Silver Island Films as Substrate for Surface-enhanced Raman Spectroscopy (SERS): A Methodological Study on Their Application to Artists' Red Dyestuffs

Alyson V. Whitney[1], Richard P. Van Duyne[1], and Francesca Casadio[2]

1: Department of Chemistry, Northwestern University, 2145 Sheridan Rd, IL. 60201-311

2: The Art Institute of Chicago, 111 S. Michigan Ave., Chicago, Il 60603-6110

ABSTRACT

Surface enhanced Raman spectroscopy (SERS) has enormous capabilities for the unambiguous identification of artists' red lake pigments and dyestuff. Until now, red lake pigments have been very poorly characterized with conventional Raman spectroscopy due to their high fluorescence and weak Raman scattering cross section. Furthermore, very small amount of dyestuff is necessary to achieve intense deep colors; therefore a characterization method with enhanced sensitivity is needed to accurately identify the dyestuff. This study focuses on two red dyestuffs, carminic acid and laccaic acid, which are extensively utilized in old masters' paintings. We propose an innovative sensing method using 6 nm silver island films (AgIFs) fabricated with electron beam (e-beam) deposition on the colorant particles under investigation. The results of an in-depth methodological investigation into the signal-enhancement achievable with various experimental conditions applied to these substrates are presented. We explore the effects of varying laser excitation frequencies (532.15 nm 632.8nm and 785.7 nm), laser power at the sample and we investigate the dependence of S/N in the spectra from different spot sizes. Finally, the sensitivity of the method is determined. The results of the semi-quantitative analysis have allowed us to determine the best experimental conditions to achieve the highest sensitivity in the investigation of this important class of artists' colorants.

Keywords: Ag island films, SERS, traditional artists' red dyestuffs

1. INTRODUCTION

Since the 1980s, Raman microspectroscopy has grown to be a well-established technique for the characterization of artists' pigments[1-5]. However, many dyestuffs, such as traditional red lake pigments and organic dyes of natural origin used for dyeing textiles, are extremely fluorescent, an effect that dominates the weak optical process of Raman scattering. Furthermore, because subnanogram levels of these dyes are needed to achieve intense coloration, normal Raman spectroscopy is often not sensitive enough to probe these materials. The Raman cross section can be amplified by Surface enhanced Raman spectroscopy (SERS). Additionally, the use of a noble metal SERS substrate can quench fluorescence. SERS is a process whereby the Raman scattering signal is increased when a 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 surfaces. The SERS signals of ensemble-averaged molecules demonstrate enhancements up to 8 orders of magnitude over normal Raman signal.[6,7]

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Artists' lake pigments were traditionally obtained by precipitation of organic dyestuffs onto an inorganic, inert substrate (usually finely divided particles of aluminum hydroxide, calcium sulfate or carbonate). Similarly, traditional textile dyeing with red natural dyestuffs was commonly achieved through the use of mordants. These compounds (usually alum – $Al_2(SO_4)\cdot K_2SO_4\cdot 24H_2O$; iron sulfate – $FeSO_4\cdot 7H_2O$ - or tin chloride -$SnCl_2$) provide a complexation site allowing bonding of the dye with the natural fiber, that otherwise would have no affinity for each other.[8] Thus, natural organic red colorants are most frequently found in works of art in the form of a metal complex. So far, successful attempts at identifying these materials have required a separation procedure that decomposes the inorganic ion- dyestuff complex into its separate components through a hydrolysis step that liberates the dyestuff. At present, the most established method of identification of these colorants is High Performance Liquid Chromatography (HPLC).[9] Normally at least 5 mm of dyed fiber are necessary for HPLC investigation, and, in the case of samples from painting layers, a substantial quantity of material is needed; unfortunately, these amounts are not always available, therefore the development of an alternative characterization method with enhanced sensitivity is very valuable for the unambiguous identification of this important class of artists' materials.

Recently, we have shown that silver island films (AgIFs) fabricated by electron beam (e-beam) deposition are a successful SERS substrate for the analysis of red lakes pigments and organic dyestuffs.[10] AgIFs are materials independent, highly reproducible, and can be applied directly on single grains of pigment without any further sample preparation step, which makes them an ideal substrate for microscopic samples from works of art.[10,11] Several reference materials including the synthetic dyestuffs alizarin, purpurin, carminic acid, and eosin as well as historic red lake pigments such as madder lake, cochineal, brazilwood, lac lake, and kermes were probed and highly detailed Raman spectra were obtained.[10] The proposed method has shown great potential for the unambiguous identification of red dyes applied in different media on a variety of substrates. The work presented here builds upon the knowledge accumulated with our previous SERS investigations of this particular class of artists' materials and expands the scope of that investigation.

Amongst the red colorants most favored by artists and craftsmen from antiquity to the 19th century, 2 dyestuffs were studied: laccaic acid and synthetic carminic acid (Figure 1). These dyestuffs were selected because very little work on their spectroscopic characterization has been done, as opposed to the much more widely investigated anthraquinone dyes alizarin and purpurine.[12,13] The compounds studied belong to the class of the coccid dyestuffs, indicating an insect origin for the dyes. Laccaic acid is the main colorant of the lac dye, originating from India. Traditionally extracted from the resin secreted by the lac insect, it was made into the pigment Indian Lake by precipitating it onto alumina trihydroxide or used to dye wool and silk, giving an intense red color, highly prized because it was very stable to light. Carminic acid is the main colorant of the traditional dye cochineal, derived from the dried bodies of insects living on the Nopal cactuses of Mexico and Central and South America. Carminic acid was processed by precipitation on aluminum or aluminum-tin substrate to make carmine lake; besides, brilliant scarlet or crimson colors were obtained when using cochineal to dye wool or silk with tin salts or alum respectively.[8]

Often when analyzing artwork, only extremely small sample sizes are available. Furthermore, the red dyestuffs are highly fluorescent making analysis by Raman spectroscopy challenging. Therefore it is imperative to optimize the experimental conditions in order to design the best protocol by which to detect these materials. The work herein presents several experiments designed to expose the optimal experimental conditions to detect red dyes with SERS. 6 nm AgIFs were e-beam deposited onto the dyestuffs and the effects induced by changing several factors including (1) excitation wavelength, (2) laser power and (3) spot size were studied. Moreover, as characterization of SERS active compounds is very fast and sensitive, we probed solutions of the analytes at different concentrations in order to assess the sensitivity of our method.

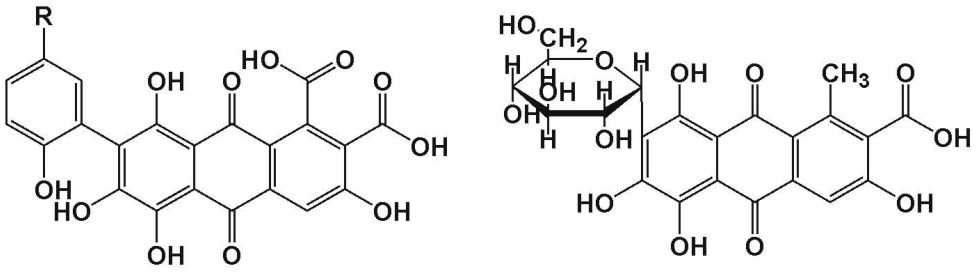

Figure 1. Molecules which contribute the red color in (A) lac dye and (2) cochineal. 5 structures of laccaic acid are present in lac dye: laccaic acid A (R = $(CH_2)_2NHCOCH_3$), laccaic acid B (R = $(CH_2)_2OH$), laccaic acid C (R = $CH_2CH(NH_2)COOH$), laccaic acid D = flavokermesic acid, and laccaic acid E (R = CH_2NH_2). Laccaic acid A is the most prominent structure in lac dye.

2. EXPERIMENTAL SECTION

2.1 Materials

Carminic acid was purchased from Aldrich (CAS # 1260-17-9), and re-crystallized from methanol. A reference sample of lac dye, made form Coccus Lacta secretion (36020) was obtained from Kremer pigments Inc and re-crystallized from methanol. Ag (99.99%) was acquired from D. F. Goldsmith (Evanston, IL). Borosilicate glass substrates, No. 2 Fisherbrand 18-mm circular coverslips were bought from Fisher Scientific (Pittsburg, PA) and KBr crystals were purchased from International Crystal Laboratories (Garfield, NJ).

2.2 Silver island Film fabrication

Solid sample pigments were mounted on KBr crystals by simply pressing the powders on the halide blocks (Figure 2A). Alternatively, the dyes were re-crystallized from methanolic

solutions on glass slides (Figure 2A). All mounted samples were then placed in a Kurt J. Lesker Axxis e-beam deposition system (Pittsburg, PA) with a base pressure of ~10^{-6} Torr and AgIFs of approximately 6 nm were fabricated on the sample surface (Figure 2C). The mass thickness and deposition rate (0.1 Å/sec) was monitored using a Sigma Instruments 6 MHz gold plated quartz crystal microbalance (Fort Collins, Colorado).

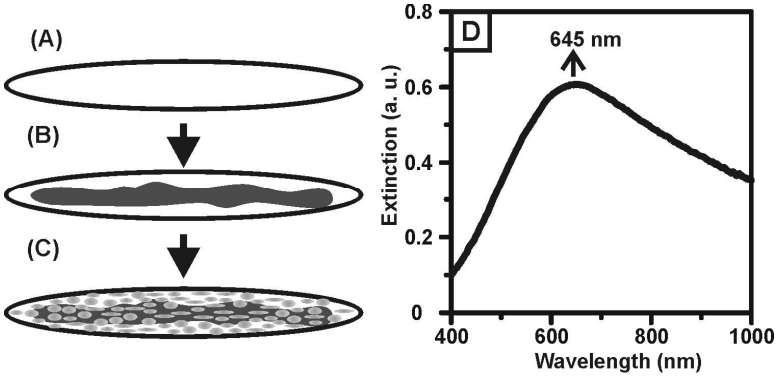

Figure 2. Schematic representation of the AgIF fabrication process which proceeds as follows: (A) clean glass or KBr substrate, (B) deposit dye solution or powder, and (C) e-beam deposit 6 nm AgIF. (D) Extinction spectrum of a typical 6 nm AgIF.

2.3 Ultraviolet-visible absorption spectroscopy

UV-vis absorption measurements were taken using an Ocean Optics (Dunedin, Fl) SD2000 fiber optically coupled with spectrometer CCD detector. All spectra of the methanolic solutions of the dyestuffs were collected with macroscopic measurements obtained from transmission mode utilizing unpolarized white light with a probe diameter of 4 mm. AgIF spectra were conducted in reflectance mode with a reflectance probe (Ocean Optics) coupled to the spectrometer. AgIF spectra were acquired against a mirror like Ag film on a glass substrate as a reference.

2.4 Surface-enhanced Raman spectroscopy

A Jobin Yvon Horiba Labram 300 confocal Raman microscope was used, equipped with Andor multichannel air cooled open electrode charge-coupled device detector (CCD: 1024x256), 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 (λ_0= 532.15 nm), He-Ne laser (λ_0=632.8 nm) and a solid state diode laser (λ_0=785.7 nm), were focused through 10x, 50x and 100x objectives on to the samples and Raman scattering was back collected through the same microscope objective. Power at the samples was controlled by interposing various neutral density filters in order to avoid any thermal and photochemical damage; the diameter of the confocal hole was set at 400 μm and collection times varied in the range of 10 to 900 seconds. Some of the spectra required post-processing involving spike removal and multi-point baseline subtraction.

3. Results and Discussion

3.1 Excitation wavelength optimization

The surface enhancement of the Raman signal depends on both the metal substrate particle size and the excitation wavelength employed. Moreover, in the case of Raman analysis of dyestuffs, two factors can contribute to the enhancement of the observed Raman signal: (1) when the excitation wavelength falls within the absorption spectrum of the compound under investigation this can give rise to the resonance Raman effect and (2) alternatively, surface enhancement is achieved by selecting an excitation wavelength that coincides with the absorption bands of the surface plasmon of the AgIF. In some cases, when both the analyte and the nanostructured noble metal substrate absorb in the same region of the spectrum where the excitation wavelength falls, the conditions of surface enhanced resonance Raman spectroscopy (SERRS) are met.

In order to evaluate the relative importance of the contribution of these two parameters, and therefore assess the most effective conditions for enhancing the weak Raman signal of the investigated red artists' dyestuffs, 3 excitation wavelengths (532 nm, 633 nm and 786 nm) were applied to AgIF coated samples. Both lac dye and carminic acid absorb visible light, thus presenting the possibility to observe resonance Raman scattering. Figure 3 depicts absorption spectra for both lac dye (3A) and carminic acid (3B) with λ_{max} = 493 nm and 496 nm respectively. Utilization of the 532 nm excitation wavelength should give rise to resonance Raman scattering. On the other hand, a typical 6 nm AgIF will have a LRPR with a λ_{max} of approximately 645 nm (Figure 2D). Applying an excitation wavelength of 633 nm will result in enhancement of the Raman spectrum by the SERS mechanism. Furthermore, because the plasmon of the AgIF is broad, exposure of the samples to an excitation wavelength of 785 may result in SERS as well.

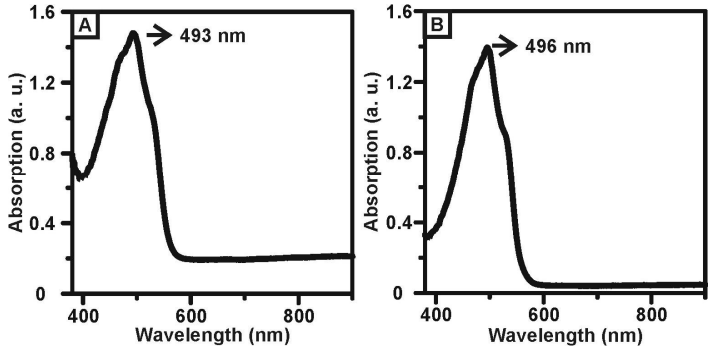

Figure 3. Absorption spectra of (A) lac dye (10 mg/ ml) and (B) synthetic carminic acid (2 X 10^{-2} M).

Figure 4 depicts spectra of both lac dye (4A) and carminic acid (4B) collected with the 3 excitation wavelengths: 532 nm, 633 nm, and 786 nm. Spectra of AgIF coated lac dye were obtained with both 532 nm (4-A1) and 633 nm (4A-2) excitation wavelengths. A 785 nm excitation wavelength was unsuccessful at recording a characteristic spectrum when applied to the lac dye. The spectrum obtained with the 633 nm excitation provides significantly more detail compared to the one collected with the 532 nm excitation, and the higher wavelength excitation source is also more effective at quenching the fluorescence of the sample. Although the 532 nm excitation produces an inferior spectrum comparatively, it is important to note that some different fundamental bands are present, as detailed in Table I. In particular, vibrational bands located at 454, 1014, 1102 and 1465 cm^{-1} appear in the spectrum collected with a 532 nm excitation and not in the spectrum collected with the 633 nm excitation; on the other hand, the peaks at 1055 and 407 cm^{-1} are observed independently of the excitation wavelength. This is understandable because, as it is well known, one can induce different molecular vibrations with different energies or excitation wavelengths.

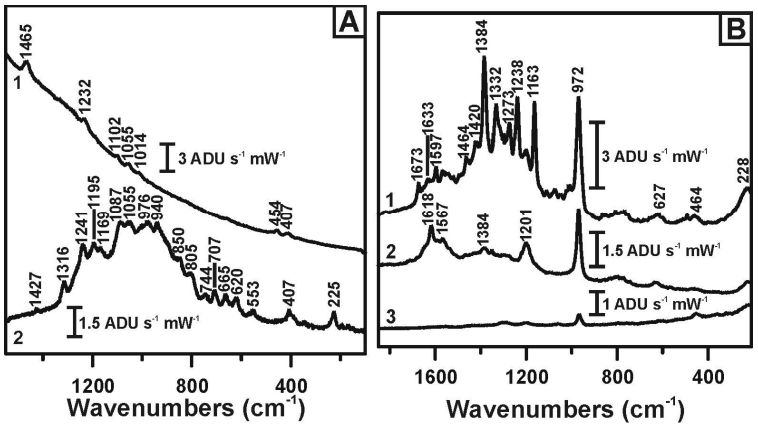

Figure 4. (A) SERS spectra of lac dye obtained with 2 excitation wavelengths: (1) 532 nm and (2) 633 nm. (A) SERS spectra of synthetic carminic acid obtained with 3 excitation wavelengths: (1) 633 nm, (2) 532 nm, and (3) 785 nm.

SERS spectra of carminic acid were collected with 532 nm (Figure 4B-2), 633 nm (Figure 4B-1), and 785 nm (Figure 4B-3) excitation wavelengths. Similarities and differences

between the 3 spectra can be observed and are detailed in Table I; however, it is particularly interesting to note that the bands at 228, 464, 617 and, most prominently, 972 cm^{-1} are observed in all spectra, while the region comprised between 1100 and 1700 cm^{-1} appears to be most affected by the choice of the excitation wavelength. Analysis of the recorded spectra leads us to conclude that the 633 nm excitation gives the most enhanced and detailed spectrum in conjunction with the silver island film substrate described, a reasonable result since SERS occurs when the excitation wavelength coincides with the LSPR of the noble metal SERS substrate.[7,14] We have thus demonstrated that the key parameter for enhancing the Raman signal from these samples (and, similarly, all other red dyestuffs used as artists' materials) lies in matching the excitation wavelength with the absorption band of the localized surface plasmon of the AgIF rather than with the absorption of the dyestuff investigated.

Table I. SERS/SERRS vibrational bands for Lac dye and carminic acid using different excitation wavelengths.

	Lac Dye		Carminic Acid		
Excitation Wavelength:	532 nm	633 nm	532 nm	633 nm	785 nm
		225	228	228	228
	407	407	464	464	464
	454			492	
		553	627	627	627
		620	972	972	972
		665		1007	
		707		1048	
		744		1070	
		805		1111	
		850		1163	
		940	1201	1201	1201
		976		1238	
	1014			1273	
	1055	1055			1296
		1087		1332	
	1102			1384	
		1169		1420	
		1195		1464	
	1232		1567	1567	
		1241		1597	
		1316	1618		
		1427		1633	
	1465			1673	

3.2 LASER power optimization

When collecting SERS spectra of organic dyestuffs containing several condensed rings, one should always keep in mind the possibility of photo-thermal oxidation of the sample. On the other hand, it is evident that employing higher laser powers at the sample can result in more intense signal for the same collection times. Therefore, we wanted to evaluate the effect of varying the laser power and spot size to determine optimal conditions of acquisition while still preserving the integrity of the sample. The experimental optimization was done by collecting spectra interposing neutral density filters of different optical density (reducing the output to 10%, 1%; 0.1% and 0.01% of the incoming laser light intensity) and varying the spot size by changing the microscope objectives employed. SERS spectra were collected from the same AgIF coated particle for each dye sample to ensure more consistent results. Figure 5 depicts SERS spectra collected with 633 nm excitation for both lac dye (5A) and carminic acid (5B) at several powers and with various microscope objectives. Spectra collected with the 100x objective and power of approximately 14 μW showed the most detail (Figure 5A-1 and 5B-1). Figures 5A-2 and 5B-2 depict spectra obtained with a 50x objective and power ~ 16 μW. While the spectra are similar to the ones collected with the 100x objective, some detail is lost. Although a larger area can be sampled with the 50x objective, the intensity of the measured signal is not significantly increased, while, on the other hand, some resolution is sacrificed. Finally, spectra collected with lower power of ~2 μW (Figures 5A-3 and 5B-3) or with the 10x objective (Figures 5A-4 and 5B-4) were poor in comparison. In summary, the optimal conditions to collect SERS spectra were found to be: highest possible magnification and a power at the sample of approximately 14 μW when using a 633 nm excitation wavelength.

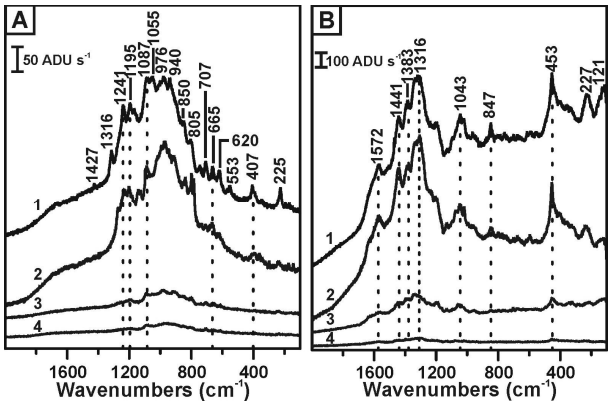

Figure 5: (A) SERS spectra of lac dye obtained with various LASER powers and microscope objectives: (1) 14.3 μW and 100X, (2) 16.2 μW and 50X, (3) 2.07 μW and 100X, and (4) 17.2 μW and 10X. (B) Synthetic carminic acid obtained with various LASER powers and microscope objectives: (1) 13.8 μW and 100X, (2) 16.9 μW and 50X, (3) 1.95 μW and 100X, and (4) 15.8 μW and 10X.

3.3 Detection capabilities of SERS with carminic acid

As a final step in this methodological study, we also wanted to determine the sensitivity of our SERS method, as in many cases material available for sampling from works of art is very

scarce. Although the method here proposed is a solid-state method, we wanted to determine what range of concentration of dyestuff could be detected with our technique. In order to do so, solutions of the dyestuffs were prepared by dissolution in methanol, at the following concentrations: 0.2 M, 0.1 M, 6.0 X 10^{-2}, 4.0 X 10^{-2}, 2.0 X 10^{-2}, 1.0 X 10^{-2}, and 2 X 10^{-3}. 2 μL of each solution of carminic acid in methanol was deposited on a glass slide and allowed to dry in ambient conditions. A 6 nm AgIF was then e-beam deposited on the diluted dyestuff. All concentrations tested allowed us to positively identify the dye. Figure 6 compares a SERS spectrum (6A) of the prepared sample derived from the solution with the lowest concentration (2 X 10^{-3} M) with a normal Raman spectrum of the undiluted carminic acid (6B). It is clear that a superior spectrum is obtained with the 2 X 10^{-3} M sample coated with the AgIF compared to the normal Raman spectrum of concentrated carminic acid. A sharp peak is consistently present at 972 cm^{-1} which can be utilized as a diagnostic band for the identification of carminic acid Furthermore because the SERS spectrum was obtained on one particle, the sample size could be extremely smaller than the one prepared for this work: further work is needed in this area to determine the absolute limit of detection of this specific method.

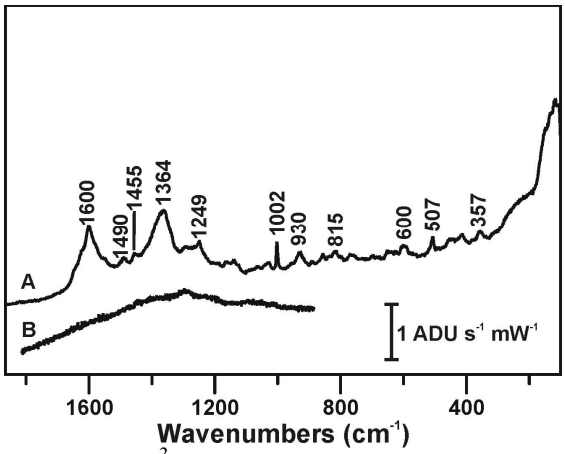

Figure6: (A) SERS spectra of 2 X 10^{-3} M synthetic carminic acid and (B) Raman spectra of powder of concentrated synthetic carminic acid.

4. CONCLUSIONS

A new sensing method has been described, which has important implications for the detection of laccaic acids and carminic acid, coloring matters of great significance in the study of artists materials. Our in-depth methodological investigation into the signal-enhancement achievable with various experimental conditions has revealed that with a 6 nm AgIF, the optimal excitation wavelength is 633 nm for both dyestuffs. Besides, use of a 100x objective and power at the sample of the order of 14 μW has been proved to give the most enhanced and detailed spectra. We have thus demonstrated that the key parameter for enhancing the Raman signal from these samples (and, similarly, most other red dyestuffs used as artists' materials) lies in matching the excitation wavelength with the absorption band of the surface plasmon of the AgIF rather

than with the absorption of the dyestuff investigated. Finally we have shown that this method has the capability to detect extremely small amounts of material (10^{-3} M).

5. ACKNOWLEDGEMNTS

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