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Analysis by surface enhanced Raman spectroscopy on silver hydrosols and silver coated filter papers*

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Abstract: Surface enhanced Raman spectrometry (SERS) is an analytical technique with a sensitivity comparable to that of conventional molecular absorption or fluorescence spectroscopy, with the additional major advantage of selectivity inherent in vibrational spectroscopies. The analytical application of flowing silver hydrosols is described. Under the controlled experimental conditions of flow injection analysis, it was possible to detect as low as 30 ng of *p*-aminobenzoic acid. The linear range was two orders of magnitude (1–100 μg ml^{-1}) with a signal reproducibility of 3.2%. Silver coated filter paper is another SERS active substrate that is simple to prepare and handle. The SERS spectra of several nitrogen-containing molecules were obtained on these substrates. The effects of laser power and paper hydration are described. The relative advantages of both substrates are compared.

Keywords: *Surface enhanced Raman spectrometry; silver hydrosols; silver coated filter papers.*

Introduction

The inelastic scattering of light by molecules, known as the Raman effect [1], results in secondary radiation of either lower (Stokes Raman transition) or higher (anti-Stokes Raman transition) radiant energy. Raman scattering is a concerted process that generally involves transitions among vibrational levels [2]. The Raman effect is a very weak phenomenon that requires the use of a powerful, monochromatic source (usually a laser) and a very sensitive photodetection system.

In 1977, Albrecht and Creighton [3] and, separately, Jeanmarie and Van Duyne [4] observed that Raman cross section for pyridine, absorbed on a roughened silver electrode, was six orders of magnitude larger than that in solution. That was the

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beginning of surface enhanced Raman spectroscopy (SERS). SERS is an analytical technique of sensitivity comparable with conventional absorption or fluorescence spectroscopy, with the additional major advantage of selectivity inherent in vibrational spectroscopies [5, 6]. Numerous interpretations have been presented to explain the SERS phenomenon [7, 8], most of them grouped into two models. In the electromagnetic model, a molecule near a conducting surface feels a stronger electromagnetic field than a free molecule. In the chemical model, the Raman polarizability of the molecule is increased by the molecule–metal interaction [9].

The first reported substrates for SERS were silver electrodes [3, 4, 10], but soon, other substrates, such as sols [11–14], thin films [15] or island coatings [16, 17], thin layer chromatographic plates [18], filter papers [19, 20], silver coated spheres [21] and prolate silica posts [22] were used. SERS on silver hydrosols is of particular interest because of the simplicity of substrate preparation, characterization and manipulation. However, the extreme dependence of the enhancement on the size and previous surface coverage of the colloidal particles imposed a rather severe restriction on the ultimate usefulness of SERS as an analytical technique.

In the first part of the present paper, it is demonstrated that silver hydrosols prepared at room temperature by activation with acid and adherence to a strict sample preparation procedure can provide satisfactory precision. Quantitative analytical applications of SERS can be obtained by the use of a flow injection configuration. In the second part of this paper, results obtained by the use of filter paper are presented and several compounds of biomedical interest are used as model analytes.

Experimental

Instrumentation

The Raman system was fully described in a previous communication [23]. Basically, it consisted of an argon ion laser (Model 171, Spectra Physics), tuned at 514.5 nm (green line) and a double monochromator (Model 1680 B, Spex Industries). The Raman scatter was collected at 90° to the excitation beam and measured with a thermoelectrically cooled photomultiplier tube (Model R-928, Hamamatsu) and a photon counting system (Model 1105/1120, SSR instruments). The laser light was vertically polarized by the use of two polarizers and a Soleil Babinet compensator (Karl Lambrecht Corp., Chicago, IL, USA). For static studies with silver sols, the samples were placed in a standard 1 × 1 cm quartz cell. For flowing silver sols, a special flow-through cell was designed. It consisted of a silica tube of 3 × 0.5 mm i.d. (volume about 0.6 μl). For filter paper studies, a laboratory-constructed holder was designed to maintain the paper surface at a 45° angle to the excitation laser beam. The laser power at the sample was 100 and 40 mW for hydrosol and filter paper studies, respectively. Each spectrum reported represents a single scan.

Chemicals

Silver nitrate and sodium borohydride were from Fisher Scientific. The analytes *p*-aminobenzoic acid (PABA), 9-aminoacridine (9-AA), 2-aminoanthracene, acridine, and 5-aminoquinoline were from Aldrich; 6-nitroquinoline was purchased from Eastman Kodak. All were used as ethanolic solutions without further purification.

Whatman No. 1 filter paper was used as supplied and was silver coated by the procedure described below.

SERS on Silver Hydrosols

Among the different methods for preparing silver hydrosols employed as SERS substrate, the reduction of silver nitrate with sodium borohydride is the most commonly employed reaction. The characteristics, stability and ability to promote intense SERS signals depend on a variety of factors including relative concentrations of both reactants, the volume ratio, reagent temperature, speed of mixing and stirring of the solutions [23, 24]. In addition, the incorporation of the sample to the hydrosols is affected almost equally by the same factors. So it is not surprising that the precision of such Raman measurements is rather poor. To obtain a reproducible procedure, a hydrosol preparation involving a continuous flow system [25] has been developed. Numerous problems had to be overcome as described in a recent paper [26]. Figure 1 shows the optimized system used in this study. Two peristaltic pumps allow the selection of the flow rate of each reactant, silver nitrate (1 mM solution) and sodium borohydride (2 mM solution). A bubble remover was introduced to trap the numerous hydrogen bubbles generated by the reduction of silver ions and by the slow reduction of water by sodium borohydride. The configuration of the system in Fig. 1 allows alteration of the silver hydrosol flow rate and composition.

Effect of hydrosol composition

The hydrosol particle size and shape depend on the volume ratio of the BH_4^- and Ag^+ solutions [24]. The SERS spectrum, obtained for a given analyte, depends on the physico-chemical structure of the hydrosol. An indication of the hydrosol particle size is obtained from its absorption spectrum [11].

Figure 2A shows the absorption spectra of two different hydrosols. Hydrosol No. 1, with a low silver content (the borohydride:silver concentration ratio was 2.5:1 or 5:1 v/v), corresponds to a partially aggregated hydrosol, as shown by the shoulder at 350 nm and the absorption from 550 to 800 nm [11]. That hydrosol had a yellow-brownish colour. The SERS spectrum of PABA, adsorbed on Hydrosol No. 1, is is presented by the full line in Fig. 2B. It was obtained by stopping the flow when the analyte reached the

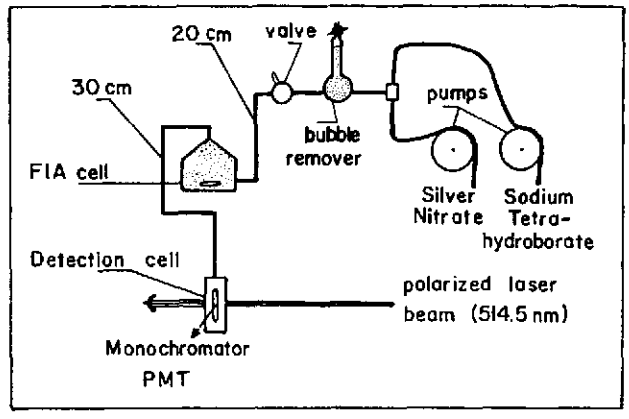

Figure 1
The flow injection configuration used to produce silver hydrosols. The volumes of the bubble remover and the flow injection analysis (FIA) cell were 1 ml and 1.21 ml, respectively. The connecting tubing is chromatographic Teflon tubing of 0.5 mm i.d.

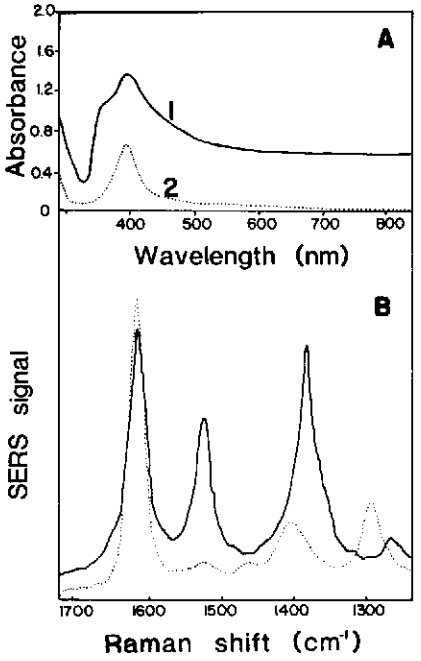

Figure 2
A. Absorption spectra of silver hydrosols obtained by mixing a solution of sodium borohydride (0.002 M) with a solution of silver nitrate (0.001 M). A1: BH_4^-:Ag^+ ratio = 2.5:1 v/v. A2: BH_4^-:Ag^+ ratio = 1:3 v/v. B. SERS spectra of PABA (about 4 μg ml). Full line: on Hydrosol No. 1; dotted line: on Hydrosol No. 2.

detection cell. The two strong bands at 1385 cm^{-1} and 1605 cm^{-1} correspond to the stretching vibration of the COO^- group and the benzene ring, respectively. The medium intensity band at 1527 cm^{-1} corresponds to another benzene ring stretching vibration.

The dotted line in Fig. 2B shows the SERS spectrum of PABA obtained with Hydrosol No. 2 (Fig. 2A). This second hydrosol, which was bright yellow, had a high proportion of silver (the borohydride:silver concentration ratio was 1:3 or 2:3 v/v) and was less aggregated than Hydrosol No. 1, shown by the symmetric absorbance band at 400 nm and the lack of absorption in the 600–800 nm range [11]. The SERS spectrum of PABA on Hydrosol No. 2 differed in some respects to that on Hydrosol No. 1 (Fig. 2B). The benzene stretching band at 1605 cm^{-1} was unchanged, the stretching band of COO^- appeared at 1405 cm^{-1} with a strong decrease in intensity and the 1527 cm^{-1} band almost disappeared. The interaction of the COO^- group with the silver surface is weakened; this may be due to a partial ionic association with the Ag^+ ions in excess.

The first conclusion is that the SERS spectrum of any analyte is strongly dependent on the physico-chemical structure of the silver hydrosol used. In this study, the best analytical conditions were: a global flow rate of about 1 ml min^{-1} with a hydrosol composition of borohydride:silver = 2.5:1 v/v.

Effect of pH

A dramatic increase of sensitivity was achieved by injecting samples acidified by the addition of nitric acid [24, 26] compared to samples at neutral pH. At pH 1.5 and 7, the spectra obtained for PABA and 9-AA were unchanged in band position and relative intensity. This excluded the primary effect of acid on the analyte molecules that was observed by Kim and Itoh [27]. The acid SERS enhancement has been related to the activation of the silver hydrosol particles by compression of the electrical double layer by protons [24].

When acidified samples were injected in the flowing system, both the analyte and the proton concentration decreased exponentially with time according to [25]:

$$C(t) = C_m \exp\{-(F/V)\,[t - (t_i + t_o)]\}$$

where:

$C(t)$ is the concentration in the dilution chamber at time t (mol l^{-1});
C_m is the concentration at time $t_i + t_o$ (mol l^{-1});
F is the global flow rate (ml s^{-1});
V is the volume of the dilution chamber (1.21 ml);
t_i is the time during which the concentration in the dilution chamber increases (injection time) (s);
t_o is the sweeping time of the dead volume (s).

Figure 3 shows that the optimum pH range for PABA is around pH 3, with a decrease in intensity above and below this value. The same optimum pH value was found for 9-AA SERS spectra [26].

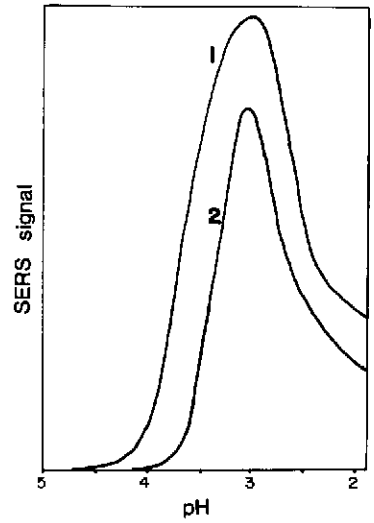

Figure 3
SERS response of PABA at a Raman shift of 1605 cm^{-1}, as a function of pH (from ref. 25). PABA concentration 32 $\mu g\ ml^{-1}$ (1) and 4 $\mu g\ ml^{-1}$ (2).

Effect of other factors

It has been shown that the SERS signal is very dependent on the hydrosol aggregation state [11]. At room temperature, the aggregation state can change with time, and so the SERS signal is dependent on the "age" of the hydrosol. Thus, the SERS signal obtained with the flowing configuration is dependent on flow rate. By keeping the ratio of silver nitrate to sodium borohydride constant (1:2.5, v/v) and varying the total flow rate, it was found that the optimum signal was obtained at 0.7 ml min^{-1}. At flow rates lower than 0.7 ml min^{-1}, the hydrosol is too "old", i.e. the aggregation state is too high, and so a weak SERS signal resulted. However, it has been shown that a partially aggregated hydrosol gives the best SERS signals [24]. When the flow rate is higher than 0.7 ml min^{-1}, the hydrosol is too monodisperse, i.e. too "young", and this results in poor SERS detection.

The effect of protons has been described. Any ions in the aqueous phase can disturb the hydrosol aggregation state, and in doing so, can influence the SERS signal. That is why the cleanliness of the glassware, the use of freshly prepared solutions, the purity of reagents and solvents are essential for the reproducibility of the Raman signals.

Quantitative analysis

To perform quantitative SERS determinations with the best analytical figures of merit, the following optimized conditions were used. The borohydride:silver ratio was 2.5:1 v/v, the global flow rate was 0.7 ml min^{-1}, and the sample was acidified to give a pH of 3 in the dilution chamber when the analyte concentration was maximum. The band that gave the best signal:noise ratio in the analysis of PABA (Fig. 2) was the 1605 cm^{-1} band. To reduce the noise due to the photomultiplier tube dark current, electronic noise and laser instability, the combination of 0.3 mm for entrance and exit spectrophotometer slits and a 10 times gain on the photon counter, which represented the optimum conditions, was used. Under these conditions, a positive drift was observed in the baseline after about 20 injections. This drift was related to the contamination of the tubing with analyte adsorbed on deposited silver. This problem was solved by cleaning the whole system with a 40% v/v nitric acid solution every 3 h.

Under these conditions, the relative standard deviation for the peak height was 3.2%. A linear relationship between the SERS signal and PABA concentration was found in the range 4–100 μg ml^{-1}. For a signal:noise ratio of 3, the detection limit of PABA was 30 ng.

SERS on Filter Papers

The use of paper as a substrate for SERS measurements was first described by Tran [19, 20] who used SERS to detect and identify some analytes separated by paper chromatography. In this study the possibilities of using silver coated filter papers as flexible SERS supports were investigated.

Silver coating

Recently, Vo Dinh reported a SERS procedure using filter paper coated with silver by thermal evaporation in a vacuum chamber [28]. The coating procedure now proposed is simpler. The filter paper (Whatman No. 1) was immersed in 0.1 M silver nitrate solution and allowed to drain for a few seconds. The wet filter paper containing silver ions was then sprayed with 0.2 M sodium borohydride solution. A nebuliser from an atomic absorption spectrophotometer was used to spray the paper from a distance of about 20 cm for 30 s. This treatment turned the white paper black. For a SERS measurement, a small portion of wet coated paper was cut to fit the cell holder and 2 μl of the alcoholic solution of the analyte under study were added. The SERS spectrum was recorded when the paper was still wet.

In the case of silver hydrosols, it has been shown that the physicochemical structure of the silver particles is very important. To characterize the coated paper, some scanning electron microphotographs were recorded. Figure 4A shows the high polydispersity of the silver clusters deposited on the cellulose fibres. At higher enlargement (×2200, Fig. 4B), the porous nature of the silver clusters or dendrites can be seen. The average size of the clusters is about 10 μm; the smallest particles that can be seen, have diameters of approximately 100 nm. Observations made by examining several samples of paper

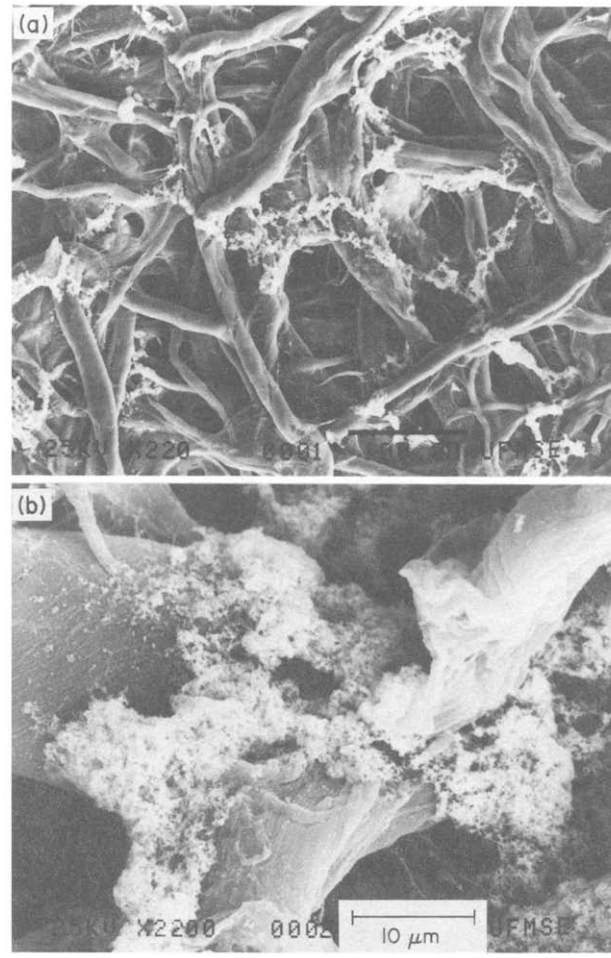

Figure 4
(a) Micrograph of a silver coated filter paper (magnification 220). (b) Magnification 2200 showing the porous structure of the silver clusters on cellulose fibres.

coated with silver by this procedure, have shown the same polydispersity of the silver clusters.

SERS spectra

Figure 5 (full line) shows the SERS spectrum of PABA on wet silver coated filter paper. The uncoated filter paper does not produce a detectable SERS signal (dotted line), even when PABA is present (dashed line). The coated filter paper does not produce any SERS signal without PABA (dash and dot line). It should be noted that the signals from coated papers (full line and dash and dot line) are recorded at 30 times the sensitivity of those from uncoated papers. Silver reduces the reflectivity of the filter paper and quenches the luminescence from some impurities in the paper. This background reduction is advantageous in SERS analysis when silver coated filter papers are used. A comparison of Fig. 5 (full line) and Fig. 2B, shows that the SERS spectrum

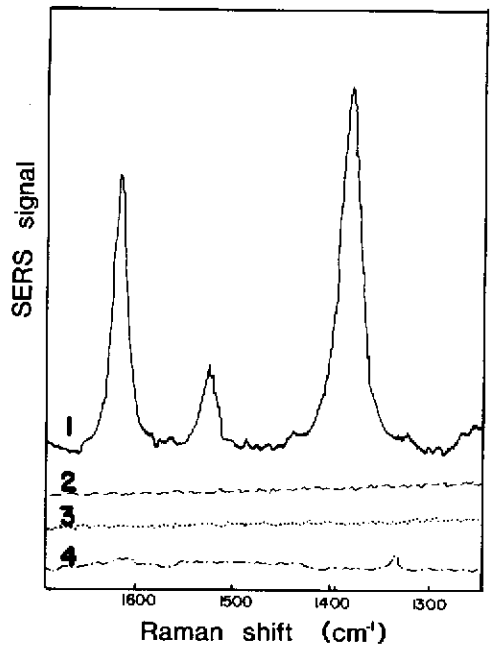

Figure 5
SERS spectrum of PABA, 1. deposited on silver coated filter paper; 2. deposited on uncoated paper; 3. uncoated paper; 4. coated paper. 1 and 4 (silver coated papers) signals were 30 times more amplified than 2 and 3.

of PABA on silver hydrosols and on silver coated filter papers are similar: the same vibrational features are present on both substrates. Therefore the PABA is adsorbed on the silver particles in water (hydrosol) or on the silver clusters (paper) in a similar way, probably through the carboxylate group.

Figure 6 shows the SERS spectra of 9-AA and 2-aminoanthracene (Fig. 6A), and of 5-aminoquinoline and 6-nitroquinoline (Fig. 6B) on a silver coated filter paper. The dissimilarity of spectra of closely related compounds shows the potential application of SERS for identification.

Effect of water

When freshly prepared, the silver coated filter paper was very dark, and upon drying, the colour slowly changed to greenish brown. These visual observations indicate a change in the hydration state of the silver clusters. This change has an important effect on the SERS signal, which decreased as the paper dried and was partially restored if the paper was dampened with 5 μl of water. It has been shown that the SERS signal is dependent on the dielectric constant of the medium surrounding the silver particles [29]. Accordingly it is not surprising that the replacement of water molecules (dielectric constant 80) by air (dielectric constant 1) decreases the observed SERS signal. This decrease, which was solute dependent, was very important with 9-AA and 2-aminoanthracene and less so with the other compounds.

Effect of laser power

Changes in the SERS spectra were observed upon irradiation with the laser beam. As SERS spectra were obtained with wet papers, it was thought that the laser beam induced a local drying of the paper. To reduce this drying effect, the laser power was decreased to 40 mW. Even with a low laser power, the vibrational structure of the SERS spectrum

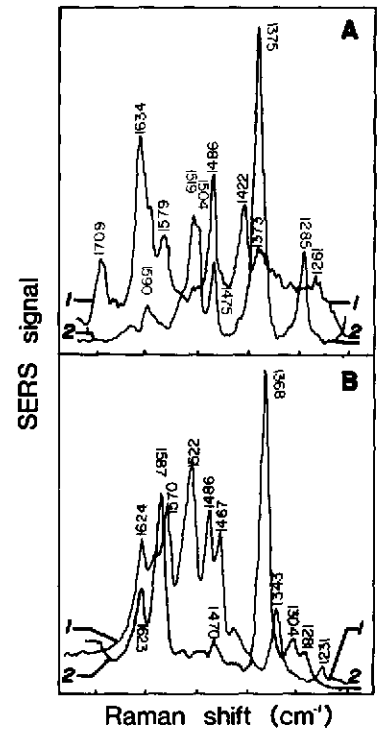

Figure 6
SERS spectrum of some nitrogen-containing compounds deposited on silver coated filter paper. A-1: 2-aminoanthracene (200 ng), A-2: 9-aminoacridine (200 ng). B-1: 6-nitroquinoline (190 ng), B-2: 5-aminoquinoline (220 ng). Labels characterize the most important vibrational features.

progressively changed and the background noise gradually increased. This phenomenon was observed for all the solutes studied. This suggests that, in addition to the drying effect, the microarrangement of the molecules on the silver surface, necessary for SERS activity, is destroyed upon irradiation. This effect and the influence of water on the SERS signal will be fully studied and described in another paper [30].

Because of those time-dependent effects and because it is very difficult to obtain an equally distributed spot on filter paper, it has not been possible to obtain quantitative results or calibration curves with SERS analysis on filter papers. However, recent results indicate that quantitative analysis will be possible with acceptable reproducibility by the use of a normalization procedure [30].

Conclusion

To conclude, the advantages and disadvantages of the two SERS substrates are as follows. The procedures used to prepare the substrates are simple, reproducible and inexpensive. For reproducible SERS spectra, the hydrosol obtained in a flowing configuration was easier to control than on coated filter paper. A relative standard deviation lower than 3.5% and a limit of detection of 30 ng were obtained with PABA on silver hydrosols. In a flowing configuration, the silver hydrosol is constantly replaced and the laser beam cannot affect the analyte adsorption as it does with filter papers. However, the advantages of filter papers are important: it is possible to obtain SERS spectra of compounds of poor water solubility, such as 2-aminoanthracene, and it is also possible to use very small volumes of sample solution. Owing to the polydispersity of

silver particles or clusters on filter papers, this substrate does not seem to be as suitable for theoretical studies of SERS phenomena as hydrosols. Nevertheless, the simplicity of these two procedures makes them analytically useful for rapid SERS identification of compounds.

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