Study of SERS efficiency of metallic colloidal systems

M Cyrankiewicz, T Wybranowski and S Kruszewski
Medical Physics Division, Biophysics Department, Collegium Medicum of Nicolaus Copernicus University, ul. Jagiellońska 13, 85-067 Bydgoszcz, Poland
E-mail: micy@cm.umk.pl

Abstract. The aim of this work was to determine the influence of colloidal silver and gold nanoparticles on the intensity of Raman scattering observed for analytes dissolved in the water. The testing analytes were rhodamine B and rhodamine 6G. A special emphasis was placed on the choice of optimal method of preparation of colloids and also on the determination of the influence of the aggregation environment on surface-enhanced Raman scattering (SERS) efficiency and stability of colloids. Potassium chloride, sodium chloride and hydrochloric acid are used as aggregating agents. Simply Raman spectrometer and UV-Vis spectrophotometer were used for measurements. The total intensity of inelastic scattering on molecules exhibited up to $10^6$-$10^8$ fold increase in the presence of metallic nanoparticles with a detection limit of analytes being shifted up to the nanomolar concentration. It was observed that SERS enhancement factor varies strongly depending on kind and concentration of used aggregating agent (especially on the concentration of Cl$^-$ ions). These changes in SERS efficiency of colloid were reflected through changes on the absorption spectra.

1. Introduction
Vibrational spectrum is specific for the chemical bonds in molecule and also is sensitive to the changes in its surrounding. So, spectroscopy methods based on Raman effect are very useful techniques for chemical and physical analysis. Raman spectroscopy is complementary and sometimes competitive to other methods. It has some advantages over various electrons (ions) spectroscopy, for example it allows to trace the surface processes without the requirement of high vacuum conditions or enables the in situ study of corrosion and catalytic processes on metal-electrolyte interface. On the other hand, Raman spectrum provides much more information specific to given compounds than UV and visible ones so it makes possible the identification of molecules. Strong water absorption in infrared region makes the Raman spectrometry more suitable in certain cases (especially for non-quantitative measurement) than infrared spectroscopy. Moreover, Raman spectroscopy usually requires very simple sample preparation.

In spite of many advantages the application of this method is limited by very low intensity of normal Raman scattering. However, much more sensitive Raman techniques have been developed such as surface-enhanced Raman spectroscopy (SERS). This method gives almost the same information on the molecules and their local interactions as normal Raman spectroscopy but ensures a great sensitivity.

The Raman scattering from a compound (or ion) adsorbed on a nanostructured metal surface (or within its proximity) can significantly increase in comparison to solution. This phenomenon was observed first time by Fleischman [1] in 1974 and was partially explained by Jeanmaire and Van
Duyne [2] in 1977. Nanostructures of noble and transition metals onto which the molecules are adsorbed can cause up to $10^{10}$ or even $10^{15}$ (on single molecule [3]) fold enhancement of the intensity of Raman scattered light. The SERS effect is strongest on silver but gold and copper are also used in practical applications.

There are two main contributions into Raman enhancement. The first is an electromagnetic (EM) enhancement related to the surface plasmon resonance (SPR). It arises when the wavelength of light couples oscillations frequency of free conduction electrons on metal surface. Molecules adsorbed or in close proximity to the surface are affected by relatively enormous electromagnetic field. Intensity of Raman bands depends on the orientation of the scattering molecules and an enhancement of vibrational modes normal to the surface is most efficient. The second mode of enhancement is related to short-range mechanism of chemical nature: the charge-transfer (CT) complex formation and chemical bonding of adsorbate to metal. It results in the increase of molecular polarizability.

The earlier reports (1970’s) focused on SERS on silver electrodes with modified roughened surfaces but since 1979 when enhancement of Raman signal on silver and gold sols was observed the first time metal colloids have become the most commonly used nanostructures for SERS. Colloidal metal nanoparticles have allowed to test theoretical models of SERS phenomenon as well as opened the window to its application as an analytical tool in chemistry and biological sciences.

There are many procedures of obtaining and further improvement of SERS activity of metallic colloids. The most widely used methods (also slightly modified by authors of this work) were subject of this study. SERS efficiency of colloidal nanoparticles and its aggregates was tested using rhodamine B and rhodamine 6G. The behavior of colloids was monitored by absorption spectroscopy.

2. Experimental

2.1. Materials

Silver nitrate (AgNO₃), trisodium citrate (Na₃C₆H₅O₇), hydroxylamine hydrochloride (NH₂OH.HCl), sodium borohydride (NaBH₄), chloroauric acid (HAuCl₄), potassium chloride (KCl), sodium chloride (NaCl) and hydrochloric acid (HCl) were obtained from POCH S.A. Rhodamine B and rhodamine 6G were purchased from Fluka. Mili-Q grade water (18.2 MΩ/cm) was used for all solutions preparation.

2.2. Silver colloids production and samples preparation

Silver colloids were produced by four ways:

(I) (the procedure based on Lee and Meisel method [4]) 100 ml of a 1 mM AgNO₃ aqueous solution was heated to 93-100°C and then 2 ml of a 1% trisodium citrate solution was added. The mixture was kept in constant (previously achieved) temperature for about 1 hour and then was allowed to cool down to room temperature. The resultant colloidal mixture was of dark grey colour.

(Ia) (a simple modification of the method (I)) The citrate was added dropwise in four portions. The first 0.2 ml was added when AgNO₃ solution was warmed to 90°C and next three portions (0.6 ml each) at 95°C at intervals over 15 min. Finally, the mixture was stirred and heated for next 30 min.

(II) (based on Creighton metod [5]) 25 ml of 1 mM AgNO₃ solution was added portionwise to 75 ml of vigorously stirred ice-cold 2 mM NaBH₄. Stirring was continued for 10 min to obtain brownish solution.

(III) (Leopold and Lendl method [6]) 4.5 ml of 0.1 M NaOH mixed with 5 ml of a 0.06 M hydroxylamine solution was added rapidly to 90 ml of 1.1 mM AgNO₃ aqueous solution. Resulting mixture was stirred until homogenous milky grey colour was obtained.

The obtained suspensions were diluted in water to the final Ag concentration of 0.2 mM.

Au nanoparticles were prepared as follows. 50 ml of an aqueous 0.5 mM HAuCl₄ solution was heated to 98°C and stirred vigorously. Then the 2 ml of 1% aqueous sodium citrate solution was added and the resultant mixture was kept at 95°C and stirred for a further 30 min. The color of the final solution was dark, deep red.
All colloids were stable and its absorption did not change over a few days or even weeks (citrate reduced Ag and Au sols)

KCl, NaCl and HCl were used as aggregating agents. All samples containing colloids with added appropriate amount of one of these compounds were shaken on vortex stirrer for 30 sec after addition. Then the analyte was added and mixing was continued for next 30 sec.

2.3. Instrumentation

Raman spectra were recorded on DK480 (Spectral Products) 0.5 m spectrometer equipped with cooled ST6 CCD camera (Santa Barbara Instrument Group). The spectra were excited by 632.8 nm line of 35 mW He-Ne laser at 30 sec exposure. The absorbance spectra were recorded on V550 (Jasco) spectrophotometer by using 10 mm path length quartz cells. Both Raman and absorbance spectra acquisitions were performed at room temperature.

3. Results and discussion

3.1. Characterization of colloids

The condition of SPR as an essential contribution to the enhancement of Raman scattering is that the real part of dielectric constant of metal must be negative and its imaginary part should be close to zero. The characteristic SPR profile is also a function of size and shape of the nanoparticles as well as depends on their aggregation pattern. While absorption spectra of metallic colloid are related to SPR they can be used to determine its microscopic features, i.e. morphology of particles and degree of aggregation. Absorbance spectra of colloidal suspensions obtained using method presented above are shown in figure 1.

![Figure 1](image)

Figure 1. Normalized absorbance spectra of borohydride (BH), hydroxylamine (HA) and citrate (method (Ia)) reduced Ag colloids and citrate reduced Au colloid (left panel). Spectra of Ag colloids prepared according to the Lee-Meisel method at various temperature (right panel).

The spectra presented in figure 1 indicate the predominant near-spherical shape of colloid particles. The mean particle diameter predicted from the wavelength of maximum absorption is about 10 nm (maximum at 390 nm), 50 nm (425 nm) and a bit more than 100 nm (460 nm) for the silver sol reduced with borohydride, hydroxylamine and obtained from modified citrate method, respectively. The absorbance spectrum of Au sol indicates the monodispersed (in size) particles of average diameter about 15 nm.

As one can see in the right panel of figure 1 the optical properties of colloid strongly depend on the temperature at which suspension was kept during preparation. The red-shift of the absorption maximum and broadening of the band is related to the larger, on the average, and more diverse in size particles.
3.2. SERS activity of aggregated colloids
Ag and Au particles obtained from methods mentioned in previous section exhibit a relatively weak SERS activity. The average intensity of Raman scattering from molecules of analytes (both rhodamines) dissolved in colloidal suspension grows up to 2 orders of magnitude. Greater enhancement is only observed for colloid prepared according to the method (Ia) – it leads to a few hundreds-fold increase of Raman signal. It is possible that the dropwise addition of trisodium citrate results in the seeding effect and, in consequence, a further growth of silver particles (of spherical as well as more irregular shape). However, in practical SERS applications a partial aggregation of colloid is necessary [7].

**Figure 2.** Absorbance spectrum of the pure citrate reduced Ag sol and spectra of colloid solution with 1 µM rhodamine 6G and colloid with 16.7 mM (both recorded in 1 min and 60 min after mixing).

Absorbance spectra of Ag sol (citrate-reduced at 98°C) with addition of rhodamine 6G (to final concentration of 1 µM) and spectra of colloid suspension containing KCl (16.7 mM) is shown in figure 2. As one can see, the addition of rhodamine only as well as KCl only does not alter the spectra. The presence of rhodamine manifests itself as a barely noticeable absorption peak centered about 528 nm and addition of KCl results only in a small decrease of absorption at 455 nm, i.e. in the plasmon resonance region. The application of NaCl and HCl gives the same effect.

The situation changes significantly when both KCl and rhodamine are added to colloid.

**Figure 3.** Evolution in time of absorbance spectrum of the citrate reduced Ag colloid with added 3.33 mM KCl and 1 µM rhodamine 6G.
As it is shown in figure 3 the absorbance spectra of sample prepared by the method described in section 2.2. evolve over time. It’s worth to notice that the five times smaller concentration of KCl (3.33 mM) with presence of rhodamine results in such radical changes (authors observed similar effect also for pyridine). During the one-hour observation the absorption of Ag colloid/KCl/rhodamine solution decreases while the plasmon resonance band shifts towards higher energy. This shift is probably related to the increase of the Fermi level due to a larger electron charge density as a result of addition of chloride ions. It also can be attributed to the substitution of residual reducing agent with Cl$^-$. Figure 3 shows also the changes of spectra in the red and nearest infrared region where the second wide absorption band arises. This band indicates appearance of closely spaced nanoparticles due to aggregation process. It is related to the localized coupled plasmon resonance between two (or more) particles [8]. These so-called “hot-spots” are characterized by the extremely high electromagnetic field enhancement and play an important role in SERS. SERS efficiency of the ensemble of colloid particles strongly depends on its geometry which is influenced by the conditions of aggregation and can vary over time. Figure 4 shows the observed changes of SERS intensity.

![Figure 4. SERS spectra of the citrate reduced Ag colloid with added 3.33 mM KCl and 1 µM rhodamine 6G. The time-dependent changes of the intensity of the most pronounced peak at 1515 cm$^{-1}$ is shown in the inset graph.](image)

It is clearly seen that the enhancement of Raman signal corresponds to the changes of red (and near infrared) band of absorption spectra. The comparison of both spectra leads to a conclusion that the tested sample exhibits the best SERS efficient 40 min after introducing analyte into colloid suspension. Unfortunately, there is no simple rule for determining the optimal moment for SERS measurement. In practice, the analysis of absorbance spectra is very helpful. Gold colloid very quickly reaches the maximum of SERS efficiency and then preserves it for a long time. Unlike it, the borohydride reduced silver colloid needs the longest time from all sols studied here. Generally, it has been observed that the process of activation proceeds faster for more concentrated colloid and for higher concentration of aggregating agent, especially in the case of smaller average size of nanoparticles or lower concentration of rhodamines.

Figure 5 shows the changes of SERS intensity over time and corresponding absorbance spectra obtained from samples of 100 nM rhodamine B in silver sol. Samples differed in concentrations of HCl which were chosen such that any flocculation and sedimentation was observed during the experiment. In the case of rhodamine B and citrate reduced silver sol the addition of HCl as an aggregating agent results in considerable increase in SERS intensity (over 10-fold growth in comparison to NaCl and KCl). Such effect isn’t observed for rhodamine 6G. As one can see the increase of the concentration of HCl results in earlier achievement of the maximum of Raman intensity. For all concentrations the changes of absorption in near infrared region are correlated with the alteration of Raman intensity. The correlation coefficient (calculated for 790 nm) ranges from 0.65 to 0.95. The highest value of correlation coefficient is obtained for 10 mM concentration of HCl.
3.3. Estimation of enhanced factor

Both rhodamine 6G and rhodamine B are fluorescent compounds so it is hard to precisely estimate an enhancement factor of Raman signal. The intensity of vibrational bands is very weak in comparison to fluorescence intensity and it causes the main difficulty. The enhancement factor can be described as

$$EF = \frac{I_{\text{SERS}}}{I_{\text{norm}}} \frac{C_{\text{norm}}}{C_{\text{SERS}}}$$

where $C_{\text{SERS}}$ and $C_{\text{norm}}$ are the concentrations of analyte for SERS and normal Raman measurement, respectively, and $I_{\text{SERS}}$ and $I_{\text{norm}}$ are the corresponding intensity of certain vibration. Enhancement factors roughly estimated with this formula are about $10^6$ for gold and borohydride reduced silver sols and about $10^7$ for the others. Almost $10^8$-fold increase of the Raman signal was observed only for rhodamine B adsorbed on citrate reduced silver colloid. The smallest amount of rhodamine B for which the distinct SERS spectra were recorded was 1 nM.

4. Conclusions

A wide field of potential applications of SERS is the reason that the methods of preparation of stable and SERS-active substrates are still developing. Silver and gold colloids are very simple in use and efficient nanostructures for SERS. The authors’ observations indicate that it is very hard to obtain the general-purpose SERS-active sols but it is possible to produce the colloid dedicated for a given application. It can be made by selection of appropriate conditions. Silver and gold nanoparticles which were the subject of this study allow to enhance efficiently the intensity of Raman scattering from molecules of rhodamine. However, some aggregation of colloid is necessary and the fundamental matter is a choice of kind as well as amount of aggregating agent.

References

[1] Fleishman M, Hendra P J and McQuillan A J 1974 Chem. Phys. Lett. 26 163-166
[2] Janmaire D L and Van Duyne R P 1977 J. Electroanal. Chem. 84 1-20
[3] Kneipp K, Wang Y, Kneipp H, Perelman L T, Itzkan I, Dasari RR and Feld M S 1997 Phys. Rev. Lett. 78 1667-1670
[4] Lee P C and Meisel D 1982 J. Phys. Chem. 86 3391-3395
[5] Creighton J A, Blatchford C G and Albrecht M G 1979 J. Chem. Soc., Faraday Trans. 2 75 790
[6] Leopold N and Lendl B 2003 J. Phys. Chem. B 107 5723-5727
[7] Bengter H, Tengroth C and Jacobsson S P 2005 J. Raman Spectrosc. 36 1015-1022
[8] Käll M, Hongxing X and Johansson P 2005 J. Raman Spectrosc. 36 510-514