Quantitative Determination of the Surface Concentration of Phenazine Adsorbed on Silver Colloidal Particles and Relationship with the SERS Enhancement Factor

Maurizio Muniz-Miranda*,† and Giuseppe Sbrana‡

*Dipartimento di Chimica, Universita' di Firenze, Via G. Capponi 9, Firenze, I-50121 Italy, and
Centro CNR di Studio sui Composti Eterociclici, Via G. Capponi 9, Firenze, I-50121 Italy*

SERS spectra of phenazine adsorbed on silver colloids have been investigated. For ligand concentrations from 10^{-4} to 10^{-6} M, it was possible to obtain the adsorption isotherm of phenazine by absorption measurements in the UV−visible region. Filtration of the ligand/hydrosol systems through suitable filters containing aluminum oxide membranes allowed a complete removal of the metal particles from the filtrate. Determination by spectral analysis of phenazine still dissolved in solution provided a quantitative estimate of the ligand adsorbed on the metal particles at different analytical concentrations. Colloids activated by chloride anions were used in these experiments to improve properly the Raman signal of the adsorbate. The results indicate that the SERS enhancement exhibits factors up to 10^6, in agreement with the previous theoretical and experimental findings.

Introduction

The Raman band enhancement of molecules adsorbed on silver colloidal surfaces is usually evaluated by referring to the concentration of the ligand in the hydrosol. Hence, the enhancement factor is generally considered as the ratio of the intensities of the SERS and the corresponding Raman bands observed in a reference aqueous solution. However, this ratio represents only an "apparent value" of the enhancement factor. The SERS spectra are closely dependent on the coverage of the colloidal surface, thus no correlation is possible with the enhancement of the Raman signal without knowing the amount of ligand really adsorbed onto the metal substrate. The methods proposed in the literature for obtaining the enhancement factor in Ag or Au colloids are based on the determination of the number of adsorbed molecules[1] and generally need the knowledge of the adsorption isotherm of the ligand.[2] A direct determination of the amount of adsorbed molecules was carried out on dyes such as rhodamine 6G and oxazine 1 in Ag and Au colloidal dispersions.[1] Fluorescence measurements, performed in an aqueous reference solution containing the ligand, were compared with the fluorescence data of the "free" molecules still present in the supernatant liquid after centrifugation of the colloidal dispersion. The applicability of this method is, however, restricted to specific compounds, such as dyes, which are characterized by strong fluorescence emission.

Here we report a different procedure, which can be extended to all organic compounds and allows satisfactory determination of the number of adsorbed molecules by means of optical measurements in the UV−visible region. The actual amount of adsorbate is determined by comparing the intensities of the absorption bands in the reference aqueous solution and in the filtrate obtained by a silver hydrosol containing the same analytical concentration of ligand.

For these experiments we have chosen, as ligand, phenazine, a heteroaromatic molecule with two nitrogen atoms, that is

strongly adsorbed on the Ag surface, giving rise to valuable surface enhanced Raman spectra. In addition, the presence of the absorption band of phenazine at 370 nm in the extinction spectrum of the aqueous solution allows measurements without overlap of nitrate and borate bands, present in the spectrum of the filtered hydrosol below 250 nm.

Experimental Section

Silver colloidal dispersions were prepared by reducing $AgNO_3$ with excess $NaBH_4$.[3] The ligand/sol systems were obtained by adding phenazine to the Ag colloids after several hours from the sol preparation in order to avoid reduction products.[4]

Phenazine, supplied by Fluka Co. (purity >98%), was purified by repeated sublimations under vacuum.

Aqueous solutions of phenazine in water were prepared with the following molar concentrations: 10^{-4}, 5×10^{-5}, 2×10^{-5}, 10^{-5}, 5×10^{-6}, 2×10^{-6}, 10^{-6}. The same concentrations were adopted for preparing the corresponding ligand/Ag hydrosols. Because of the scarce solubility of phenazine in water, it was impossible to increase the concentration of the solutions. In this range of concentrations it was difficult to have a satisfactory Raman signal without the addition to the sols of NaCl (10^{-3} M), which improved considerably the SER scattering. The addition of salt decreased the aggregation of the silver particles[5] and increased the stability of the colloids, which remained unaltered during the experiments.

All of the Raman spectra were registered with the 514.5 nm exciting line of an Ar^+ laser equipped with a Jobin-Yvon HG-2S monochromator and a cooled RCA-C31034A photomultiplier. A power of 200 mW was monitored at the base of a 10 mm quartz cell containing the sample.

SERS intensity measurements were carried out without the addition to the colloidal dispersion of an internal standard which could alter the sol stability.[16] A methanol solution of phenazine (5×10^{-2} M) was used as external standard.

The colloidal samples were filtered through ANOTOP filters (Anotec, U.K.) containing aluminum oxide membranes with 0.02 μm filtration diameter, which are able to retain the overall Ag

---

* Corresponding author fax: +39-055-2476961. E-mail: muniz@chim.unifi.it.
† Universitá di Firenze.
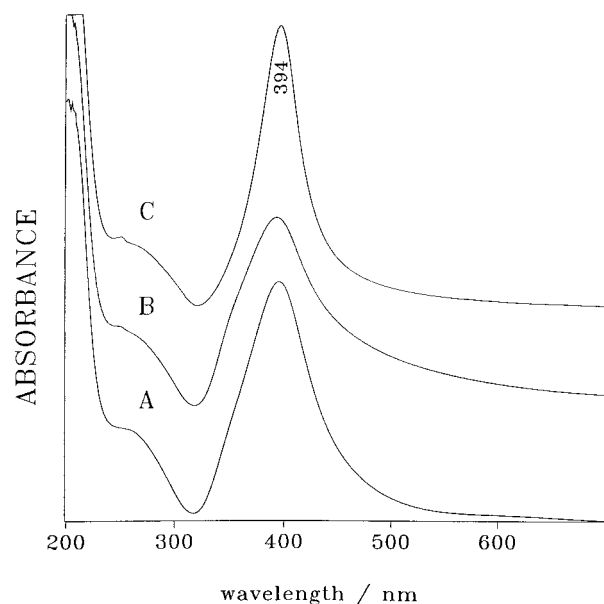‡ Centro CNR di Studio sui Composti Eterociclici.

Figure 1. Extinction spectra of a salt-free Ag colloidal dispersion without ligand (A); with 10^{-6} M phenazine (B); with 10^{-6} M phenazine and 10^{-3} M NaCl (C).

Figure 2. Absorption spectra of 10^{-4} M phenazine in aqueous solution (A) and in the filtered Ag colloidal dispersion (B); in the inset the absorption spectra of 10^{-6} M phenazine are reported in the same environments.

particles.[6] This procedure was repeated twice up to the disappearance of the plasmon band at 394 nm, due to the nonaggregated particles.

UV−visible absorption spectra were recorded in the 800−200 nm region with the aid of a Cary 5 spectrophotometer using cells from 1 to 10 mm path lengths.

Results

The extinction spectra of silver colloids are shown in Figure 1. Spectrum A refers to a freshly prepared colloid, spectrum B to a colloid containing phenazine (10^{-6} M), spectrum C to the same ligand/colloid system with addition of NaCl (10^{-3} M).

The addition of phenazine induces aggregation of the silver particles, which results in a broadening of the plasmon band at 394 nm toward longer wavelengths. Addition of NaCl causes sharpening of this band, suggesting the presence of nonaggregated and nearly uniform Ag particles. In Figure 2 the UV−visible extinction spectrum of a 10^{-4} M aqueous solution of phenazine is compared with that of the filtrate obtained from a silver colloidal suspension containing the same concentration of ligand. Apart from the occurrence below 250 nm of absorption bands due to borate and nitrate ions deriving from the colloid preparation, spectrum B closely matches spectrum A in the 250−500 nm region, because of the total disappearance of the plasmon band at 394 nm. This confirms that, via a double filtration, it is possible to remove the overall metal particles and to consider the band of phenazine at 370 nm for absorbance measurements. From the different values detected in the reference and filtered solutions it results that only 1.8% of phenazine dissolved in the Ag hydrosol is really adsorbed on the metal substrate. In the inset of Figure 2, spectra A and B refer to a 10^{-6} M concentration of ligand. In this condition the percentage of phenazine really adsorbed corresponds to 37%.

These measurements are not extended to more concentrated solutions since the percentage of adsorbed ligand is negligible with respect to the amount dissolved in the colloidal dispersion and cannot be estimated. On the other hand, more diluted solutions cannot be employed because UV and SERS spectra are too weak to provide reliable information.
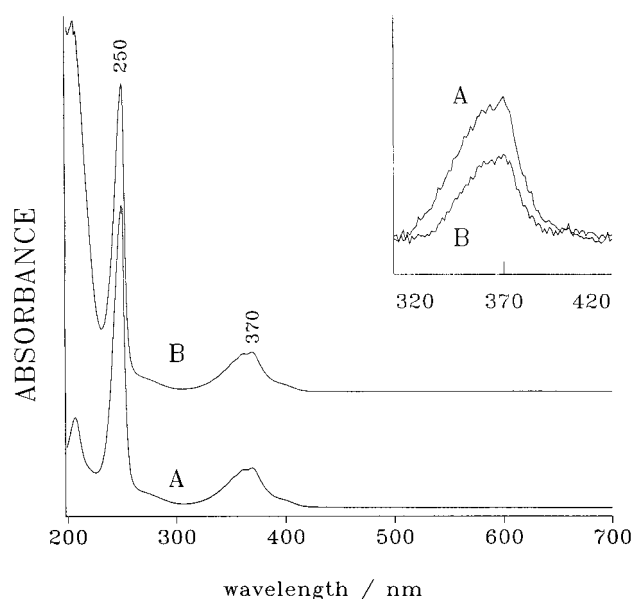
This simple technique is very effective since it allows a complete separation between the free ligand and the ligand really adsorbed on the silver particles. As alternative to the centrifugation or precipitation employed until now in the separation procedure,[1,2] the filtration appears more suitable since it does not alter the equilibrium between adsorbate and substrate. Actually, the SERS spectra obtained from colloidal dispersions or coated filters show a close similarity,[7] since the morphology of the dry Ag layer closely resembles that of the colloidal particles.[6]

Our results could be affected with the adsorption of the ligand onto the alumina membrane of the filter. The comparison between the absorbance of the filtrate with those of the solutions obtained by centrifugation or collapse of the colloidal particles shows that only a negligible amount of phenazine (less than 0.1% of the analytical concentration) is really adsorbed on alumina.

The compared analysis of seven solutions of phenazine, in the 10^{-4} to 10^{-6} M concentration range, with the corresponding filtered solutions of the equimolar ligand/hydrosols samples identifies a number of points sufficient to draw satisfactorily the curves reported in Figure 3.

The surface concentration (C_s) and the percentage of phenazine really adsorbed on the metal surface are plotted versus the analytical concentration of phenazine dissolved in the Ag colloidal dispersions (C_a). The plot of the C_s values is depicted by a curve showing the typical shape of the adsorption isotherm, according to Langmuir's equation

$$C_s/C_s^{max} = kC_a/(1 + kC_a) \tag{1}$$

C_s^{max} represents the surface concentration at saturation of the active sites and k, the adsorption coefficient. For dilute solutions, as in the present case, the molar concentrations satisfactorily replace the corresponding activities of phenazine. According to the UV−visible absorption data, the following values are obtained: $C_s^{max} = 2.1 \times 10^{-6}$ M; $k = 6 \times 10^{4}$ M^{-1}.

The indications drawn from the plots of Figure 3 confirm that the surface concentration of adsorbate increases by increasing the amount of ligand dissolved in the colloidal dispersion
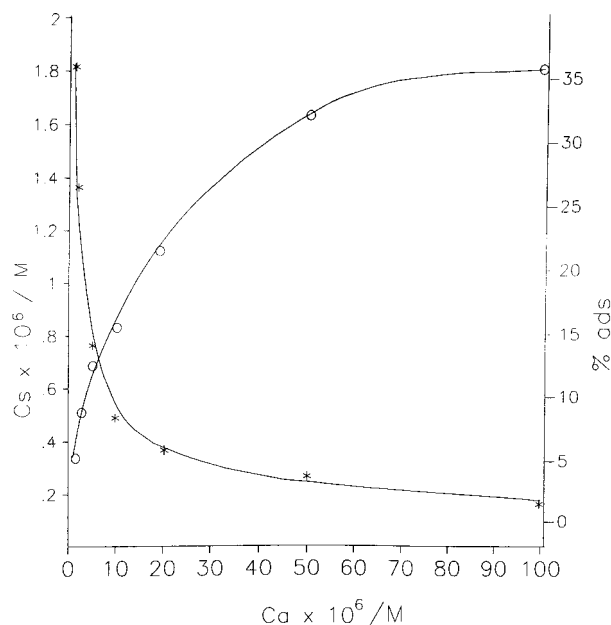
Phenazine Adsorbed on Silver Colloidal Particles

J. Phys. Chem. B, Vol. 103, No. 48, 1999 **10641**

Figure 3. Surface concentration (C_s), [O], and percentage (%ads), [*], of phenazine adsorbed on Ag particles versus the analytical concentration of phenazine dissolved in the Ag hydrosols (C_a).

Figure 4. Raman spectra of phenazine (A) in methanol (5×10^{-2} M) and from Ag hydrosols containing NaCl (10^{-3} M) with 10^{-6} M (B), 10^{-5} M (C), 10^{-4} M (D) ligand concentrations.

following the adsorption isotherm. On the contrary, the percentage of adsorbed phenazine drastically decreases from 37% at $C_a = 10^{-6}$ M to 8% at $C_a = 10^{-5}$ M up to 1.8% for a 10^{-4} M analytical concentration of ligand.

The C_s concentration, described as ratio between moles of adsorbed ligand and volume V of the aqueous dispersion of Ag particles taken with uniform size, can be expressed as

$$C_s = n_p s d / (VN) \tag{2}$$

where n_p is the number of colloidal particles in the volume V, n is the number of molecules adsorbed on a single metal particle, N is Avogadro's number, s is the surface of the colloidal particle, and d is the surface density of the adsorbate, defined as n/s.

In the hypothesis of a colloidal particle with spherical shape, the surface density of the adsorbate can be obtained by assuming $s = 4\pi r^2$. The average radius of the colloidal particles generally changes from 10 to 100 nm depending on the aggregation degree. Hence, a first indication of the value r can be achieved from the inspection of the plasmon band in the UV−visible extinction spectrum, whose wavelength is closely dependent on the size of the silver particles. This allows determination of the number of metal particles in the volume V by considering the concentration of the AgNO$_3$ solution used in the colloid preparation.

In Figure 4 the Raman spectrum of phenazine in methanol is compared with the SERS spectra of the molecule adsorbed on silver colloids activated by chloride anions. The SERS spectra of phenazine at different analytical concentrations (10^{-4} to 10^{-6} M) exhibit the same pattern and closely match the normal Raman spectrum. Hence no change in the substrate−molecule interaction is expected by varying the ligand concentration.

The SERS spectra shown in Figure 4 are similar to that obtained on an Ag electrode at applied potential $E = -0.05$ V in aqueous solution of phenazine with 10^{-1} M KCl.[8] At more negative potential ($E < -0.3$ V), a quite different spectrum was observed, due to reduction products of phenazine. No photoreduction product was observed during our experiments in air, contrary to the photoreduction process which phenazine undergoes in deaerated ligand/Ag hydrosols.[8]
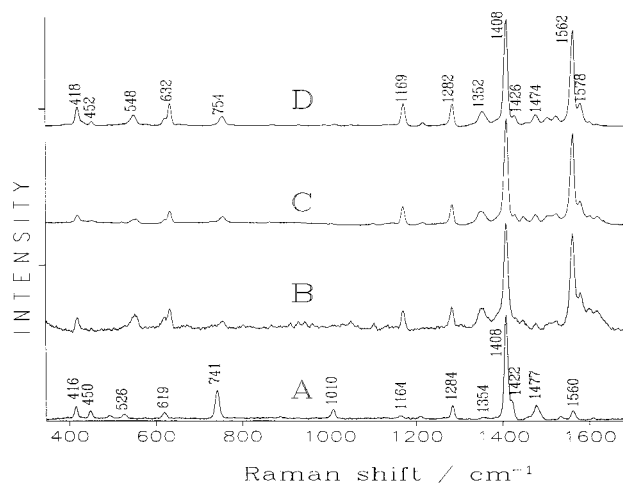
Determination of the SERS Enhancement Factor

In previous papers,[9−11] the enhancement effect was widely discussed from the quantitative point of view and it was found that the Raman cross section undergoes enhancement of 10^6 in the SERS. Actually, the enhancement factor F can be written as

$$F = F_{EM} F_{CH} \tag{3}$$

where F_{EM} and F_{CH} are the electromagnetic and chemical enhancement factors, which contribute to the total SERS intensity. The electromagnetic effect is accepted as improving the intensity of the Raman signal from 100 up to 1000 times.[2,13−16] For the chemical mechanism, which the molecule undergoes when adsorbed on silver colloids activated by halide ions, an enhancement factor of ∼100 is generally predicted[17] and ascertained.[2,18] The addition of these anions to Ag sols is expected to induce severe changes in the chemical and physical properties of the metal substrate.[19,20] The effect of increasing the Raman signal and the anion-activated SERS effect are discussed in ref 1 on the basis of both electromagnetic and chemical mechanisms.

We have calculated the overall SERS enhancement factors of phenazine by determining the amount of ligand really adsorbed on the colloids and by measuring the SERS spectra of phenazine in the same colloidal dispersions.

The enhancement factor of the Raman signal, due to the molecules adsorbed on the metal surface, can be expressed as

$$F = \frac{I_{SERS} C_{sol}}{I_{Raman} C_s} \frac{1}{f_{sh}} \tag{4}$$

where I_{SERS} and I_{Raman} are the Raman intensities of the ligand in the Ag colloidal dispersion and in the aqueous reference solution, respectively. C_{sol} and C_s indicate the concentrations of the ligand in solution and adsorbed onto the substrate, respectively. C_s is determined, by means of absorption measurements, as the difference between the concentrations of phenazine dissolved in the hydrosol and phenazine still present in the filtrate. The f_{sh} factor represents the shielding, which exciting and scattered radiations undergo by the metal particles. A value of 0.25 has been chosen in agreement with the works of Moskovits[21] and Hildebrandt.[2]
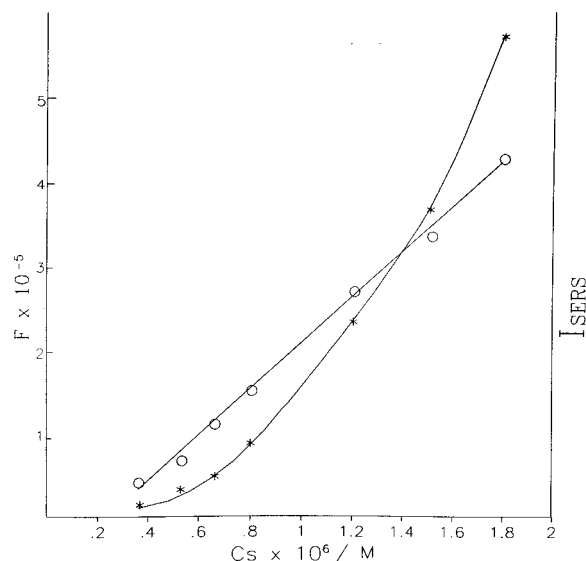
Figure 5. Enhancement factor (F), [O], and SERS intensity (I_{SERS}), [*], versus the surface concentration of phenazine adsorbed on the Ag colloid (C_s). F and I_{SERS} are referred to the 1408 cm^{-1} band of phenazine; I_{SERS} in arbitrary unit.

As shown in Figure 5, the enhancement factor seems to depend on the surface concentration of adsorbate (C_s), according to a linear relationship. An enhancement factor of 4.4×10^5 is found when 1.8% of phenazine is adsorbed in Ag hydrosol with 10^{-4} M analytical concentration.

The choice of suitable SERS bands for determining the enhancement factor plays a nonnegligible role. Different values of the enhancement factor are obtained by measuring the intensity of the strongest band observed at 1408 cm^{-1} in both SERS and Raman spectra or that of the most enhanced SERS band at $\sim$1560 cm^{-1}. In the latter case, the enhancement factor displays the same trend but with an increment of 1 order of magnitude.

Actually, many factors, besides the surface coverage, can affect the SERS enhancement, such as modifications of both substrate and adsorbate. For example, the aggregation of the metal particles with increasing ligand concentration generally improves the SERS enhancement. In our samples, however, the presence of chloride anions in the Ag hydrosols provokes reversal of aggregation and more stability of the colloidal particles. Thus, no change in the substrate is expected to alter the Raman enhancement. On the other hand, no change in the adsorbate is detected at different ligand concentrations (Figure 4). Hence the linear relationship between the enhancement factor and the surface coverage cannot be considered casual.

With regard to the plotting of Figure 5, showing the correlation between the SERS intensity (I_{SERS}) and the surface concentration (C_s), I_{SERS} increases with the square of the concentration C_s. This can be also deduced from eq 4 by assuming I_{Raman} proportional to the concentration of the reference solution (C_{sol}) and by considering a direct proportionality between the factor F and the C_s concentration. The relation between SERS enhancement and surface coverage was widely discussed in several papers. A nonlinear dependence was found for ligands adsorbed on Ag films;[22-26] more recently, a direct proportionality between SERS intensity and surface coverage on Ag or Au electrodes was established on the basis of electrochemical measurements.[27,28] In this latter case the absence of chloride anions in the electrolyte avoided charge-transfer effects induced by coadsorption of these ions. The different relationship, obtained from the present experiments, can rely
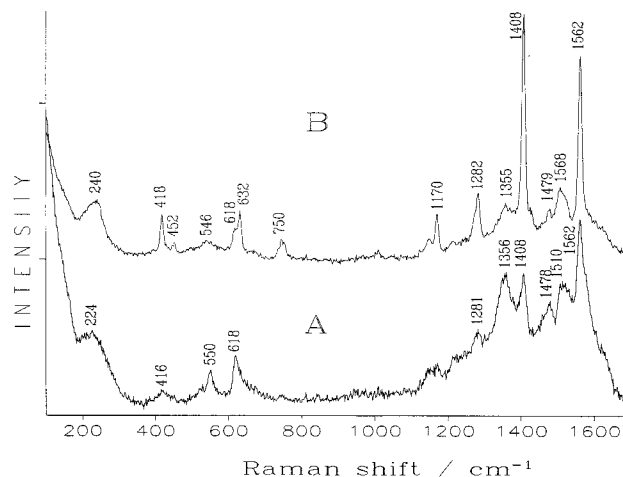

Figure 6. SERS spectra of phenazine adsorbed on dry Ag layers, obtained by filtration of salt-free ligand/colloid (A) and of ligand/colloid activated by chloride anions (B). 10^{-4} M ligand concentration in hydrosols.

on the different type of substrate, formed by colloidal particles activated by chloride anions.

We have also extended our investigation to phenazine adsorbed on salt-free colloids with the same analytical concentrations, but we failed, because of the scarce intensity of the SERS bands. However, by filtering the salt-free Ag colloidal dispersion containing 10^{-6} M phenazine, we found that only $\sim$25% of the ligand is really adsorbed, against $\sim$37% detected in the activated colloids. Hence, the further enhancement of the SERS signal in activated colloids can arise also from an increase of ligand adsorbed on the metal surface, which is favored by the formation of Ag$^+$−chloride anion complexes, as SERS-active sites.

Finally, the Ag-coated filters, obtained by filtration of silver hydrosols, provide a SERS-active substrate, which can be employed in comparison with the usual Ag colloidal dispersions or as alternative to them.[6,7] Hence the appropriate use of these filters not only provides a quantitative determination of the effective adsorbate but, at the same time, allows obtaining SERS spectra of the ligand adsorbed on dry Ag layers. SERS spectra of phenazine adsorbed on Ag-coated filters in the presence or in absence of chloride anions are shown in Figure 6.

Conclusions

From the present investigation it is evident the general validity of our procedure for molecules adsorbed on silver colloids. The amount of adsorbed ligand is determined, without the need of Raman measurements, by a simple comparison of the UV−visible absorption spectra of the aqueous solution and of the equimolar ligand/hydrosol after filtration with ANOTOP filters.

Contrary to the other procedures previously mentioned, this method could be applied to all organic molecules, provided that the relative absorption bands are sufficiently intense. The study of compounds with absorption bands below 250 nm, where nitrate and borate anions deriving from the colloid preparation exhibit intense bands, is strongly impaired.

The knowledge of the surface concentration allows estimating the adsorption isotherm of phenazine and the SERS enhancement factors. Quantitative data about the relation between the surface-enhanced Raman intensity and the surface coverage of adsorbate on silver particles can provide a better understanding of the SERS mechanism.

The addition of coadsorbed chloride anions to Ag hydrosol gives rise to a further increase of the SERS intensity by

Phenazine Adsorbed on Silver Colloidal Particles

J. Phys. Chem. B, Vol. 103, No. 48, 1999 **10643**

activation of the colloidal surface. Moreover, it ensures adequate stability of the colloids by avoiding the rapid decay of the Raman signal with time.

References and Notes

(1) Hildebrandt, P.; Keller, S.; Hoffmann, A.; Vanhecke, F.; Schrader, B. *J. Raman Spectrosc.* **1993**, *24*, 791.

(2) Hildebrandt, P.; Stockburger, M. *J. Phys. Chem.* **1984**, *88*, 5935.

(3) Creighton, J. A.; Blatchford, C. G.; Albrecht, M. G. *J. Chem. Soc., Faraday Trans. 2* **1979**, *75*, 790.

(4) Muniz-Miranda, M.; Neto, N.; Sbrana, G. *J. Mol. Struct.* **1986**, *174*, 351.

(5) Heard, S. M.; Grieser, F.; Barraclough, C. G.; Sander, J. V. *J. Phys. Chem.* **1985**, *89*, 389.

(6) Muniz-Miranda, M.; Neto, N.; Sbrana, G. *J. Mol. Struct.* **1997**, *410−411*, 205.

(7) Neto, N.; Muniz-Miranda, M.; Sbrana, G. *J. Mol. Struct.* **1995**, *348*, 277.

(8) Takahashi, M.; Goto, M.; Ito, M. *J. Electroanal. Chem.* **1989**, *51*, 177.

(9) *Surface Enhanced Raman Scattering*; Chang, R. K., Furtak, T. E., Eds.; Plenum Press: New York, 1982.

(10) Otto, A. In *Light Scattering in Solids IV*; Cardona, M., Güntherodt, G., Eds.; Springer-Verlag: Heidelberg, 1984.

(11) Schatz, G. C. In *Fundamentals and Applications of Surface Raman Spectroscopy*; Garrell, R. L., Pemberton, J. E., Cotton, T. M., Eds; VCH: Dearfield Beach, 1993.

(12) Wokaun, A. In *Solid State Physics: Advances in Research and Applications*; Ehrenreich, H., Turnbull, D., Eds.; Academic: London, 1984; Vol. 28, p 223.

(13) Yamada, H. In *Infrared and Raman Spectroscopy;* Tsuboi, M., Tanaka, S., Tasumi, M., Eds.; Namkodo: Tokyo, 1983; Vol. 2, p 1.

(14) Numata, H. *J. Phys. Soc. Jpn*, **1982**, *51*, 2575.

(15) Chen, M. C.; Tsai, S. D.; Chen, M. R.; Ou, S. Y.; Li, W.-H.; Lee, K. C. *Phys. Rev. B* **1995**, *51*, 4507.

(16) Aubard, J.; Bagnasco, E.; Pantigny, J.; Ruasse, M. F.; Levi, G.; Wentrup-Byrne, E. *J. Phys. Chem.* **1995**, *99*, 7075.

(17) Wang, X.; Wen, H.; He, T.; Zuo, J.; Xu, C.; Liu, F.-C. *Spectrochim. Acta A* **1997**, *53*, 2495.

(18) Liang, E. J.; Kiefer, W. *J. Raman Spectrosc.* **1996**, *27*, 879.

(19) Wetzel, H.; Gerischer, H. *Chem. Phys. Lett.* **1980**, *76*, 460.

(20) Muniz-Miranda, M.; Sbrana, G. *J. Raman Spectrosc.* **1996**, *27*, 105.

(21) Suh, J. S.; DiLella, D. P.; Moskovits, M. *J. Phys. Chem.* **1983**, *87*, 1540.

(22) Rowe, J. E.; Shank, C. V.; Zwemer, D. A.; Murray, C. A. *Phys. Rev. Lett.* **1980**, *44*, 1770.

(23) Arunkumar, K. A.; Bradley, E. B. *J. Chem. Phys.* **1983**, *78*, 2882.

(24) Pockrand, I. *Surf. Sci.* **1983**, *126*, 192.

(25) Murray, C. A.; Bodoff, S. *Phys. Rev. Lett.* **1984**, *52*, 2273.

(26) Murray, C. A.; Bodoff, S. *Phys. Rev. B* **1985**, *32*, 671.

(27) Foresti, M. L.; Funtikov, A. M.; Guidelli, R.; Muniz-Miranda, M. *J. Electroanal. Chem.* **1994**, *367*, 223.

(28) Brolo, A. G.; Irish, D. E.; Szymanski, G.; Lipkowski, J. *Langmuir* **1998**, *14*, 517.