 17th century. Figure 8 shows the SER spectra of this textile wool sample, which is a very good match for lac dye.[17] This finding is significant as examples of early

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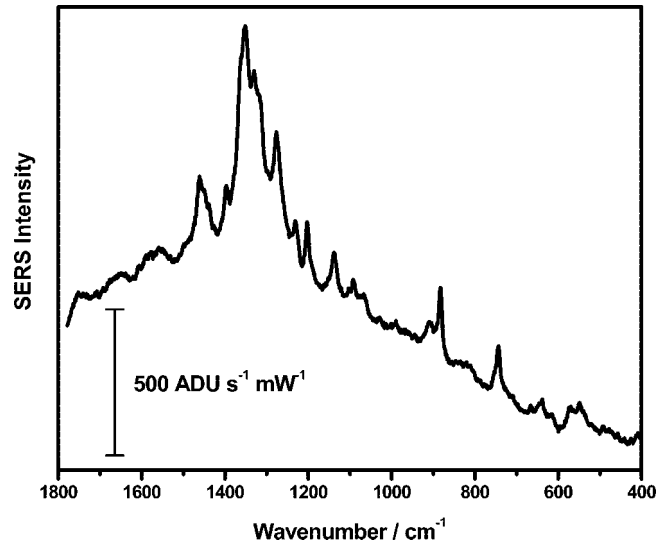

Figure 6. In-situ *on the fiber* SER spectrum of the red embroidery sampled from the historical textile shown in Supporting Information, Figure S-3.

Figure 7. Image of a portion of a carpet from Bursa/Turkey/Istanbul which dates to the late 16th/early 17th century (AIC 1964.554; 104.1 × 294.3 cm, silk, wool and cotton; gift of Mrs. Siegfried G. Schmidt). A red wool fiber from the ground weft was sampled.

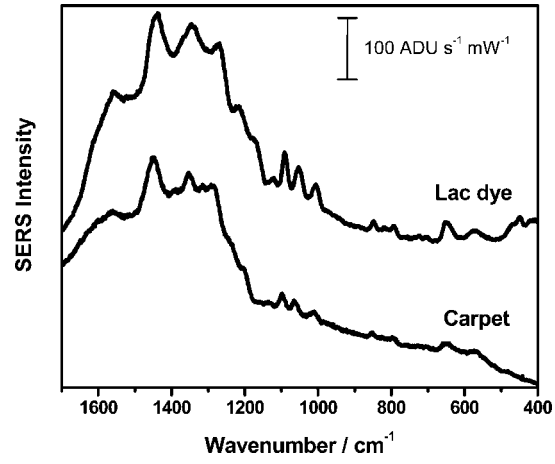

Figure 8. Comparison of the in situ *on the fiber* SER spectra for a wool fiber dyed with lac dye and the red fiber sampled from the historical carpet shown in Figure 7.

European textiles dyed with lac are very rare. Supporting Information, Figure S-4 is an image of a long shawl from France, which

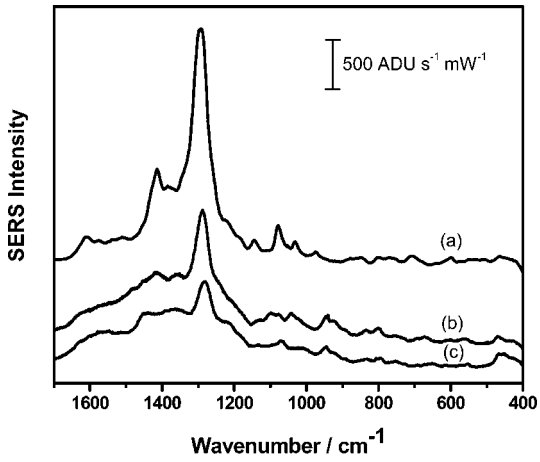

Figure 9. Comparison of the in situ *on the fiber* SER spectra for a wool fiber dyed with carminic acid (curve a) and the red (curve b) and pink (curve c) fibers sampled from the historical textile shown in Supporting Information, Figure S-4.

dates between 1850 and 1900 A.D. Both a red and a pink weft fiber were sampled, and the SER spectra from these fibers are shown in Figure 9. It is obvious that in this case, the reds and pinks were created in this textile using the red dye cochineal (main dye component = carminic acid). These results indicate the feasibility of using in situ *on the fiber* SERS for detecting natural organic pigments contained in historic textiles and artworks, using very minute quantities of textile, and minimal sample preparation.

CONCLUSIONS

Results presented here indicate that silica gel with silver colloid substrates for SER spectroscopy provide excellent quality spectra of natural organic dye components with minimal background interferences and very good resolution. In addition, these substrates are cost-effective and easy to prepare, so that any museum laboratory equipped with a Raman spectrometer may successfully implement this technique. Coupling of TLC and SERS (TLC-SERS) for the separation and identification of organic dyes of interest to art conservation was demonstrated, significantly reducing the amount of material and sophisticated equipment needed if compared to HPLC, and the applicability of this technique to dyed textile samples has been elucidated. However, it should be realized that in some cases, calculation of relative ratios of dye components is necessary to make distinctions between chemically closely related dyes belonging to different biological sources, a task for which HPLC is ideally suited.[39] These initial experiments show great promise for TLC-SERS in the field of art conservation and restoration. Finally, in cases where a dyed textile was believed to contain only one dye component, or where sample extraction would not be possible, given the extremely small dimensions of the sample available and the risk of dye degradation during extraction, in situ *on the fiber* extractionless, non-hydrolysis SERS was used to give excellent SER spectra of the colorant, with very minimal preparation. This methodology was used on three historical textile samples, and for the first time in situ *on the fiber* SERS was used to identify lac dye and cochineal in red and pink dyed fibers from historical artworks.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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