A New Method for Fast Preparation of Highly Surface-Enhanced Raman Scattering (SERS) Active Silver Colloids at Room Temperature by Reduction of Silver Nitrate with Hydroxylamine Hydrochloride

Nicolae Leopold[†,‡] and Bernhard Lendl*[,‡]

Faculty of Physics, Babes-Bolyai University, Kogalniceanu 1, 3400 Cluj-Napoca, Romania, and Institute of Chemical Technologies and Analytics, TU Vienna, Getreidemarkt 9-164/AC, 1060 Vienna, Austria

A very effective and simple way to produce silver colloids for surface-enhanced Raman scattering (SERS) is reported. Reduction of silver nitrate with hydroxylamine hydrochloride at alkaline pH and at room temperature yields highly sensitive SERS colloids within a short time. The so-produced colloids can be used for SERS spectroscopy immediately after preparation. The overall procedure is fast, simple, and characterized by a high preparation success rate. Changing the mixing order and rate of the two involved solutions, silver nitrate and hydroxylamine hydrochloride containing sodium hydroxide, one can control the size and dispersion of the produced colloids. The obtained colloids have been characterized by UV−vis spectroscopy, transmission electron microscopy, and SERS using a 1064 nm laser line on a Fourier transform and a 785 nm laser line on a dispersive Raman spectrometer. The SERS enhancement factor of the hydroxylamine-reduced silver colloids was tested using crystal violet, rhodamine 6G, methylene blue, and 9-aminoacridine. It was found that for both excitation lines sensitivities comparable to those achievable with a Lee−Meisel silver colloid were obtained thus rendering the new colloid advantageous because of its significantly simpler and faster synthesis.

1. Introduction

The possibility of utilizing colloidal dispersions of Ag or Au in aqueous solutions as a method for enhancing Raman scattering was first demonstrated by Creighton and co-workers[1] in 1979. Experimentally, it was found that the most intense surface-enhanced Raman scattering (SERS) was obtained from molecules adsorbed to silver surfaces, although almost comparable intensities have also been observed for many other metal surfaces such as copper, lithium, sodium, potassium, aluminum, or indium.[2] For SERS, the most frequently used silver colloids are produced either by reduction of silver nitrate with sodium citrate, the so-called Lee−Meisel[3] method, or by reduction of silver nitrate with sodium borohydride following the Creighton[1] procedure. Apart from these, other silver reducing methods have also been reported for preparing SERS-active silver colloids.[4−8] Despite the apparent simplicity of the published procedures, the preparation of a highly active SERS colloid is not a trivial subject. Citrate reduction of silver requires addition of the reducing agent to the silver nitrate solution during vigorous stirring and refluxing. For the Creighton silver colloid, a solution of sodium borohydride is mixed with a solution of silver nitrate during intensive stirring in an ice-cold bath. If due care is not exercised during silver colloid synthesis, the colloid aggregates, eventually precipitating the metal. This leads to changing properties of the silver colloid and thus also to varying enhancement factors.[9]

In this work, we report a simple and fast method for preparing stable, highly SERS-active silver colloids by reduction of silver nitrate with hydroxylamine hydrochloride at alkaline pH and at room temperature. The so-produced colloids have been characterized by UV−vis spectrometry, transmission electron microscopy (TEM), and SERS. The SERS activity of these colloids was tested using various analytes and compared with those exhibited by the Lee−Meisel silver colloid. Hydroxylamine as reducing agent is widely known,[10,11] but the use of hydroxylamine to produce silver colloid suspension as SERS substrate has not been reported yet. The advantages of the hydroxylamine hydrochloride reduced silver colloid are seen in its fast preparation at room temperature and its immediate applicability for SERS spectroscopy.

2. Experimental Section

Chemicals. Solutions of silver nitrate (Merck), hydroxylamine hydrochloride (Fluka), sodium hydroxide (Sigma-Aldrich), sodium chloride (Merck), 9-aminoacridine hydrochloride monohydrate (Merck), crystal violet chloride (Sigma-Aldrich), rhodamine 6G (Sigma-Aldrich), and methylene blue (Sigma-Aldrich) were prepared with distilled water.

Methods. UV−vis spectra were recorded on an UV−vis diode array spectrometer (HP 8452A, Hewlett-Packard). Transmission electron micrographs were taken on an analytical transmission electron microscope JEOL JEM-100CX operating at 100 kV. The particle diameters have been estimated from the TEM micrographs using the imageJ 1.22d software from Wayne Rasband, National Institute of Health, U.S.A. Fourier transform (FT) SERS spectra were recorded in backscattering geometry on a Bruker FRA 106 Raman accessory attached to a Bruker IFS 66 FT-IR spectrometer equipped with a liquid nitrogen cooled Ge detector. The 1064 nm Nd:YAG laser was used as excitation source, and the laser power was set to 100

---

* To whom correspondence should be addressed. Web address: http://www.iac.tuwien.ac.at/cavs/.
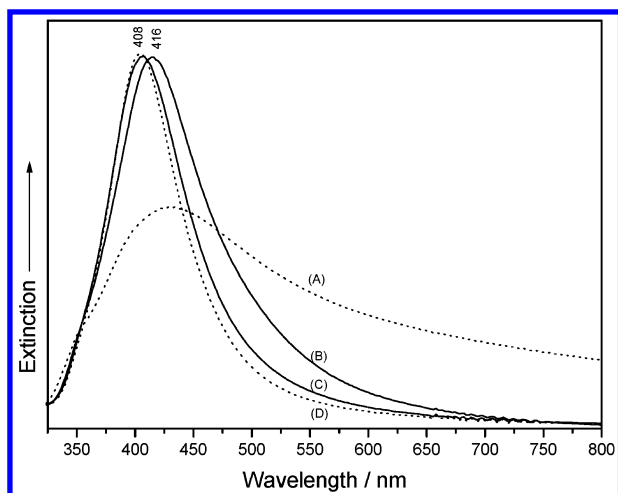† Babes-Bolyai University.
‡ TU Vienna.

Figure 1. Extinction spectra of hydroxylamine-reduced silver colloids 1 day after preparation: 10 mL of hydroxylamine/sodium hydroxide added (A) dropwise or (B) rapidly to 90 mL silver nitrate; 10 mL silver nitrate added (C) rapidly or (D) dropwise to 90 mL of hydroxylamine/sodium hydroxide.

mW. FT-SERS spectra were recorded with a resolution of 8 cm^{-1} by coadding 32 scans each. SERS spectra were recorded with a Kaiser Optics HLR 5 Raman spectrometer equipped with a "NIR-R-CCD ", near-infrared (NIR)-optimized detector. The excitation wavelength was 785 nm from a diode laser, and the laser power was set to 47 mW on the probe. All SERS spectra were collected in backscattering geometry with one scan of 5 s for each spectrum and a spectral resolution of 4 cm^{-1}. All FT-SERS and SERS experiments were carried out in a quartz cuvette with an optical path of 1 cm using 1 mL of silver colloid.

3. Results and Discussion

Preparation of the Silver Colloids. In concordance with the Lee−Meisel silver colloid, the concentration of silver nitrate in the prepared reaction mixtures was set to 10^{-3} M. The concentration of hydroxylamine hydrochloride was chosen to obtain a concentration of 1.5×10^{-3} M in the final reaction mixture. When using hydroxylamine hydrochloride for reduction of silver nitrate, an initial alkaline pH of the hydroxylamine hydrochloride solution is required. The actual reaction sequence that takes place during reduction is complex and involves most likely complex formation between the hydroxylamine and the silver ions, in addition to the observed silver reduction. Gaseous reaction products are observed too. Recently, it has been reported that reduction of silver using hydrazine[5] produces N_2 whereas when studying the oxidation products released upon reduction of iron(III) with hydroxylamine N_2O and again N_2 were found.[12] In our experiments, a decrease in pH was observed, which indicates the release of protons during the reaction. The final pH of the colloidal solution can be adjusted by varying the amount of sodium hydroxide added to the hydroxylamine solution. In the silver sols reported here, a final pH of 7 was measured. The overall reaction was completed within a few seconds in any case with a preparation success rate greater than 90% as revealed by the recorded SERS spectra.

UV−Visible Spectroscopy and Transmission Electron Microscopy (TEM). To characterize the morphology of the produced colloids, UV−vis spectroscopy and TEM were used. The absorption maximum of the measured UV−vis spectrum of the colloidal solution provides information on the average particle size, whereas its full width at half-maximum (fwhm) can be used to estimate particle dispersion.[13] It was found that colloids with different particle size and dispersion could be
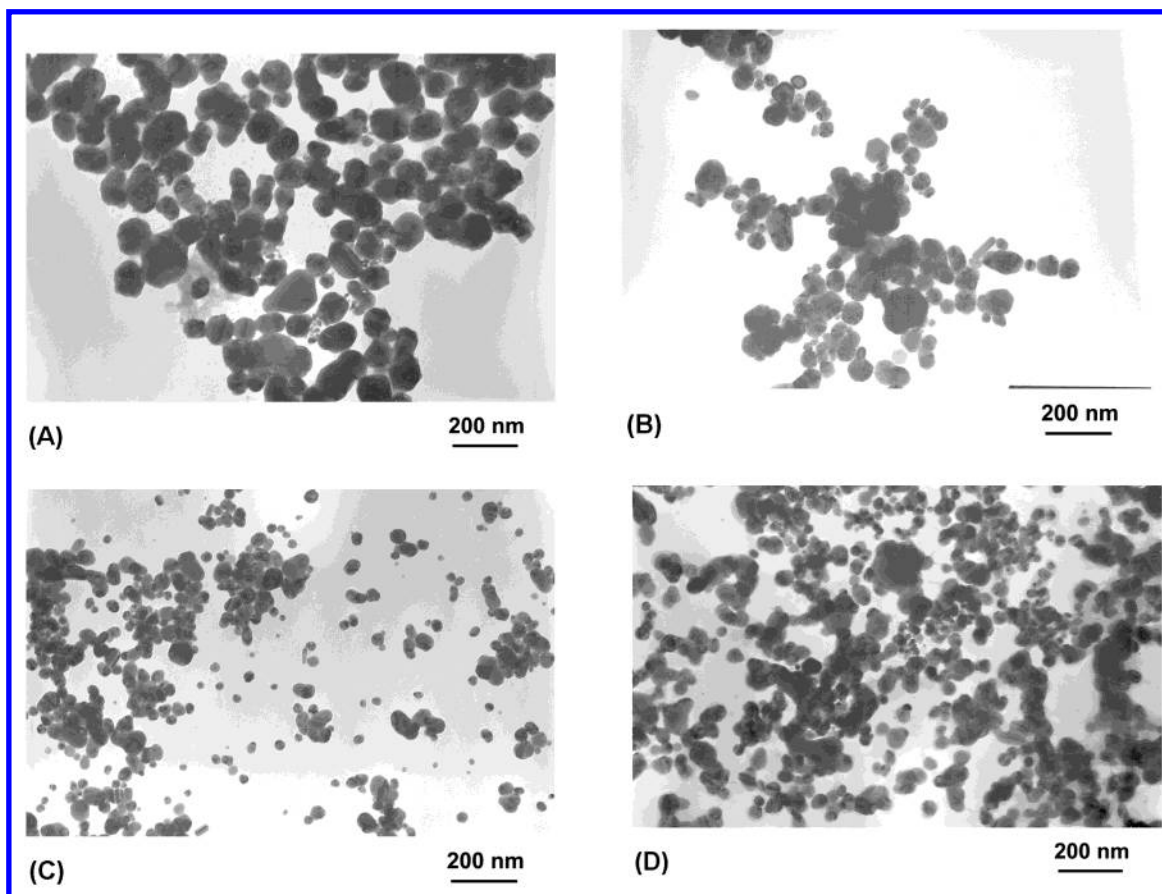

Figure 2. TEM images of hydroxylamine-reduced silver colloids presented in Figure 1.

Fast Preparation of SERS-Active Silver Colloids

J. Phys. Chem. B, Vol. 107, No. 24, 2003 **5725**

obtained reproducibly by changing the mixing order and the mixing rate of the silver nitrate and the hydroxylamine hydrochloride/sodium hydroxide solution involved in silver colloid synthesis. Four different examples are shown in Figure 1. In all cases, the concentration of silver nitrate and hydroxylamine hydrochloride/sodium hydroxide was calculated to be 10^{-3} and $1.5 \times 10^{-3}/3 \times 10^{-3}$ M in the finally obtained reaction mixture. A volume of 100 mL was prepared in all cases. All colloids were prepared under vigorous stirring and found to be SERS-active right after preparation.

As can be seen in Figure 1C,D, the most monodispersed distribution of the particle size was obtained when 10 mL of silver nitrate solution (10^{-2} M) was added to 90 mL of a hydroxylamine hydrochloride solution (1.67×10^{-3} M) containing 3.33×10^{-3} M sodium hydroxide. The absorption maximum was found at 408 nm with a fwhm of approximately 80 nm. As can be seen, rapid (Figure 1C) and dropwise (Figure 1D) addition of a small volume of a concentrated silver solution to a large volume of a less-concentrated hydroxylamine hydrochloride/sodium hydroxide solution produced a similar distribution in the particle size of the prepared colloids. To support this observation, TEM micrographs of the corresponding colloids were recorded as well (Figure 2).

As can be seen in Figure 2C,D, the obtained micrographs corroborate the results obtained by UV−vis spectroscopy. Using the imageJ 1.22d software, we could estimate the average diameter of the silver colloid particles to be in the range of 23 nm in both cases.

However, upon addition of 10 mL of a more-concentrated hydroxylamine hydrochloride/sodium hydroxide solution (1.5×10^{-2} M/3×10^{-2} M, respectively) to 90 mL of a less-concentrated silver nitrate solution (1.11×10^{-3} M), the situation is different. In case of rapid mixing of the two solutions (Figure 1B), an absorption maximum at 418 nm with a fwhm of approximately 100 nm was obtained. This result is also indicative of a rather monodispersed size of the silver particles. From the corresponding TEM micrograph (Figure 2B), a mean diameter of approximately 34 nm was revealed. If the hydroxylamine hydrochloride/sodium hydroxide solution is added dropwise to the silver nitrate solution, the maximum of the absorption band is shifted to 430 nm. The large fwhm extending over more than 100 nm (Figure 1A) is indicative of polydispersed silver particles. Also in this case, the TEM micrograph (Figure 2A) verifies the greater size of the particles; here the averaged diameter of the silver particles was estimated to be about 67 nm. The dropwise addition of the reducing agent probably leads to a seeding effect resulting in a growth of the silver particles.[11] From the obtained TEM micrographs of all hydroxylamine-reduced silver colloids, it can be seen that the particles are predominantly spherical. The colors of the colloids differ from greenish gray if the particle size is rather monodisperse to milky gray when the particles are polydispersed. Comparing the absorption spectra of several hydroxylamine-reduced silver colloids prepared under the same conditions, we found a good reproducibility. For colloids prepared by the same procedure, the variations of the fwhm are less than 10%. Also different preparation procedures can be clearly distinguished by the different color tinge of the colloids. The SERS enhancement of a 3 months aged hydroxylamine-reduced silver colloid was successfully tested as well. It is also worth noticing that during the synthesis of the colloids stirring is not necessary. However, the colloids produced in this study were stirred to ensure a reproducible preparation protocol.

SERS Measurements. The surface-enhanced Stokes Raman signal is proportional to the Raman cross section of the adsorbed molecule, the excitation laser intensity, and the number of
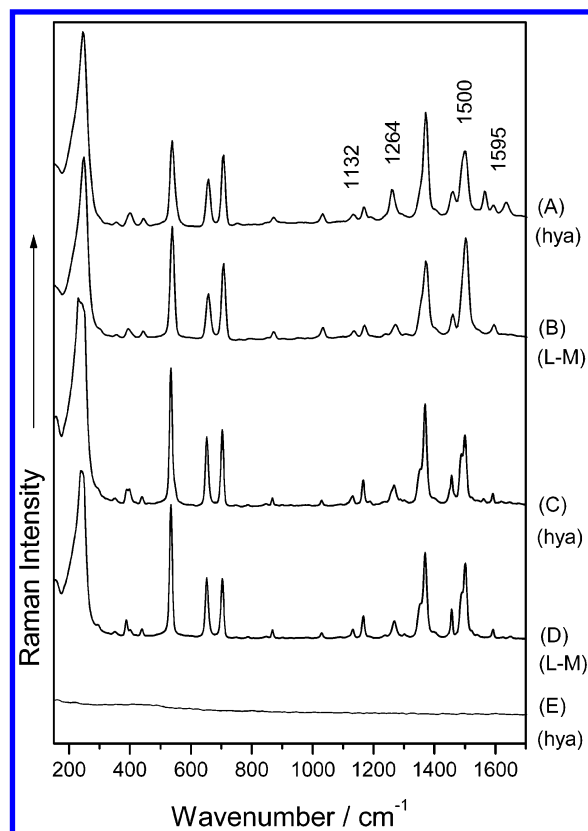

Figure 3. FT-SERS spectra (employing the 1064 nm laser line) of 9-aminoacridine adsorbed on (A) the hydroxylamine-reduced (hya) and (B) Lee−Meisel (L−M) silver sol and SERS spectra (employing the 785 nm laser line) of 9-aminoacridine adsorbed on the (C) hydroxylamine-reduced (hya) and (B) Lee−Meisel (L−M) silver sol and (E) a typical black spectrum obtained of a hydroxylamine-reduced (hya) silver sol using the 1064 nm laser line.

molecules that are involved in the SERS process. The SERS effect occurs because of the very strong electromagnetic fields and field gradients available in the so-called "hot spots" of the fractal colloidal silver cluster.[14] Therefore, the molecules involved in the SERS effect are predominantly those adsorbed on aggregates that are favorable for surface plasmon resonances. The existence of "hot spots" at a given excitation wavelength depends strongly on the geometry of the aggregates. High SERS intensities occur when the exciting laser line overlaps the longitudinal surface plasmon resonance.[15] Therefore, to increase sensitivity, when using laser lines at 785 or 1064 nm, it is necessary to shift the absorption maximum of the silver colloid to higher wavelengths. The addition of a surplus of sodium chloride leads to aggregation of the silver particles and to formation of clusters resulting in a shift of the absorption profile of the colloids to longer wavelengths. The differently produced colloids (Figures 1 and 2) exhibit comparable sensitivities using either a 785 or a 1064 nm laser line. To characterize the SERS activity of the hydroxylamine-reduced silver colloids, the colloid (B) from Figure 1 that was produced by fast mixing of 90 mL of a 1.11×10^{-3} M silver nitrate solution with 10 mL of a hydroxylamine hydrochloride/sodium hydroxide solution (1.5×10^{-2} M/3×10^{-2} M) was selected as a representative model. Because the Lee−Meisel colloid[3] is one of the most often used SERS substrates, it was chosen here as a reference for the SERS activity of the hydroxylamine-reduced silver colloid. Figure 3A,B shows FT-SERS spectra of 9-aminoacridine obtained with the hydroxylamine-reduced and the Lee−Meisel silver sols using the 1064 nm laser line. The concentration of the analyte 9-aminoacridine was 2×10^{-6} M in both cases.
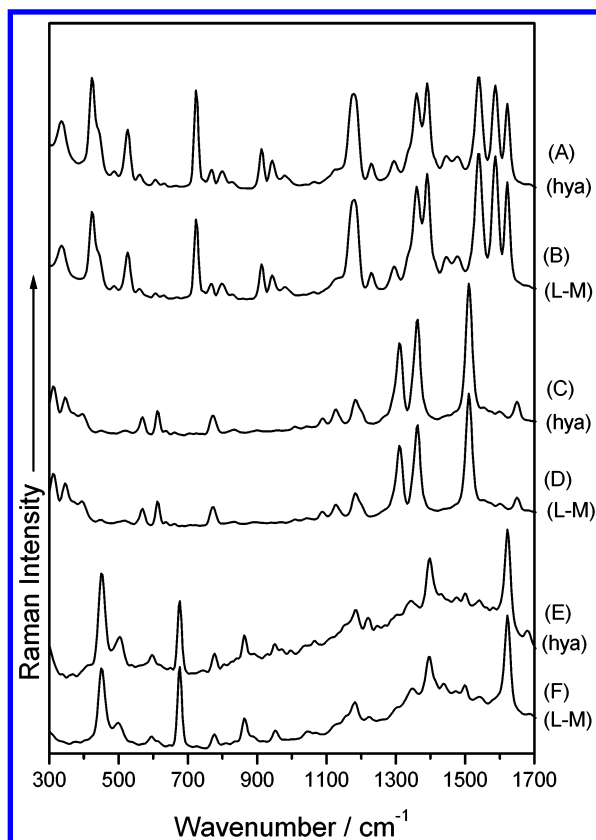
Figure 4. FT-SERS spectra of 3.8×10^{-7} M crystal violet employing (A) the hydroxylamine-reduced (hya) and (B) the Lee−Meisel (L−M) silver sol, 7×10^{-8} M rhodamine 6G employing (C) the hydroxylamine-reduced (hya) and (D) the Lee−Meisel (L−M) silver sol, and 3.8×10^{-7} M methylene blue employing (E) the hydroxylamine-reduced (hya) and (F) the Lee−Meisel (L−M) silver sol.

In Figure 3C,D, SERS spectra of 9-aminoacridine (2.5×10^{-7} M) are shown that were obtained with both colloid types but using the 785 nm laser line. The concentration of sodium chloride in the suspensions used for measurement was in all four cases 8×10^{-3} M. The performed study reveals that the observed surface-enhanced Raman scattering yields similar spectra for the Lee−Meisel and the hydroxylamine-reduced silver colloid. Furthermore, the shape of the recorded spectra is comparable for the 785 and 1064 nm laser line. Small differences in the relative intensities can be attributed to a different morphology of the silver particles due to different reduction procedures or colloid age. The morphology of the particles is a significant factor, determining the distribution of the local enhanced electromagnetic field above the aggregates and the adsorbed molecules.[14] In the Lee−Meisel colloid, a great variety of shapes, from spheres and cubes to rods and needles can be found,[5] whereas the particles in the hydroxylamine-reduced silver colloid are predominantly spherical with some variation in size. From the FT-SERS and SERS spectra, it can be further concluded that the adsorption geometry of 9-aminoacridine to the hydroxylamine-reduced or the Lee−Meisel silver colloid is the same, being in both cases the imino tautomer. This adsorption geometry is favored by the strong interaction with the active sites existing on the silver surface due to a charge transfer from the nitrogen atom to the metal. The strong band at 1500 cm^{-1} (C=N and C=C stretching), as well as further less intense bands at 1595, 1264, and 1132 cm^{-1}, are indicative of the imino form.[16] To check the general applicability of the hydroxylamine-reduced silver sol, FT-SERS spectra of crystal violet, rhodamine 6G, and methylene blue were recorded and

compared with those obtained using the Lee−Meisel silver colloid, again using the 1064 nm laser line (Figure 4).

For both types of silver colloids, comparable scattering intensities were recorded for all investigated analytes. The signal-to-noise ratio did not differ by more than a factor of 2 for the same analyte. This deviation can be attributed to several factors such as variations in the aggregation of the silver particles, variation in time that had elapsed between colloid preparation, addition of the analytes, and measurement, as well as to different morphology of the two types of silver colloids. Most important, however, was the observation that for each analyte the recorded peak positions and the relative intensities in the recorded spectra were independent of the preparation method used to produce the silver colloids.

4. Conclusion

A new, fast, and simple procedure at room temperature for preparing stable, highly SERS-active silver colloids based on reduction of silver nitrate with hydroxylamine hydrochloride at alkaline pH has been described. In function of the mixing order and mixing rate of the two reagent solutions, different particle size distributions around average particle diameters between about 23 and 67 nm can be obtained. TEM micrographs of the hydroxylamine-reduced silver colloids show that the silver particles are predominantly spherical. In comparison of the hydroxylamine-reduced silver sol with the Lee−Meisel silver sol, SERS spectra that are comparable in intensity and shape were obtained. Therefore, it can be concluded that the analyte molecules adsorb in similar geometries and that sensitivities as known for Lee−Meisel colloids can also be obtained using hydroxylamine-reduced silver colloids. Important advantages of the hydroxylamine-reduced silver colloids over the Lee−Meisel colloid are, however, fast and simple preparation at room temperature combined with high preparation success rates while maintaining good reproducibility in the obtained enhancement factors. These characteristics of the new colloid allow fast synthesis followed by immediate application also in on-line measurements. We therefore consider the hydroxylamine-reduced silver colloid highly promising for a range of different applications in physical, as well as analytical, chemistry.

References and Notes

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

(2) Creighton, J. A. Selection Rules for Surface-Enhanced Raman Spectroscopy. In *Spectroscopy of Surfaces*; Clark, R. J. H., Hester, R. E., Eds.; Advances in Spectroscopy, Vol. 16; John Wiley & Sons: New York, 1988; p 39.

(3) Lee, P. C.; Meisel, D. *J. Phys. Chem.* **1982,** *86*, 3391.

(4) Li, Y. S.; Cheng, J.; Coons, L. B. *Spectrochim. Acta A* **1999,** *55*, 1197.

(5) Nickel, U.; Castell, A. Z.; Pöppl, K.; Schneider, S. *Langmuir* **2000,** *16*, 9087.

(6) Kim, H. S.; Ryu, J. H.; Jose, B.; Lee, B. G.; Ahn, B. S.; Kang, Y. S. *Langmuir* **2001,** *17*, 5817.

(7) Ahern, A. M.; Garell, R. L. *Anal. Chem.* **1987,** *59*, 2816.

Fast Preparation of SERS-Active Silver Colloids

J. Phys. Chem. B, Vol. 107, No. 24, 2003 **5727**

(8) Kneipp, K. *Spectrochim. Acta A* **1994**, *50*, 1023.

(9) Brandt, E. S.; Cotton, T. M. In *Investigations of Surfaces and Interfaces-Part B*, 2nd ed.; Rossiter, B. W., Baetzold, R. C., Eds.; John Wiley & Sons: New York, 1993; Vol. IX B, p 667.

(10) Turkevich, J.; Stevenson, P. C.; Hillier, J. *Discuss. Faraday Soc.* **1951**, *11*, 55.

(11) Brown, K. R.; Natan, M. J. *Langmuir* **1998**, *14*, 726.

(12) Bengtsson, G.; Fronæus, S.; Bengtsson-Kloo, L. *J. Chem. Soc., Dalton Trans.* **2002**, *21*, 2548.

(13) Mie, G. *Ann. Phys.* **1908,** *25*, 377.

(14) Kneipp, K.; Kneipp, H.; Itzkan, I.; Dasari, R. R.; Feld, M. S. *Chem. Rev.* **1999**, *99*, 2957.

(15) Laserna, J. J. *Anal. Chim. Acta* **1993**, *283*, 607.

(16) Shelton, R. D.; Haas, J. W.; Wachter, E. A. *Appl. Spectrosc.* **1994**, *48*, 1007.