The *in-situ* analysis of lipsticks by surface enhanced resonance Raman scattering

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C. Rodger, V. Rutherford, D. Broughton, P. C. White* and W. E. Smith

Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK G1 1XL

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The use of surface enhanced resonance Raman scattering (SERRS) spectroscopy is reported for the *in-situ* characterization of chromophores in lipstick smears on glass and cotton surfaces. A surfactant is required to obtain SERRS spectra of the dyes and pigments in these waxy samples. Of the surfactants tested, poly(L-lysine) is preferred for this purpose and serves a dual function, since it also produces the required aggregation of the silver colloid. The method is quick, effective and sensitive, and with the silver colloid distributed on the surfaces tested, no appreciable background fluorescence from the substrates is detected. For six commercial lipstick samples examined by this *in-situ* SERRS method, discrimination between the samples could be achieved and it was possible to identify some of the individual pigments present, thus indicating the potential of the technique for forensic and quality control applications.

Introduction

A common problem in forensic science is to identify and establish the provenance of trace samples of lipstick smears deposited on a variety of surfaces including glass, paper, cigarette butts and garments. In addition, the manufacturer of lipstick requires methods which can provide quick and effective sample identification for quality control. A lipstick typically consists of 65% castor oil, 15% beeswax, 10% carnuba wax, 5% lanolin, a number of soluble and insoluble dyes, pigments and perfume.[1] Standard methods of analysis either involve an assessment of the perceived colour by microscopy and microspectrophotometry,[2,3] or separation techniques such as thin-layer and high-pressure liquid chromatography.[3,4] However these techniques are not entirely satisfactory since they are insensitive and either involve human opinion, or require a complicated extraction procedure to isolate the dyes and pigments from the waxy matrix. Contamination or incomplete extraction can result due to the insolubility of any pigments and the nature of the waxy matrix. Additionally, during the extraction process the sample is susceptible to dissolution, modification, evaporation and absorption of contaminants. Therefore, a technique which does not require an extraction step or the use of solvents would be of value.

Modern Raman spectroscopy is now simple and effective but fluorescence from both the matrix and the chromophores in lipsticks limits the use of resonance Raman scattering to determine the chromophore mix selectively and *in-situ*. However, by using the SERRS technique, fluorescence is usually quenched and therefore, should overcome these problems. SERRS is obtained when a molecule with a chromophore is adsorbed onto or is in close proximity to a suitable metal surface and the excitation wavelength is tuned to the molecular resonance frequency of the analyte. The enhancement obtained is very much greater than with either resonance or surface enhancement alone and the spectra obtained are unique. Since a SERRS spectrum is characteristic of the molecule the technique has been used to discriminate between dyes and identify dyes in mixtures, even when the dyes have very similar chemical structures.[5,6]

Recently, an *in-situ* SERRS method has been reported for the detection of a reactive dye covalently bound to cotton, whereby a fibre was treated with colloid.[7] Since the scattering from the chromophores is much stronger than that from any other component on or close to the colloid surface, and SERRS can discriminate mixtures of structurally similar compounds, an *in-situ* determination of the chromophores used in lipsticks without any separation procedures was considered to be feasible.

To confirm this an *in-situ* method was devised to enable SERRS detection of the colourants in lipstick smears on both glass and cotton surfaces. SERRS analyses were also performed on dye extracts from lipsticks and the results obtained from these studies are presented and discussed. Results generated from the analysis of five other lipsticks are also included to illustrate potential forensic and quality control applications of this *in-situ* technique.

Experimental

Raman scattering was recorded using the Renishaw 2000 Micro Probe Raman Spectrometer and a modified Cary 81 system described previously.[8] The Renishaw system was used to study solid samples of each lipstick. An argon laser with an excitation wavelength of 514.5 nm and power output of approximately 20 mW was used to irradiate samples and the Raman scattering was collected using ten, ten second accumulations. Six different areas were analysed from each sample. Any fluorescent background was subtracted using the standard background subtraction programme provided with the software. The Cary 81 system was used to collect the spectra from lipstick extracts contained in a cuvette with a pathlength of 1 cm. The slit width and amplification were set to '4' and '300 K' respectively and an argon laser (100 mW) was used to provide the excitation wavelength of 514.5 nm.

Silver colloid was prepared using a modified Lee and Meisel procedure.[9,10] The nature of this colloid varies from laboratory to laboratory. In this laboratory, the colloid is almost mono disperse and consists of hexagonal particles with a longest dimension of 36 nm. Quality control is obtained by testing every batch using UV/VIS spectroscopy. An absorption maximum between 404 and 410 nm with a half width of less than 60 nm is required. This colloid is stable and usable for a period of at least six months. For *in-situ* SERRS analyses the aqueous colloid was concentrated by centrifuging an aliquot of colloid (15 ml) at 2750 rpm for 30 min, then removing 98% of the supernatant and resuspending the colloidal silver in the

remainder of the supernatant to give approximately 300 μl of the concentrated silver colloid solution.

The lipstick samples examined in this study included; Yardley '28' Holly Red, Outdoor Girl '37' Cocktail Cherry, Outdoor Girl '64' Summer Fruits and three samples (A, B and C), which were supplied by Boots (Airdrie, Scotland).

For the *in-situ* analyses, lipstick samples were smeared onto glass microscope slides or samples of cotton (1 cm × 1 cm). The smears were treated with approximately 70 μl of an aqueous 0.01% (v/v) solution of the surfactant poly(L-lysine) hydrobromide (Sigma, Poole, Dorset, UK). Concentrated colloid (300 μl) was added directly to the surfactant treated surface and allowed to dry out naturally. SERRS spectra were collected directly from six separate areas of the surface on each sample using the microscope to focus on a specific area. Other surfactants examined included aqueous and ethanolic solutions of hexamine at a concentration of 10^{-4} M and an aqueous solution of dodecylamine hydrochloride (10^{-4} M). These surfactants were obtained from BDH, Poole, Dorset, UK.

To prepare extracts of the lipsticks, a sample was smeared on the inside of a beaker into which 10 ml of ethanol was added. Extraction of the dyes and pigments occurred upon vigorous shaking. No attempt was made to determine the concentration of the lipstick dyes and pigments and consequently the method remains qualitative. SERRS was obtained by adding a small aliquot of the extract to the aqueous silver colloid which had been aggregated with an aqueous poly(L-lysine) solution [0.01% (v/v)]. Ultimately, the size of the aliquot required depended upon the concentration of the dyes and pigments in the extract. In these particular studies, where the concentrations were unknown a standard experimental procedure was adopted. With an aliquot of typically 150 μl the volumes of colloid and poly(L-lysine) used were 2 ml and 150 μl respectively.

Results and discussion

All the lipsticks studied were red in colour and observation of the smears with the microprobe revealed that they contained a very fine distribution of red particles with a few larger aggregates randomly distributed throughout. Resonance Raman scattering could not be obtained readily from these smears or from the extracts due to strong fluorescence. However, using the procedures discussed below, fluorescence quenching was achieved and good SERRS was obtained either by addition of colloid onto the surface of a smear or into a solution of an extract.

Initially, SERRS from the surface of the smear was obtained by painting the concentrated silver colloid suspension onto the surface and allowing it to dry at ambient temperature. Since the colloidal particles are charged and surrounded by a polar layer their adherence to the waxy surface of the lipstick is poor and, although good scattering could be obtained by focusing the microprobe on one of the small aggregates of silver on the surface, it was difficult to obtain reproducible results. To overcome this problem, the surface was treated with a surfactant before the colloid was introduced.

Using 'Holly Red' lipstick smeared on both glass and cotton as the test samples, three surfactants were assessed to identify which of these were the most effective in achieving adherence between the lipstick and colloid. The surfactants tested included poly(L-lysine) hydrobromide, hexamine, and dodecylamine hydrochloride, but some experimental problems were encountered in their application. With the exception of hexamine prepared in ethanol, there was a tendency for the aqueous solutions of the surfactants to roll off the waxy sample. Consequently, great care was required during the application to achieve reproducible coverage. Observation of the coated samples under the microscope indicated areas where the colloid had covered the sample completely and uniformly. These areas appeared grey and shiny and SERRS signals collected from them were strong with little or no fluorescence. Areas of incomplete colloid coverage produced poor SERRS and strong fluorescent backgrounds. In addition, the absolute intensity of the SERRS was reproducible from any one spot but varied from spot to spot. However, the relative intensities of the peaks were approximately constant from spot to spot.

The effects of using each surfactant on the SERRS signals generated from 'Holly Red' samples of lipstick on glass and cotton surfaces are shown in Fig. 1 and 2. All of the surfactants produced good quality SERRS signals but poly(L-lysine) and dodecylamine, when applied under the conditions as described in Experimental, were found to be the most effective. Hexamine was applied both as an aqueous solution and an ethanolic solution. By using the latter, an improvement in the surface wetting of the sample was expected with an enhancement of the SERRS signals, but only minor improvements were obtained. Poly(L-lysine) was therefore selected as the surfactant to be used for further studies because of its effectiveness and that it has been used successfully on previous occasions as an aggregating agent for solution and *in-situ* SERRS spectroscopic studies.[5,7]

SERRS from the ethanol extract of 'Holly Red' (Fig. 3) were collected using a Cary 81 instrument fitted with an argon laser producing an excitation wavelength of 514.5 nm (power 100 mW). This instrument was used because it was equipped to measure solution or suspension Raman scattering quantitatively. Poly(L-lysine) was selected as the aggregating agent because it enables SERRS to be obtained from a wide range of dyes, irrespective of the charge on the dye. Many other aggregants are unsuitable because they tend to discriminate against some analytes, particularly if the analyte has the same charge as the silver surface.

Under these conditions the lipstick extract gave acceptable SERRS, although a fluorescence background was observed.

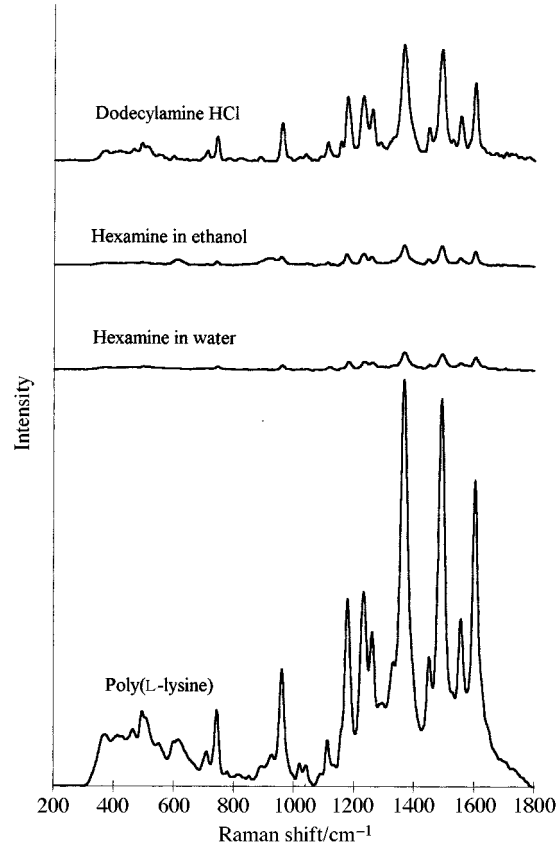

Fig. 1 Comparison of *in-situ* SERRS of 'Holly Red' on glass using four different surfactants collected using 514.5 nm excitation.

Provided the silver colloid surface is correctly treated so as to attract the dyes, the concentration in the extract can be low, with concentrations of 10^{-8} M or lower capable of being analysed routinely.[8,11,12] The fluorescence quenching observed in SERRS requires surface adhesion of the analyte normally at monolayer coverage or below, therefore the fluorescent background observed suggested that some of the chromophores did not adhere. A modern system such as the Renishaw (Renishaw plc., Wotton-under-Edge, Gloucestershire, UK) equipped with a solution cell could comfortably remove this degree of background thus allowing the analysis of sample extracts. However, this approach was considered unsuitable because of the problem in obtaining complete extraction of all the chromophores from the sample. Nonetheless, six replicate analyses from the 'Holly Red' sample did produce spectra with identical peak positions and relative intensities and reasonably reproducible absolute intensities at low concentration. With further development of this method it may be possible to provide a simple way of detecting products from more conventional assays for which separation steps are a prerequisite, and in particular, in more routine analyses where the dye compositions are better defined.

Examples of the SERRS spectra obtained from 'Holly Red' lipstick extracted from cotton and glass surfaces are shown in Fig. 4. From these results it can be observed that there are some additional peaks in the *in-situ* spectra that may represent an insoluble component not extracted by the ethanol. The ability of SERRS to provide a good molecular fingerprint for dyes and to discriminate between mixtures of closely related chromophores has been demonstrated for mixtures of four or five azo dyes.[6] Therefore discrimination between the dyes and pigments used in lipsticks should be possible without separation of the components. A comparison with data from previous SERRS results indicate that the colourants present in the lipstick are typical of the rhodamine class of dyes or pigments.[8,11,13]

The *in-situ* studies from the cotton and glass samples gave spectra with very similar relative intensities of the SERRS signals, thus indicating no specific surface effects which

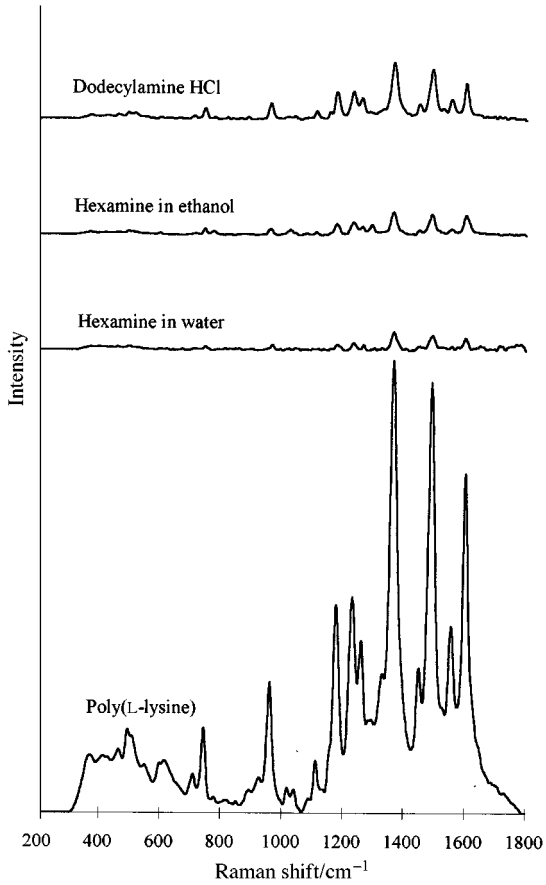

Fig. 2 Comparison of *in-situ* SERRS of 'Holly Red' on glass using four different surfactants collected using 514.5 nm excitation.

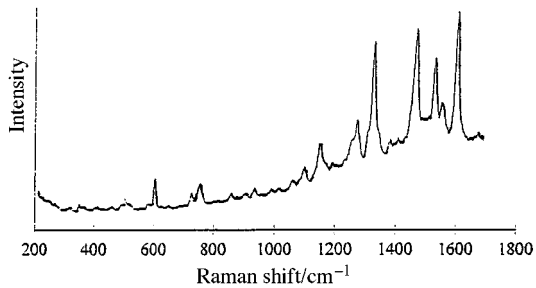

Fig. 3 Solution SERRS from an ethanol extract of 'Holly Red' lipstick collected using 514.5 nm excitation.

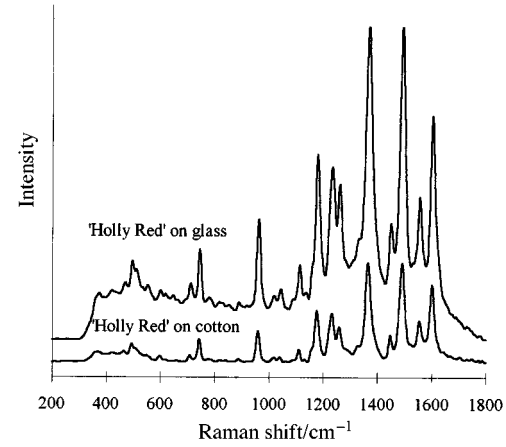

Fig. 4 *In-situ* SERRS from 'Holly Red' lipstick smeared on cotton and glass collected using 514.5 nm excitation.

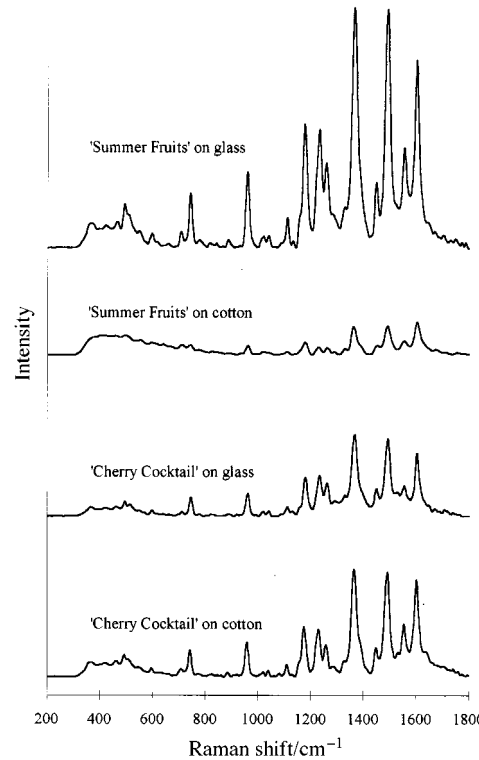

Fig. 5 *In-situ* SERRS collected from 'Cherry Cocktail' and 'Summer Fruits' lipstick samples smeared on glass and cotton using 514.5 nm excitation.

prevent comparison or recognition of the chromophores on these different surfaces. Some variations of the intensities were observed when these results were compared with the spectra obtained for the extracted samples but the frequencies of the main peaks remained the same. This variation in intensities can possibly be attributed to the degree of control on the angle of the dye to the silver colloid surface under the solution and *in-situ* conditions. In a recent study of rhodamine dyes in solution it was reported that the nature of the chemisorption process and, in particular the angle the dye subtends from the surface, can affect the relative intensity of the SERRS signals.[14] Under the *in-situ* conditions, the matrix is more likely to control the angle of the dye but some variation in intensity of the scattered light would still be expected because there would still be a random distribution of angles between the dye and the surface of the silver colloid.

To establish the generality of the results, a further five lipsticks were investigated using the *in-situ* method and in each case good SERRS spectra were obtained from smears deposited on glass and cotton surfaces (Fig. 5 and 6). With some of the samples a background fluorescence can be observed. Since the spectra indicate that the lipsticks contain similar chromophores, this fluorescence probably arises from areas of the substrate where there has been incomplete coverage with the silver colloid. Although fluorescence background can be removed from spectra these results emphasise the need for efficient adsorption of the colloid onto the sample surface.

These results do however show that each lipstick generates a different SERRS 'fingerprint' thus indicating the discriminative power of the technique. Since this has been achieved on extremely small quantities of samples, and without any prior separation of the chromophores, this *in-situ* method offers considerable advantages over techniques that are currently in use.

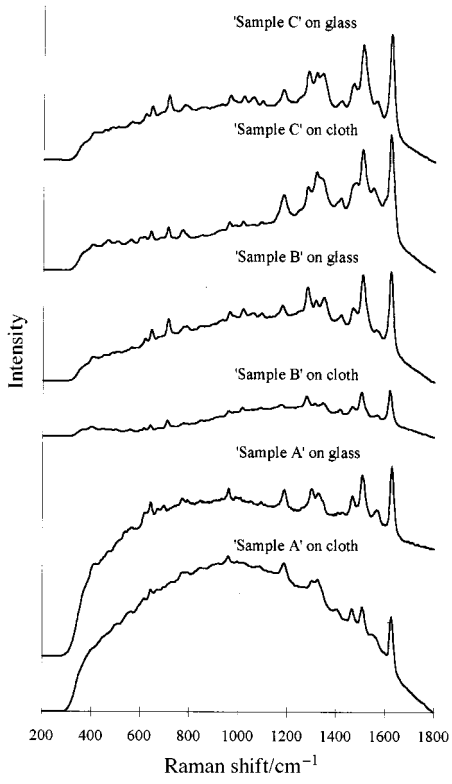

Fig. 6 *In-situ* SERRS collected from 'A', 'B' and 'C' lipstick samples smeared on glass and cotton using 514.5 nm excitation.

Conclusions

SERRS spectroscopy was used successfully for the *in-situ* analysis of lipstick smears on glass and cotton surfaces. To obtain good quality spectra from the waxy samples a surfactant was required before the colloid was introduced. Of the surfactants studied poly(L-lysine) was determined to be the most favourable to use in conjunction with the concentrated colloid. The reduction of background fluorescence from a sample can be achieved by ensuring good adsorption of the colloid onto the chromophore in the sample area being analysed.

The technique is simple, fast, sensitive, selective and requires a minute amount of sample and there are no pre-separation steps. Spectra characteristic of each lipstick tested were obtained indicating the discriminative power of the technique and a degree of universality not common with techniques based on Raman scattering.

A comparison of this *in-situ* method with an extracted sample illustrated the difficulties inherent in any analytical method which requires complete extraction and identification of a mixed dye and pigment system of chromophores from a waxy matrix. However, such methods are employed to analyse the total chromophore mix and SERRS could be used effectively to obtain a quick indication of the success of the extraction procedure required to separate the chromophores from the matrix without the need for further and difficult steps required to isolate each component.

Overall, the results from this study clearly indicate that this *in-situ* SERRS spectroscopic method has considerable advantages over other qualitative analytical techniques used currently for the examination of lipsticks by forensic scientists and manufacturers.

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