# Improvement in Fingerprinting Capability of Surface-Enhanced Raman Spectrometry by Simultaneous Measurement of Scattering Signal and Transmitted Light

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**A method for simultaneous measurement of surface-enhanced Raman spectra (SERS) and absorbance changes occurring in colloid substrates is reported. The method provides information on the aggregation state of the colloid while the SERS spectrum is recorded. The relationship between colloid aggregation and SERS intensity is thus readily established, allowing recording of SERS spectra under the most favorable circumstances. Anomalous bands, which appear and disappear mainly at low concentration, are detected and studied.**

## INTRODUCTION

Since the discovery[1] of surface-enhanced Raman spectrometry (SERS) most theoretical and practical work has been directed to the elucidation of enhancement mechanisms and description of applicable substrates.[2-5] Most recently, novel aspects of the technique have been investigated, including the relationships between the SERS activity and surface morphology of the substrate[6,7] or chemical structure of the aggregating substance,[8] attenuation of SERS spectra by protein adsorption,[9] and effects of formation of silver clusters and changes in optical properties of silver colloid.[10] The results reported allowed the development of qualitative methods of analysis by SERS.[11-15] However, it has been difficult to obtain reproducible enhancements (signals) mostly when hydrosols are used. Although semiautomatic processing of samples and colloid substrates in flow injection systems[16] could improve precision up to levels of ca. 5%, the poor reproducibility observed in batch systems hinders acceptation of SERS as a practical technique for quantitative trace analysis. In addition, poor reproducibility makes difficult interpretation of SERS spectra.

Silver hydrosols are easy to characterize in terms of particle size by electronic absorption spectroscopy. This issue is of relevance in SERS studies since the enhancement factor for a given adsorbate is closely related to the particle size.[17] The use of absorption spectroscopy as a diagnostic tool in characterization of colloids is of limited scope, however. Crystal growth in colloidal systems depends on a variety of experimental parameters and it is inherently a dynamic process. As a result the colloid examined spectrophotometrically is usually at a different aggregation degree than that used for the SERS measurement.

In this work a method for simultaneous measurement of SERS spectra and absorbance changes occurring in colloid substrates is reported. The method employs a medium-power He–Ne laser for excitation and a low-power He–Ne laser for probing the scattering volume. The lasers are used in a crossed-beam configuration. The intensity of the transmitted probe beam is detected with a silicon photodiode. The method provides information on the aggregation state of the colloid while the SERS spectrum is being recorded. The relationship between colloid aggregation and SERS intensity is thus readily established, allowing recording of SERS spectra under the most favorable circumstances.

## EXPERIMENTAL SECTION

**Instrumentation.** The excitation source consisted of a He–Ne laser (Siemens, Model LGK 7626S), tuned at 632.8 nm, releasing about 30 mW at the sample. The sample was placed in a standard 1-cm square cell (four optical faces). In order to match illumination–collection geometries, the excitation beam illuminated the sample in a vertical configuration. This method allows a 9-fold improvement in signal-to-noise ratio regarding the standard horizontal illumination geometry.[18,19] A 0.5-mW He–Ne laser, also at 632.8 nm, crossed the excitation beam at the center of the scattering volume, in such a way that the excitation, probing, and collection axes were at 90° (ortho configuration). Both lasers were collimated (unfocused). The Raman scatter was dispersed with a 0.22-m double-grating spectrometer (Spex Industries, Model 1680B), and detected with a thermoelectrically cooled photomultiplier tube (Hamamatsu, Model R928) and a photon-counting system (Stanford Research Systems, Model SR400). Data acquisition, storage, processing, and plotting were controlled by an IBM AT compatible microcomputer using the Stanford Research SR465 software. Spectral data were generated in binary code and converted to ASCII for processing in standard graphics software. The intensity of the transmitted probe laser was attenuated with a neutral-density filter and detected with a silicon photodiode. A voltage mode preamplifier was utilized to convert the photocurrent to voltage. A standard chart recorder was used to acquire the transmitted signal. Absorption spectra were recorded in a Hewlett Packard, Model 8452A, diode array spectrophotometer.

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**Chemicals and Procedure.** Analytical reagent-grade chemicals and distilled, deionized water were used throughout the study. 9-Aminoacridine, sulfanilamide, and furosemide were from Sigma and used as methanolic solutions. Silver colloids were prepared at room temperature by adding 1 mL of $1 \times 10^{-3}$ M silver nitrate to 3 mL of $2 \times 10^{-3}$ M sodium tetrahydroborate. After mixing, 0.1 mL of the analyte solution was added. The SERS signal was measured at the most prominent peak of each compound, i.e., 1370 $cm^{-1}$ for aminoacridine and 948 $cm^{-1}$ for sulfanilamide. The intensity of the transmitted probe beam was monitored simultaneously vs the baseline corresponding to the stopped beam.

## RESULTS AND DISCUSSION

The characteristics, stability, and ability to promote intense SERS signals of silver colloids depend on a variety of experimental factors including relative concentrations of reactants, their volume ratio, reagent temperature, and speed of mixing and stirring of the solutions.[20] 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. Additionally, vibrational modes in SERS spectra change with the evolution of the colloid during the aggregation process.[21,22] Specific bands appear and disappear depending on the aggregation state of the colloid which is being recorded. Figure 1 shows the changes in the SERS spectrum of furosemide on colloidal silver at three different stages of aggregation. A band centered at about 1120 $cm^{-1}$ is observed at 10 min, the band disappearing after 20 min. The initial band at about 840 $cm^{-1}$ disappears after 10 min. It should be noted that for this particular sample the intensity of a band at about 1000 $cm^{-1}$ remains unchanged. The situation depicted in Figure 1 is often encountered when a SERS spectrum is obtained and causes difficulties in spectral interpretation. Measurement of the intensity of the probe beam transmitted by the silver colloid simultaneously recorded as the SERS signal is being obtained provides data on this incident.

Metal particles in aqueous colloidal dispersion carry a negative charge due to adsorbed anions. Repulsive electrostatic forces between the particles hinder aggregation and under these circumstances the colloid is stable. Adsorbate molecules added to the stable colloid displace the adsorbed ions from the surface, decrease the charge of the particles, and result in particle collisions by diffusional movement. Particle growth and aggregation now take place. It has been shown that large enhancement factors on metal colloids are observed when the exciting laser line overlaps the longitudinal surface plasma resonance of the metal.[17] Since the excitation wavelength employed here was in the red (632.8 nm), high aggregation was required to obtain large SERS intensity. Aggregation can be achieved by altering the composition and conditions of preparation of the colloid or by increasing the adsorbate concentration. However, it should be stressed that the aggregation kinetics depends to a large extent on the chemical structure of the adsorbate under study.[8] Compounds with large SERS activity produce large aggregation rates at low concentrations, while inactive compounds cause no aggregation at all even at large concentrations. In this work two model compounds (sulfanilamide and 9-aminoacridine) with SERS activity in between both extremes permitted the study of colloid aggregation by comparing the SERS intensity with the intensity of the probe beam transmitted through the colloid.

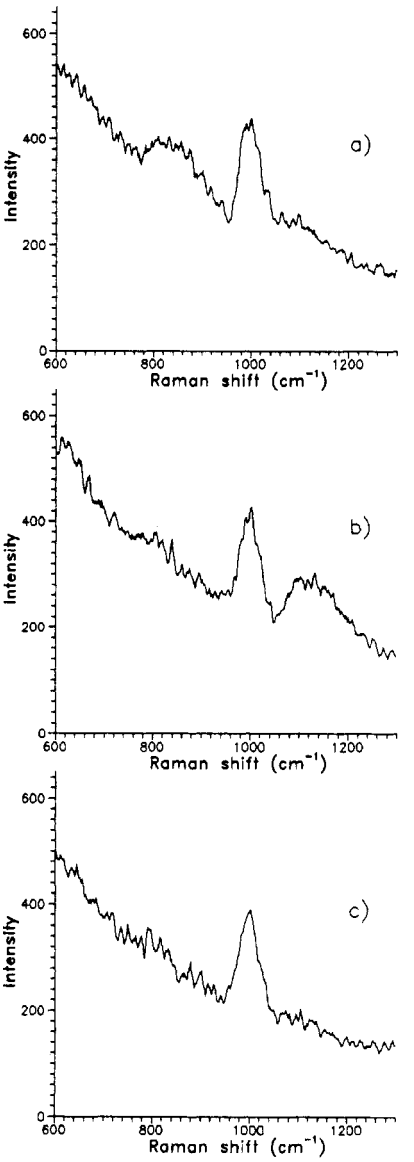


**Figure 1.** SERS spectrum of furosemide on colloidal silver: (a) initial; (b) after 10 min; (c) after 20 min.

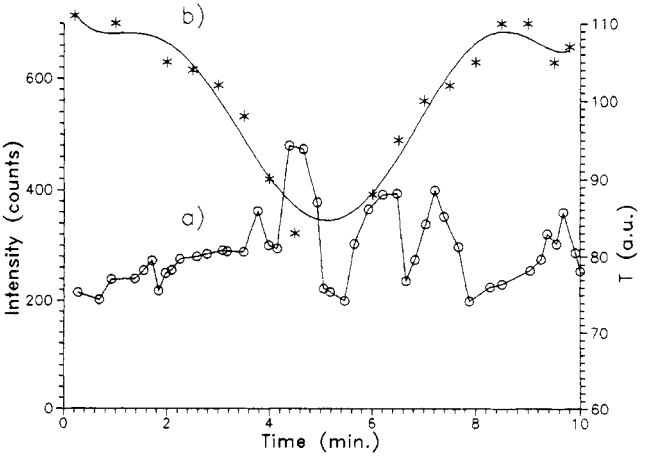


**Figure 2.** (a) SERS intensity and (b) transmitted light of the aggregation kinetics of a silver hydrosol with 12.5 μg/mL sulfanilamide.

Figure 2 shows the kinetics of aggregation of a silver hydrosol in the presence of 12.5 μg/mL sulfanilamide. Curve a represents the scattering Raman signal at 948 $cm^{-1}$ while curve b corresponds to the signal of the probe beam, both vs time. The sample was placed in the holder when the

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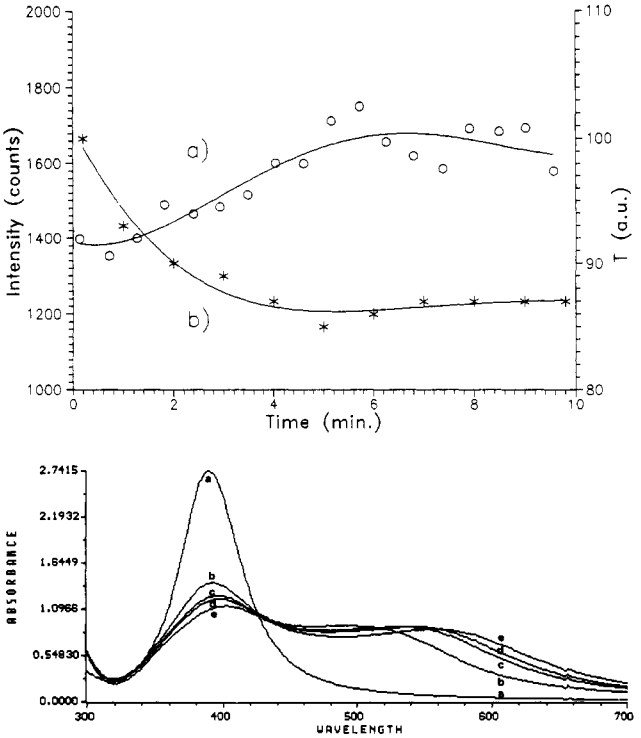


**Figure 3.** (Top) (a) SERS intensity and (b) transmitted light of the aggregation kinetics of a silver hydrosol with 12.5 μg/mL sulfanilamide stirred manually. (Bottom) absorption spectra of a silver hydrosol following the addition of 12.5 μg/mL sulfanilamide: (a) immediate; (b) 0.5 min; (c) 1 min; (d) 1.5 min; (e) 6 min.

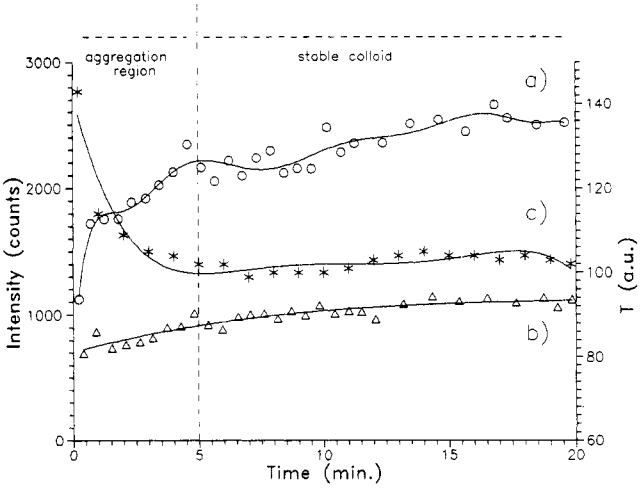


**Figure 4.** (a) SERS intensity, (b) background intensity, and (c) transmitted light of the aggregation kinetics of a silver hydrosol with 25 μg/mL sulfanilamide.

aggregation had not yet started, the colloid showing a homogeneous yellow color. After a few minutes, a simple visual inspection of the sample cuvette revealed the presence of dark blue color zones forming on the top of the cuvette, indicating that aggregation is taking place. In the absence of stirring, these colored zones diffuse slowly from top to bottom in an irregular fashion (heterogeneous aggregation) and they can be readily detected by the probe beam, as shown in Figure 2b. This curve shows a minimum at about 5 min, indicating that large particle aggregates are blocking partially the probe beam. After 5 min the transmitted light increased to recover the original level, since the zones of inhomogeneous aggregation diffused to the sample. SERS intensity follows the pattern of the transmitted light, as depicted in Figure 2a. Particle aggregates passing through the exciting beam raise the SERS intensity at about 5 min. However, the observed

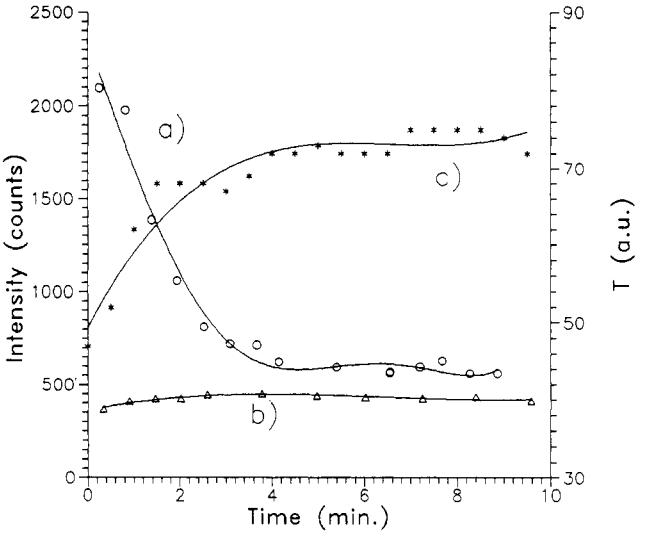


**Figure 5.** (a) SERS intensity, (b) background of SERS intensity, and (c) transmitted light of the aggregation kinetics of a silver hydrosol with 1.25 μg/mL 9-aminoacridine.

intensity is unstable since the colloid is not homogeneous. Because of the optical configuration used, the degree of heterogeneity detected by the probe beam could be different than that detected by the SERS excitation beam. This fact causes small deviations in the behavior of curves a and b in Figure 2 between 4 and 8 min.

Figure 3 (top) shows the kinetics of aggregation of a sample (12.5 μg/mL sulfanilamide) stirred manually prior to spectral observation. The SERS signal was larger than that in Figure 2a since stirring favored collisions between small particles. Nevertheless, aggregation is still proceeding as manifested by both an increase in SERS intensity and a decrease in the transmitted light. Figure 3 (bottom) shows time-dependent absorption spectra of a sample prepared under the same experimental conditions as that monitored in Figure 3 (top). The correlation between the increase in absorbance at 632.8 nm and the decrease in transmission of the probe laser is evident. The stability of the SERS signal is much improved regarding that in Figure 2a since the observed sample is homogeneous.

For concentrations of adsorbate molecules larger than 50 μg/mL aggregation was rapid and leads to complete precipitation. At intermediate concentrations, new insights into the process taking place in the colloid can be observed. Figure 4 shows the kinetics of aggregation of a silver hydrosol with 25 μg/mL sulfanilamide. The cuvette was stirred and placed in the holder when the color changed from yellow to wine red. Curve a corresponds to absolute counts of the peak at 948 $cm^{-1}$, curve b corresponds to the background of SERS signal where no peak occurs, and curve c corresponds to the transmitted light. During the first 5 min absolute SERS intensity increased while transmitted light intensity decreased as expected. After 5 min the difference between the SERS signal at 948 $cm^{-1}$ and the background remained stable and the colloid transmittance showed no significant changes (curve c). It should be noted that only the net peak signal remains stable after constant transmitted light (5 min on), while both the absolute peak signal and the background keep growing. This fact is probably due to Rayleigh scattering by very large silver particles unable to cause enhancement of Raman signals because of their large size. For this concentration the stability of the SERS signal is improved regarding that at lower concentrations (Figure 3). The stability of the transmitted signal confirmed the absence of inhomogeneous zones in the sample.

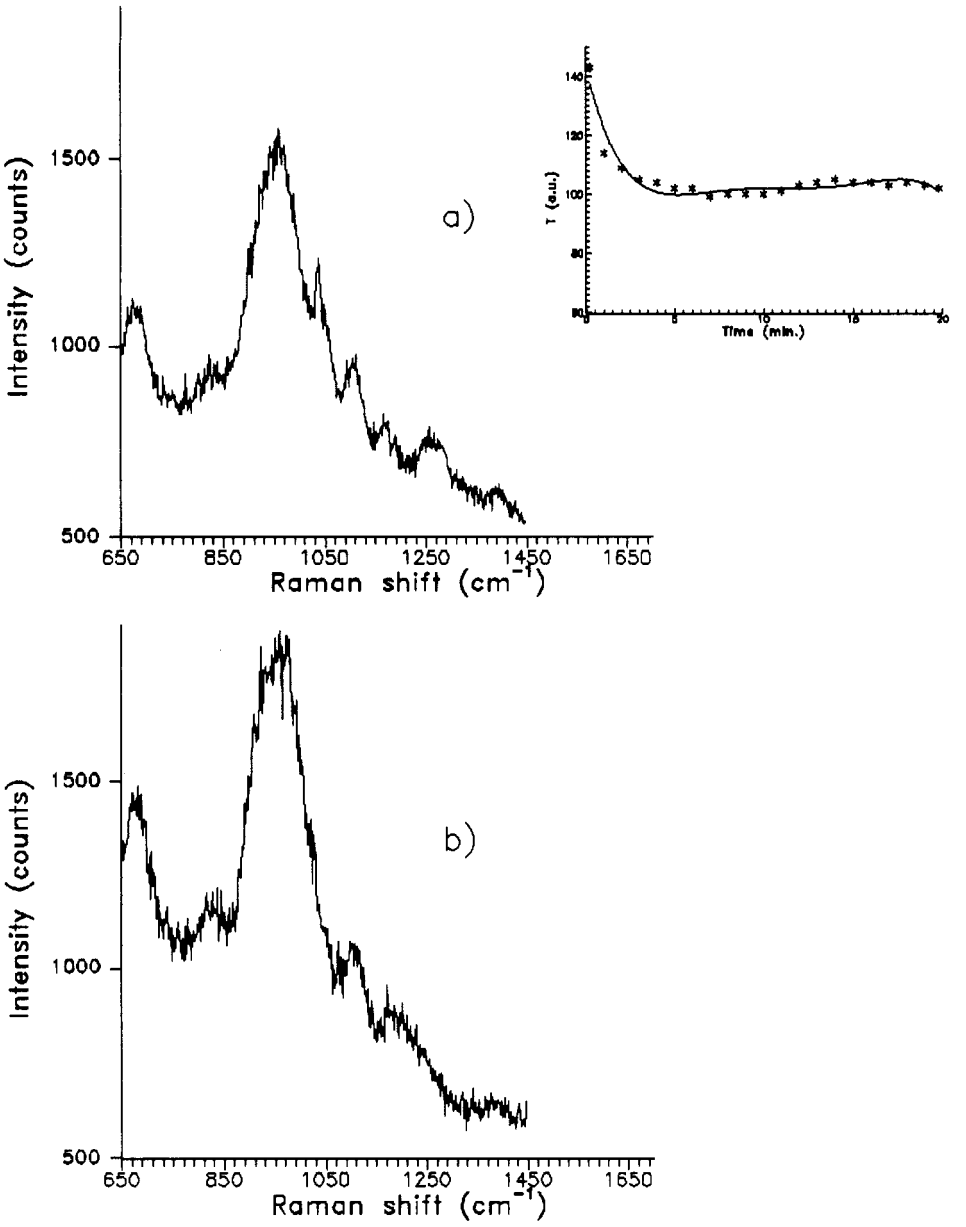


**Figure 6.** SERS spectra of sulfanilamide (25 μg/mL) on colloidal silver. Scan started (a) immediately after mixing colloid and adsorbate and (b) after 5 min later. The inset shows the variation of the transmitted light with time.

**Applications.** The extent of the aggregation process and, consequently, the SERS intensity depends on the chemical structure of the adsorbate. So, adsorbates with different SERS-active functional groups show particular aggregation kinetics which are difficult to predict on the sole basis of chemical structure. Figure 5 shows a practical example of how a rapid aggregation leading to useless spectral data can be readily detected with the system proposed here. With 1.25 μg/mL 9-aminoacridine (a compound with large SERS activity), the SERS intensity decreases rapidly (Figure 5a) to levels similar to the background signal (Figure 5b). No spectral information can be obtained from this sample after 4 min. The evident rise in transmitted light (Figure 5c) clearly indicates that spectral data would be recorded under non-optimal experimental conditions.

The use of monochannel spectrometers for spectral data acquisition in dynamic samples such as those generally found in colloid SERS may cause spectral misinterpretation. At aggregation rates in the same time frame as the spectrometer scan speed what one observes in the abscissa axis is a convolution of time and Raman shift. In SERS, the relative intensity of certain vibrational modes changes in the course of colloid aggregation in response to reorientation of adsorbate molecules at the silver surface. Thus to avoid the dependence of spectral features on data acquisition conditions one should be able to scan the spectral zone of interest at least during the time the colloid is stationary. Figure 6 shows two SERS spectra from the same sample (sulfanilamide at 25 μg/mL). The inset shows the variation of the transmitted intensity with time. Spectrum a, whose scan started immediately after mixing the colloid and adsorbate, shows peaks at 1050 and 1260 $cm^{-1}$. These peaks do not appear in spectrum b, obtained 5 min later. These peaks could correspond to transient vibrational modes, evolving in the same time frame as the spectral acquisition. The intensity of the transmitted light reveals the dynamic character of the system during the first 5 min and shows that data collected during this time period may be affected by dynamic components. After constant transmitted light, spectral features and intensities are reproducible.

In conclusion, inhomogeneous aggregation and slow adsorption kinetics in colloidal systems may cause severe distortions in SERS intensities. These effects are more prone to occur for low adsorbate concentrations. The measurement

of the transmitted light as proposed here provides information in real time on the aggregation status of the sample being examined and it can be used as a diagnostic tool for unstable and transient situations. This approach is conceptually simple and it is easy to implement in any spectrometer.

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