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New advancements in SERS dye detection using interfaced SEM and Raman spectromicroscopy (μRS)

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In the past decade Surface Enhanced Raman Scattering (SERS) has emerged as a powerful technique for the analysis of artistic, historical and archaeological material culture. However, the identification of organic compounds in complex samples using SERS can be challenging owing to the complexity in optimizing the adsorption of target analytes onto the plasmonic substrate and the difficulty to identify proper areas on the sample for robust SERS analysis using optical systems. Scanning electron microscopy (SEM) interfaced with Raman spectromicroscopy (μRS) provides an ideal hyphenated system to overcome the last challenge by: (1) evaluating the nanoparticles coverage/distribution on the sample and (2) locating suitable areas for successful and reproducible SERS analysis. In this paper we demonstrate the potential of a system interfacing SEM and μRS for single fiber, extractionless analysis in the characterization of dyes from reference collections and archaeological textiles. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: SERS; SEM; archaeological textiles; cochineal; alizarin

Introduction

The identification of dyestuff in ancient textiles provides a major analytical challenge. High-Performance Liquid Chromatography (HPLC) coupled with diode-array detection and/or mass spectrometry is the most common technique for the identification of dye molecules. However, this technique has limitations in the study of ancient dyed fibers because of: (1) its sample destructive protocol and (2) the relatively large amount of sample required.[1] Moreover, chemical extraction processes necessary for HPLC analysis can be detrimental to the size-limited and irreplaceable archaeological materials.

In recent years Raman spectromicroscopy (μRS) has emerged as a powerful alternative technique for the analysis of artistic, historical and archaeological material culture. μRS is a powerful tool for chemical and phase characterization. It is a non-destructive vibrational technique probing the molecular structure with micron resolution, and it therefore requires only a minute sample or can be used to analyze whole objects, without need of taking a sample. Owing to its non-destructive nature, it can be used in combination with other techniques, making the same sample reusable for subsequent analyses after μRS has been performed.

A versatile non-destructive technique enabling *in situ* morphological characterization, elemental identification and structural analysis integrates μRS with scanning electron microscopy (SEM) at variable pressure (VP) and energy dispersive X-ray spectroscopy (EDS) in the 'hyphenated' SEM–EDS–μRS system. Introduced for the first time in the late 80s,[2,3] this system takes full advantage of the high spatial resolution offered by the SEM for non-destructive *in situ* nanoscale morphological and topographic mapping, and the atomic and molecular micro-probing of the EDS and μRS for spatially resolved elemental characterization and structural analysis of materials on the same platform. These capabilities exceed what can be achieved when Raman spectrometer is coupled with an optical microscope and when each instrument is used independently. The laser power for the μRS (transferred through a fiber optic cable) is, however, significantly reduced in the SEM configuration and reduces the Raman response generated by the samples. Furthermore, Raman analysis can also be frustrated by naturally fluorescent organic materials, or by fluorophores that have become incorporated into artifacts from handling, burial or other processes. To address the loss in laser intensity and circumvent fluorescence with an increase of the signal-to-noise ratio, Surface Enhanced Raman Scattering (SERS), a surface-sensitive technique, can be employed *in situ* within the SEM chamber for the characterization of materials. SERS occurs by molecules adsorbed on, or in close proximity to, the surface of certain nanostructured metal (normally

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silver or gold) substrates[4–6] including microfluidic plasmonic platforms.[7–9] It has been shown that the SERS signal exhibits up to eight orders of magnitude enhancement over the normal Raman signal.[10] One of the problems of SERS is that the high enhancement factor is not always reproducible. It was noted that for the identification of organic compounds in complex samples SERS is challenging owing to the difficulty of optimizing the adsorption of target analytes onto the plasmonic substrate because it is a process which depends on the surface chemistry of the substrate and on the properties of the target molecule.[11]

There are only few published works dedicated to direct on-the-fiber SERS analysis of dyes without the need of extraction or hydrolysis pre-treatments.[12–14] In these studies SERS was used on traditional platform for μRS-optical systems. The main predicament for on-the-fiber application lies on the difficulty of obtaining a uniform, thin and well adhered to the fiber coating of silver nanoparticles and identifying the best area to perform the measurement. VPSEM interfaced with a μRS provides an ideal hyphenated system to overcome this challenge by: (1) evaluating the nanoparticles coverage/distribution on the fiber and (2) locating suitable areas for successful and reproducible SERS analysis. In our paper we demonstrate the potential of a system interfacing SEM and μRS for single fiber extractionless analysis in the characterization of dyes from reference collections and archaeological textiles.

Experimental procedure

Materials and method

In this research we have tested reference cochineal and alizarin solutions and two different dyed fiber samples to show the ability of this method to distinguish between different dyestuff molecules when present at very small concentrations. The first sample is an alpaca fiber from the M. Saltzman collection (and now at the Cotsen Institute of Archaeology, University of California Los Angeles) dyed with cochineal without any mordant. Cochineal is the name used for the coccid dyestuff and the scale insect (*Dactylopius coccus Costa*) from Mexico and South America, from which the crimson-colored natural dye carmine is derived.[15] The main coloring matter in the cochineal dyestuff is carminic acid (94–98%).[15] This dye was used in Central and South America since antiquity for coloring fabrics and became an important export good, during the colonial period. With the introduction of synthetic dyes in the late nineteenth century, the natural-dye production was gradually diminished. The second sample is a wool fiber obtained from H. Schweppe collection (of the Getty Conservation Institute) dyed with 'Indian madder' obtained from the chay roots (*Rubia* species). Madder dyestuffs are red colorants with main coloring matters alizarin and purpurin. Also present together with alizarin and purpurin are the anthraquinones: xanthopurpurin, pseudopurpurin, rubiadin and munjistin.[15] Indian madder is distinguishable from other madder species in that the main coloring matter is alizarin, while purpurin is absent in the extracts of this species.[15,16] This wool fiber was mordanted with 25% alum and 6% tartar.

For our non-destructive analysis using SERS for the detection of the fiber dyestuff, a small segment (about 5 mm) of a single fiber was treated with a silver colloid obtained following the protocol adapted from Lee and Meisel.[17] A silver colloid was prepared by boiling a solution of $AgNO_3$ and sodium citrate for 1 h under reflux. Prior to analysis, the colloid was concentrated to 20-fold. The fiber was mounted on a SEM stub using adhesive copper tape on either side to keep the fiber stretched. Silver colloid (0.5 μl) was deposited on a very small area of the fiber and mixed with 0.5 μl of 0.2 M KNO_3 to induce aggregation of the silver nanoparticles (AgNPs). After 2 min, the excess of the liquid was removed and dried leaving a thin coating of AgNPs aggregated on the fiber surface. The SEM evaluation of the dried colloid demonstrates NPs with size between 30 and 150 nm. The reference samples 0.1 mM aqueous solutions of pure alizarin and carminic acid have been purchased from SIGMA-ALDRICH. For the analysis of these samples, 1 μl of standard solution was deposited on an aluminized glass slide and mixed with 1 μl of the concentrated silver colloid and 1 μl of 0.2 M KNO_3. After 2 min, the excess of the liquid was removed and dried leaving a thin residue on the aluminized glass slide.

Instrumentation and analysis

All samples including the reference materials were first tested using the inVia (Renishaw) Raman spectrometer, and a 785-nm laser probe. The Raman spectrometer was equipped with a Leica microscope with a 50× long-distance objective. Spectra were recorded in extended mode in the range of 100 to 2500 cm^{-1}. To avoid thermal damage of the fibers, the laser power on the sample was maintained below 0.01 mW (most of the results were collected using 0.001 mW). For each measurement, an integration time of 10–30 s was selected. The number of scans was adjusted according to the SERS response of the different samples. Measurements were also performed on the fiber samples using the Nova 230 (FEI) variable pressure SEM, coupled with inVia system through the Structural and Chemical Analyzer (SCA) from Renishaw.

Results and discussion

Figure 1 shows the SEM and the respective optical microscope images of alpaca fiber dyed with cochineal with AgNPs deposition. The selection of areas to be analyzed using μRS is significantly improved using SEM rather than traditional optical microscopy because of the superior depth of field and resolution the SEM provides. The only disadvantage of the SEM images compare to the optical microscope is that it doesn't show the true color of

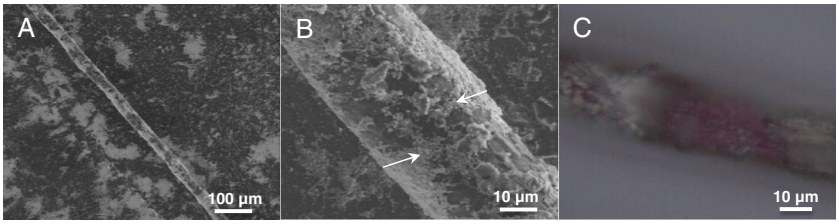

Figure 1. SEM (A, B) and light optical microscope (C) images of wool alpaca fiber with deposited silver nanoparticles. Arrows on the image B highlight the areas optimal for the μRS analysis. Those are not distinguishable on the optical micrograph, C.

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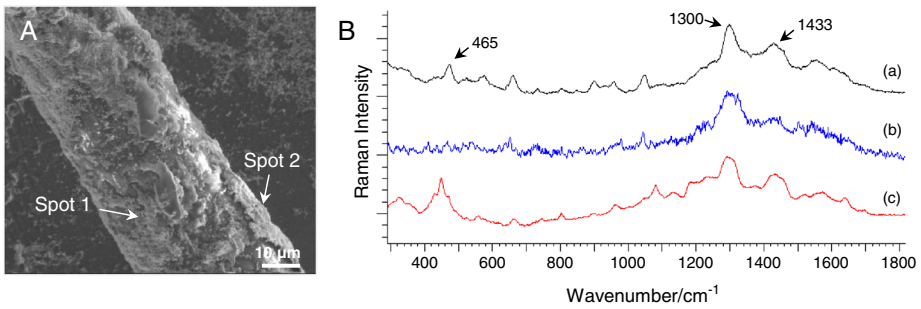

Figure 2. (A) SEM image of an alpaca fiber dyed with cochineal. (B) Stack of the SERS spectra measured in the SEM using SCA on the spot 1 (a) and 2 (b) shown in (A) and SERS spectrum of the reference sample of carminic acid obtained in the confocal optical microscope (c).

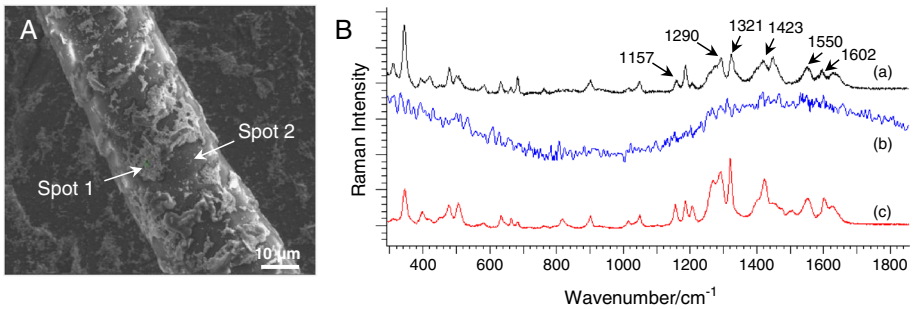

Figure 3. (A) SEM image of a wool fiber dyed with chay root. (B) Stack of the SERS spectra measured in the SEM using SCA on the spot 1, (a), and 2 (b) shown in (A) and SERS spectrum of the reference sample of alizarin obtained using the confocal optical microscope (c).

the fiber. However, SEM images showed that when metal colloids are deposited on the fiber surface to enable SERS analysis, the distribution of AgNPs is not uniform and the thickness of the deposited layer may vary largely. It is worth noticing that even on the flat surface of the SEM stub, the distribution of the nanoparticles is highly non-uniform.

Figure 2(A) illustrates an example of two areas (Spot 1 and Spot 2) on an alpaca fiber dyed with cochineal, which were selected in the SEM for SERS analysis. Measurements were carried out in these areas without moving the sample in the SEM. The resulting spectra are shown in Fig. 2(B). The analysis of areas with a thick deposition of AgNPs (Spot 2) resulted in low signal-to-noise ratio or no signal at all. SEM image also shows that thick layers of AgNPs very often get detached from the fiber surface that negatively impacts the signal-to-noise ratio. The areas with a thin and uniform deposition of AgNPs (like Spot 1) were found to be more suitable for the identification of the red dyestuff used to color the alpaca fiber, leading to optimal conditions for reproducible spectra characteristic of cochineal dye. The spectra of the experimental sample show characteristic bands at 465, 1300 and 1433 cm^{-1} similar to what was observed on the reference sample of carminic acid, and it is in agreement with published results obtained using SERS on cochineal dyestuff.[13,14]

Figure 3 shows the results obtained from the analysis of a wool fiber dyed with Indian madder. In this case SEM images also showed a non-uniform distribution of AgNPs on the fiber surface. Figure 3(A) illustrates the areas selected for SERS analysis (Spot 1 and Spot 2). Figure 3(B) shows the SERS spectra obtained from the analysis of these areas. The results of the analysis in the area with lack of AgNPs deposition (Spot 2) resulted in low signal-to-noise ratio or no signal. The area with a thin layer of AgNPs (Spot 1) was confirmed to be ideal to obtain optimal and reproducible spectra characteristic of the dye molecules. Observed bands on the experimental sample at 1153, 1290, 1316, 1457 and 1617 cm^{-1} as well as their relative intensities are in agreement with results in the reference sample of alizarin shown in Fig. 3(B). Our data are also in good agreement with earlier published results using SERS of alizarin.[18–20] It is important to note that Raman spectroscopy alone would not work on fibers dyed with alizarin because of high fluorescence emission, which limits the application of μRS as an investigation method. SERS, however, helps quench the fluorescence and increase the signal-to-noise ratio achieving better detection.[18]

Conclusions

Our results clearly illustrate the potential of this non-destructive methodology (in a sense that it does not consume the sample and the sample can be further analyzed by other techniques) for on-the-fiber extractionless analysis of natural organic colorants using SERS. The interfaced SEM–μRS provides a powerful tool for the analysis of unique and irreplaceable samples and small artifacts in fast time and cost effectively. Areas of the fiber with a thin uniform deposition of AgNPs easily detected using SEM showed optimal and reproducible SERS spectra.

This approach addresses the issue of microscopic spatial resolution[11] of the analyzed area on the sample making SERS–SEM a tool of high specificity. Utilizing the improved depth of field and high imaging resolution of the SEM, it is easy to locate areas of optimal deposition of metal nanoparticles enabling successful and reproducible SERS spectra and unbiased identification of the dyestuff. It is also possible to identify the species of the fiber based on their morphology, which can be supported by SEM. Moreover, EDS, which has become a standard attachment to the SEM in recent years, can significantly compliment the molecular information

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provided by SERS with qualitative and quantitative elemental data obtained on the same areas analyzed by SERS. This optimized methodology is currently tested for the identification of different dyestuff (other than cochineal and alizarin) and also for the study of a collection of Peruvian archaeological textiles including samples from the site of Huaca Malena. Our research will also expand to include basic research to further improve the understanding of SERS.

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