Study of optical and physicochemical properties of colloidal silver nanoparticles as an efficient substrate for SERS

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Abstract. The unique optical and physicochemical properties of the noble metal colloidal nanoparticles enable their use in a wide range of applications, especially as a substrate in SERS and MEF study. The aim of this work is to characterize the conditions for the enhancement of Raman scattering by molecules adsorbed on silver surface. Silver sol is prepared by slightly modified Lee-Meisel’s method and rhodamine 6G is used as a probe adsorbate. Pure colloidal silver suspension containing isolated nanoparticles exhibits relatively poor SERS efficiency. The extremely large electromagnetic field is induced in the junctions between two or more metallic nanocrystalites so some degree of their aggregation is necessary. The influence of potassium chloride and nitric acid as the aggregating agents is investigated here. The experiments show that both of them can promote the controlled aggregation process but chloride anions, unlike nitrate, much more effectively affect both electromagnetic and chemical mechanisms contributing to SERS. Due to the co-adsorption with rhodamine 6G they allow the dye molecules to directly interact with metallic surface. Moreover, the results clearly indicate that chloride in the presence of silver particles can induce the dimerization of the dye molecules.

1. Introduction
The noble metal nanocrystals due to their unique properties have recently attracted considerable interest of scientific world. They still remain the subject of fundamental research but also have begun to be widely employed for microanalytical applications in many fields, especially in biotechnological and biomedical research.

The great interest in colloidal silver and gold is related mainly to their ability to amplify the scattered light and fluorescence emission from adsorbed species. The first experimental and theoretical reports on surface-enhanced Raman scattering (SERS) [1][2] and metal-enhanced fluorescence (MEF) [3] appeared in the 70’s of 20th century. Both phenomena manifest themselves in signal enhancement which, in the case of SERS, can reach at least six orders of magnitude [4] or even more on specific “hot spots” [5]. The earlier reports focused on SERS on silver electrodes with modified roughened surfaces [6] but since 1979 when enhancement of Raman signal on silver and gold sols was observed the first time colloidal metal particles have become the most commonly used substrates for SERS measurements (more recently also for MEF applications).

The generally accepted explanation of SERS is based on the combination of two mechanisms. The main contribution to the SERS originates from electromagnetic (EM) mechanism [7] and is related to the resonance of surface plasmons localized on the metallic nanostructures (LSPR). LSPR arises when
the wavelength of light couples oscillations frequency of free conduction electrons of metal. Molecules adsorbed on the surface of silver or gold nanoparticles are affected by relatively enormous electric field. The EM enhancement is not chemically selective but 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 [8]. The second contribution to the enhancement is a short-range resonant mechanism of chemical nature leading to the increase of molecular polarizability, mainly due to the charge-transfer (CT) and the adsorbate-metal complex formation. The prerequisite for the chemical enhancement is the adsorption of molecule directly on the metal, in so-called “active sites” [9]. The contribution of CT to the enhancement depends on the work function of metal and the energy of the HOMO or LUMO (highest occupied and lowest unoccupied molecular orbital) levels of the molecule (for transfer to or from the metal, respectively) [10]. The relative contribution of EM and CT effects can considerably differ for various compounds [11].

There are many procedures for obtaining silver sol exhibiting the desired plasmonic properties dependent on particle size, shape and surface charge. The method proposed by Lee-Meisel [12] was applied in this work. The further improvement of SERS activity of metallic colloid is achieved by its aggregation. The reason of such proceeding is the fact that the extremely large EM field is induced in the junctions between two or more metallic nanocrystals.

Rhodamine 6G (Rh6G) is used here as a probe adsorbate. Rh6G is a cationic xanthene dye that has a variety of applications, especially in biochemical and biomedical fields. Moreover, extremely SERS activity of Rh6G allowed the first single-molecule SERS observation in 1996 [5].

2. Experimental

2.1. Materials
Silver nitrate (AgNO₃), trisodium citrate (Na₃C₆H₅O₇·2H₂O), nitric acid (HNO₃) and potassium chloride (KCl) were obtained from POCH S.A (Poland). Rhodamine 6G (Rh6G) was purchased from Fluka (Switzerland). Mili-Q grade water (18.2 MΩ/cm) was used for the preparation of all solutions.

2.2. Silver colloids production and samples preparation
The nanoparticles used in experiments were prepared by chemical reduction of silver nitrate with trisodium citrate using a modified Lee and Meisel method [12]. Mechanism of reaction can be expressed as follows:

\[ 4Ag^+ + Na₃C₆H₅O₇ + 2H₂O \rightarrow 4Ag^0 + H₅C₆H₅O₇ + 3Na^+ + H^+ + O₂↑ \]

Silver colloid was obtained by addition of 2 ml of a 1% (w/v) trisodium citrate solution to 100 ml of a 1 mM AgNO₃ aqueous solution. First (0.2 ml) portion of citrate was added when AgNO₃ solution was heated to 60°C. Then the mixture was warmed up and kept at about 95°C. Next three portions (each of 0.6 ml) were added at an interval of 15 min (the first of them was added about 20 min after the addition of the initial small portion). Finally, the mixture was stirred for 30 min (still at 95°C). The resultant mixture was cooled down to room temperature and pure water was added to compensate the losses due to evaporation. pH of the mixture was about 6.5. The obtained suspension was diluted with water to the final concentration of Ag atoms of 0.167 mM in each measured sample.

KCl and HNO₃ were used as aggregating agents. All samples containing colloids with added appropriate amounts of these compounds were shaken on vortex stirrer for 30 sec after addition. Then Rh6G was added and mixing was continued for next 30 sec.

2.3. Instrumentation
Raman spectra were recorded on DK480 (Spectral Products Inc., USA), 0.5 m spectrometer equipped with ST6 CCD camera (Santa Barbara Instrument Group Inc., USA). Both devices were controlled by KestrelSpec software (Catalina Scientific Corp., USA). The spectra were excited by 632.8 nm line of
35 mW He-Ne laser (Coherent Inc., USA) at 30 sec exposure. The absorbance was measured with a two-beam V550 (Jasco Inc., USA) spectrophotometer. The particle sizing was performed by photon correlation spectroscopy. Fluctuations of the dynamically scattered light (DLS) intensity were analyzed on the system composed of a photon counting unit Photocor-PC1 (based on Hamamatsu R6358-10 photomultiplier) and 128 channels Photocor-FC correlator working in linear spacing mode (both devices with dedicated software were purchased from Photocor Instruments Inc., USA). The incident beam (632.8 nm light from a He Ne laser source) was polarized perpendicularly to the scattering plane. The scattered light was accumulated for 15 sec. Obtained autocorrelation functions were analyzed using DynaLS software (Alango Ltd., Israel). The steady-state emission fluorescence spectra of Rh6G diluted in silver colloidal suspension were recorded with a PTI (Photon Technology International, USA) spectrofluorometer working in typical “L-format” configuration. In order to improve the spectral purity of the light outgoing from excitation monochromator (of the wavelength 500 nm) the short pass 520 nm filter was mounted in excitation channel.

All measurements were performed in standard four-sided optical glass cell (10x10 mm inside width) in room temperature. They were started immediately after preparation of samples

3. Results and discussion

3.1. Characterization of silver sol.

Fig. 1. shows the optical absorption spectra of pure silver sol and Rh6G/silver sol system both without and with KCl addition. Silver nanoparticles dispersed in aqueous medium exhibit plasmon resonance at 453 nm. On the basis of Mie theory and optical data taken from CRC Handbook [13] authors have found that the dominant radius of nanoparticles is about 42 nm but the width of the resonance band points out to some diversification in size. Its worth to notice that both mean size of Lee-Meisel’s colloid and its polydispersity strongly depends on the temperature at which suspension was kept during preparation [4].

![Absorbance spectra](image1.png)

![Fluorescence spectra](image2.png)

**Figure 1.** (Left panel) Aborbane spectra of the pure silver colloidal suspension and colloid with 1.7 mM KCl and 0.8 μM Rh6G added separately or together. (Right panel) Fluorescence spectra of 0.8 μM Rh6G on silver sol containing various amounts of KCl. The highest curve is related to the pure water solution of Rh6G.

According many authors particles obtained with the presented method are characterized by nearly spherical shape with smoothed multifaceted surface [14] whereas the others claim that up to 20% of them can be of different shape (mostly rods) [9]. However, the presence of elongated or non-ellipsoidal particles in the measured sample should manifest itself in the appearance of additional band
in longer wavelengths [15] but in the presented case the absorbance beyond the plasmon resonance maximum smoothly and monotonically decays with the increase of wavelength.

As is shown in Fig. 1., after addition of the small amount of chloride ions the main absorption peak of silver sols is slightly suppressed, broadened and blue-shifted. This shift of the extinction maximum can probably be related to the changes in refractive index of medium surrounding the silver particles and formation Ag-Cl surface complexes.

3.2. The influence of small amount of chloride.
The used amount of Rh6G (the concentration of 0.8 μM) in the absence of chloride was insufficient to detect Raman signal from the dye molecules as well as it did not affect the resonance energy of surface plasmon on silver nanoparticles. The only change is the appearance of absorption peak of the dye at 527 nm. However, it should be mentioned here that Rh6G at higher concentration can alone induce the aggregation of colloid and considerable changes in its optical response. Author observed such effect for the Rh6G concentration of the order of 0.1 mM or higher.

The extinction spectrum changes significantly when Rh6G is added to the silver sol previously treated with chloride. After addition of the dye further decrease of main maximum is observed and new spectral band arises at about 500 nm, as one can see in Fig. 1.

It is remarkable that the presence of chloride in silver sol/Rh6G solution also results in the reduction of fluorescence emission from the dye molecules (see right panel in Fig. 1.). This effect is most evident for the concentration of KCl between 0.7 mM and 2 mM. Rh6G molecules due to their cationic properties easily adsorb on negatively charged metallic nanoparticles and tend to concentrate in their surroundings. Considering this fact the fluorescence quenching can be interpreted as a result of the resonant dye-metal energy transfer [16] but this kind of interaction should not have an effect on the absorption spectrum of the dye. The quenching of fluorescence emission can also be contributed by nonradiative deactivation of Rh6G dimmers (similarly as in reference [17]) due to dipole-dipole intermolecular interactions. The last mentioned relaxation process requires an appropriate distance and a specific mutual spatial orientation of the interacting molecules, i.e. a “sandwich” structure shown schematically in the Fig. 2. Theoretical calculations performed using a total energy minimization scheme [18] shows that the preferred orientations of adsorbed molecules with respect to the silver surface can promote the complexation of dye. One can suppose that the local increase of Rh6G concentration and partial ordering of molecules placed on or near the nanoparticles can result in the observed spectral effects.

![Figure 2](image)

**Figure 2.** Simplified diagram for electronic transition in Rh6G dimers depending on the geometric arrangement of monomer units. Radiative transitions are represented by linear arrows, nonradiative - by waved ones. \( V_M \) stands for a transition energy in isolated Rh6G molecules corresponding to about 527 nm. (diagram created on the basis of ref. [19])
According to the above diagram the appearance of new band (at about 500 nm) in the extinction spectra of colloidal silver/KCl/Rh6G system can be attributed to the formation of H-type dimers of Rh6G molecules.

3.3. Determination of size and aggregation extent of colloid.

The dynamic light scattering (DLS) technique allows to determine the particle size (more precisely: hydrodynamic radius) distribution of silver colloid and also provides information about the extent of its aggregation. The basic distribution obtained from a DLS measurement is an intensity - the other distributions can be (in the theory) generated from this using Mie theory. Nevertheless, the authors’ experiences suggest that a posteriori modifications (reweighting) of regularized solution very often lead to the unrealistic results so only the primary distributions (i.e. size distributions by the scattering intensity obtained from DynaLS software) are presented here.

Despite the differences between absorbance spectra of pure silver sol and colloidal suspension with a small amount of Rh6G or KCl the particle sizing measurements gave almost identical results. It indicates that both Rh6G as well as KCl at low concentration does not cause distinct changes in the structure of colloid and a large majority of nanoparticles remain unaggregated.

3.4. Aggregation and SERS-activation of silver sol.

According to the fact that the extremely large EM field is induced in the junctions between silver nanocrystals some aggregation is desired in order to improve the SERS efficiency of silver sol. Initially the simple assemblies consisting of a few nanoparticles and then larger aggregates are formed with the increasing concentration of chloride ions. Due to the aggregation the decrease of extinction band related to the dipole plasmon resonance occurs (see Fig. 3.). Simultaneously, a new band arises beyond 600 nm and then increases and shifts towards longer wavelength. 1.7 mM concentration of chloride turned out to be insufficient to the effective SERS activation of silver sol and no Raman scattering of Rh6G was detected. As the content of chloride increased the SERS intensity of Rh6G on silver nanoparticles also rapidly increased and reached the maximum at 33.3 mM KCl concentration (see middle panel in Fig. 3.). The chloride at such high concentration strongly affected the stability of colloidal solution and after a few minutes a noticeable degradation of the sample, i.e. the gradual precipitation of colloid was observed. It has also resulted in considerable decrease of SERS intensity. From the other hand, the silver sol activated with smaller amount of chloride needs much more time to achieve the maximum of SERS efficiency (see [4]). In many cases this time are longer than the duration of measurements in the presented experiments.

As one can see in Fig. 3. the results of consecutive DLS measurements are correlated with the changes in absorption spectra. The increase of KCl concentration from 3.33 mM to 33.3 mM leads to the broadening of the particle size distribution function but the modal size varies relatively little. It suggests that most of the nanoparticles still remain unaggregated or form only small clusters consist of two or thee silver particles. One can notice that the coexistence of KCl and Rh6G in colloid results in a slightly greater extent of its aggregation than in the case of silver sol without Rh6G added.

Fig. 4. shows that also strong acids affect the Rh6G/silver sol system. The extinction and corresponding SERS spectra were studied at various concentrations of HNO₃ which was chosen to avoid the influence of chloride ions. The absorption spectra in Fig. 4. essentially differ from previously presented ones. The main difference is a smaller width of the blue band and the absence of secondary maximum in the red region. Instead, as pH decreases the absorption beyond 550 nm increases as the wide spectral wing monotonically decaying towards the longer wavelengths. For HNO₃ concentration changing stepwise from 0.83 mM to 5 mM the dipolar plasmon resonance band gets lower and shoulder peak attributed to the absorption of Rh6G (at 527 nm) becomes more distinct. It should be underlined that any spectral band indicating the complexation of dye molecules is observed. The sample containing 5 mM HNO₃ is very unstable and further addition of nitric acid leads to the fast (in just teens of seconds or even seconds) destruction of colloid what is indicated by darkening of the solution and sedimentation of large metallic aggregates.
It is noteworthy that not presented here spectra of KCl/silver sol system (without Rh6G) resemble spectra of silver colloid aggregated with nitric acid. The results of DLS measurements of colloid aggregated by acidification with HNO$_3$ show that the range of pH values between 3 and 2 is critical for citrate reduced silver sol. This observation is consistent with [20]. As pH of the silver colloid containing 0.8 μM Rh6G decreases the average hydrodynamic radius of aggregates strongly increases. The right graph in Fig. 4. also shows that, unlike the case of the aggregation with chloride, the growth of aggregates leads to the fast decrease of the number of remaining isolated nanoparticles.

![Figure 3](image-url). Extinction (left panel) and SERS (midle panel) spectra and the corresponding results of DLS measurements of 0.8 μM Rh6G on silver colloid with various amounts of KCl added to solution. The DLS intensity distribution function are normalized to equal maximum.

![Figure 4](image-url). Extinction (left panel) and SERS (midle panel) spectra and the corresponding results of DLS measurements of 0.8 μM Rh6G on silver colloid after addition of various amounts of HNO$_3$. pH of the samples is equal to 2.92, 2.55, 2.26, 2.10, 2.05, respectively.

3.5. A discussion of the influence of chloride and acidity. Chloride ions exhibit much larger complexation affinity to silver than nitrate and adsorb directly on the surface, replacing other anions. Detailed visual inspection of the SERS spectra of Rh6G on colloid aggregated with HNO$_3$ allows to perceive a weak hump at 1400 cm$^{-1}$ (assignable to the carboxylate symmetric stretching vibrations in the citrate ion) which disappears in the presence of KCl (compare...
SERS spectra shown in Fig. 5.). Also the changes in the intensity of fluorescence emission of Rh6G shown in Fig. 1. can suggest that KCl promotes chemisorption of Rh6G on silver only when the concentration of chloride ions does not exceed some critical value. The comparison of absorbance spectra of silver sol/Rh6G samples with the KCl concentration of 3.3 mM and 33.3 mM (see left and middle graph in Fig. 5., respectively) leads to the similar conclusion regarding the dimerization of dye molecules. The second derivative spectra clearly show that the band assignable to the H-dimers of Rh6G disappears at higher concentration of KCl.

![Graph](image)

**Figure 5.** (Left panel) Extinction spectra of 0.8 μM Rh6G on silver colloid with 3.3 mM KCl and 0.83 mM HNO$_3$ added, both together or separately. Extinction (middle panel) and corresponding SERS (right panel) spectra of 0.8 μM Rh6G on silver sol with addition of 5 mM HNO$_3$ and 3.3 mM KCl (a), 33.3 mM KCl (b) and 5 mM HNO$_3$ (c).

Due to strong chemical interaction with silver, chloride ions can induce changes of surface morphology resulting in the creation “active sites” [9][21]. The co-adsorption of chloride with Rh6G allows the dye molecules to directly interact with silver surface and leads to the additional SERS enhancement. It is the main reason of the differences in the intensity between SERS spectra shown in Fig. 3. and Fig. 4. One can also suppose that the appearance of the second extinction band at the longer wavelengths is not only the manifestation of the aggregation of nanoparticles but also is an indirect evidence for the chemisorption of Rh6G and CT transition between dye molecules and silver surface [22]. From the other hand, most researchers agree that the aggregation of silver sol leads to the formation of fractal assemblies [21] and theoretical calculations predict that such pattern of aggregation is necessary for the occurrence of low-frequency plasmon modes [23].

Unlike nitric acid, chloride ions effectively affect both mechanisms contributing to SERS. Moreover, the comparison between the Raman spectrum marked as (a) in Fig. 5. and the spectra shown in Fig. 3. suggests that the influence of the small amount of chloride on SERS efficiency of silver sol increases when colloidal solution is previously acidified with HNO$_3$. The resultant effect of chloride measured as the ratio between intensity of the most pronounced Raman band at about 1505 cm$^{-1}$ obtained for sample with KCl and without KCl addition decreases with the increasing of acidity. From the other hand, before the addition of chloride a role of chemical Rh6G-Ag interaction (“first-layer” effect) in the amplification of the SERS signal seems to be very limited probably due to the presence of the citrate coating on the surface. It is also worth to notice that the extinction spectrum of Rh6G on silver sol after 5 mM HNO$_3$ and subsequently 3.3 mM KCl addition, considerably differs from the ones obtained for the greatest amounts of both HNO$_3$ and KCl added separately. Despite the expected highest degree of aggregation and lowest stability, the mixture is more stable and exhibits
high extinction coefficient below 600 nm and relatively low in infrared region. It is possible that in this case chloride ions partly reduce the influence of nitric acid.

All vibrational bands of molecules adsorbed on the silver sol are liable to the EM selection rules but some of them (stretching band at 614 and 775 cm\(^{-1}\) in the case of Rh6G) are additionally coupled to molecular and CT resonance. The excitation wavelength (632.8 nm) in presented experiments seems to bee too far away from resonance condition so the observed differences between SERS spectra shown in Fig. 5. (see right panel) should be rather attributed to the reorientation of Rh6G molecules with respect to surface of particles.

4. Conclusions

Results presented in this work show that the aggregation of silver nanoparticles plays the crucial role in SERS efficiency of colloid. The experiment shows that both potassium chloride and nitric acid can promote this process but among these two aggregating agents chloride exhibits better properties from point of view SERS of Rh6G on silver. Colloidal solution with the addition of small amount of chloride ions is characterized by greater stability in comparison to the silver particles aggregated with HNO\(_3\). Moreover, unlike nitrate, chloride ions effectively affect not only electromagnetic but also chemical mechanism of SERS. It results in additional enhancement of Raman scattering. This work also indicates that chloride in the presence of silver particles can induce the dimerization of Rh6G molecules.

Rhodamine 6G as well as the other cationic compounds can easily adsorb on the negatively charged silver surface but in the case of neutral and anionic molecules further modification of the surface potential is needed. Our preliminary results show that it can be successfully achieved with the addition of appropriate amount of poly(L-lysine) but the further studies are needed.

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