Effect of Substrate Optical Absorption on Surface-Enhanced Raman Spectrometry on Colloidal Silver

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Quantitative analytical applications of surface-enhanced Raman spectrometry (SERS) on colloidal substrates are hindered by the experimental difficulties in measuring intensities with precision comparable to that of state-of-the-art spectrochemical techniques. Several factors, both adsorbate- and substrate-dependent, contribute to the poor reproducibility of SERS. Since the observed enhancement factor is related to the sample-induced aggregation of the colloid, the several possible aggregation degrees achieved when samples of identical adsorbate concentration are added to colloidal dispersions result in poor reproducibility. Large local concentrations of adsorbate when mixing sample and colloid cause variable crystal-growth rates and a range of final aggregation states. Crystal growth in colloidal systems is a dynamic process in nature, so the timing in the measurement process must be carefully controlled. On the other hand, the SERS spectrum of an adsorbed species corresponds to the spectrum of the first adsorbed monolayer. Subsequent layers of adsorbate undergoing enhancement due to long-range electromagnetic effects have the tendency to generate the ordinary Raman spectrum.[1,2] As a result, competitive molecular adsorption by the limited amount of active sites on the metal surface may result in uncontrolled intensities for a given adsorbate. Several authors have discussed the effects of coadsorption of nitrate and borate on SERS intensities on colloidal silver.[3–5] Differences in enhancement factors may also result from inhomogeneities in the distribution of molecules at the surface, their several possible orientations, and various adsorption energies.[6] Temperature, pH, aging of solutions, and colloidal preparations as well as laboratory practices contribute certainly to the observed reproducibilities.[3,7]

Some of these problems have been solved by processing both the adsorbate solution and the colloid on a semiautomatic system such as those used in flow injection analysis.[8,9] A relative standard deviation of 3% was reported for replicate injection of *p*-aminobenzoic acid on a stream of colloidal silver. However, problems associated with clogging of the tubing and memory effects on the flow cell were observed. After these reports, other applications of colloid SERS on flowing systems have been described.[10–12]

SERS on colloidal dispersions is generated in a medium of high optical absorbance. Aggregated colloids show broadband absorption through the visible spectrum as a result of longitudinal plasmon resonance on the silver aggregates.[13] Thus, even in the case that the adsorbate does not absorb in the visible, attenuation of both the exciting laser and the SERS light is extensive in any colloid SERS experiment. Consequently, variations in excitation and collection path lengths may also result in large variations in SERS intensities. The effect of analyte absorbance on Raman intensities has been addressed for unenhanced resonance Raman scattering. Expressions to correct Raman intensities for self-absorbance[14–17] and to calculate absolute Raman cross sections and excitation profiles in resonance Raman spectroscopy[17] have been presented. The present study establishes the effect of colloid optical absorption on SERS measurements of model compounds not absorbing significantly at the exciting wavelengths. The choice of the appropriate configuration for sample illumination and SERS scattering collection contribute significantly to optimize the quality of SERS measurements and to improve the signal-to-noise ratio and limit of detection. The model proposed is valid for a particular sample geometry, but can be easily extrapolated for both batch and flowing systems.

EXPERIMENTAL SECTION

Instrumentation. The Raman instrument consisted of a 0.22-m focal distance double-grating spectrometer (Spex Industries, Model 1680B) with 1200 grooves/mm classically ruled gratings, a cooled photomultiplier tube (Hamamatsu, Model R-928), a fast preamplifier (Stanford Research Systems, Model SR440), and a two-channel gated photon counter (Stanford Research Systems, Model SR400). Operation of the photon counter was controlled by an AT personal computer with Stanford Research SR465 software. The laser sources all running in single mode and TEM_{00} operation were a helium-neon laser (Siemens, Model LGK 7626S) tuned at 632.8 nm and an argon ion laser (Coherent, Innova 70-5) tuned at 488 or 514.5 nm. The power at the sample was 30 mW in each case.

Chemicals and Procedure. Analytical reagent-grade chemicals and distilled, deionized water were used throughout the study. 9-Aminoacridine (Sigma) and sulfanilamide (Sigma) were used without further purification. Silver colloids were prepared at room temperature by adding 1 mL of 1×10^{-3} M aqueous silver nitrate to 3 mL of 2×10^{-3} M aqueous sodium tetrahydroborate. The tetrahydroborate solution was aged for 6 h at room temperature before the experiments. In order to study the effect of excitation path length, standard 1-cm^2 quartz cells were filled with variable volumes of silver hydrosols containing a fixed amount of adsorbate. The laser beam entered the sample vertically, and the scattered light was collected at a right angle. The laser beam was displaced laterally at constant filling of the

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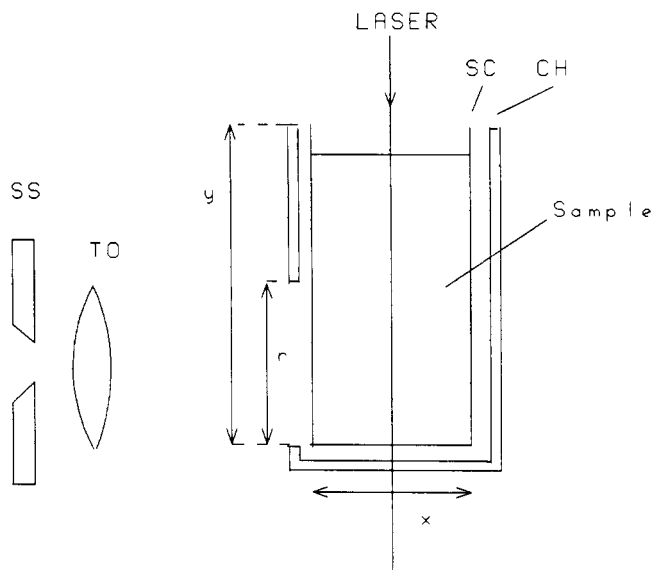
Figure 1. Front view of the sample cell holder: r is the effective excitation path length collected by the transfer optics (TO) in the spectrometer; y, excitation path length; x, collection path length; SC, sample cuvette; CH, cuvette holder; SS, spectrometer entrance slit.

sample cuvette for the study of the effect of collection path length on the scattered intensity.

RESULTS AND DISCUSSION

Surface-enhanced Raman scattering of molecular adsorbates using colloidal dispersions as substrates is generated in a medium of high optical absorbance. This situation arises since colloid aggregation by the adsorbate of interest is needed for a large SERS signal, as recognized in the electromagnetic theory of enhanced optical processes at rough metallic surfaces. Unaggregated, monodisperse silver colloids show a single absorption band in the visible region centered at about 400 nm, the exact position depending on the size of the silver particle. However, aggregated silver colloids show broad-band absorption in the range 450–900 nm with the position of the maximum (if any) depending on the aggregation degree, i.e., on the particle size. Under these circumstances, both the exciting laser beam and the SER scattered light are strongly attenuated by the colloid.

The degree to which colloid absorption affects a SERS measurement will depend on the geometry of the scattering experiment. As is common in many spectrometers, the entrance and exit slits of the monochromator used here are vertical to the optical plane. In this case large improvements in scattered light collection can be achieved by matching the sample image with the entrance-slit geometry. The signal-to-noise ratio (SNR) is improved by about 1 order of magnitude by using an optical configuration in which the exciting laser enters the sample vertically rather than using the conventional horizontal illumination configuration. The resulting sample illumination-collection geometry is shown in Figure 1. The SERS light is collected at a right angle through a constant aperture, r, designed to match the collection angle of the transfer optics to the spectrometer (TO). The variables of concern here are the excitation optical path length (y) and the collection optical path length (x). The first is directly related to the filling of the sample cuvette with the colloid and can be measured in terms of sample volume in milliliters since 1-cm² cells are used. The second is related to the lateral position of the laser beam with regard to the spectrometer.

In the absence of colloid absorption, the total Raman intensity (photons sr⁻¹ s⁻¹) from a sample illuminated by a
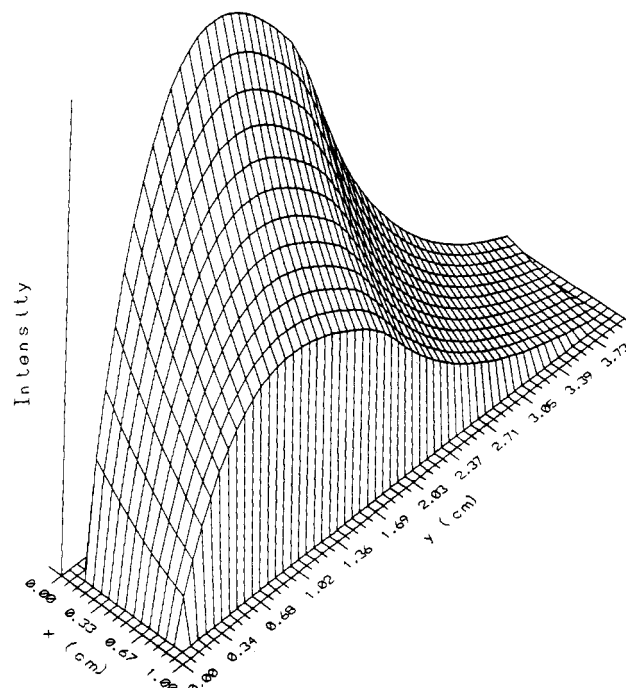
Figure 2. Raman intensity profile generated by a collimated laser beam passing through a sample cuvette (1 cm × 4 cm) using the geometry in Figure 1. The laser beam illuminates the sample cuvette along the y axis. The spectrometer is situated along the x axis. Calculated from eqs 4 and 5 for an absorption coefficient of 1 cm⁻¹.

collimated laser beam of power I_0 (photons s⁻¹) is given by

$$I = I_0 \sigma N r \qquad (1)$$

where σ is the Raman cross section (cm² sr⁻¹ molecule⁻¹), N is the number density of scatterers (molecules cm⁻³), and r is the excitation path length (cm) of the laser beam in the sample observed by the spectrometer. In the expression above it is assumed that both the excitation and collection path lengths are constant. For a nonresonant SERS experiment on aggregated colloidal silver, the transmittance decreases exponentially with collection path length, x (cm), so the intensity observed as a function of the lateral movement of the laser beam will be

$$I_{OBx} = I e^{-\gamma x} = I_0 \sigma N r e^{-\gamma x} \qquad (2)$$

where γ is the colloid coefficient of absorption (cm⁻¹) at the Raman band of interest. The effect of variations in excitation path length on the observed intensity can be conveniently studied by modification of the filling of the cuvette with the sample, as shown in Figure 1. For cuvette fillings above the effective path length, r, the observed intensity as a function of excitation and collection path lengths, x and y, will be

$$I_{OBxy} = I_0 \sigma N R e^{-\beta y} e^{-\gamma x} \quad \text{for } y > r \qquad (3)$$

where β is the colloid coefficient of absorption (cm⁻¹) at the exciting wavelength. For aggregated colloids showing broad-band absorption, β and γ can be considered equal in value when the Raman shift is not too large, so

$$I_{OBxy} = I_0 \sigma N r e^{-\alpha(y+x)} \quad \text{for } y > r \qquad (4)$$

where $\alpha = \beta = \gamma$. For excitation path lengths smaller than r, the observed intensity will be

$$I_{OBxy} = I_0 \sigma N y e^{-\alpha(y+x)} \quad \text{for } y < r \qquad (5)$$

Figure 2 shows a calculation of the SERS intensity vs excitation optical path length (y axis) and collection optical path length (x axis) according to eqs 4 and 5 for an absorption
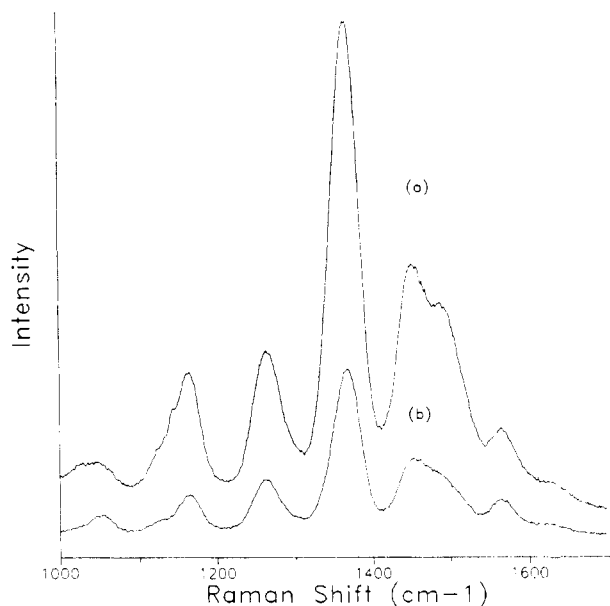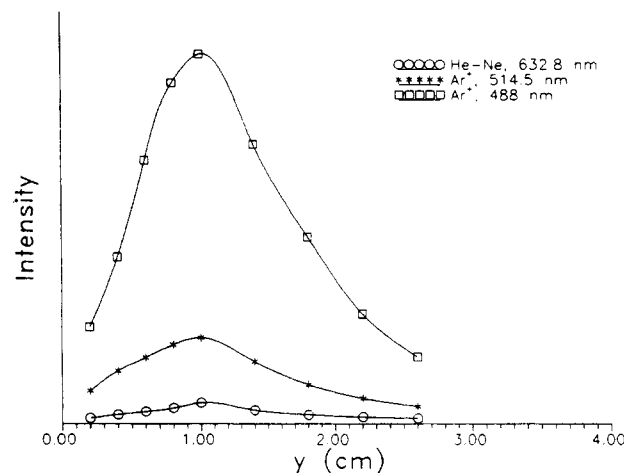
Figure 3. Surface-enhanced Raman spectra of 9-aminoacridine on colloidal silver. (a) Spectrum obtained with the laser beam positioned at $x = 0.1$ cm. (b) Spectrum obtained with the laser beam positioned at $x = 0.8$ cm.


Figure 4. Effect of excitation optical path length on the observed surface-enhanced Raman intensity of 9-aminoacridine on colloidal silver at three different excitation wavelengths. Power at the sample 30 mW. Raman shift 1370 cm⁻¹.

Figure 5. Surface-enhanced Raman intensity vs sulfanilamide concentration. Full line obtained with a silver colloid prepared from 1.0 × 10⁻³ M silver nitrate. Dashed line, same from 0.5 × 10⁻³ M silver nitrate. Variation bars represent the range for three replicate measurements. Raman shift 1108 cm⁻¹.

coefficient of 1 cm⁻¹. Figure 2 predicts the intensity would be maximum close to the cuvette wall facing the transfer optics. Also, maximum intensity would be obtained at a given excitation path length, i.e., at a definite filling of the cuvette with the sample. It is interesting to note that according to eq 5 the cuvette filling for maximum intensity (y_{opt}) will depend on the absorption coefficient of the colloid. This means that strongly absorbent colloids (large α) would produce maximum intensity for small sample volumes, while less absorbent colloids would allow larger sample volumes into the cuvette. Figure 2 clearly indicates that selection of sample volume and illumination contributes to optimize the observed SERS intensities.

Two model compounds have been used for the present study, 9-aminoacridine (AA) and sulfanilamide (SA). The SERS band of AA on colloidal silver at 1370 cm⁻¹ and that of SA at 1108 cm⁻¹ have been monitored. As discussed above, the absorption profile of aggregated colloids is broad enough for the individual SERS bands to be almost equally attenuated. Figure 3 shows the SERS spectra of AA obtained at two extreme points of the x axis (Figure 1). As shown, the only effect observed is an attenuation to the same extent of the SERS intensities through the whole spectral region investigated. Generation of an experimental plot as that shown in Figure 2 is extremely difficult (if possible at all) in SERS owing to the evolving nature of the colloidal system. However, a study of the SERS intensity vs the excitation and collection optical paths independently can be performed in a time frame compatible with colloid stability. Figure 4 shows the effect of variations in excitation path length on SERS intensity of SA at three different excitation wavelengths. The maximum intensity is observed at an excitation path length below the effective optical path length, $y_{opt} < r$ ($r = 1.8$ cm for the present configuration). The curves in Figure 4 show their maximum at the same value of y, regardless of the excitation wavelength used in each case. This fact verifies that the value of the colloid absorption coefficient was rather similar in the range 488–632.8 nm.

Decreased SERS intensities as a result of large collection path lengths or nonoptimum excitation path lengths will cause a decrease in SNR's and a corresponding increase in limits of detection. For an aggregated colloid with 25 μg/mL AA,
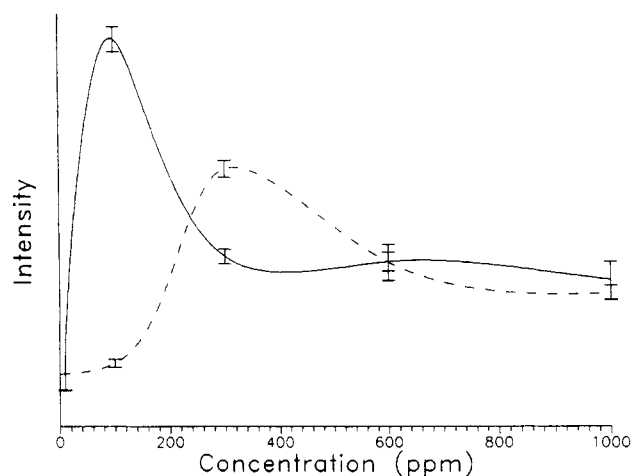
the SNR degrades from 235 to 180 (ca. 25%) when the collection path length changes from 0.1 to 0.7 cm. In addition, it should be noted that small variations in the volume of sample deposited into the cuvette may cause large variations in SERS intensities. The precision associated with variations in cuvette filling from 2.6 to 3.0 mL ($n = 5$) was found to be 18% RSD for AA at 25 μg/mL on colloidal silver. The precision for replicate measurements of SERS at a constant filling (3.0 mL) was improved up to the 7% RSD level.

An increase in analyte concentration will cause a linear increase in SERS intensity. However, colloid aggregation and hence sample absorbance will also increase accordingly. In addition, saturation of the limited amount of SERS active sites at the surface of the silver colloid would also contribute to deviations from the linearity. Figure 5 shows the effect of SA concentration on observed SERS intensity. The same spectrometer conditions and sample preparation protocol was used, the two curves only differing in that the concentration of the silver solution used for colloid preparation was twice as concentrated in one case than in the other. The curve obtained with 1.0 × 10⁻³ M silver shows maximum intensity at 100 μg/mL SA, while that obtained with 0.5 × 10⁻³ M silver shows its maximum at 300 μg/mL SA. These data would indicate that the larger the colloid absorption the shorter the range for linear response of SERS intensity with adsorbate

concentration. However, a complete description of the shape of the curve should consider in addition several SERS specific factors, including kinetics of aggregation at different adsorbate concentrations, instability of colloid systems at high adsorbate concentrations, and saturation of active sites on the colloid. Unfortunately, deconvolution of colloid absorption from SERS specific factors is not straightforward, and work is currently being done in such direction.

In conclusion, enhanced Raman scattering at silver colloidal surfaces is a technique of short path length. All portions of the sample volume between the laser beam and the transfer optics are detrimental for SERS intensity and detectability. It is evident that colloid optical absorption cannot be avoided in a SERS experiment. However, the results reported here indicate that selection of sample volume and an appropriate illumination configuration contribute significantly to optimize the quality of SERS measurements.

Registry No. Silver, 7440-22-4; 9-aminoacridine, 90-45-9; sulfanilamide, 63-74-1.