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# Cysteine mediated assembly of gold nanoparticles

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Citrate capped gold nanoparticles of controlled size in aqueous solution were prepared and their interaction with L-cysteine was investigated, monitoring their UV-Vis spectra, for different cysteine concentrations and various mixing ratios, as well as by transmission electron microscopy and atomic force microscopy. The interaction of gold nanoparticles with cysteine is quite strong, due to the peculiar affinity of S-atoms for gold. Cysteine is probably bound to the gold surface by its thiol group. AFM images evidence ordered gold nanoparticles assemblies mediated by cysteine. Our findings are in substantial agreement with existing data for other self-assembled organosulphur compounds on gold surfaces.

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*Keywords:* Gold nanoparticles; Self-assembly; L-Cysteine; S-Au interaction

## 1. Introduction

The interest for gold nanoparticles is continuously increasing, as a result of new discoveries by interdisciplinary research teams, being related to potential applications in the field of nanotechnology, self-assembly, catalysis and molecular electronics Particularly, biofunctionalized and self-assembled gold nanoparticles are in the focus of research in biomedical and bioanalytical areas, such as controlled drug delivery, medical diagnosis and biosensors [1-4].

In the biofunctionalization of gold nanoparticles, amino acids are considered as suitable agents for use as protective layers and for their assembly, due to the presence of different functional groups (-SH, $-NH_2$) with affinity for gold. Therefore, an amino acid, containing both functional groups, such as L-cysteine, is a promising compound to be used in this study for biofunctionalization of gold nanoparticles and for their self-assembly. Amino acid capped gold surfaces are also considered to represent the simplest mimics for protein surfaces [5].

Amino acids can be adsorbed on the gold particle surface already during the formation of particles, using the amino acid itself as reduction agent [6, 7], or in a latter stage, by ligand exchange reactions or binding on the former adsorbed stabilizing molecules. Amino acids can also be used in the assembly formation of gold nanoparticles. The properties of such assemblies could be designed rationally by choosing the initial amino acid [8].

The binding of cysteine and lysine to gold nanoparticles was communicated [9] and a review on amino acid interactions with metallic nanoparticles was given [10]. Among amino acids, used as reduction and capping agents for silver or gold nanoparticles, cysteine is found [10]. Further, the S-Au interaction in cysteine capped gold nanoparticels was discussed [7, 11, 12] and the binding of cysteine to Au was compared with that of leucine and asparagine [7]. Gold-silver nanocomposites were prepared from gold nanorod seeds in amino acid solutions, including cysteine [13]. Moreover, cysteine adsorbed on a gold surface was used to immobilize protein molecules [14, 15]. Maniu et al. [16] have investigated by density functional theory the adsorption of p-aminothiophenol molecules on gold nanoparticles.

In previous works, we synthesized gold nanoparticles in aqueous solutions of citrate and used them to be functionalized with various biomolecules, such as globular protein extracted from aleurone cells of barley [17] and amino acids, namely lysine [18].

The aim of the present investigation is to gain insights into the assembly formation of gold nanoparticles and interparticle interactions in the presence of L-cysteine, which could have potential application for the analytical detection of amino acids found in various media. This investigation concerns the effect of cysteine concentrations on the surface plasmon resonance (SPR) band evolution of gold nanoparticles. The cysteine mediated aggregates of gold nanoparticles are visualized via TEM and AFM images. In literature, AFM images for gold nanoparticles functionalized with cysteine are lacking.

L-cysteine is an amino acid with a thiol group (-SH) on the side chain (Fig. 1). It is a neutral, polar amino acid, with only limited hydrophilic properties [19]. Its $pK_a$ values are 1.96, 8.18 and 10.28 respectively [20].

## 2. Experimental

The colloidal gold solution was prepared by a method adapted from [21]. An 0.0125% aqueous solution of gold chloride (solution A) and a mixture of 4 ml 1% $Na_3C_6H_5O_7 \cdot 2H_2O$, 1 ml 1% tannic acid, 1 ml 25 mM potassium carbonate and 14 ml $H_2O$ (solution B) were separately warmed up to 60°C and then mixed while stirring. After the red color appeared (almost instantly) the mixture was heated up to 95°C under stirring and then cooled on ice. The gold content in the final sol is 92 mg/L

The solution of colloidal gold particles was stored in brown bottles and kept at 4 °C.

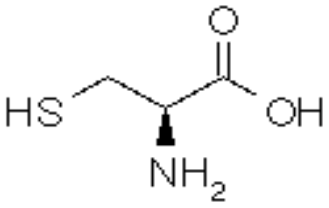


*Fig.1. L-Cysteine formula.*

$AuCl_3$ was purchased from Merck (high purity above 99.5 %). The trisodium citrate dihydrate was obtained from Sigma Aldrich (high purity above 99%). Tannic acid, pure powder, was purchased from Merck. $K_2CO_3$ pro analysis was obtained from Reactivul Bucharest. L-cysteine was purchased from Merck (high purity). All chemicals were used without further purification. L-cysteine was dissolved in deionized water in order to prepare 0.001 and 0.01 M solutions. Deionized water with resistivity of 18 MΩ·cm was used in all experiments and it was obtained from an Elgastat water purification system.

Tannic acid was added in the preparation with the aim to obtain smaller gold nanoparticles. With increasing amounts of tannic acid, the mean size of the particles decreases. Tannic acid is itself a reducing agent and increases the rate of nucleation for gold nanoparticles, while potassium carbonate is added in order to neutralize the acid.

The UV/Vis absorption spectrum of the solutions was studied using a Jasco UV/Vis V-530 spectrophotometer, with 10 mm path length quartz cuvettes in the 190 – 900 nm wavelengths range.

The investigated mixtures were obtained from the gold colloidal solution ($c_{Au}$) and the cysteine solutions (concentration $c_C$), by successive removal of small amounts of the previous mixture and adding of equal amounts of amino acid solution. The gold to cysteine content ratios reported to ($c_{Au}/c_C$) are given in the figures representing their spectra.

The gold nanoparticles suspension in the absence and in the presence of cysteine was deposited and air dried on the specimen grid and observed with a transmission electron microscope (TEM: JEOL – JEM 1010). TEM specimens consist of carbon or collodion coated copper grids. TEM images have been recorded with a JEOL standard software.

Atomic force microscopy (AFM) investigations were executed on the gold nanostructured films made from gold nanoparticles functionalized with arginine, using a commercial AFM JEOL 4210 equipment operating in tapping (noted *ac*) mode on thin adsorbed gold films on glass plates, that are optically polished and silanized with 3-aminopropyl-trietoxysilane. Standard cantilevers, non-contact conical shaped of silicon nitride coated with aluminium, were used. The tip was on a cantilever with a resonant frequency in the range of 200 - 300 kHz and with a spring constant of 17.5 N/m. AFM observations were repeated on different areas from 10 x 10 $\mu m^2$ to 250 x 250 $nm^2$ of the same gold film. The images were obtained from at least ten macroscopically separated areas on each sample. All images were processed using the standard procedures for AFM. AFM images consist of multiple scans displaced laterally from each other in y direction with 512 x 512 pixels. Low pass filtering was performed to remove the statistical noise without to loose the features of the sample. All AFM experiments were carried out under ambient laboratory conditions (about 20 °C) as previously reported [22].

## 3. Results and discussion

The visible absorption spectra of the gold aqueous colloidal solutions present a well-defined absorption band with a maximum at the wavelength $\lambda_{max}$ = 521-522 nm. This value is characteristic for plasmon absorbance for nanometric Au particles.

The size of the colloidal gold particles has been measured by TEM imaging (for example Fig. 2). From the sizes of a great number of particles, measured on the TEM images, the following characteristics were calculated: average size (diameter): 6.9 nm; standard deviation: 1.3 nm; average mass of a particle (considered spherical): $3.3 \cdot 10^{-18}$ g; number of gold atoms in a particle: $10^4$; number of particles per $cm^3$ of solution $2.8 \cdot 10^{13}$. A histogram providing the size distribution of gold nanoparticles, obtained from TEM pictures is given in Fig. 3. For comparison, the curve indicating the expected normal distribution was added.

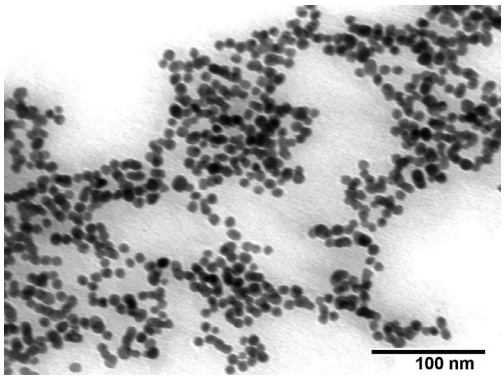


*Fig.2. TEM images of gold nanoparticles.*

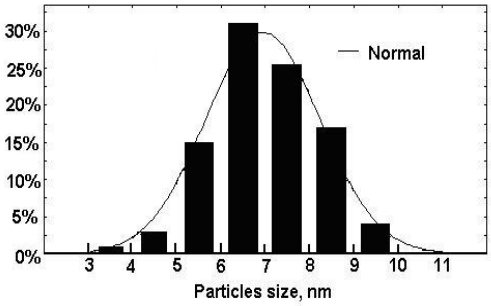


*Fig.3. Histogram of size distribution for particles.*

*L-cysteine* has a strong effect on ***UV-Vis spectra*** of gold nanoparticle solutions, more than other amino acids, for instance lysine [18]. Already, a $10^{-3}$ M solution (Fig. 4) added to the colloidal gold solutions leads to a rapid broadening of the adsorption peak, which is shifted toward higher wavelengths. This is a consequence of the coupling of surface plasmon resonance of two adjacent nanoparticles and is an indication of the anisotropic optical properties of the gold nanoparticles aggregates [23]. The peak for the broad band at longer wavelength surpasses rapidly the peak of the initial non-aggregated gold particles and the maximum wavelength goes till 620 nm. Finally, for the initial individual gold particles only a shoulder remains. The addition of 0.01 M cysteine solution leads to the color change in blue already at the first amount added. The time evolution of the aggregation is also observable from the figures.

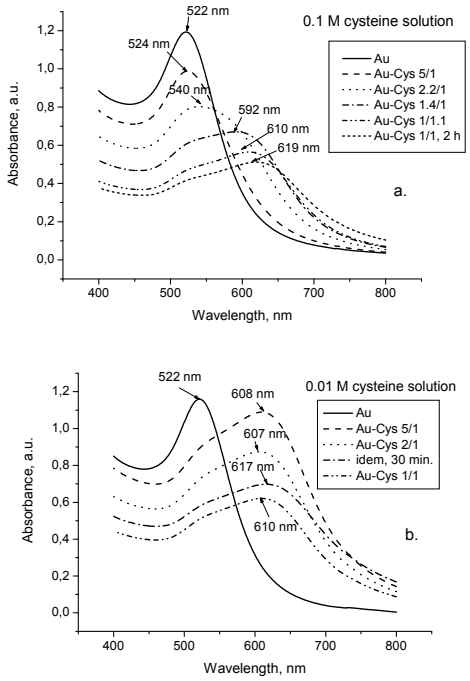


*Fig.4. Optical spectrum of colloidal gold solutions with 0.001 M (a) and 0.01 M (b) cysteine solution in different ratios, expressed in terms of the $c_{Au}/c_C$ ratio.*

This kind of color change as an effect of aggregation is a well-understood phenomenon [24]. When the interparticle distance in the aggregates decreases to less than about the average particle diameter, the electric dipole–dipole interaction and coupling between the plasmons of neighboring particles in the aggregates results in the bathochromic shift of the absorption band

These results support the observations about the interaction of cysteine with gold nanoparticles [11], where, based on the analysis of FT-IR and Raman spectra, the authors affirm the existence of covalent interaction of sulphur and gold. The formation of a covalent bond Au-S is also assumed by other authors [12, 25]. Further, it was suggested [26] that the positively charged amino group in cysteine ($-NH_3^+$) should interact with the negative charge on the surface of other gold nanoparticles through electrostatic binding, thus forming assemblies. Also, our findings are in substantial agreement with reported data on the adsorption of homocysteine on gold nanoparticles [27].

***TEM images*** for gold nanoparticles functionalized with 0.001 M cysteine solution (1:1 volume ratio) show the aggregates of nanoparticles (Fig. 5). As a characteristic for the cysteine mediated assembly of gold nanoparticles, it is to be observed that the nanoparticles are mostly linearly arranged, but also united in a more complex rather ordered network.

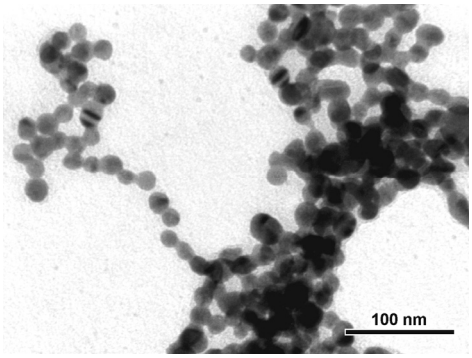


*Fig. 5. TEM image of gold nanoparticles with 0.001 M cysteine solution.*

The ***AFM images*** for gold nanoparticles functionalized with cysteine, adsorbed as monolayers on hydrophobic glass, evidence ordered self-assembly of gold nanoparticles, as seen in Fig. 6. A tendency of linear arrangements is also evident from these AFM images in agreement with TEM observations (Fig. 5).

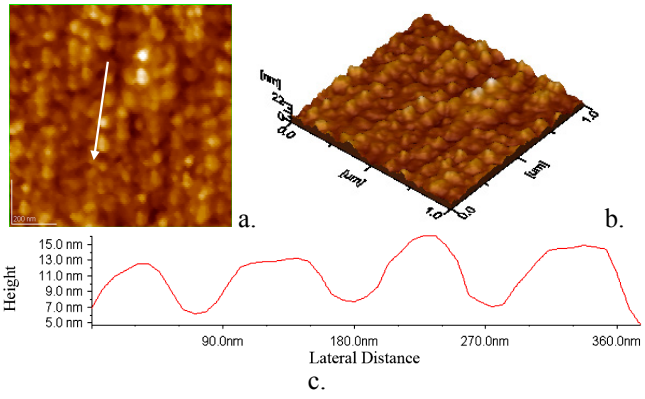


*Fig.6. (a) 2D-topographic AFM image of the assembly of gold nanoparticles, mediated by 0.001 M cysteine, deposited on hydrophobic glass after 2 min. deposition time; scanned area 1000 x1000 nm$^2$; 3D-view (b) of the topography (a); (c) cross section profile along the arrow in panel a.*

Cysteine is probably bound to the gold nanoparticles by its thiol (-SH) group, as was also suggested by the study of $^1$H NMR spectra [10,14]. After its adsorption on the gold nanoparticles, the cysteine molecule has still two functional groups free to form bonds between particles. A

model illustrating both the possible cysteine bindings to gold nanoparticles and the formation of particle aggregates is schematized in Fig. 7.

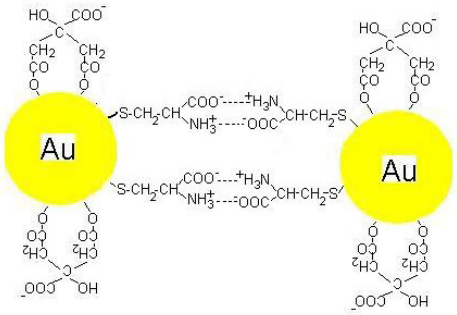


*Fig. 5. Schema of cysteine binding to citrate capped gold nanoparticles and bonds formation between particles*

## 4. Conclusion

Our data indicate that the assembly of gold nanoparticles can be induced by cysteine, an amino acid possessing an additional thiol functional group besides the alpha-amine. The cysteine assembly effect could be explained primarily through the zwitterion-type electrostatic interactions between the charged amine and acid groups of cysteine molecules, bound to the gold nanoparticles by their –SH groups. The unusual affinity makes cysteine more effective than other amino acids in its adsorption and binding process on gold nanoparticles.

The strong affinity of gold nanoparticles towards cysteine, as well as towards other amino acids, can lead to the development of new detection methods for analytical purposes, medical diagnostics and biosensors and to potential controlled drug delivery applications. The use of cysteine both in the functionalization of gold nanoparticles and in the cross-linking of amino acid capped gold nanoparticles leading to stable self-assemblies are promising ways to the synthesis of nanostructured biomaterials. Certainly, the functionalization of gold nanoparticles through amino acids is also important for the understanding of complex phenomena involved in the formation of new biomaterials by binding of proteins with gold nanoparticles with important implications in nanoscience and nanotechnology.

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