

# Activation of Silver Colloids for Enhancement of Raman Scattering

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Obtaining of systems that provide ever-increasing enhancement of the Raman scattered light is a big challenge. The silver colloids obtained by reduction of silver nitrate by trisodium citrate are the most promising enhancement systems. Nanoparticles of silver forming fresh colloid obtained by this way exhibit in extinction spectrum the absorption band proving of the surface plasmons excitation, but do not enhance the Raman scattered light, are not surface enhanced Raman scattering active. Adding of KCl or  $\text{HNO}_3$  causes the surface enhanced Raman scattering activation, i.e. causes that the conditions for aggregation of nanoparticles and for adsorption on their surface of molecules providing Raman scattering are created. Increasing amount of added KCl or  $\text{HNO}_3$  leads to significant changes in the extinction spectrum and to significant increase in intensity of surface enhanced Raman scattering. The observed changes in extinction spectrum are mainly due to progressive aggregations of nanoparticles. In junctions between nanoparticles existing inside aggregates the condition for single molecule surface enhanced Raman scattering are fulfilled and therefore aggregation leads to increase in average surface enhanced Raman scattering intensity. Silver colloid activated by using KCl provides about 10-fold higher surface enhanced Raman scattering than that treated with  $\text{HNO}_3$ .

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## 1. Introduction

In 1974 Fleischman et al. [1] observed that the intensity of the Raman scattered light from molecules adsorbed on electrochemically roughened silver electrodes is 6 order higher than that from free molecules. This promising phenomenon was repeated by other research groups and in 1977 called surface enhanced Raman scattering (SERS) [2, 3]. In 1979 enhancement of the Raman signal on silver and gold sols was observed [4] and starting from this year the silver and gold colloids became most commonly used substrates for SERS. During eighties and next years the SERS has been intensively studied, its theories have been created and it has been widely used in chemical, biological, biomedical, pharmaceutical and other studies. Number of publications about SERS and its application has grown every year. The majority of these publications which appeared till 2006 are gathered in monograph of Aroca [5].

On the basis of experimental and theoretical studies it was proven that the SERS is a result of two independent resonance enhancement mechanisms: electromagnetic (which is dominating) and chemical. The electromagnetic (EM) enhancement is a result of electric field enhancement of both incident and scattered lights on the metal nanoparticles, e.g. on nanostructures forming the

rough surface [6] or on the metal colloids. EM enhancement is related with resonance excitation of surface plasmons localised in metal nanoparticles. Plasmons excited in metal nanoparticles have the characteristic frequencies dependent on sizes, shapes and dielectric functions of nanoparticles and surrounding medium [7]. Conditions for occurring of large EM enhancement are fulfilled for silver, gold and copper, only. If metallic nanoparticles are in the field of light and if frequency of this light is equal or close to frequency of surface plasmons on the surface of these nanoparticles and in vicinity of it, the resonance enhancement of electric field of light occurs [8]. If molecules are placed in these sites, where electric field of light is enhanced, the intensity of the Raman scattered light is enhanced up to  $10^6$  fold. Chemical mechanism provides additional enhancement. It is associated with the increase in polarizability of molecules adsorbed on metal — for adsorbed molecules exciting light can cause charge transfer transition from (or to) the Fermi level of metal to (or from) levels of adsorbed molecules and thus condition for resonance Raman scattering can be fulfilled. The total enhancement provided by chemical mechanism may be equal up to  $10^2$ .

Since on the surface of nanoparticles not in every place conditions for maximum enhancement are fulfilled, hence for colloids containing isolated nanoparticles, total enhancement does not exceed  $10^6$ . Enhancement properties of colloid essentially change by nanoparticles aggregation. Then, the large enhancement of electric field oc-

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curs in “junctions” between nanoparticles. Value of this enhancement depends on width of gaps (smaller width — larger enhancement) and on curvature of nanoparticles (larger curvature — larger enhancement). If in the internal “junctions” in aggregates the molecules are placed and number of “junctions” are enough large then the intensity of Raman scattered light can be enhanced even  $10^{15}$ -fold. It means that the presence of few molecules placed in “junctions” and in field of exciting light is sufficient for SERS spectrum recording. Phenomenon of such giant enhancement was for the first time observed in 1996 by two independent research groups [9, 10] and was called single molecules SERS (SM SERS), and sites into which molecules should be placed for occurring of such big enhancement were called “hot spots”.

Obtaining of colloid containing aggregated nanoparticles with large number of “hot spots” is a big challenge. The subject of studies described in this paper is aggregation process of silver colloids and the influence of aggregation on extinction spectra of colloids and on intensity of recorded SERS spectra of rhodamine 6G. Observed increase in SERS intensity during aggregation progress may prove forming “junctions” into which conditions for SM SERS are fulfilled.

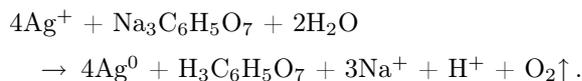
## 2. Experimental

### 2.1. Materials

Silver nitrate ( $\text{AgNO}_3$ ), trisodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ), nitric acid ( $\text{HNO}_3$ ) and potassium chloride (KCl) were obtained from POCh S.A. (Poland). Rhodamine 6G was purchased from Sigma-Aldrich. Milli-Q grade water ( $18.2 \text{ M}\Omega/\text{cm}$ ) 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 [11]. Mechanism of reaction can be expressed as follows:



The Lee and Meisel method permits to produce the nanoparticles with desired sizes. For studies carried out in this work silver nanoparticles are obtained by mixing of 50 ml of 1 mM silver nitrate with 1 ml of 1% aqueous solution of trisodium citrate. The last solution was added in 4 portions. First portion (0.1 ml) was added after silver nitrate solution heating to temperature of  $90^\circ\text{C}$ . Next, solution was heated to  $95\text{--}97^\circ\text{C}$  and after 20 min, the second portion equal to 0.3 ml was added. The next portions (3th and 4th) each equal to 0.3 ml were added after next 15 and 30 min, respectively. Temperature of solution was kept at  $95\text{--}97^\circ\text{C}$ . After adding the last portion, the temperature was kept at  $95\text{--}97^\circ\text{C}$  for about 30 min, and content of flask was occasionally stirred. Finally,

the resultant mixture was cooled down to room temperature and pure water was added to compensate the losses due to evaporation. Obtained mixtures were milky gray-green color and remained stable for a long time (up to several months). For further studies (extinction, DLS, SERS) they were 6-fold diluted in water.

### 2.3. Instrumentation

The extinction spectra of colloids were recorded with a two-beam V550 (Jasco Inc., USA) spectrophotometer in standard 10 mm path length optical glass cells. The particle sizes were determined by photon correlation spectroscopy. Fluctuations of the dynamically scattered light (DLS) intensity were analysed on the system composed of a photon counting unit Photocor-PC1 (based on Hamamatsu R6358-10 photomultiplier) and 288-channels Photocor-FC correlator working in multiple-tau spacing mode (both devices with dedicated software were purchased from Photocor Instruments Inc., USA). The power of the incident beam (632.8 nm light from a He-Ne laser source) was adjusted with circular variable neutral density filter (Thorlabs Inc., USA) and a width of the beam was reduced by using lenses and pinholes. Scattering angle was set to  $90^\circ$ . The laser light was polarized perpendicularly to the scattering plane. The scattered light was accumulated for 15 s. Obtained autocorrelation functions were analyzed using DynaLS software (Alango Ltd., Israel).

The 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 s exposure.

## 3. Results and discussion

The violet-blue extinction band of silver colloids proves existence of silver nanoparticles and strong interaction of violet-blue light with surface plasmons localised in these nanoparticles. Extinction maximum position and its bandwidth depend on the sizes of nanoparticles forming colloid. Figure 1A presents the extinction spectra of silver colloids obtained by methods described in previous part. Curve 1 (solid line) presents extinction spectrum of pure silver colloid 6-fold diluted in water. Figure 1B (top part) presents the DLS spectrum of sizes of nanoparticles forming this pure colloid from which it follows that nanoparticles with hydrodynamic radius about 40 nm dominate in this colloid. Curve 2 (dotted line) in Fig. 1A represents the extinction spectrum of the same colloid to which rhodamine 6G was added. Final concentration of rhodamine 6G in the colloid was equal to  $0.8 \mu\text{M}$ . The extinction spectrum of colloid with Rh 6G is almost the same as extinction of pure colloid. One can suspect that Rh 6G does not adsorb on nanoparticles, so nanoparticles in fresh prepared colloid are not SERS-active. Figure 1B (middle part) shows that the DLS

spectrum of sizes did not change. Curve 3 (dashed line) in Fig. 1A shows extinction spectrum of silver colloid to which KCl was added (final concentration of KCl in colloid was equal to 1.7 mM) and next Rh 6G (final concentration  $0.8 \mu\text{M}$ ). In this case the extinction spectrum of colloid is significantly changed. The new absorption band with maximum at about 500 nm appears and it is not absorption band of Rh 6G. This new absorption band at about 500 nm proves the dimerization of Rh 6G [12]. It differs significantly from absorption band of free Rh 6G, which exhibits maximum at about 527 nm. Absorption spectrum of Rh 6G as dot-dash line is also presented in Fig. 1A. Probability of Rh 6G dimerization increases significantly near the silver surface [13], so one can conclude that the adding of KCl causes the adsorption of Rh 6G molecules on silver nanoparticles. DLS studies showed that adding of small amount of KCl (1.7 mM) and Rh 6G ( $0.8 \mu\text{M}$ ) did not cause the change in size spectrum of nanoparticles (lower part in Fig. 1B). On the basis of results presented in Fig. 1, one can conclude that adding of KCl causes the adsorption of Rh 6G which means the SERS-activation of colloid. Adding of small amount of KCl as it shows inset in Fig. 1A does not change the extinction of colloid. In the case of such small amount of added KCl the significant changes in extinction spectrum appear after adding Rh 6G.

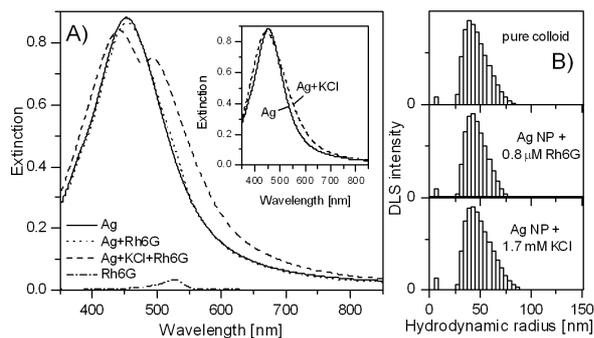


Fig. 1. (A) Extinction spectra of the pure silver colloid and silver colloid with 1.7 mM KCl and  $0.8 \mu\text{M}$  Rh 6G added separately or together. (B) The distribution of the dynamic light scattering intensity with respect to the size of silver particles in the studied colloids.

Figure 2A shows the influence of amount of added KCl on the extinction spectrum of silver colloid. After adding KCl, the Rh 6G was added (final concentration of Rh 6G in colloid was always  $0.8 \mu\text{M}$ ). Decreasing and broadening of violet-blue band and the appearance of second absorption band in the red region is observed with the increase of amount of added KCl. Figure 2B shows how do the sizes of nanoparticles change by adding of KCl. Maximum of sizes spectrum shifts to the larger value and significant broadening of this spectrum is observed. One can conclude that adding of KCl causes not only SERS-activation by adsorption of Rh 6G on nanoparticles but also (or first of all) the aggregation of these nanoparticles.

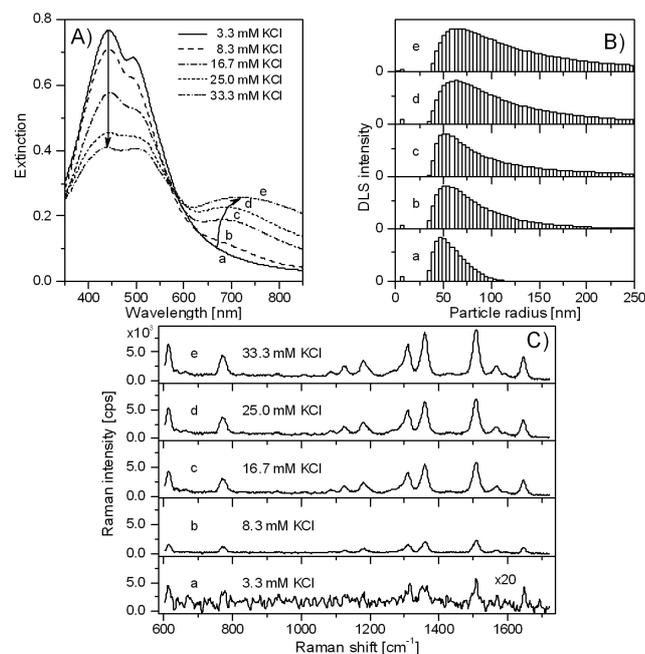


Fig. 2. (A) Extinction spectra of silver colloids containing  $8 \times 10^{-7} \text{ M}$  Rh 6G after adding of various amounts of KCl. (B) Corresponding results of DLS measurements and (C) SERS spectra.

Figures 1A, 2A and Figs. 1B, 2B present extinction spectra and sizes spectra (DLS spectra) of silver colloids in dependence of amount of added KCl. Using considered above colloids the SERS spectra of Rh 6G were recorded. Fresh colloid (without adding KCl) does not provide the SERS — such colloid is SERS-inactive. Colloids to which KCl was added start to exhibit the SERS. Figure 2C shows that SERS intensity rises as amount of added KCl increases.

On the basis of experimental results summarized in Fig. 2 one can conclude: (1) If the sizes spectrum of silver colloid is narrow with maximum at about 40 nm (hydrodynamic radius), then extinction spectrum of this colloid has only one band in violet-blue region, and such colloid exhibits weak SERS-activity; (2) If the colloid sizes spectrum broadens together with rising of dominant nanoparticles (more precisely — aggregates) size, then in extinction spectrum the violet-blue band decreases and broadens and in the red region the second extinction band appears. (3) The largest SERS is observed for colloid, extinction spectrum of which characterizes by intense red band and its sizes spectrum is broad.

Observed results can be interpreted as follows: (1) Adding of small amount of KCl and Rh 6G to fresh colloid causes SERS activation i.e. adsorption of Rh 6G on nanoparticles. Nanoparticles are isolated and hence SERS signal is not large; (2) Adding ever larger amounts of KCl together with constant amounts of Rh 6G ( $0.8 \mu\text{M}$ ) leads to nanoparticles aggregation. Many junctions exist in formed aggregates and molecules of Rh

6G placed in these junctions provide strong SERS because in such case (molecules placed in junctions) conditions for SM SERS are fulfilled.

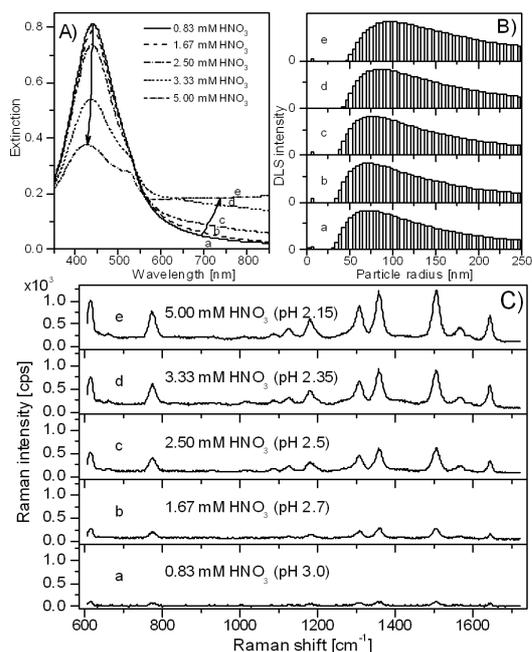


Fig. 3. (A) Extinction spectra of silver colloids containing  $8 \times 10^{-7}$  M Rh 6G after adding of various amounts of  $\text{HNO}_3$ . (B) Corresponding results of DLS measurements and (C) SERS spectra.

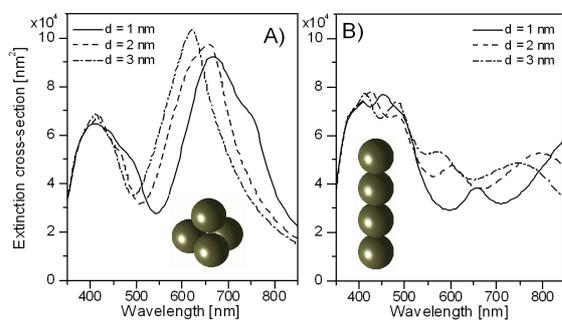


Fig. 4. Extinction spectra of 4-element clusters of silver nanoparticles averaged over various spatial orientation calculated on the basis of generalized multiparticle Mie-solution (GMM) [17] for two types of cluster geometry. Radius of individual nanoparticle is taken as 40 nm and interparticle distance ranging between 1 nm and 3 nm.

Figure 3 shows how using a nitric acid as activation compounds influences the spectra of extinctions, sizes and SERS. Nitric acid was added just before adding of Rh 6G ( $0.8 \mu\text{M}$ ). Intensity of blue-violet extinction band decreases as amount of added nitric acid rises. Extinction band in red region does not appear, however in this spectral region, as it shows Fig. 3A, extinction systematically rises.

DLS spectra of sizes shown in Fig. 3B, look quite surprisingly. Colloid into which even small amount ( $0.83 \text{ mM}$ ) of nitric acid was added is characterized by a broad spectrum of sizes. Increasing the amount of added nitric acid, leads to further broadening of the spectrum of sizes and to the shift of the dominant size towards larger values.

Figure 3C shows Rh 6G SERS spectra provided by colloids activated by nitric acid. It shows that increase of amount of added nitrogen acid causes the rise in SERS intensity. It is necessary to note that the SERS-activation of colloid by nitrogen acid is not so efficient as activation by KCl. Intensity of SERS is 10-fold smaller than in case of using KCl. The key to understanding of observed differences in SERS intensity lies in red extinction band. Colloids activated by KCl exhibit this red band, and colloids activated by nitrogen acid do not. It is open question — how this red band can be interpreted. One of possible explanation is assumption that activation by KCl leads to situation in nanoparticles aggregates in which beside of EM enhancement the chemical enhancement occurs. The chemical enhancement, if occurs, is associated with charge transfer metal — Rh 6G molecules. Observed red extinction band may have source in light absorption by charge transfer transition [14–16]. It may also suppose the activations by KCl and activation by nitric acid lead to formation of aggregates with different geometry. They can differ by numbers of “junctions” and widths of “gaps”. Our theoretical calculations of extinction spectra based on generalized multiparticle Mie approach [17] prove that above last hypothesis is probable.

Results of theoretical calculations of extinction spectra of two kind of 4-nanoparticles aggregates are presented in Fig. 4. This calculations show that the grape-shape aggregates exhibit the red extinction band, while the linear aggregates do not have distinct extinction band in red. By comparison of experimental extinction spectra from Fig. 2A and Fig. 3A with theoretical extinction spectra presented in Fig. 4 one can suppose that the using of KCl leads to preferential forming of grape-shaped aggregates, while the using of nitric acid leads to preferential formation of linear aggregates. Because the intensity of SERS provided by KCl activated colloids is about 10-fold higher than provided by nitric acid activated colloids, one can conclude that the aggregates in the first colloids (KCl activated) are more efficient for SERS. It can be supposed that in these aggregates parameters of junctions (number, widths of “gaps”) are more advantageous for SM SERS than in aggregates formed by using nitric acid.

#### 4. Conclusion

Silver colloids containing isolated nanoparticles with average hydrodynamic radius equal to 40 nm were obtained by Lee and Meisel method. Recorded violet-blue extinction band proves surface plasmon excitation

in these isolated nanoparticles. Nanoparticles in obtained colloids are surrounded by citrate and are not able to adsorb other molecules. Thus fresh colloids are not SERS-active. Adding of small amount of KCl or HNO<sub>3</sub> causes the SERS activation of colloid, but SERS provided by them is weak. Molecules of Rh 6G are able to adsorb on nanoparticles, but these nanoparticles remain isolated and thus only some molecules can provide Raman signal enhanced about 10<sup>6</sup>-fold. Increasing of amount of added KCl or HNO<sub>3</sub> causes the rise in SERS intensity as a result of aggregation of nanoparticles. Molecules situated in junction inside aggregates are in the places where conditions for SM SERS are fulfilled. Aggregates formed by using KCl are more efficient for SERS than that ones formed by using of HNO<sub>3</sub>. Most probably in the junctions of first aggregates the total enhancement of Raman scattered light is higher than in the junctions of last aggregates.

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### References

- [1] M. Fleischman, P.J. Hendra, J. McQuillan, *Chem. Phys. Lett.* **26**, 163 (1974).
- [2] D.L. Jeanmaire, R.P. Van Duyne, *J. Electroanal. Chem.* **84**, 1 (1977).
- [3] M.G. Albrecht, J.A. Creighton, *J. Am. Chem. Soc.* **84**, 5215 (1977).
- [4] J.A. Creighton, C.G. Blatchford, M.G. Albrecht, *J. Chem. Soc. Faraday Trans. II* **75**, 790 (1979).
- [5] R. Aroca, *Surface-Enhanced Vibrational Spectroscopy*, Wiley, Chichester 2006.
- [6] S. Kruszewski, *Surf. Interface Anal.* **21**, 830 (1994).
- [7] A. Derkachova, K. Kolwas, *Eur. J. Phys. Special Topics* **144**, 93 (2007).
- [8] H. Ditlbacher, N. Felidj, J.R. Krenn, B. Lamprecht, A. Leitner, F.R. Aussenegg, *Appl. Phys. B* **73**, 373 (2001).
- [9] K. Kneipp, Y. Wang, H. Kneipp, L.T. Perelman, I. Itzkan, R.R. Dasari, M.S. Feld, *Phys. Rev. Lett.* **78**, 1667 (1997).
- [10] S. Nie, S.R. Emory, *Science* **275**, 1102 (1997).
- [11] P.C. Lee, D. Meisel, *J. Phys. Chem.* **86**, 3391 (1982).
- [12] V.M. Martinez, F.L. Arbeloa, J.B. Prieto, I.L. Arbeloa, *J. Phys. Chem. B* **109**, 7443 (2005).
- [13] M.A. Noginov, M. Vondrova, S.M. Williams, M. Bahoura, V.I. Gavrilenko, S.M. Black, V.P. Drachev, V.M. Shalaev, A. Sykes, *J. Opt. A, Pure Appl. Opt.* **7**, 219 (2005).
- [14] H. Hamada, H. Nagata, K. Toba, Y. Nakao, *Surf. Sci.* **182**, 269 (1982).
- [15] B. Park, S.D. Lee, M.S. Kim, Y.D. Kim, *J. Korean Phys. Soc.* **26**, 693 (1993).
- [16] S. Kruszewski, *J. Raman Spectrosc.* **27**, 513 (1996).
- [17] Y. Xu, B.Å.S. Gustafson, *Recent Res. Devel. Opt.* **3**, 599 (2003).