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An innovative surface-enhanced Raman spectroscopy (SERS) method for the identification of six historical red lakes and dyestuffs

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Surface-enhanced Raman spectroscopy (SERS) was used in this work to obtain highly detailed spectra of artists' red lake pigments and colorants. In the past, Raman spectroscopy has been successfully employed to identify many pigments and modern synthetic dyes. Unfortunately, red lake pigments and dyes commonly employed in artistic production from antiquity to the mid-nineteenth century are often extremely fluorescent, making identification with Raman spectroscopy difficult or impossible. This work presents an innovative SERS technique that quenches fluorescence, significantly enhances the weak Raman scattering effect, and requires very little sample material and minimal sample handling. A silver island film (AgIF), approximately 6–8 nm thick, is deposited on the substrate by electron beam (e-beam) deposition. The SERS-active surface is then analyzed with a confocal dispersive Raman microscope, at an excitation wavelength of 632.8 nm. Reference materials including the synthetic dyestuffs alizarin, purpurin, and eosin, high-purity carminic acid, and historic red lake pigments such as madder lake, cochineal, brazilwood, lac lake, and kermes were studied. The proposed method has great potential for the unambiguous identification of red dyes applied in different media on a variety of substrates, as demonstrated by the highly detailed Raman spectra presented here.

KEYWORDS: alizarin; brazilwood; carminic acid; cochineal; eosin; kermes; lac lake; madder lake; surface-enhanced Raman spectroscopy; Ag island film

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, found in traditional red lake pigments and organic dyes of natural origin, are extremely fluorescent, an effect that dominates the weak optical process of Raman scattering. Furthermore, because only subnanogram levels of these dyes are needed to achieve intense coloration, normal Raman spectroscopy is often not sensitive enough to probe these materials. Red lakes were extremely prized for their transparent, yet intense, colors and are often found in paintings and illuminated manuscripts as thin glaze layers, usually characterized by a high proportion of oil medium. Lake pigments are obtained by precipitation of deeply colored dyestuffs onto inert, finely divided particles of alumina trihydrate or, alternatively, calcium carbonate or gypsum. The dyestuffs themselves have been used for dyeing textiles from the earliest times of human history, bound to the fibers through formation of a metal complex with metal salts (called mordants, most commonly alum – $Al_2(SO_4) \cdot K_2SO_4 \cdot 24H_2O$; iron sulfate – $FeSO_4 \cdot 7H_2O$; and tin chloride – $SnCl_2$). Previously, normal Raman spectroscopy has been successfully employed to characterize the historical red lake madder, which contains both the anthraquinone alizarin and purpurin.[6] FT-Raman spectroscopy has also been a successful technique to study madder as well as brazilwood.[3,7,8] However, no other red dyestuff has been successfully characterized owing to the combined effect of high fluorescence and the relatively small Raman scattering cross section of these compounds. The Raman cross section can be amplified by surface-enhanced Raman scattering (SERS). Furthermore, the use of a noble metal SERS substrate can quench fluorescence. SERS is a process whereby the Raman scattering signal is increased when

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a Raman-active molecule is spatially confined within the electromagnetic fields generated upon excitation of the localized surface plasmon resonance (LSPR) of nanostructured noble metal surfaces. The SERS signals of ensemble-averaged molecules demonstrate enhancements up to 8 orders of magnitude over the normal Raman signal.[9,10] A variety of SERS substrates have been successfully employed to detect and study analytes including: (1) citrate-reduced Ag colloids,[11–13] (2) porous Ag electrodes,[14] (3) Ag films over nanospheres (AgFON),[15,16] (4) nanosphere lithography (NSL) derived Ag nanoparticles,[9] and (5) Ag island films (AgIF).[17]

Guieneau and Guichard first explored SERS as a technique to study the fluorescing anthraquinone dyes in 1987.[14] The authors demonstrated significant S/N enhancement obtained by performing SERS measurements on a porous Ag electrode cooled with liquid nitrogen on synthetic alizarin and extracts from an eighth-century textile sample dyed with madder.[14] However, nearly 20 years passed before a significant body of study was published on SERS analysis of dyes, with most of the studies presented to date still focused on alizarin and purpurin.[11,12]

Citrate-reduced Ag colloids have been successfully employed as SERS substrates to detect alizarin and purpurin[11–13]; however, significant spectral changes have been described for alizarin and purpurin as functions of pH.[12] Because SERS is extremely distance dependent, where the molecule in question must be confined within the decay length of the electromagnetic fields (0–4 nm),[18] one must have molecules that can replace the citrate surfactant and bind to the Ag surface, which can prove challenging. Furthermore, the Ag colloids must be aggregated, which is difficult to reproduce and can have significant effects on the SERS spectra. An alternate SERS substrate is AgIF, which is highly reproducible and can be applied directly on single grains of pigment, as is typically found in works of art.

The work presented herein describes the use of electron beam (e-beam) deposited AgIFs on samples and successive analysis of the SERS-active surface with a confocal, dispersive Raman microscope. The work conducted allowed us to optimize several parameters of the analysis, including thickness of the AgIF, attenuation of laser power at the sample to limit thermal and photochemical damage, dimension of the total volume sampled (via reduction of the diameter of the confocal hole), and optimal laser excitation line (initially $\lambda_0 = 532$ nm; 632.8 nm and 785.7 nm were evaluated), thus achieving significant improvement in S/N and resolution of peaks over normal Raman spectra.

SERS spectra of complex molecules such as anthraquinoid or flavonoid dyes will show different band positions and selective enhancement of particular peaks compared to normal Raman or FT-Raman spectra. The band position shift may be due to the chemisorption of the molecule with the SERS surface. Because these dyes absorb visible light, there may be a resonant effect, which will provide further peak enhancement and will result in surface-enhanced resonance Raman scattering (SERRS). However, experimental work carried out by the authors and described elsewhere has demonstrated that the maximum enhancement of the Raman signal from these samples of red dyestuffs used as artists' materials occurs when the excitation wavelength matches the absorption band of the localized surface plasmon of the AgIF rather than the absorption of the dyestuff investigated.[19] Finally, the orientation of the molecule (perpendicular or parallel) with the SERS surface will result in selective enhancement of certain peaks. This renders interpretation of the spectra quite complex. Therefore, owing to a lack of published reference SERS spectra of traditional artists' dyes and colorants, we focused on red lake pigments and collected extremely detailed, reproducible spectra for 6 dyestuffs and selected lakes. In some cases, in order to support the interpretation of the spectra, single-molecule theoretical computation of vibrational normal modes were performed, confirming good correlation between the calculated and actual band positions.

Natural and early synthetic organic colorants

The materials presented herein were chosen because they are the most commonly encountered red lakes and dyes in artistic production from antiquity to the nineteenth century.[20] All these red natural organic compounds are complex structures that share a composition of condensed aromatic rings with hydroxyl, carbonyl, and carboxylic acid functions and various other substituents, including sugars (cochineal) and amino groups (lac) (Fig. 1).

Madder is one of the oldest and most utilized dyestuff, with ample geographical distribution, spanning the East and West cultures since antiquity. In Europe, it was extracted from the roots of the *Rubia Tinctorium L.* herb. When precipitating the dye on aluminum trihydrate, one obtains a pigment of orange-red hue. Purpurin ($C_{14}H_8O_5$, 1,2,4-trihydroxyanthraquinone, C.I. 58205) and alizarin ($C_{14}H_8O_4$, 1,2-dihydroxyanthraquinone, C.I. 58000) are the main coloring substances of the madder root. Synthetic alizarin was introduced into the market in 1871, and soon after the use of the natural root extract ceased. Among the coccid dyestuffs kermes, cochineal and lac dye were examined. When used as lake pigments, each produce different shades of blue-toned, intense scarlet crimson color. Kermes has been used since antiquity, and one of the earliest mentions of this material can be found in the Old Testament. Kermes and cochineal are obtained by water or alcohol extraction of the dried wingless female scale insects of two different species: *Kermes vermilio* Planch (kermes) and *Dactylopius coccus* L. Costa (cochineal) either cultivated or wild. Host plants for kermes are various types of oaks from Europe and Asia, while cochineal was collected from the Nopal cactus, in Mexico, and Central and South America.[21] Both were very expensive commodities owing to the laborious process of harvesting the insects. Imported to Europe since the 1540s, cochineal quickly supplanted kermes. Cochineal is still commercially

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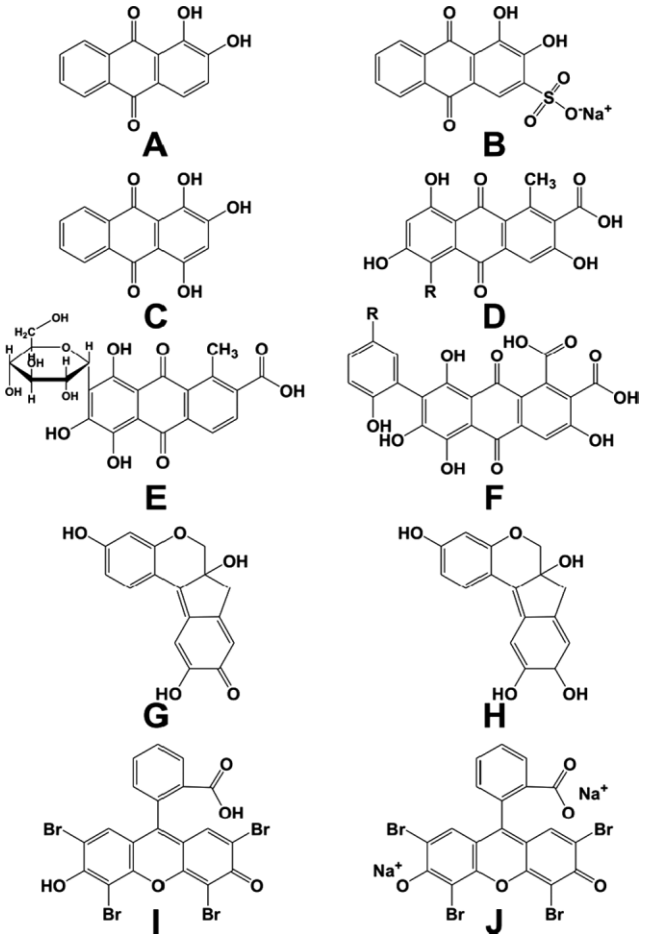

Figure 1. Natural and organic red organic compounds: (A) alizarin, (B) alizarin red S, (C) purpurin, (D) kermesic acid (R═OH); flavokermesic acid (R = H), (E) carminic acid, (F) 5 structures of laccaic acid present in lac dye: laccaic acid A (R = $(CH_2)_2NHCOCH_3$), laccaic acid B (R = $(CH_2)_2OH$), 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, (G) brazilein, (H) brazilin, (I) eosin Y free acid, (J) eosin Y.

available today; however, because of its scarce light-fastness is no longer found in paintings and is instead used in the cosmetic industry. The coloring matter of kermes and cochineal are kermesic acid ($C_{16}H_{10}O_8$, C.I. 75460) and carminic acid ($C_{22}H_{20}O_{13}$, C.I. 75470), respectively.

Laccifer lacca Kerr is another important scale insect, found on trees in India and southeast Asia, from which lac dye is extracted. Used in India from ancient times to dye silk, it is not widely mentioned in European recipes for textile dyeing in the fifteenth and sixteenth century, but instead became more popular in the nineteenth century. The use of lac dye as an artists' pigment began in the thirteenth century and enjoyed widespread and extensive use in fifteenth century Italy and onwards. Laccaic acids (C.I 75450) are the primary colorants in lac lake and are characterized by a hydroxyanthraquinone chemical structure associated with those of kermesic and carminic acid.

Brazilwood, a redwood from the tropical trees of the senna genus *Caesalpinia*, a native of Brazil, Nicaragua, and southeast Asia, was imported into Europe beginning in the 1500s. It was used to dye wool and silk and for manufacturing red inks. Although brazilwood was more economical than carmine, it appears to have been applied less frequently in paintings probably because it has a less satisfactory hue and poor color permanence. The red color's principal component is brazilin ($C_{16}H_{14}O_5$, C.I. 75280), readily oxidized by light and air exposure to brazilein ($C_{16}H_{13}O_5$).

Eosin Y is an early synthetic dye that was utilized among nineteenth century artists. The potassium or sodium salt of 2,4,5,7-tetrabromofluorescein was first made in 1871 and used for making inks. As a lake pigment, it makes a brilliant bluish red and was sold under the name of geranium lake. Van Gogh used the dye quite extensively, but unfortunately it has almost invariably faded in many of his paintings.

EXPERIMENTAL

Raman microspectroscopy

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); a holographic notch filter; and an 1800 grooves/mm dispersive grating.

The excitation lines of an air-cooled, frequency doubled, Nd : YAG solid state laser (λ_0 = 532.15 nm), and a He–Ne laser (λ_0 = 632.8 nm) were focused through a 100× objective on to the samples and Raman scattering was back-collected through the same microscope objective. Power at the samples was kept at 10 μW by interposing a neutral density filter, 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 s. Some of the spectra required postprocessing, involving spike removal and multipoint baseline subtraction.

Silver island film fabrication

Solid sample pigments were mounted on KBr crystals (International Crystal Laboratories) by simply pressing the powders on the halide blocks. Alternatively, the dyes were recrystallized from methanolic solutions on glass slides or aluminum-coated slides (Thermo Electron Scientific). All mounted samples were then placed in a Kurt J. Lesker Axxis e-beam deposition system (Pittsburgh, PA) with a base pressure of 10^{-6} Torr, and AGIFs of approximately 6–8 nm thickness were fabricated on the sample surface. The mass thickness and deposition rate (1 Å/s) were monitored using a Sigma Instruments 6-MHz, gold-plated QCM (Fort Collins, Colorado).

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Samples' suppliers and preparation

Eosin Y high purity (CAS# 17372-871), eosin Y free acid 90–95% purity (CAS# 15086-94-9), alizarin 97% (CAS# 72-48-0), and purpurin (CAS# 81-54-9) were all purchased from ACROS organics and used as received without further purification; carminic acid was purchased from Aldrich (CAS # 1260-17-9) and recrystallized from methanol. Samples of red extracts from brazilwood and lac dye, made from *Laccifer lacta* secretion, were purchased from Kremer pigments Inc (36020; indicated as extracted from *Coccus lacta* on the supplier's original label), together with the pigment alizarin Crimson dark PR83 (2361). Historical samples of pink madder dark (Newman), cochineal carmine lake (F. Weber) and the distilled water extract of Kermes scale insects were obtained from the Forbes collection of pigments. Comprising over 1600 specimens assembled in the 1930s by E.W. Forbes, former director of the Fogg Art Museum at Harvard University, Boston, this collection is widely used as a source of reference materials for museum research.[22]

Colorants were also extracted from wool fibers dyed with alizarin and alizarin red S (9,10-dihydro-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid, sodium salt; $C_{14}H_7NaO_7S$ C.I. 58005; sodium alizarinsulphonate), and madder.

Extraction of colorants from dyed fibers

A solution of hydrochloric acid (Aldrich, reagent grade 37%)/distilled water/methanol (Aldrich) was prepared, with volume ratios of 2:1:1. A small segment of the dyed fiber, measuring approximately 3 mm, was placed in a conical vial and covered with 0.1 ml of the solution. The vial was then positioned in a hot water bath and after approximately 10 min, the extract from the fiber was pipetted onto a glass or aluminum-coated slide and let dry in air.[23]

Calculations

All geometry optimization and theoretical calculations of normal vibrational modes were conducted with Gaussian 2003 edition.[24] The B3LYP/6-311G^{++} (d,2p) basis set of the DFT method was used to calculate the normal vibrational modes for alizarin, purpurin, and brazilein. The B3LYP/6-311G(d) basis set of the DFT method was used to calculate the normal vibrational modes for brazilin and kermesic acid.

RESULTS AND DISCUSSION

In this work, all the materials examined have been grouped according to their common origin and are discussed separately. Tables are presented for each compound, reporting the wavenumbers of the observed bands in each SERS spectrum and normal Raman spectrum (when applicable). The tables also contain a comparison of the experimental values, with values found in the literature and calculated bands (when available). Attribution for the vibrational modes are also proposed, derived from the literature. Bands observed that are considered most suitable as markers for unambiguous identification of the individual colorants have also been indicated.

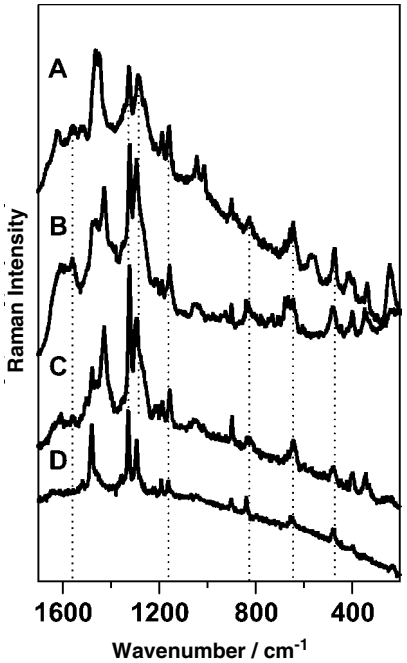

Figure 2. SERS spectrum of synthetic alizarin (A) excited with 632.8 nm laser, and spectra of specialty artists' pigments pink madder dark (B) and alizarin crimson dark (C). The normal Raman spectrum of pink madder dark is denoted by D.

Madder dyestuffs and synthetic derivatives (alizarin, purpurin, pink madder dark, alizarin crimson, and alizarin red S)

As previously mentioned, both the normal Raman spectra[6] and SERS spectra of solutions of red alizarin dye have been published[11–14]: our results are in good agreement with the earlier published data. Slight variations in the intensity ratios of selected bands with the synthetic compound compared to actual pigment powders were observed. A SERS spectrum of synthetic alizarin was obtained with the 632.8 nm excitation (Fig. 2(A)). While some residual fluorescence occurs, the peaks due to the dye are very intense and sharp. Vibrational bands were observed at 243, 339, 396, 414, 473, 564, 645, 826, 900, 1013, 1042, 1157, 1186, 1284, 1323, 1448, 1461, 1556, and 1623 cm^{-1} (Table 1). The usefulness and ready applicability of the reference SERS spectrum of the pure dye is demonstrated by successful examination of the lake pigments from specialist pigment suppliers including those of pink madder dark (Fig. 2(B) and (D)) and alizarin crimson dark (Fig. 2(C) and can be compared with the normal Raman spectrum of pink madder dark (Fig. 2(D)). Significant enhancement is observed, as well as more detailed spectra. In particular, the normal Raman spectrum of the powder, acquired on the historic pigment pink madder dark, shows a more significant fluorescence background, and fewer bands

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Table 1. Principal experimental and calculated Raman vibrations, and previously published SERS spectra of synthetic Alizarin

SERS (cm^{-1})	Assignments[11]	Published bands[11] (cm^{-1})	Calculated (cm^{-1})
243m			243
339w	Skeletal vibration	336vw (Normal Raman – alkaline)	340
396	Skeletal vibration	398m (SERS pH 11.8)	382
414w	Skeletal vibration	417vw (Normal Raman – alkaline)	414
*473m	Skeletal vibration	473vw (Normal Raman – alkaline)	475
564w,br	Skeletal vibration	577w (SERS pH 11.8)	563
645m,br			651
826w	γ(C–H)/γ(C–O)	827vw (pH 5.5)	821
*900w	γ(C–H)	901vw (Normal Raman – alkaline)	890
1013m	υ(CC)/δ(CCC)	1011w (Normal Raman – solid)	1016
1042m	δ(CCC)	1044s (Normal Raman – alkaline)	1033
*1157s	υ(CC)/δ(CH)	1157w (SERS pH 11.8)	1151
1186s	υ(CC)/δ(CH)/δ(CCC)	1185m (SERS pH 11.8)	1177
1284s	Y(CO)/υ(CC)/δ(CCC)	1282s (Normal Raman – alkaline)	1278
1323s	Y(CC)	1323m (Normal Raman – alkaline)	1317
1448s,br	υ(CO)/δ(COH)/δ(CH)	1451 (SERS pH 5.5)	1452
1461s,br	υ(CO)/υ(CC)/δ(CH)	1461vs (Normal Raman – alkaline)	1461
1558w,br	υ(CC)		1560
1623w,br	υ(C═O)	1624s (Normal Raman – alkaline)	1626

vw: very weak; w: weak; m: medium; s: strong; vs: very strong; sh: shoulder; br: broad; υ: stretching; δ: in-plane bending; γ: out-of-plane bending. Bands marked with an asterisk are suggested as markers for unambiguous identification of alizarin red dye in an unknown sample. Vibrational assignments were obtained from Cañamares *et al.* where the authors utilized Ag colloids to collect SERS spectra of alizarin

are observed at 239vw, 401vw, 479w, 659w, 860w, 901w, 1160w, 1190w, 1219vw, 1292m, 1326vs, 1355w, 1679s and 1517w cm^{-1}. The identification of the colorant of a wool fiber dyed with Alizarin red S was also obtained with SERS (Fig. 3), which demonstrates that AgIFs have the potential to be utilized in investigations on works of art in the future. Furthermore, all spectra recorded show good agreement with the reference spectrum of alizarin.

A highly reproducible SERS spectrum of purpurin is shown in Fig. 4(A). Substantial improvement of S/N with respect to the spectrum recorded with normal Raman spectroscopy was achieved (Fig. 4(B)). The fluorescence background is virtually completely quenched by using the excitation line 632.8 nm. SERS spectra of purpurin in solution of aggregated, citrate-reduced silver colloids have been reported recently, showing substantial pH dependence.[12] Our results compare well with data reported for purpurin at pH 6.2, i.e. when the dye is dissociated in the monoanionic form. This may be due to the fact that in the powder inter- and intramolecular interactions of the carbonyl and phenol groups occur.

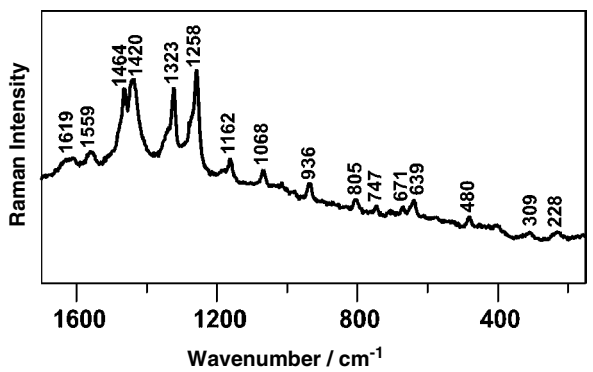

Figure 3. SERS spectrum of the colorant extract from a sample wool fiber dyed with alizarin red S and deposited on an aluminum-coated slide, excited with 632.8 nm (accumulation time: 180 s).

The calculated Raman bands and observed SERS bands are shown in Table 2. On the basis of the vibrational data collected, we can distinguish between alizarin and purpurin by the presence of the marker bands centered at 243, 473 and 1186 cm^{-1} (alizarin) and 971, 1063, 1401 and 1606 cm^{-1} (purpurin). In order to differentiate these two dyes from all other red dyestuffs discussed in this paper, we propose to use bands at 473, 900, and 1157 cm^{-1} for alizarin and 366, 606, 970, 1020 (also observed for carminic acid at 1021), and 1401 cm^{-1} for purpurin.

Coccid dyestuffs (kermes, cochineal, and lac dye)

SERS spectra of kermes were taken for the distilled water extracts of the kermes scale insects (Fig. 5(A)). It is important to note the dramatic increase in S/N with respect to the normal Raman spectrum, both experimentally observed in

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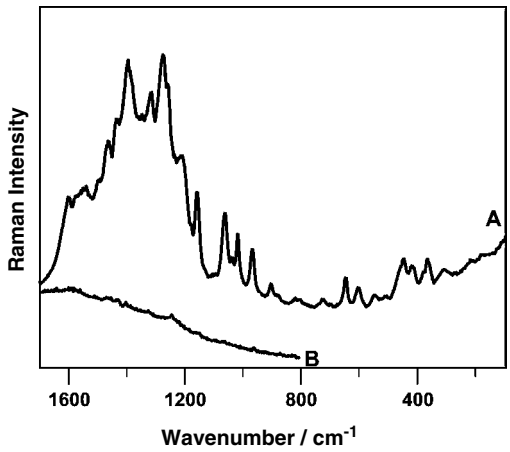

Figure 4. SERS spectrum (A) and the normal Raman spectrum (B) of synthetic purpurin, excited with 632.8 nm (accumulation time: 60 s, for two cycles).

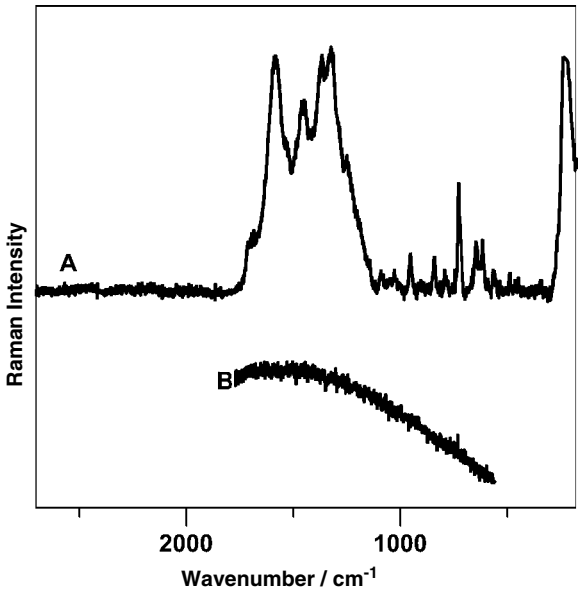

Figure 5. SERS spectrum(A) recorded on distilled water extract from kermes scale insects, successively deposited on an aluminum-coated slide, excited with 632.8 nm (accumulation time: 60 s, after multipoint baseline correction), and the normal Raman spectrum (B).

this work (Fig. 5(B)) and previously published normal[25] and FT-Raman spectra.[3] The spectra collected correlate well with calculated bands (Table 3).

The 632.8 nm excited SERS spectrum for carminic acid (Fig. 6(A)) is compared with the normal Raman spectrum in Fig. 6(B). The SERS spectrum depicts vibrational bands at 122, 225, 454, 756, 847, 962, 1021, 1043, 1196, 1319, 1380, 1441, and 1570 cm^{-1}, while the normal Raman spectrum provides little vibrational information. A normal Raman spectrum of carminic acid has been published,[3] with excitation line of

Table 2. Principal experimental and calculated Raman vibrations, and previously published SERS spectra of synthetic purpurin

SERS (cm^{-1})	Published (SERS, pH 6.2)[12] (cm^{-1})	Calculated (cm^{-1})
313vw	309	334
*366m	364	356
422m	425	421
451m	447	445
513vw	512	519
549w	551	579
606m	603	622
648m	647	644
728w	737	721
806w	–	810
824w	–	823
905w	900	890
*970s	968	975
*1020m	1015	1015
1040w	–	1047
1064s	1068	1079
1160s	1153	1162
1213m	–	1224
1277s	–	1271
1319s	1326	1318
*1401s,br	1407	1415
1439vw,sh	1435	1438
1468s	1471	1463
1545w,br	–	1561
1606m	1616	1597

vw: very weak; w: weak; m: medium; s: strong; vs: very strong; sh: shoulder; br: broad; υ: stretching; δ: in-plane bending; γ: out-of-plane bending. Bands marked with an asterisk are suggested as markers for unambiguous identification of purpurin in an unknown sample.

780 nm and bands at 465w, 474w, 1108w, 1257 m, 1314 s, 1440 m, 1489 m, 1592 m, and 1645 m cm^{-1}. Despite the excellent quality of the spectrum of carminic acid, attempts at recording a good spectrum of the historical pigment cochineal only resulted in a spectrum still dominated by high fluorescence and with poorly resolved bands (Fig. 7). Nonetheless, the spectrum shows a drastic improvement compared to the normal Raman spectrum observed in our experiment and shows a characteristic profile that is believed to provide sufficient indications for the identification of unknowns.

Figure 8 depicts both a normal Raman spectrum (B) and SERS spectrum (A) of the lac dye. While an adequate normal Raman spectrum of lac dye is obtainable, the SERS spectrum provides enhanced detail, especially in the wavenumber range 1200–1600 cm^{-1}. Low fluorescence levels were observed and the spectra were easily reproducible, with

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Table 3. Principal experimental bands observed for kermes scale insects and their water extract, compared with calculated Raman vibrations of kermesic acid

Calculated (cm^{-1})	SERS (cm^{-1})
244	240vs
372	–
410	–
479	–
499	–
539	–
556	564vw
576	–
597	615m
627	–
653	646m
710	–
736	729s
760	–
785	794vw
811	–
863	842m
868	–
922	–
940	953m
1013	–
1028	1029w
1085	1091w
1139	–
1168	–
1205	–
1317	1330vs
1391	1371vs
1448	–
1458	1457vs
1484	–
1603	1590vs
1672	1703sh

vw: very weak; w: weak; m: medium; s: strong; vs: very strong; sh: shoulder; br: broad.

observed bands at 408w, 452m, 655w, 1059m, 1100m, 1185m, sh, 1232m, sh, 1281s, 1356m, 1386m, 1425m, 1461s, 1518m, and 1579s cm^{-1}. On the basis of our observation, we propose that bands at 452 (keeping in mind that carminic acid has a band at 454 cm^{-1} and purpurin at 451 cm^{-1}), 1059, 1100, 1281 and 1579 cm^{-1} can be used for unambiguous identification of this colorant in unknown artifacts.

Redwoods (brazilwood)

The red extract of brazilwood has been analyzed with both SERS and normal Raman (Fig. 9) and compared with theoretical calculations of both brazilin and brazilein

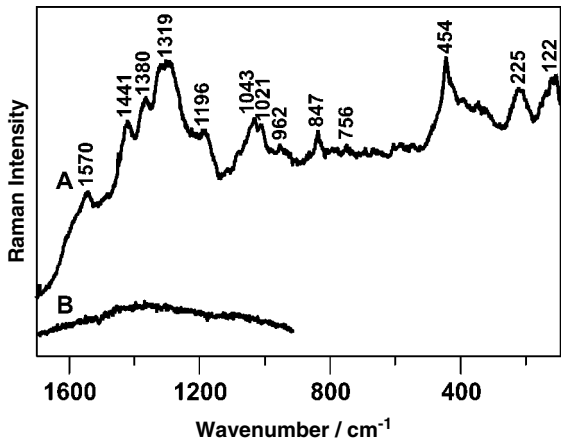

Figure 6. SERS spectrum (A) and the normal Raman spectrum (B) of high purity carminic acid, excited with 632.8 nm (accumulation time: 60 s for two cycles).

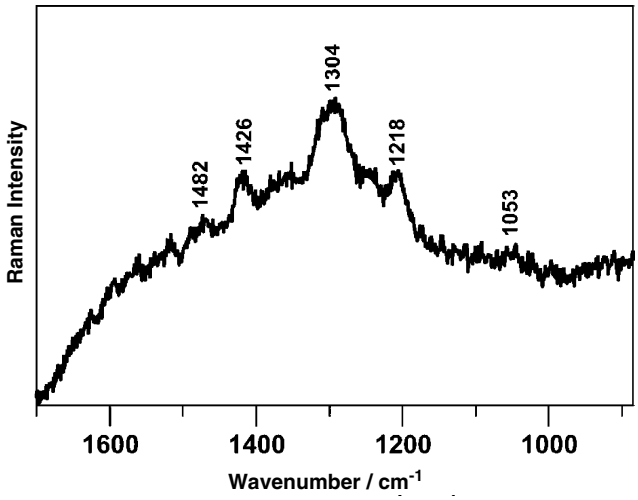

Figure 7. SERS spectrum of cochineal lake excited with 632.8 nm (accumulation time: 60 s for two cycles).

(Table 4). SERS spectra were recorded with two excitation wavelengths (532 in Fig. 9(A) and 632.8 nm in (Fig. 9(B)) on the same dyestuff particle and important variations in the vibrational modes were observed. Similarly, it was possible to record a normal Raman spectrum (Fig. 9(C)) of the dye, but this presented some differences with the SERS spectra, in particular, with bands at 886m, 919m, 1233s, 1281s, and 1553w cm^{-1} that are not evident in the 632.8 nm excited SERS spectrum (Fig. 9(B)) and variation in intensity of bands centered at 993w, 1135w, 1204m, br, and 1398s cm^{-1}. It is important to note that observation of the dye powder under a stereomicroscope in visible light showed a mixture of bright ruby-red particles, transparent particles, and dark grains, and therefore it is possible that different colors (and different spectra) account for the mixture of redwood dyestuffs (different molecular formulas that constitute this colorant). In fact, some of the spectra presented a combination of the bands

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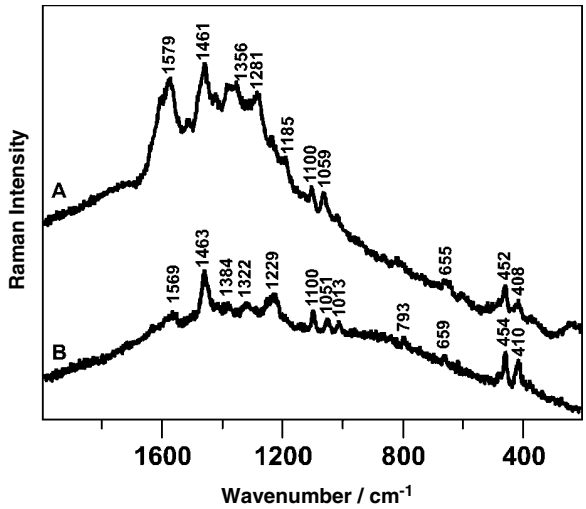

Figure 8. SERS spectrum of the lac dye (A), excited with 632.8 nm, compared with normal Raman spectrum of the same material (B) (632.8 nm excitation, accumulation time: 120 s for eight cycles).

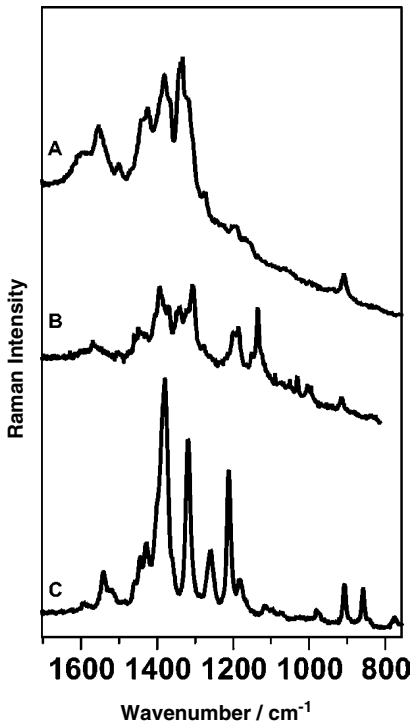

Figure 9. SERS spectra of brazilwood excited with 632.8 nm (accumulation time: 60 s for three cycles) (A) and 532 nm (accumulation time: 60 s for three cycles) (B) nm, compared with normal Raman spectrum of the same material (C) excited with 632.8 nm (accumulation time: 120 s for eight cycles).

shown in Fig. 9 for the normal and SERS spectrum, possibly supporting this hypothesis. Finally, both the experimental SERS and normal Raman spectra are in good agreement with the calculated vibrational bands (Table 4).

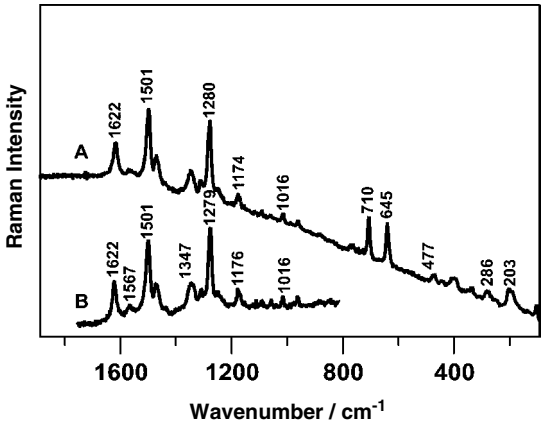

Figure 10. Normal Raman spectrum of synthetic eosin Y of high purity in its disodium salt form (A) (accumulation time: 80 s twice), compared with the SERS spectrum (B), excited by 632.8 nm, of the same material (accumulation time: 300 s for three cycles).

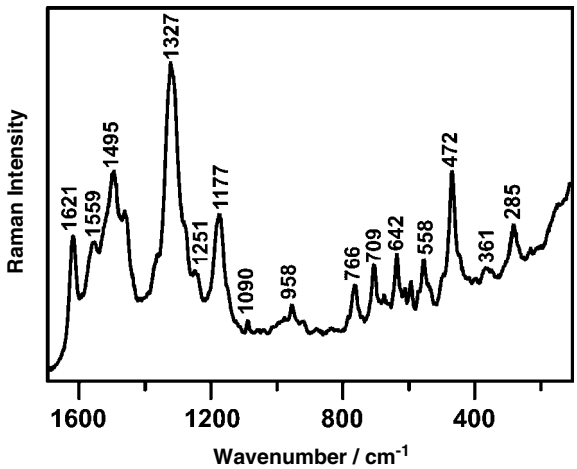

Figure 11. SERS spectrum of synthetic eosin Y free acid (632.8 nm excitation, accumulation time: 90 s for two cycles).

Early synthetic dyes (eosin Y)

The disodium salt of this dye has a deep red color and showed an intense normal Raman spectrum (Fig. 10(A)) on a slightly sloping fluorescence background. Fabrication of AgIF on the synthetic dye caused total elimination of this residual fluorescence (Fig. 10(B)). An excitation wavelength of 632.8 nm was used to obtain a normal Raman spectrum and an SERS spectrum. The normal Raman spectrum had the following vibrational bands: 109w, 203w, 286,w, 343w, 407w, 448 vw, 477w, 645m, 710m, 772w, 963w, 1016w, 1090, 1174w, 1253w, sh, 1280vs, 1314w, 1349br, m, 1474m, 1501vs 1570w, 1620m cm^{-1} (Fig. 10(A)). The SERS spectrum correlated well with the normal Raman spectrum with bands located at 962w, 1016w, 1059w, 1091w, 1176w, 1251w, sh, 1279vs, 1312w, 1347m, 1473m, 1501vs, 1567w, and 1622m cm^{-1} (Fig. 10(B)). Surprisingly, no normal Raman spectrum could

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Table 4. Principal experimental (at two excitation wavelengths), calculated Raman vibrations, and previously published SERS spectra of brazilwood

Normal Raman (cm^{-1})	SERS 633 (cm^{-1})	SERS 532 (cm^{-1})	Assignments[7,8]	Published (FT-Raman)[7,8] (cm^{-1})	Brazilin calculations (cm^{-1})	Brazilein calculations (cm^{-1})
777w	–	–	γ(CO) + γ(CH)	767w	775	–
866m	–	–	–	–	865	866
–	913	910m	–	–	902	–
919m	–	–	–	–	–	923
993w	–	–	υ(C–C) + υ(C–O)	990vw	981	–
–	1002m	–	–	–	1010	1009
–	1030m	–	Ring stretching	1032	–	1045
1092vw	–	–	δ (C–H) i.p. wood cellulose	1092w	–	–
1115vw	–	–	δ (C–H) i.p. wood cellulose	1115w	–	–
1135vw	1134vs	–	–	–	1133	–
–	1189s	–	–	–	1191	–
1204m	1200s	1198br	δ (C–C)	1203w	1200	–
1233vs	–	–	υ(C–O) + υ(C–C)	1230sh	1235	1231
1281s	1280sh	1278sh	–	–	1279	1276
–	1307vs	–	–	–	1311	1302
1339vs	1340m	1339s	–	–	1334	–
–	1370sh	–	–	1377	1372	1374
–	–	1383s	–	–	1348	–
1398vs	1393s,br	–	–	–	1411	–
–	–	1427s	–	–	–	1429
1445m	1449m	1441sh	–	1443w	1438	1436
1461m	1460w	–	–	–	1465	1455
1475sh,br	–	–	–	–	–	1478
–	1505vw	1503w	–	1502[b]	1490	–
1553m	–	1554	–	1550w	–	1548
–	1567s	–	υ (C=C)	1564s	1562	–
1600w	1598vs	–	υ(C–C)	1600s	–	1599

vw: very weak; w: weak; m: medium; s: strong; vs: very strong; sh: shoulder; br: broad; υ: stretching; δ: in-plane bending; γ: out-of-plane bending.

be acquired on the yellow powder of the free acid of 2,4,5,7-tetrabromofluorescein, while an enormous enhancement was achieved with SERS. Bands were observed at 285m, 361w, 472s, 558m, 642m, 709m, 766m, 926vw, 958w, 1090w, 1177s, 1251sh, w, 1327vs, 1462 sh, m, 1495s, 1559 sh, m, 1621s cm^{-1} (Fig. 11), which correlate well with previously published SERS results (288s, 473s, 619w, 639w, 709w, 1177s, 1235w, 1281w, 1333vs, 1457w, 1501s, 1563w, 1617s cm^{-1}).[26] We therefore propose to use bands at 203, 645, 710, 1280, 1501 cm^{-1} to identify eosin Y disodium salt, and 285, 472 (also present in the spectrum of alizarin) 642, 709, 1177, 1327, and 1495 cm^{-1} to identify the free acid.

CONCLUSIONS

In this paper, we have described an innovative SERS technique based on e-beam deposition of AgIFs for the analysis of organic red colorants. On the basis of the spectral evidence presented here, it seems possible to differentiate the most common historical artists' red lake pigments and dyestuffs, given the highly detailed SERS spectra obtained. The work presented here may constitute a first step in the direction of enabling researchers to identify unknown pigments on historical artifacts without further sample manipulation such as dye extraction. However, when analyzing actual paint layers, unknown aspects such as sample preparation, influence of other components (mineral pigments and fluorescent organic compounds such as varnishes and binding media), and possible use of mixtures of red lakes in a single brushstroke or adjacent layers are to be taken into account and may cause interference in the analysis. A certain amount of success has been obtained by the authors in analyzing woolen fibers dyed with alizarin and alizarin red S; however, the experiments underscored the necessity of developing finely tuned, less aggressive extraction procedures than those currently in use

for high-performance liquid chromatographic analysis of these materials (HPLC). In fact, SERS is a surface method and is, therefore, highly sensitive to impurities and matrix effects due to components of the degraded proteinaceous structure of the wool that are present in higher proportion than the colorant of interest.

Other areas of future work may involve the expansion of the range of examined dyestuffs to the natural yellow and green dyes, and the study of the effects on the SERS spectra recorded of precipitation of the different dyestuffs on a variety of inorganic substrates.

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Suppliers

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REFERENCES

1. Guineau B. *Stud. Conserv.* 1984; **29**(1): 35.
2. Bell IM, Clark RJH, Gibbs PJ. *Spectrochim. Acta, Part A* 1997; **53A**(12): 2159.
3. Burgio L, Clark RJH. *Spectrochim. Acta, Part A* 2001; **57A**(7): 1491.
4. Smith GD, Clark RJH. *Rev. Conserv.* 2002; **2**: 92.
5. Vandenabeele P, Moens L, Edwards HGM, Dams R. *J. Raman Spectrosc.* 2000; **31**(6): 509.
6. Bussotti L, Castellucci E, Matteini M. *Sci. Tech. Cult. Heritage* 1996; **5**: 13.
7. de Oliveira LFC, Edwards HGM, Velozo ES, Nesbitt M. *Vib. Spectrosc.* 2002; **28**(2): 243.
8. Edwards HGM, de Oliveira LFC, Nesbitt M. *Analyst (Cambridge, U.K.)* 2003; **128**(1): 82.
9. Haynes CL, Van Duyne RP. *J. Phys. Chem. B* 2003; **107**(30): 7426.
10. Haynes CL, Yonzon CR, Zhang X, Van Duyne RP. *J. Raman Spectrosc.* 2005; **36**(6/7): 471.
11. Cañamares MV, Garcia-Ramos JV, Domingo C, Sanchez-Cortes S. *J. Raman Spectrosc.* 2004; **35**(11): 921.
12. Shadi IT, Chowdhry BZ, Snowden MJ, Withnall R. *J. Raman Spectrosc.* 2004; **35**(8/9): 800.
13. Leona M. In *Proceedings of the 6th Infrared and Raman Users Group Conference*. Florence, Italy, 2006; 105.
14. Guineau B, Guichard V. *ICOM Committe for Conservation: 8th Triennial Meeting, Sydney, Australia, 6–11 September, 1987*, Preprints, vol. 2. The Getty Conservation Institute: Marina del Rey, CA, 1987; 659.
15. Stuart DA, Yonzon CR, Zhang X, Lyandres O, Shah NC, Glucksberg MR, Walsh JT, Van Duyne RP. *Anal. Chem.* 2005; **77**(13): 4013.
16. Zhang X, Young Matthew A, Lyandres O, Van Duyne Richard P. *J. Am. Chem. Soc.* 2005; **127**(12): 4484.
17. Van Duyne RP, Hulteen JC, Treichel DA. *J. Chem. Phys.* 1993; **99**(3): 2101.
18. Shafer-Peltier KE, Haynes CL, Glucksberg MR, VanDuyne RP. *J. Am. Chem. Soc.* 2003; **125**(2): 588.
19. Whitney AV, Van Duyne Richard P, Casadio F. *Proc. SPIE* 2005; **5993**: 117.
20. Kirby J, White R. *National Gallery Technical Bulletin* 1996; **17**: 56.
21. Schweppe H, Roosen-Runge H. *Artists' Pigments. A Handbook of their History and Characteristics*, vol. 1. Oxford University Press: New York, 1996; 255.
22. Gary CW, Omecinsky D. *J. Am. Inst. Conserv.* 1983; **22**: 68.
23. Hofenk de Graaff JH. *The Colorful Past; Origins, Chemistry and Identification of Natural Dyestuffs*. Abegg-Stiftung and Archetype Publications Ltd.: Riggisberg, Switzerland, London, 2004; 40.
24. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA Jr, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA. *Gaussian 03, Revision C.02*. Gaussian, Inc: Wallingford, CT, 2004.
25. Clark RJ, Huxley K. *Sci. Tech. Cult. Heritage* 1996; **5**(2): 98.
26. Narayanan VA, Stoke DL, Vo-Dinh T. *J. Raman Spectrosc.* 1994; **25**(6): 415.

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