(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2012 (05.04.2012)

PCT

(10) International Publication Number
WO 2012/041262 A1

(51) International Patent Classification:
G01N 21/65 (2006.01) ***B82Y 15/00*** (2011.01)
G01N 33/58 (2006.01) ***B82Y 30/00*** (2011.01)
G01N 33/543 (2006.01)

(21) International Application Number:
PCT/CZ2011/000083

(22) International Filing Date:
29 August 2011 (29.08.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PV 2010-708 29 September 2010 (29.09.2010) CZ

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

(54) Title: METHOD FOR ACTIVATION OF AQUEOUS SILVER NANOPARTICLE DISPERSIONS FOR SURFACE ENHANCED RAMAN SPECTROSCOPY

(57) Abstract: The invention provides a method for activation of aqueous dispersions of silver nanoparticles for purposes of surface enhanced Raman spectroscopy, wherein a solution of chloride ions is added to the aqueous dispersion of silver nanoparticles in the presence of oxygen in such an amount that the final chloride ion concentration in the activated dispersion ranges from 0.1 $mol.dm^{-3}$ to 1 $mol.dm^{-3}$. Preferably, oxygen is added to the dispersion in the course of activation. This method leads to recrystallization of silver particles, which is faster and more reproducible for the purposes of surface enhanced Raman spectroscopy than the particle aggregation so far known in the art.'

Method for activation of aqueous silver nanoparticle dispersions for surface enhanced Raman spectroscopy

Field of Art

The present invention relates to a method for activation of aqueous silver nanoparticle dispersions for purposes of surface enhanced Raman spectroscopy, which represents a very promising and sensitive analytical method.

Background Art

The discovery of surface enhanced Raman scattering (SERS) on a silver electrode by Fleischmann in 1974, and especially its re-discovery on colloidal silver particles by Creighton in 1977 started the extensive development of a new and very sensitive analytical method that allows the detection of molecules in the concentration range from pico- to femtomols (Doerning W. E. and Nie S. M., J Phys Chem B, 106, 2002). A high enhancement of SERS, reaching the values of up to 10^{15}, even allowed the detection of individual molecule adsorbed on a single silver nanoparticle (Michaels M. et al. *J Am Chem Soc*, 121, 1999; Nie S. and Emory S. R., Science, 275, 1997). Some studies have shown that so high a value of the enhancement can be achieved only on particles of a certain sizes which are referred to as “hot particles”. These particles’ optimum size depends on the wavelength of the laser used for the excitation and ranges approximately from 70 nm to 200 nm for the excitation wavelengths in the range from 488 nm to 647 nm (Emory S. R.et al., J Am Chem Soc 120, 1998). For the commonly used argon lasers (wavelength of 514.5 nm), silver particles of the size of about 90 nm are the most effective ones. Based on the mentioned dependence of the size of the “hot particles“ for a given laser wavelength, it can be expected that when using lasers in the red (785 nm) and near-infrared area (1064 nm), the maximum enhancement of Raman signal should be obtained on silver particles of the size of around 400 nm. Unfortunately, particles of this dimension are unstable in solution and they usually sediment within a few hours. Silver nanoparticles of the size ranging from units of nanometers to tens of nanometers can be stable for several months or years, even without any extra stabilization. However, these small particles themselves usually do not provide surface enhancement of the Raman signal. For this purpose, they must be activated for example by the addition of some inorganic ion solution. The most frequently used activation agents of the silver nanoparticles include halide ions, particularly chlorides. However, the mechanism of the activation of these ions is not yet fully explained; one of possible explanations of this effect is based on a partial aggregation of silver nanoparticles caused by mentioned ions. The state-of-art procedures of silver nanoparticle activation by means of chloride ion solutions employ the final concentrations in the range of from 0.001 mol.dm^{-3} to 0.02 mol.dm^{-3} (Leopold, N. and Lendl, B. J Phys Chem B 107, 2003; Michaels A. et al., J Am Chem Soc 121, 1999; Leng W. N. et al., J Raman Spectrosc 37, 2006; Zhang P. X. et al., J Raman Spectrosc 21, 1990; Campbell M. et al., J Raman Spectrosc 30, 1999; Doering W. E. and Nie S. M., J Phys Chem B 106, 2002). But in these cases, a slow aggregation of silver nanoparticles occurs, resulting in Raman signal irreproducibility and often also in a long activation time.

Disclosure of the Invention

The object of the invention is a method for a fast activation of aqueous dispersions of silver nanoparticles for purposes of surface enhanced Raman spectroscopy, wherein a solution of chloride ions is added to the aqueous dispersion of silver nanoparticles in the presence of oxygen in such an amount that the final chloride ion concentration in the activated dispersion ranges from 0.1 mol.dm^{-3} to 1 mol.dm^{-3}.

The advantage of the method of activation according to the present invention is that it allows to prepare silver particles suitable also for the application in surface enhanced Raman spectroscopy that uses lasers with the wavelength in a near-infrared area.

In case of the method according to the present invention, particularly when using a higher final chloride ion concentration (above 0.4 mol.dm^{-3}), not only an aggregation but especially gradual recrystallization of nanoparticles occurs. After about 15 minutes following the addition of the chloride anions with the final concentration of 0.4 mol.dm^{-3}, particles of the size of about 400 nm are already present in the activated dispersion. This particle size modification process caused by the recrystallization is reproducible. Hence another advantage of the present invention is that it allows a fast and reproducible activation of silver nanoparticles for the purposes of SERS through their recrystallization, which is different from the state of the art methods which lead to a slow and often irreproducible aggregation of silver particles.

It is an aspect of the invention that the re-crystallization and/or activation occurs in the presence of oxygen. The presence of oxygen in the activated dispersion is an important requirement for the nanoparticle recrystallization and activation. Usually, the amount of oxygen naturally dissolved in water under standard conditions (ambient temperature, atmospheric pressure) is sufficient for a successful course of the activation. Where necessary, more oxygen can be added into the dispersion during the activation. The adding of oxygen can be performed, e.g., by several short shakings of the dispersion during the activation, by a vigorous agitation, by adding oxygen from a container, by chemically preparing oxygen (e.g., using a chemical source of oxygen) or by any other suitable method. In total absence of oxygen in the activated dispersion, large aggregates of the initial nanoparticles are formed that do not manifest any surface enhancement of Raman signal.

When using a lower final chloride ion concentrations in the activated dispersion (about 0.1 $mol.dm^{-3}$), also in the presence of oxygen, the activation and recrystallization process is not as fast and reproducible as in the case of the higher final chloride anion concentrations. Moreover, in these cases the particles recrystallize only partially within the same time period. For that reason, it is preferable to use higher final chloride ion concentrations, i.e. 0.4 $mol.dm^{-3}$ and higher.

The final chloride ion concentration in the activated silver nanoparticle dispersion is in the range of from 0.1 $mol.dm^{-3}$ to 1 $mol.dm^{-3}$, more preferably 0.4 to 0.8 $mol.dm^{-3}$.

For the activation of silver nanoparticles for the spectroscopy using a laser with the wavelength of 1064 nm, it is appropriate to use higher final chloride ion concentrations in the final activated dispersion, particularly the concentrations of from 0.4 $mol.dm^{-3}$ to 0.8 $mol.dm^{-3}$.

The concentration of chloride ions in the chloride ion stock solutions used for the activation is preferably in the range of from 0.125 $mol.dm^{-3}$ to saturated solution concentration, more preferably 1 mol.dm^{-3} to saturated solution concentration, most preferably 1 mol.dm^{-3} to 4 mol.dm^{-3}.

Any water-soluble salt containing the chloride anion can be used as the source of chloride ions, provided that its stock solution can be prepared with a sufficient concentration, i.e. if the concentration of the chloride ions in the saturated solution thereof is sufficient for achieving the required final chloride ion concentration. Also, the cation contained in that salt should not interact with the activated silver nanoparticle dispersion. For example, chlorides of alkali metals (i.e. metals from the IA group of the periodic table of elements) and ammonium chloride are suitable salts.

The initial size of silver nanoparticles in the dispersion before the activation is typically in the range of from 2 nm to 300 nm, preferably 5 nm to 200 nm, more preferably 10 nm to 100 nm.

The final Ag concentration in the activated silver nanoparticle dispersion is typically in the range of from 10^{-7} mol.dm^{-3} to 0.05 mol.dm^{-3}, preferably 10^{-6} mol.dm^{-3} to 0.01 mol.dm^{-3}, more preferably 10^{-4} mol.dm^{-3} to 10^{-3} mol.dm^{-3}.

Brief Description of Drawings

Figure 1 shows the transmission electron microscopy images of the primary silver nanoparticles and the transformed silver particles formed during 15 minutes after the addition of the sodium chloride solution with the concentration of 4 mol.dm^{-3} (the final concentration of 0.4 mol.dm^{-3}). (A) primary silver nanoparticles with the size of approx. 20 nm; (B) silver particles formed after 30 seconds; (C) after 2 minutes; and (D) after 15 minutes following the addition of NaCl solution with the concentration of 4 mol.dm^{-3}. Figure 2 represents transmission electron microscopy images of recrystallized particles (A, B, C) with various morphologies of the size of several hundred nanometers formed 15 minutes after the addition of sodium chloride solution (the final concentration of 0.4 mol.dm^{-3}) and transmission electron microscopy images of primary non-activated silver nanoparticles (D, E, F) with the same crystal shapes (see insets) before the addition of sodium chloride solution.

Figure 3 shows the time-dependent change of the average size of silver particles recorded during 20 minutes after the addition of 4M sodium chloride solution (the final concentration of 0.4 $mol.dm^{-3}$). The data presented in the chart are an average obtained from 12 independent measurements performed during one year, wherein the error bars represent maximum and minimum determined values of silver particle average sizes. The average particle sizes were determined by DLS method.

Figure 4 shows transmission electron microscopy images of silver nanoparticles of the initial size of approx. 20 nm transformed during 15 minutes following the addition of the sodium chloride solution with the concentration of 1 $mol.dm^{-3}$ (the final concentration of 0.1 $mol.dm^{-3}$).

Figure 5 shows (A) transmission electron microscopy images of silver particle aggregates obtained after 15 minutes following the addition of sodium chloride solution (the final concentration of 0.4 $mol.dm^{-3}$) to particles of the size of approx. 20 nm in the absence of oxygen in the activated dispersion, and (B) the detail of these aggregates' surface.

Figure 6 shows Raman spectra and surface enhanced Raman spectra of adenine recorded using a laser with the wavelength of 1064 nm. (A) Surface enhanced Raman spectrum of adenine with the concentration of 10^{-5} $mol.dm^{-3}$ recorded in the presence of silver particles transformed by means of sodium chloride solution with the final concentration of 0.4 $mol.dm^{-3}$. (B) Raman spectrum of adenine with the concentration of 10^{-5} $mol.dm^{-3}$ recorded using silver nanoparticles of the size approx. 20 nm without the sodium chloride solution addition. (C) Raman spectrum of adenine of concentration equal to 10^{-1} $mol.dm^{-3}$.

Examples of Carrying Out the Invention

Sodium chloride was obtained from Sigma-Aldrich. Silver nanoparticles were prepared by means of reduction of silver ion ammonium complex with maltose, glucose, ascorbic acid and sodium tetrahydridoborate (Kvitek et al., J Mater Chem 15, 2005, Panacek et al., J Phys Chem B 110, 2006, Creighton et al., J Chem Soc Faraday Trans II 75, 1979). The starting compounds were obtained from Sigma-Aldrich.

The size of nanoparticles was measured by Zetasizer Nano-ZS (Malvern, UK) instrument using the DLS (Dynamic Light Scattering) principle. The prepared dispersions were also characterized by UV-Vis absorption spectroscopy using Specord

S600 spectrophotometer (Analytic Jena AG, Germany). The size of silver nanoparticles and their polydispersity was also verified by transmission electron microscopy (TEM) by JEM 2010 instrument (Jeol, Japan) using the accelerating voltage of 160 kV. The surface enhanced Raman spectra were measured on Jobin-Yvon T 64 000 instruments with the excitation wavelengths of 488 nm and 514.5 nm (Jobin-Yvon, France), HORIBA iHR550 with the excitation wavelength of 532 nm, Thermo Scientific DXR Microscope with the excitation wavelengths of 633 nm and 780 nm (Thermo Scientific, USA) and Nicolet FT-IR 6700 (Nicolet, USA) equipped with Raman module (NXR FT-Nicolet, USA) with the excitation wavelength of 1064 nm.

Example 1

Recrystallization of silver nanoparticles with an average size of 25 nm using sodium chloride solution with a concentration of 4 mol.dm^{-3}

0.2 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 3 ml cuvette. Then 0.7 ml of distilled water was added. After stirring the mixture, 0.1 ml of NaCl solution with the concentration of 4 mol.dm^{-3} was added. The final concentration of sodium chloride in the solution was 0.4 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then the changes in the silver particle dispersion characteristics were measured using the following methods: DLS, UV-Vis absorption spectroscopy, and TEM. After 15 minutes following the addition of chloride anions, particles with the size of 400 nm were already present in the activated dispersion (Fig. 1, 2). The process of the particle size change caused by the recrystallization is reproducible, as it was shown in the course of 12 measurements carried out during one year (Fig. 3).

Example 2

Transformation of silver nanoparticles with an average size of 25 nm using sodium chloride solution with a concentration of 1 mol.dm^{-3}

0.2 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 3 ml cuvette. Then 0.7 ml of distilled water was added. After stirring the mixture, 0.1 ml of NaCl solution with the concentration of 1 mol.dm^{-3} was added. The final concentration of sodium chloride in the solution was 0.1 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then the changes of the silver particle dispersion characteristics were measured using the following methods: DLS, UV-Vis absorption spectroscopy, and TEM. When the final chloride ion concentration in the activated dispersion is 0.1 mol.dm^{-3}, the particles recrystallize only partially and incompletely within the same time as in Example 1 (Fig. 4).

Example 3

Transformation of silver nanoparticles with an average size of 25 nm using sodium chloride solution with a concentration of 4 mol.dm^{-3} in the absence of oxygen in reaction solutions

0.2 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 3 ml cuvette. Then 0.7 ml of distilled water was added. After stirring the mixture, 0.1 ml of NaCl solution with the concentration of 4 mol.dm^{-3} was added. The final concentration of sodium chloride in the solution was 0.4 mol.dm^{-3}. All air oxygen has been removed from all solutions by a thorough bubbling with argon and the transformation was carried out in the absence of air. In this case, the primary particle recrystallization did not occur, but only their aggregation. On TEM images, we can observe aggregates of the size of several units of micrometers (Fig. 5); these aggregates prepared in the absence of oxygen are not suitable for use in the SERS.

Example 4

Recrystallization of silver nanoparticles with an average size of 25 nm using sodium chloride solution with a concentration of 4 mol.dm^{-3}

0.1 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 3 ml cuvette. Then 0.8 ml of distilled water was added. After stirring the mixture, 0.1 ml of NaCl solution with the concentration of 4 mol.dm^{-3} was added. The final concentration of sodium chloride in the solution was 0.4 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then the changes of the silver particle dispersion characteristics were measured using the following methods: DLS, UV-Vis absorption spectroscopy, and TEM. The course of the particle size changes was similar to the data presented in Example 1 (Fig 3). However, after 15 minutes following the addition of chloride anions, the final particle size determined by DLS method was 250 nm.

Example 5

Recrystallization of silver nanoparticles with an average size of 25 nm using sodium chloride solution with a concentration of 4 mol.dm^{-3}

0.8 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 3 ml cuvette. Then 0.1 ml of distilled water was added. After stirring the mixture, 0.1 ml of NaCl solution with the concentration of 4 mol.dm^{-3} was added. The final concentration of sodium chloride in the solution was 0.4 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then the changes of the silver particle dispersion characteristics were measured using the following methods: DLS, UV-Vis absorption spectroscopy, and TEM. The course of the particle size changes was similar to the data presented in Example 1 (Fig 3). However, after 20 minutes following the addition of chloride anions, the final particle size determined by DLS method was 700 nm.

Example 6

Activation of silver nanoparticles with an average size of 25 nm using sodium chloride solution with a concentration of 4 mol.dm^{-3} for the purpose of surface enhanced Raman spectroscopy (with a laser excitation wavelength of 1064 nm)

0.2 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 3 ml cuvette. Then 0.7 ml of distilled water was added. After stirring the mixture, 0.1 ml of NaCl solution with the concentration of 4 mol.dm^{-3} was added. The final concentration of sodium chloride in the solution was 0.4 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then 10 μl of adenine solution (or alternatively another analyzed or examined substance) with the concentration of 0.001 mol.dm^{-3} was added to the mixture. Surface enhanced Raman spectrum was recorded after 15 minutes following the sodium chloride solution addition (Fig. 6). For the evaluation of enhancement, the Raman spectrum of adenine solution (concentration equal to 0.1 mol.dm^{-3}) was measured without the presence of activated silver particles. The enhancement factor was determined by comparing the intensities of the most intensive peak in spectrum of adenine at 734 cm^{-1} and it was estimated to approx. 10^5.

Example 7

Activation of silver nanoparticles with an average size of 25 nm using potassium chloride solution with a concentration of 4 mol.dm^{-3} for the purpose of surface enhanced Raman spectroscopy (with a laser excitation wavelength of 488 nm)

1 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 10 ml flask. Then 3.5 ml of distilled water was added. After stirring the mixture, 0.5 ml of KCl solution with the concentration of 4 mol.dm^{-3} was added. The final concentration of potassium chloride in the solution was 0.4 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then 10 μl of adenine solution (or alternatively another analyzed or examined substance) with the concentration of 0.0005 mol.dm^{-3} was added to the mixture. Surface enhanced Raman spectrum was recorded after 3 minutes following the potassium chloride solution addition. The surface enhanced Raman spectrum of adenine was similar to spectrum presented in Example 6 (Fig 6). The enhancement factor was estimated to approx. $5 \cdot 10^5$.

Example 8

Activation of silver nanoparticles with an average size of 60 nm using sodium chloride solution with a concentration of 1 mol.dm^{-3} for the purpose of surface enhanced Raman spectroscopy (with a laser excitation wavelength of 514 nm)

1 ml of a dispersion of silver nanoparticles with the average size of 60 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 10 ml flask. Then 3.5 ml of distilled water was added. After stirring the mixture, 0.5 ml of NaCl solution with the concentration of 1 mol.dm^{-3} was added. The final concentration of sodium chloride in the solution was 0.1 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then 10 μl of adenine solution (or alternatively another analyzed or examined substance) with the concentration of 0.001 mol.dm^{-3} was added to the mixture. Surface enhanced Raman spectrum was recorded after 15 minutes following the sodium chloride solution addition. The surface enhanced Raman spectrum of adenine was similar to spectrum presented in Example 6 (Fig 6). The enhancement factor was estimated to approx. $2 \cdot 10^5$.

Example 9

Activation of silver nanoparticles with an average size of 200 nm using sodium chloride solution with a concentration of 1 mol.dm^{-3} for the purpose of surface enhanced Raman spectroscopy (with a laser excitation wavelength of 1064 nm)

0.2 ml of a dispersion of silver nanoparticles with the average size of 200 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 3 ml cuvette. Then 0.7 ml of distilled water was added. After stirring the mixture, 0.1 ml of NaCl solution with a concentration of 1 mol.dm^{-3} was added. The final concentration of sodium chloride in the solution was 0.1 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then 10 μl of adenine solution (or alternatively another analyzed or examined substance) with the concentration of 0.001 mol.dm^{-3} was added to the mixture. Surface enhanced Raman spectrum was recorded after 10 minutes following the sodium chloride solution addition. The surface enhanced Raman spectrum of adenine was similar to spectrum presented in Example 6 (Fig 6). The enhancement factor was estimated to approx. $5 \cdot 10^4$.

Example 10

Activation of silver nanoparticles with an average size of 25 nm using sodium chloride solution with a concentration of 4 mol.dm^{-3} for the purpose of surface enhanced Raman spectroscopy (with a laser excitation wavelength of 532 nm)

0.2 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 3 ml cuvette. Then 0.6 ml of distilled water was added. After stirring the mixture, 0.2 ml of NaCl solution with a concentration of 4 mol.dm^{-3} was added. The final concentration of sodium chloride in the solution was 0.8 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then 10 μl of adenine solution (or alternatively another analyzed or examined substance) with the concentration of 10^{-6} mol.dm^{-3} was added to the mixture. The final concentration of sodium chloride in the solution was 0.8 mol.dm^{-3}. Surface enhanced Raman spectrum was recorded after 6 minutes following the sodium chloride solution addition. The surface enhanced Raman spectrum of adenine was similar to spectrum presented in Example 6 (Fig 6). The enhancement factor was estimated to approx. $6 \cdot 10^7$.

Example 11

Activation of silver nanoparticles with an average size of 25 nm using potassium chloride solution with a concentration of 4 mol.dm^{-3} for the purpose of surface enhanced Raman spectroscopy (with a laser excitation wavelength of 633 nm)

1 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 10 ml flask. Then 3.5 ml of distilled water was added. After stirring the mixture, 0.5 ml of KCl solution with the concentration of 4 mol.dm^{-3} was added. The final concentration of potassium chloride in the solution was 0.4 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then 10 μl of adenine solution (or alternatively another analyzed or examined substance) with the concentration of 0.001 mol.dm^{-3} was added to the mixture. Surface enhanced Raman spectrum was recorded after 6 minutes following the potassium chloride solution addition. The surface enhanced Raman spectrum of adenine was similar to spectrum presented in Example 6 (Fig 6). The enhancement factor was estimated to approx. 10^5.

Example 12

Activation of silver nanoparticles with an average size of 25 nm using potassium chloride solution with a concentration of 4 mol.dm^{-3} for the purpose of surface enhanced Raman spectroscopy (with a laser excitation wavelength of 780 nm)

1 ml of a dispersion of silver nanoparticles with the average size of 25 nm and Ag concentration of 0.001 mol.dm^{-3} in the dispersion was transferred into a 10 ml flask. Then 3.5 ml of distilled water was added. After stirring the mixture, 0.5 ml of KCl solution with the concentration of 4 mol.dm^{-3} was added. The final concentration of potassium chloride in the solution was 0.4 mol.dm^{-3}. The mixture was vigorously stirred for 10 seconds and then 10 μl of adenine solution (or alternatively another analyzed or examined substance) with the concentration of 0.001 mol.dm^{-3} was added to the mixture. Surface enhanced Raman spectrum was recorded after 6 minutes following the potassium chloride solution addition. The surface enhanced Raman spectrum of adenine was similar to spectrum presented in Example 6 (Fig 6). The enhancement factor was estimated to approx. $8 \cdot 10^4$.

CLAIMS

1. A method for activation of aqueous dispersions of silver nanoparticles for purposes of surface enhanced Raman spectroscopy, characterized in that a solution of chloride ions is added to the aqueous dispersion of silver nanoparticles in the presence of oxygen in such an amount that the final chloride ion concentration in the activated dispersion ranges from 0.1 mol.dm^{-3} to 1 mol.dm^{-3}.

2. The method according to claim 1, characterized in that the final chloride ion concentration in the activated dispersion ranges from 0.4 mol.dm^{-3} to 0.8 mol.dm^{-3}.

3. The method according to claim 1, characterized in that oxygen is added to the dispersion in the course of the activation.

4. The method according to claim 1, characterized in that the initial size of silver nanoparticles in the dispersion before the activation is in the range of from 2 nm to 300 nm, preferably 5 nm to 200 nm, more preferably 10 nm to 100 nm.

5. The method according to claim 1, characterized in that a water-soluble salt containing the chloride anion is used as the source of chloride ions.

Fig. 1

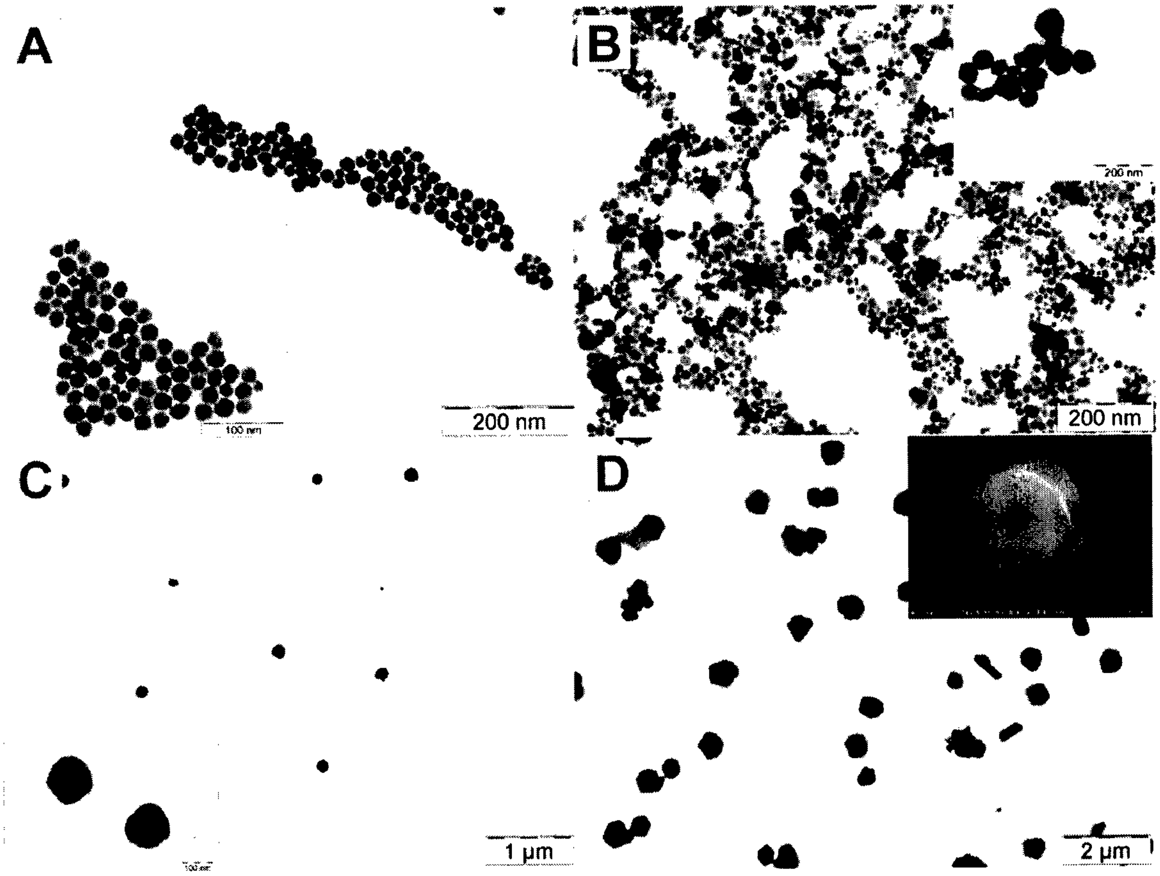


Fig. 2

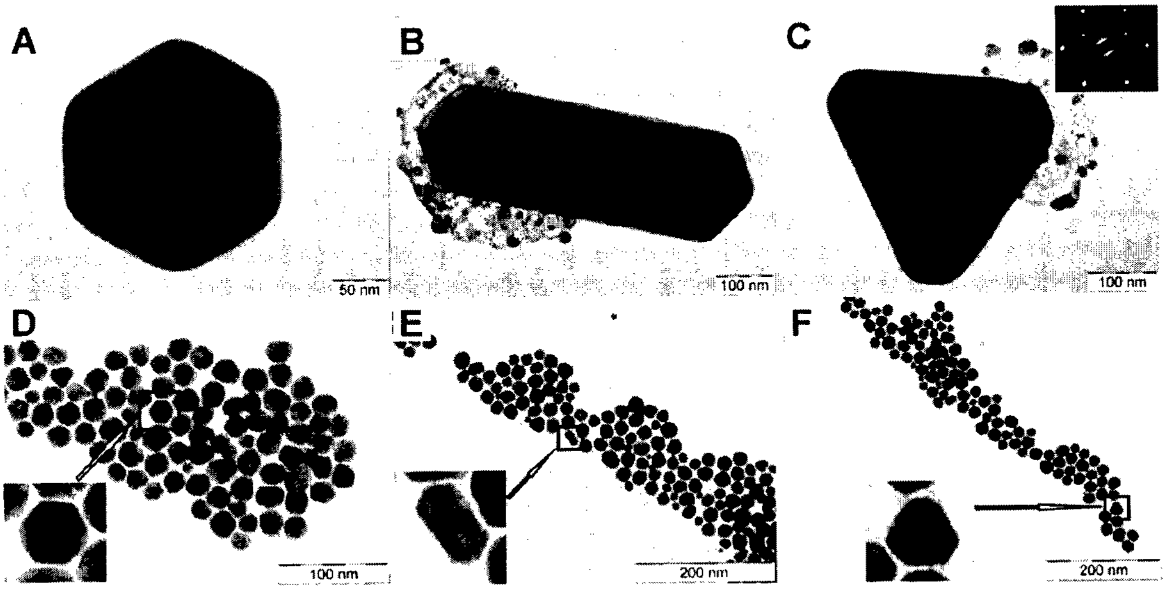


Fig. 3

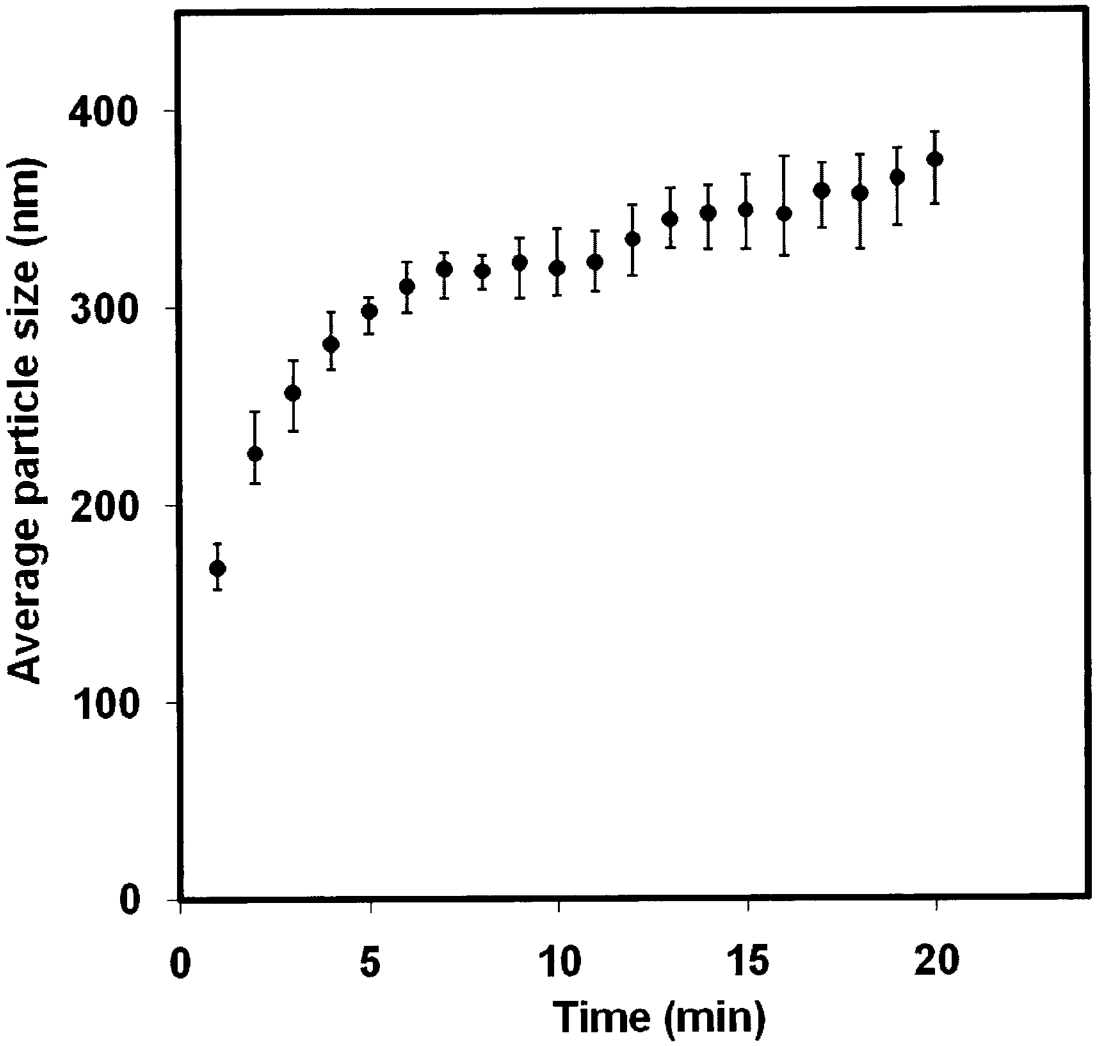


Fig. 4

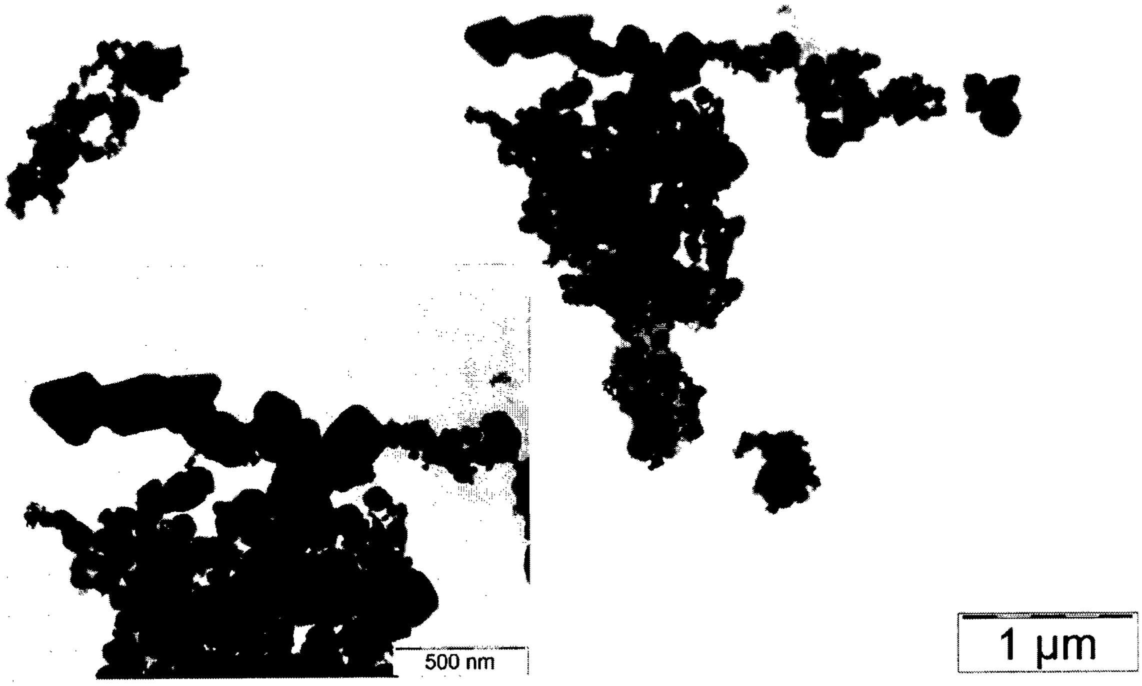


Fig. 5

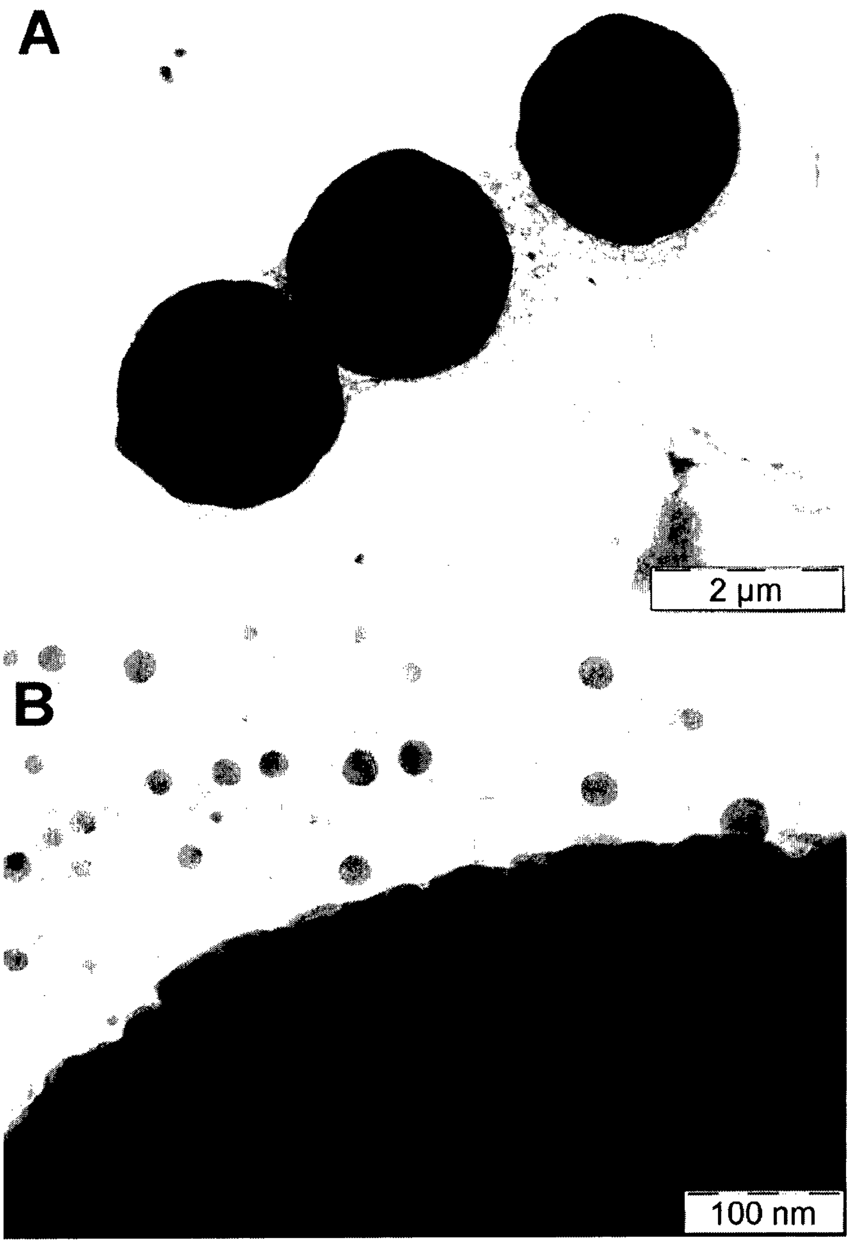
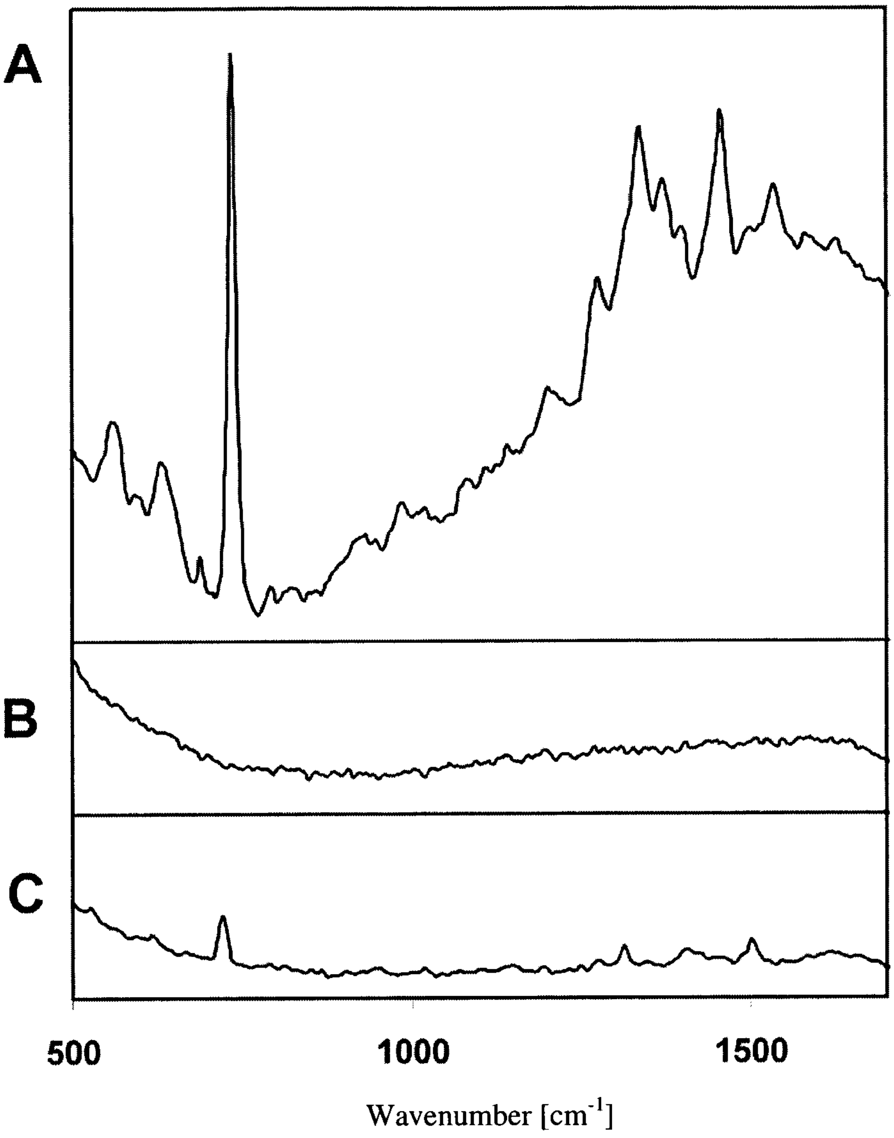


Fig. 6



INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2011/000083

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/65 G01N33/58 G01N33/543 B82Y15/00 B82Y30/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N B82Y

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KVITEK L ET AL: "The influence of complexing agent concentration on particle size in the process of SERS active silver colloid synthesis", JOURNAL OF MATERIALS CHEMISTRY R. SOC. CHEM. UK, vol. 15, no. 10, 14 March 2005 (2005-03-14), pages 1099-1105, XP055015704, ISSN: 0959-9428	1,3-5
Y	page 1100, column 2, last paragraph - page 1101, column 2, line 2; table 1 page 1103, column 2, last paragraph - page 1104, column 2, last line; figures 8,9 -----	2

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Date of the actual completion of the international search 12 January 2012	Date of mailing of the international search report 18/01/2012
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Consalvo, Daniela

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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