

EVALUATION OF CAPPING AGENTS FOR SILVER NANOPARTICLES

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ABSTRACT. We have synthesized silver nanoparticles capped with: citrate, mercaptosuccinic acid, and thioctic acid respectively. Each of the obtained nanoparticles were characterized by means of: UV-Vis and ATR-FT-IR spectroscopy, dynamic light scattering (DLS), atomic force microscopy (AFM) and were found to be spherical in shape and aqueous stable.

Keywords: *silver nanoparticles, capping agents, citrate, mercaptosuccinic acid, thioctic acid*

INTRODUCTION

Silver nanoparticles (AgNPs), with their localized surface plasmon resonance (SPR) and remarkable antimicrobial properties have been a research focal point in the field of life sciences, for the past decade [1-4].

Several studies showed that the activity of noble metal nanoparticles, such as AgNPs, is strongly correlated with their colloidal stability [5, 6]. Special consideration must be given to the stabilization agents used for the synthesized nanoparticles. For example, divalent cations can induce AgNP aggregation by displacing the capping citrate layer [7], also pH significantly influences the stability of a AgNPs suspensions [8]. Adequate functionalization strategies must be undertaken in order to obtain nanostructures with specific characteristics for each biomedical application envisioned. These strategies can be divided into four

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groups: a). covalent binding; b). non-covalent binding; c). using electrostatic charges between the biomolecules and the surface of the nanoparticles and d). ligand mediated binding (such as chemisorption) [4, 9, 10].

In the present paper we have synthesized aqueous stable AgNPs with capped with citrate (cit), mercaptosuccinic acid (MSA), and thiocetic acid (TA) respectively. The obtained silver colloids were investigated in terms of their size, shape, SPR band localization and stability by employing several analytical techniques: UV-Vis and FT-IR spectroscopies, dynamic light scattering (DLS) and atomic force microscopy (AFM).

RESULTS AND DISCUSSION

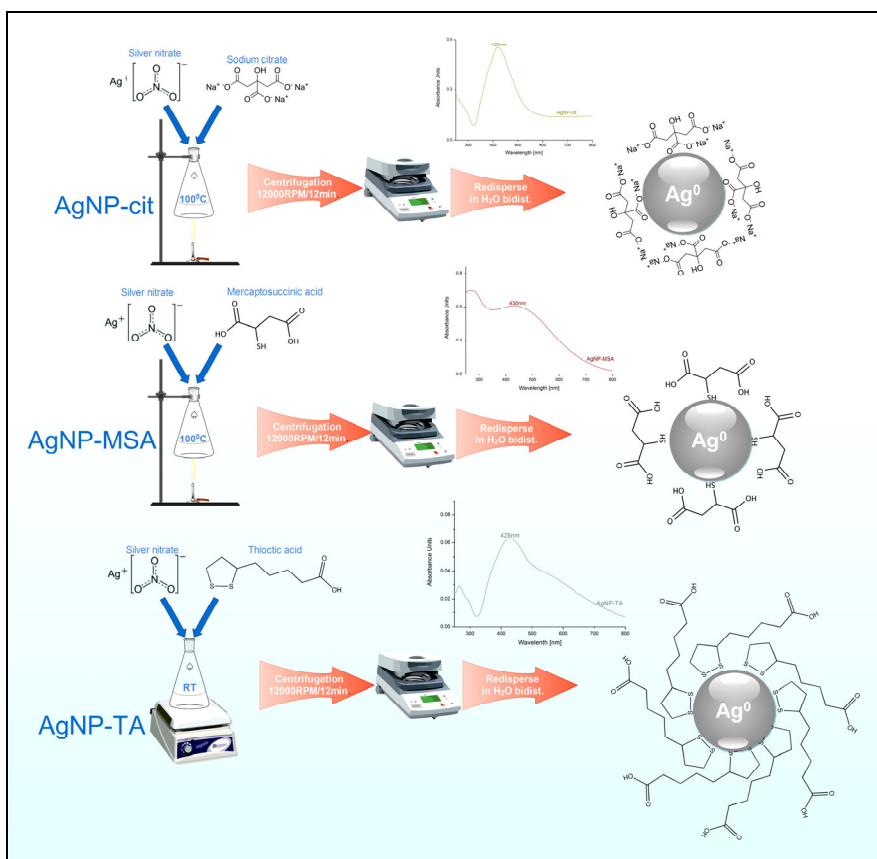


Figure 1. Graphical abstract for the synthesis routes employed for AgNP-cit, AgNP-MSA and AgNP-TA

We have proposed to evaluate 3 types of capping agents for AgNPs: citrate, mercaptosuccinic acid and thioctic acid. The synthesis routes undertaken are illustrated in fig.1. For citrate stabilized AgNPs a modified Turkevich method was used, where the citrate ions act as both reducing and stabilizing agents [11]. The same principle, where the capping agent acts both as a reducing and stabilizing agent, was employed with the mercaptosuccinic acid, yielding AgNP-MSA nanoparticles where the capping agent is linked to the nanoparticle surface through its thiol group [12]. While in the case of thioctic acid capping of AgNPs the reducing agent was NaBH_4 [13]. All three variants of AgNPs obtained were characterized in terms of aqueous stability, surface plasmon resonance band, surface chemistry, size and shape.

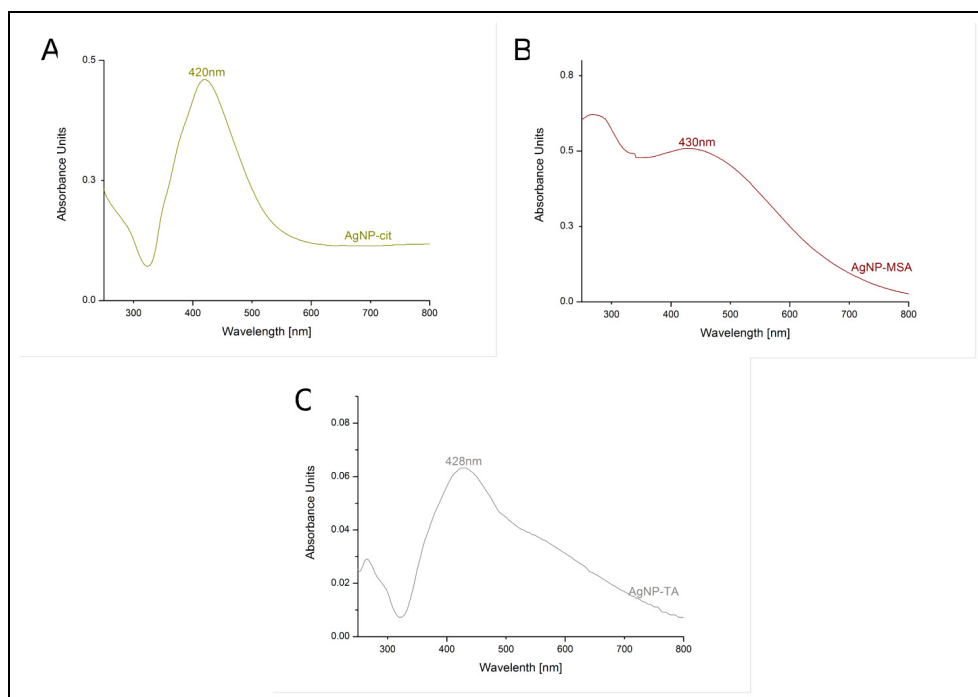


Figure 2. UV-Vis spectra of AgNP-cit (A), AgNP-MSA (B) si AgNP (C)

UV-Vis spectroscopy was used in order to determine the position of the surface plasmon band for AgNPs. In figure 2 the spectra for AgNP-cit (A), AgNP-MSA (B) si AgNP (C) are presented. The citrate stabilized silver nanoparticles presented an absorption peak at $\lambda_{\text{max}}=420$ nm, the MSA capped AgNPs had a wide peak centered at $\lambda_{\text{max}}=430$ nm, while the TA capped nanoparticles had a peak a $\lambda_{\text{max}}=428$ nm. These data are in good agreement with literature [14, 15].

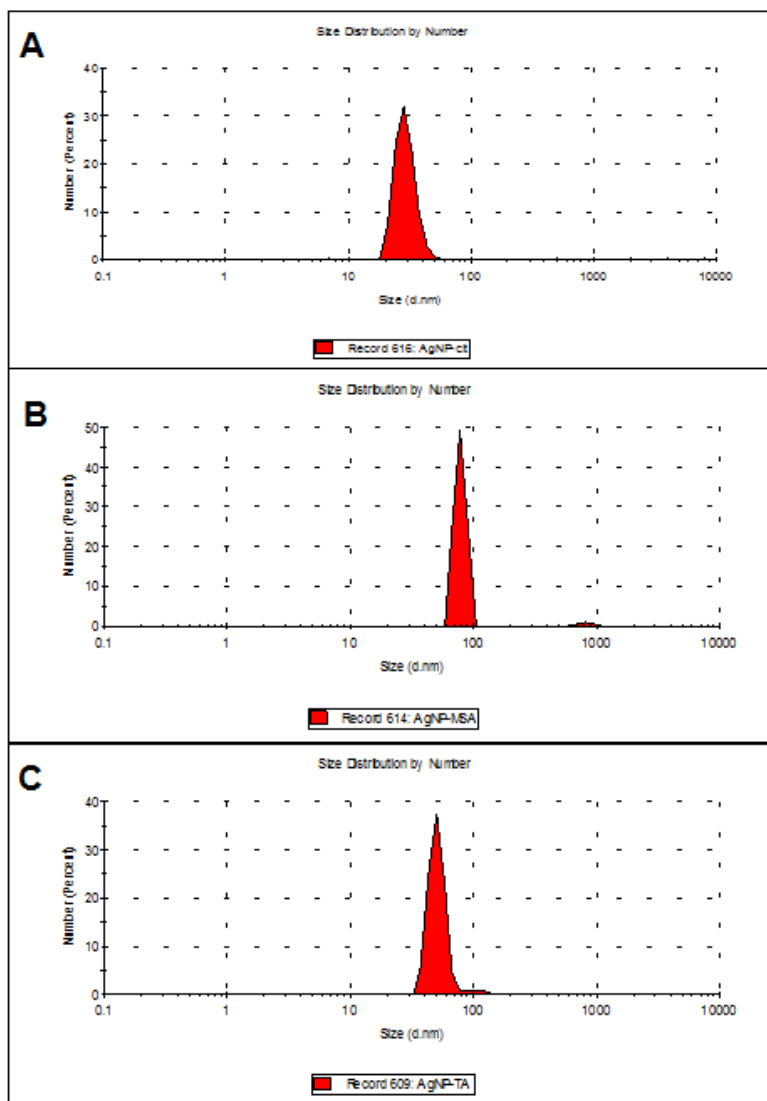


Figure 3. DLS size distribution curves AgNP-cit (A), AgNP-MSA (B) si AgNP-TA (C)

Figure 3 shows the size distribution of the hydrodynamic diameter for the citrate (A), MSA (B) and TA stabilized silver nanoparticles as measured by the DLS technique. AgNP-cit had a mean diameter of 29 nm, AgNP-MSA 69 nm and the AgNP-TA 51 nm. All three samples presented themselves as aqueous stable.

In order to confirm the presence of citrate, MSA and TA on the surface of the AgNPs the IR 'fingerprint' was acquired for each sample and are shown in figure 4. The AgNP-cit sample presented two absorption bands at 1582 and 1356 cm^{-1} which correspond to the antisymmetric and symmetric stretching vibrations of the COO^- from the citrate ions present on the surface of the silver nanoparticles. In the case of the AgNP-MSA sample the absorption band attributed to COO^- were recorded at 1567 and 1358 cm^{-1} , also the absence of an absorption band at 2550 cm^{-1} indicates the fact that the mercaptosuccinic acid is bonded on the surface of the nanoparticles via its thiolic group. For the AgNP-TA the bands at 2916 cm^{-1} , 2850 cm^{-1} , 1601 cm^{-1} si 1356 cm^{-1} are consistent with the characteristic IR spectra of thioctic acid [16].

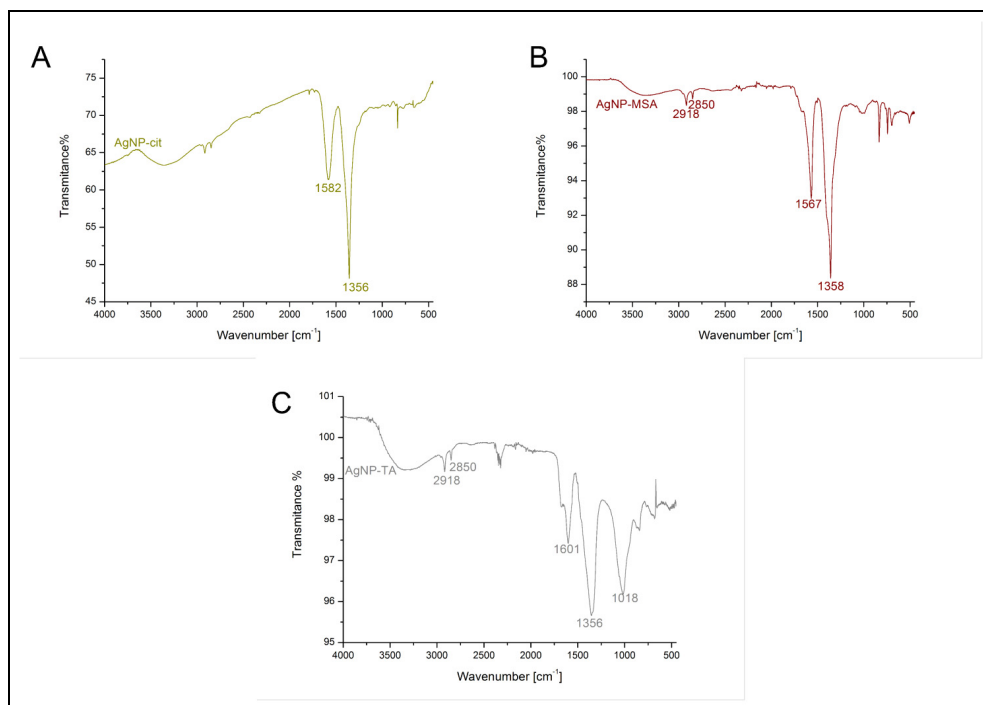


Figure 4. ATR-FT-IR spectra for AgNP-cit (A), AgNP-MSA (B) and AgNP-TA (C).

In figure 5 the AFM images, 2D and 3D representations, for the three samples are given. All capping agents used yielded nanometric, spheroidal particles. The sizes registered were: for AgNP-cit a size of ~ 23 nm; ~ 65 nm for AgNP-MSA and ~ 44 nm for AgNP-TA. The differences in sizes measured by AFM and DLS can be explained by the fact that dynamic light scattering technique provides slightly larger values due to the fact that this method

provides a mean hydrodynamic diameter of the nanoparticles surrounded by the capping agents [17].

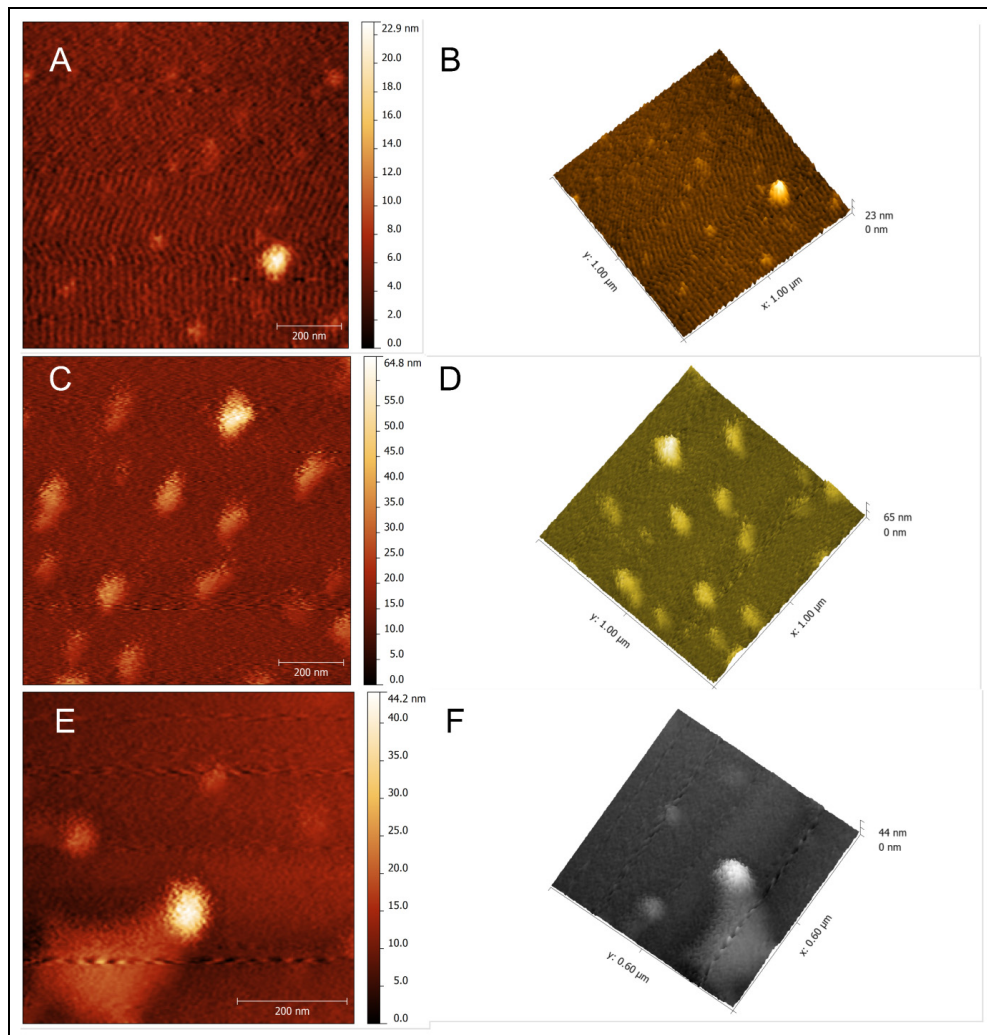


Figure 5. AFM image of r AgNP-cit (A- 2D; B- 3D), AgNP-MSA (C- 2D; D- 3D) and AgNP-TA (E- 2D; F 3D).

Each of the obtained samples can be further functionalized with proteins, peptides, drugs, and so on, depending on the biomedical application envisioned. For example for the AgNP-cit sample place exchange reactions can be employed, while for the AgNP-MSA and AgNP-TA samples EDC/NHS coupling can be used.

CONCLUSIONS

Aqueous stable silver nanoparticles were synthesized and capped with citrate, mercaptosuccinic acid, and thiocetic acid respectively. All three types of capping agents investigated yielded spherical AgNPs with different sizes. For the AgNP-cit a size of ~23 nm was registered, the AgNP-MSA had a mean size of ~65 nm, while the AgNP-TA sample ~44 nm. The surface plasmon resonance band for each sample was identified by means of UV-Vis spectroscopy. For the confirmation of the presence of each capping agent on the surface of the AgNPs IR spectra were recorded and compared. The different sizes and surface chemistries of the synthesized AgNPs can be used in different areas of biomedical research such as: antibacterial, drug delivery and sensor applications.

EXPERIMENTAL SECTION

Silver nitrate (AgNO_3 99.9%), tri-sodium citrate ($\geq 99\%$), mercaptosuccinic acid and the thiocetic acid were purchased from Sigma-Aldrich (Darmstadt, Germany) and used as received, without further purification. All glassware was cleaned with aqua regia ($\text{HCl}:\text{HNO}_3$, 3:1 v/v) prior to its use.

The citrate stabilized silver nanoparticles were synthesized by employing a modified Turkevich method. For this, 18 mg AgNO_3 were dissolved in 100 mL dist. H_2O and the solution was heated to 100°C under constant stirring. Afterwards, 2 mL sodium citrate 0.5% were rapidly injected and the reaction was allowed to continue until the solution turned pale-yellow in color. The AgNP-cit solution was allowed to cool to room temperature and subjected to a centrifugation step at 12000RPM/12min. The obtained sediment was re-dispersed in dist. H_2O with the aid of a 'in-probe' sonicator.

MSA capped AgNPs were obtained by reducing Ag^+ to Ag^0 with the aid of the mercaptosuccinic acid. Briefly, 50 mL sol. AgNO_3 0.6mM was brought to boiling and the 6 mL MSA sol. 28mM was added, where the MSA sol. was previously neutralized with NaOH 0.1M. The reaction was allowed to perfect for 30 minutes and after cooling the obtained solution was subjected to centrifugation and re-dispersing steps as described above in the case of AgNP-cit.

Thiocetic acid stabilized AgNPs were synthesized by mixing 2.5 mL AgNO_3 75 μM with 2.5 mL thiocetic 75 μM and then rapidly injecting 5 mL of freshly prepared NaBH_4 2.5mM. The reaction was allowed to perfect for 12h at room temperature and then the solution was centrifuged at 15000RPM/30min and the resulting pellet re-dispersed in dist. H_2O with the aid of a 'in-probe' sonicator.

The synthesized AgNP-cit, AgNP-MSA and AgNP-TA nanoparticles were evaluated on a UV-Vis spectroscopy Shimadzu UV-1800 spectrophotometer instrument. The spectra were recorded from 800nm to 200 nm with a spectral resolution of 0.5nm and normalized with the aid of OriginLab® software v7.0.

For dynamic light scattering (DLS) measurements of the samples a Nano ZS90 instrument (Malvern Instruments, Westborough, UK) was used at 25°C, a refractive index of 0.135 and an absorption of 3.99.

A Perkin-Elmer Spectrum Two® instrument equipped with an UATR single reflection diamond was used for the IR spectroscopy measurements. All spectra baseline corrections were done with the aid of the Spectrum10™ software.

Atomic force microscopy (AFM) data were recorded with a Workshop TT-AFM® instrument (AFMWorkshop, CA, USA), equipped with ACTA-SS (AppNano, CA, USA) cantilevers operated in vibrating mode. Samples were deposited on a mica substrate with a KLM® SCC spin-coater. The raw data collected were processed with the Gwyddion® software v2.36.

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