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# Synthesis of Fluorescent Ag Nanoclusters for Sensing and Imaging Applications

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**Abstract.** Metal nanoparticles have attracted more and more attention in the last years due to their unique chemical and physical properties which are very different from the metal bulk material. In particular, when the size of nanoparticles decreases below two nm, nanoparticles can be described as nanoclusters (NCs), and they present peculiar optical properties. The excited electrons in addition to specific absorption bands show also a bright luminescence related to the quantum size effect which produce discrete energy levels. Optical properties (absorption and fluorescence) of these NCs are widely used in many different applications in science and engineering, such as chemical sensors, fluorescent probes for bio imaging or in environmental issues. In the present study, we report on the synthesis of silver nanoclusters (AgNCs) in aqueous phase using silver nitrate as precursor salt and L-Glutathione (GSH) as stabilizer. AgNCs were characterized using absorption and fluorescence spectroscopy, and transmission electron microscopy (TEM). The strong absorption and luminescence shown by these NCs are very promising for a possible exploitation both as label for bioimaging and for optical sensors for heavy metal ions.

## Introduction

The study and development of nanomaterials for specific applications in different fields such as sensors, imaging, energy, medicine, photonics and biology have received an incredible boost in the last years [1,2,3,4,5,6,7,8,9,10,11,12].

The driving force of the research on these materials is related mainly to the possibility to change and tailor specific properties that are not owned by the material itself but that appear for their reduced size. One example is metal nanoclusters (MNCs) consisting of tens or few hundreds of atoms with diameters less than 2 nm, which show molecules-like properties including discrete electronic transitions and strong fluorescence [13,14] instead of the metal like behaviour. Thus, MNCs can be exploited not only for scientific purposes, but also for practical technological applications in many fields of science. Optical properties of metals depend of sizes; for bulk, electrons can move inside it freely (conduction band is half-empty), then the metals are good reflectors. When the mean diameter approaches to tens of nanometers (nanoparticles – NPs), electrons can move mostly on the particles surface. When an electromagnetic wave (visible light) interacts with NPs the physical phenomena of Surface Plasmon Resonance (SPR) occurs, i.e. a collective oscillation of conduction electrons upon interaction of the light. Colloidal suspensions of metal NPs display intense colours, thus in the absorption spectrum appears a characteristic band that

depends on the type of metal (silver, gold, copper, titanium) and on the dimension and shape. There are many interesting uses of NPs in different fields of science for their large surface-to-volume ratio that allows surprising interaction with the external environmental [15,16], moreover they are optimal candidates for catalysis [17,18], energy [19,20] and sensors [21,22] applications. In the case of NPs, for optical sensing, the phenomena monitored are the modifications of the SPR in presence of contaminants (change of SPR wavelength maximum, change of the intensity and the Full Width Half Maximum (FWHM) of the absorption band) [22].

If the size of metal NPs decreases below two nanometers, the structures are called nanoclusters (NCs), and they show a molecules-like electronic levels behaviour following the quantum mechanics theory [23]. Colloidal suspensions of metal NCs are characterized by a strong absorption and luminescence and these properties can be both exploited as optical sensor for environmental monitoring [24,25,26,27] or for bioimaging [28,29]. As reported recently for quantum dots of graphene oxide [30,31] the presence of contaminants can alter at the same time both the optical absorption and luminescence spectra giving a higher sensitivity and selectivity to the system.

In our research we synthetized silver nanoclusters (Ag-NCs) stabilized by L-glutathione (GSH), following a previous work [32]. We studied optical properties of the Ag-NCs system as optical absorption and luminescence. Ag-NCs have a very sharp absorption centred at 350 nm (full width half maximum is about 17 nm) and a fluorescent emission peak at around 450 nm and a shoulder at about 480 nm when excited with a $\lambda = 364$ nm. Through Transmission Electron Microscopy (TEM) we have determined the mean diameter, which is $1.6 \pm 0.4$ nm.

## Experimental

**Materials.** Reduced glutathione (GSH), silver nitrate ($AgNO_3$) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich and were used as received without further purification. All reagents were solubilized in deionized water, electrical conductivity less than 1 $\mu\Omega$/cm at room temperature, obtained from a Millipore Milli-Q water purification system.

**Ag nanoclusters synthesis.** In a typical synthesis, we used 1,5 ml of $AgNO_3$ water solution with a concentration of 20 mM (Solution 1), freshly prepared; 6 ml of GSH water solution with a concentration equal to 50 mM (Solution 2). All steps of the synthesis are carried out in the dark; the only light source is a yellow lamp, because $AgNO_3$ is very sensible to UV light. Solution 2 is placed into a beaker under vigorous mechanical stirring, after that we trickled the Solution 1, suddenly the mixture turns to opaque from a clear and transparent solution. This change is referred to the formation of Ag-GSH complexes that are insoluble in water (initial pH= 2), to increase the solubilization, we added some drops of NaOH (1M) up to a pH = 5, at this stage the solution changes its aspect to transparent and clear indicating that Ag-GSH complexes are solubilized [33] and very small particles are formed. The solution is stored in the dark at T = 4°C.

**Apparatus.** We have characterized AgNCs solutions by optical absorption and fluorescence spectroscopy; in the first case, we recorded spectra with *Perkin-Elmer Lambda 19* spectrometer in the range of 300-700 nm; in the second case, we have excited the solutions at 364 nm with an argon Laser (*Coherent, Innova 90C*) and we have recorded the photoluminescence with an *OceanOptics Flame* spectrometer. The laser beam strikes with grazing angle (< 30°) the sample inside the quartz cuvette, the emitted light is collected by an optical fibre (*OceanOptics, QP600-2UV-BX*). The fibre is equipped with a lens, which enhances the collection of emitted light from the sample. The lens and the fibre are arranged at 90° respect to the surface of the cuvette. Photoluminescence spectra are recorded in the range 350-1100 nm. The morphological characterization of the AgNCs has been accomplished with a Transmission Electron Microscope (TEM). The experimental apparatus is a FEI TECNAI 12 G2 (120 KeV) equipped with an energy filter (GATAN GIF model) and a Peltier cooled SSC (slow scan charged coupled device) multiscan camera (794 IF model).

## Results and Discussions

**Optical and structural characterization.** After the synthesis, we have recorded the optical absorption of the AgNCs as a function of time. Figure 1(a) shows the optical absorption of the solution at different times after the synthesis. In 90 minutes after the synthesis, the absorption band presents a blushift from 354 nm to 351 nm, an increment of the intensity up to 2 O.D. and a corresponding reduction of the FWHM from 45 nm to 29 nm. The values as a function of time (for the first 90 minutes) of the maximum of the absorption band and the corresponding change of the FWHM are reported in figure 1(b). These changes can be ascribed to the formation of additional AgNCs as a function of time. It has to be underlined that the nucleation reaction goes on for some days and it stops after 6 days. In these conditions the maximum wavelength of the band was 350 nm, the intensity 3.5 O.D. and the FWHM 17 nm. After this time the system is completely stable for over a month if kept at t = 4°c and in the dark. Once stability of AgNCs was achieved, we performed luminescence measurements exciting the solution at 364 nm. Figure 2 shows emission spectra of AgNCs (black curve) and of only the capping agent (red curve). Ag nanoclusters present a peak at about 450 nm and a shoulder at approximately 480 nm.
The aqueous solution of the bare GSH does not present any emission peaks as shown in the figure. To confirm the existence of AgNCs, we have performed a morphological characterization byTEM. The figure 3 shows the presence of NCs. The images were analysed by the *ImageJ* program and we have estimated the mean diameter of the nanocolusters, obtaining an average size of 1.6 ± 0.4 nm. Usually a reducing method is important in order to synthesize the nanoclusters, the most common are the exposition to UV light or the use of ultrasounds. However, in our case we found that even without the application of a specific reduction procedure the AgNCs are effectively formed and their quality regarding the morphology and the optical properties are excellent. We tried to produce AgNCs also with a photoreduction process exposing the solution after the synthesis to a UV light at 254 nm for different times or using a sonochemical process exposing the solution to ultrasounds for several minutes after the synthesis. In both case, we obtained AgNCs that are comparable regarding the shape and the optical properties with those synthesized with the procedure exposed in the present paper.

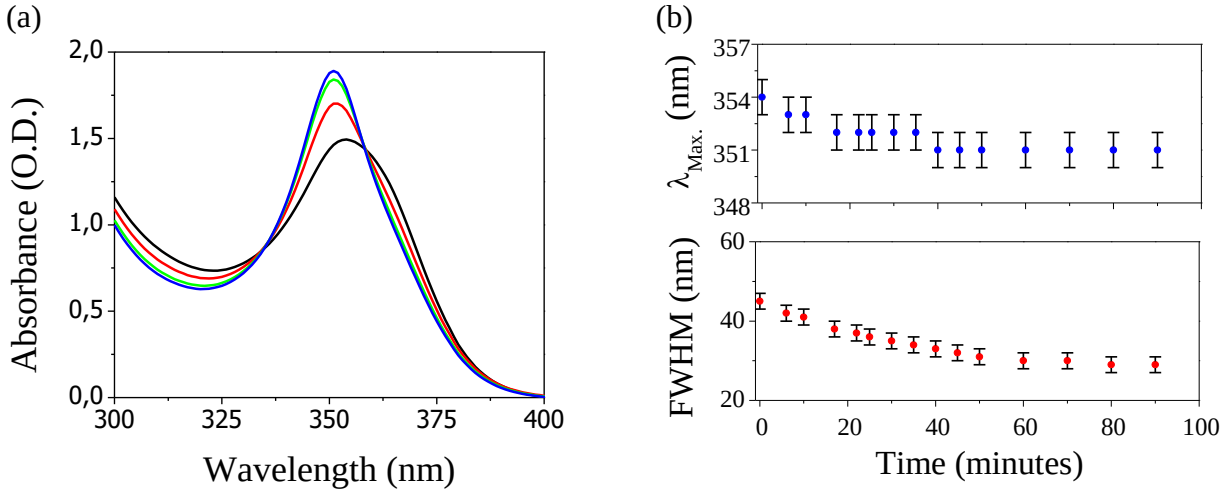


**Fig. 1** (a) UV-Vis spectroscopy of the solution of AgNCs at different times after synthesis, 0, 30, 60 and 90 minutes, black, red, green and blue curve, respectively; (b) behaviour of $\lambda_{max}$ and FWHM as function of time, for the first 90 minutes after the synthesis.

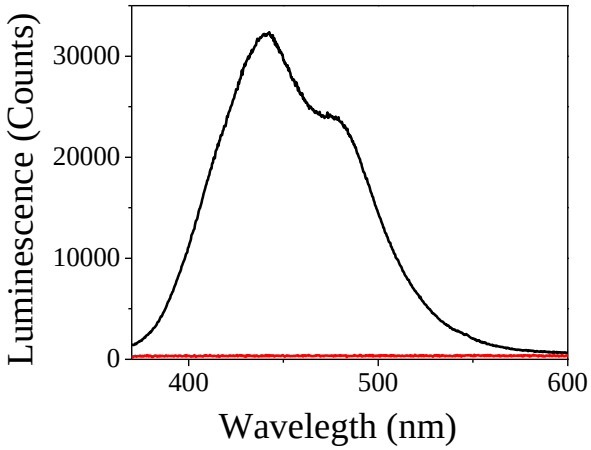


**Fig. 2** Luminescence emission spectra of AgNCs aqueous solution with GSH as capping agent (black line) and of aqueous solution of GSH alone (red line), the excitation wavelength was 364 nm for both systems.

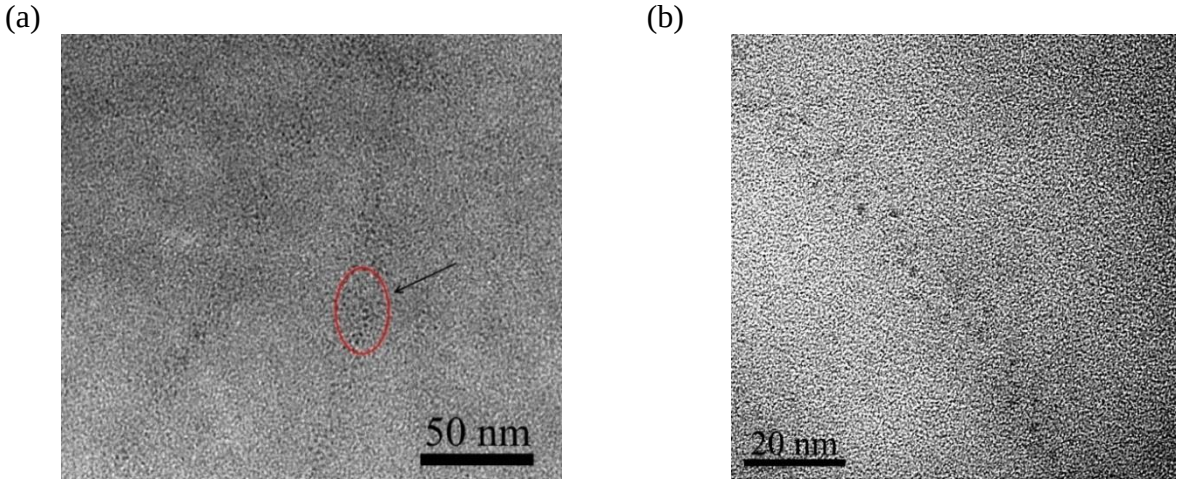


**Fig.3** Transmission Electron Microscopy images of AgNCs with GSH: (a) the arrow and the circle underline the presence of nanoclusters; (b) a magnification of previous image. The mean diameter of nanoclusters is about 1.6 nm.

## Conclusions

In conclusion, we have synthesized very stable L-Glutathione capped silver nanoclusters in aqueous solution, which are characterized by a small size (1.6 ± 0.4 nm). AgNCs in addition to optical absorption peak centred in $\lambda$ = 350 nm, present also an intense fluorescence emission related to their small dimensions, which have a maximum at $\lambda$ = 450 nm when excited at 364 nm. The absorption and emission properties of such nanoclusters are very promising to be exploited as biolabel for imaging and for optical sensing of heavy metal ions in water.

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