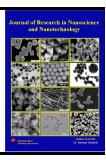


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## **Optically Active Gold Nanoparticles**

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#### **ABSTRACT**

Nanoparticles play a key role in our daily life from high-end quantum technologies to medical diagnostics and therapies. Chiral nanoparticles have emerged as a promising class of nanomaterials for their application in asymmetric catalysis, biosensing, spintronics and chiroptical devices. We have previously shown that cysteine-capped silver nanoparticles achieve strong optical activity. Herein we apply the same synthetic approach with the goal to form chiral gold nanoparticles. The chiroptical properties of the obtained nanostructures were investigated by circular dichroism spectroscopy in the ultraviolet and visible wavelength range. We show that using a ligand exchange procedure, exchanging the citrate ligands with amino acids (L-glutathione) keeps the structure of the spherical nanoparticles mostly unchanged failing to generate optical activity in the optical transitions of the metal. While direct reduction of gold salt to from nanoparticles does not lead to transfer of chirality in gold NPs, supramolecular polymeric chiral nanoparticles are formed at higher relative concentrations of cysteine that can show relatively high optical activities. Promoting the reduction of gold with a more potent reducing agent in presence of cysteine seems to be necessary to form chiral plasmonic gold nanoparticles opening new possibilities for the design of biocompatible chiral plasmonic materials.

*Keywords:*Chirality; optical activity; chiral nanoparticles; chirality transfer; induced chirality

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#### 1. Introduction

Nanomaterials have become an essential part of life. An important class of nanomaterials are chiral nanoparticles (CNPs) that have gained much attention recently due to their current and potential applications in asymmetric catalysis [1-3], spintronic devices [4-5] and bio-sensing [6-7] to name a few. Although there are various methods to form chiral nanoparticles [8-14], wet-chemical synthesis still is the mostly used approach [15-16]. Recently we have reported on synthesis of chiral silver nanoparticles through reduction of silver nitrate by chiral D/L Cysteine [17]. One important



question while considering the transfer of chirality from chiral reductants to the fabricated nanoparticles is the mechanism by which this takes place. There are commonly three possible mechanisms for chirality transfer proposed: (i) a chiral arrangement of the ligands on an achiral metal core (chiral footprint) (ii) formation of a chiral metal core due to the influence of chiral ligand molecules on the formation of the cluster (chiral growth), and (iii) electronic interaction between the chiral ligands and achiral metal core electrons (chiral polarisation) [18-22]: While almost always a combination of these mechanisms is responsible for the observed chirality transfer, one of them is usually the dominant one. In the case of the reported Ag CNPs [17], the dominant mechanism was found to be a mixture of a chiral adsorption pattern and chiral polarisation of the electric field, caused by the attached ligands. While plasmonic Ag CNPs are of high interest for their use in pharmaceutical and medical applications such as antibacterial or tracing agents [23-27], the demand for CNPs of gold is arguably larger, mainly due to the expected inertness of gold NPs and their widespread role in multiple applications [28]. For instance, it was recently shown that gold NPs can act as efficient vehicles for DNA vaccine delivery [29].

Motivated by the simplicity and efficiency of the approach we attempt to use the same approach to synthesize Au CNPs. We have used two routes which were successful in case of silver CNPs. First, we performed a simple ligand exchange reaction by adding L-glutathione to spherical gold NPs. Second, we attempted the direct reduction of auric acid using L/D cysteine.

#### 2. Materials and Methods

Gold(III) chloride trihydrate (≥99.9%), sodium citrate tribasic dehydrate (>99.5%), tannic acid (≥97%), L-cysteine (≥97%), D-cysteine (≥99%) and L-glutathione (≥98%) were obtained from Sigma-Aldrich and used without further purification. Ultrapure water (UPW) was obtained from a Direct-Qs 5 UV purification system from Merck Millipore.

CD and UV/VIS spectra were recorded on a J-815 spectrophotometer from Jasco. Brightfield TEM images were recorded using a JEM-1400 microscope from JEOL and processed with ImageJ.

We have performed two different wet chemical synthetic routes to generate Au NPs in search for of an easy method to form CNPs with high optical activity. These include (i) a single ligand exchange after formation of metallic NPs, (ii) direct reduction of the salt by cysteine as a chiral reducing agent, In the first case, a single ligand exchange reaction was performed by adding L-glutathione to already prepared Au NPs. Briefly, 1.99 mg of gold(III) chloride trihydrate is dissolved in 48 mL of UPW. The solution is stirred at 500 rpm and heated until it starts to reflux. Then 10 mg trisodium citrate and 2.5 mg tannic acid are dissolved in 1 mL UPW each, mixed and added to the boiling gold solution. The mixture is heated for further 10 min and rapidly cooled to 0 °C afterwards. The resulting red solution is stored at 8 °C. Subsequently, a ligand exchange reaction is performed with L-glutathione. Therefore, 5 mL of the above prepared nanoparticle solution is put into a round bottom flask and 5 mg of L-glutathione is added. The mixture is stirred at 500 rpm, whereas a color change to violet can be observed after 2 h reaction time.

In the second case, samples were prepared by mixing aqueous solutions of gold(III) precursor salt and L/Dcysteine with varying reactant ratio. Here 4.76 mg cysteine (40  $\mu$ mol) dissolved in 2 mL UPW are added to a solution of 3.98 mg gold(III) chloride trihydrate (10  $\mu$ mol) in 3 mL UPW and the mixture is stirred for 24 h at 500 rpm. The resulting cloudy solution is stored at 8 °C until further use.



#### 3. Results and Discussion

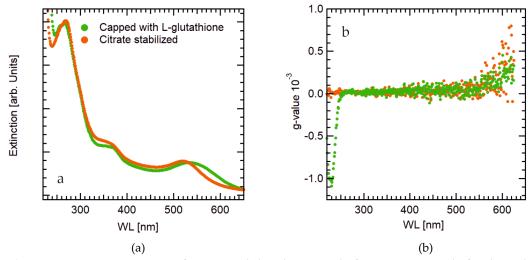
This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

## 3.1. Ligand Exchange with L-glutathione

Unlike silver NPs, changing the ligands of citrate stabilized plasmonic gold NPs does not lead to chirality transfer as shown in Figure 1. In Figure 1(a) the extinction spectra of citrate stabilized plasmonic Au NPs before and after ligand exchange with L-glutathione are shown. Citrate stabilized gold NPs show multiple bands. The peaks at ~270 and ~380 nm belong to the interband transitions of gold NPs and thus, are not expected to be much affected by the ligand exchange. The peak at ~380 nm can be attributed to the transition from the uppermost occupied d state to states just above the Fermi level and the peak at ~270 nm originates from transitions from lower d states into the conduction band [30]. The peaks above 500 nm are due to the surface plasmon resonances (SPR) in gold NPs. Figure 1(b) depicts the g-value spectra of the samples as defined in the following equation (1):

$$g = \frac{A_L - A_R}{\frac{1}{2}(A_L + A_R)} = 2\frac{A_L - A_R}{(A_L + A_R)} \tag{1}$$

where  $A_{L/R}$  denotes the absorbance (more precisely, extinction) recorded for *Left/Right* circularly polarized light and g-value is the unitless quantity given by the ratio of the difference between the two to their average [31-33]. It should be emphasized that with this definition, the values for the g-value are bound between -2 and +2 or -200% and 200%.

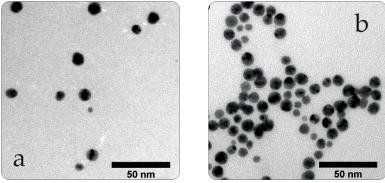


**Fig. 1.** (a) Extinction spectra of citrate stabilized Au NPs before (orange) and after ligand exchange with L-glutathione (green) are shown. (b) g-values of the samples (same color code) are shown.

As expected, spherical citrate stabilized Au NPs do not possess any optical activity. L-glutathione capped Au NPs, however, show optical activity at wavelengths below 250 nm, while no optical activity is observed for the plasmonic transitions above 500 nm. The optical activity in the UV end of the spectrum can be confidently assigned to the chiral L-glutathione ligands. Looking at the extinction



spectra and comparing the SPR we notice a slight red shift from ~520 nm in the case of citrate stabilized Au NPs to ~540 nm for SPR of L-glutathione capped particles. This shift can be explained by the change in the refractive index of the surrounding medium of the particles or by particle growth during the ligand exchange process [34-35]. Consulting the TEM images of the samples presented in Figure 2, it appears that the particles' size and shape have not been affected much in the process. Due to the absence of optical activity in Au NPs capped with L-glutathione, it can be concluded that despite the strong interaction between the ligands and gold atoms, these seem to be lacking any asymmetrical pattern in their adsorption, fail to influence the collective plasmon oscillations asymmetrically or cause any chiral foot print on the particles. Considering the fact that Au-S bond is stronger than the Ag-S bond, it is surprising that in contrast to Ag NPs the SPR of Au NPs is neither quenched nor chiralized by addition of L-glutathione [17]. This can be understood through the much lower polarizability of the SPR in gold compared to silver [36].



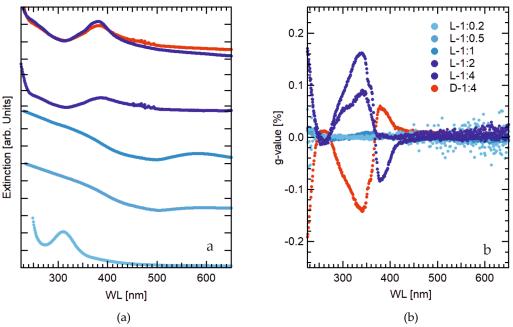
**Fig. 2.** TEM images for citrate stabilized Au NPs (a) and for Au NPs capped with L-glutathione after ligand exchange. The particles' shape and size appear to be very similar in both cases.

## 3.2. Direct Reduction of Auric Acid with D/L Cysteine

In the second approach we performed direct reduction of auric acid using L/D cysteine. The extinction and g-value spectra for various concentration ratios between auric acid and L/D cysteine are presented in Figure 3. Changing the gold to cysteine ratios leads to qualitatively different behaviours. At low relative concentrations of cysteine (Au:Cys = 1:0.2) spectroscopically no interaction between the educts is observed, as the extinction spectra of aqueous solution of auric acid remains unaltered as shown in Figure 3a. When the relative concentration of cysteine is increased to (Au:Cys = 1:0.5), a very broad absorbance band from 225 nm (the UV limit of our measurements) to 500 nm and another broad band centred at ~580 nm which can be interpreted as SPR indicating the reduction of gold salt to plasmonic gold NPs. At a ratio of Au:Cys = 1:1 the assigned SPR peak gains intensity while the broad band between 225 nm and 500 nm loses on oscillator strength. The optical response of the synthesized material qualitatively changes again when the concentration ratio is changed in favour of cysteine. At a gold to cysteine ratio of Au:Cys = 1:2 a new peak at ~390 nm appears, while the broad SPR band and the band between 225 nm to 500 nm are diminished. It seems that when cysteine becomes abundant in the mixture the formation of plasmonic particles is not promoted. Further increasing the share of cysteine to Au:Cys = 1:4 leads to the pronounced enhancement of the optical transition at ~ 390 nm. This band can be attributed to ligand-to-metal charge transfer (LMCT; S→Au) coupled with a ligand-to-metal-metal charge transfer (LMMCT; S→Au...Au) in densely stacked polynuclear Au(I) chains. [37-39] The appearance of this absorption



band is accompanied by the emergence of optical activity in the samples, which is known to be due to the helical arrangement of Au(I) in polymeric supramolecular structures [38].



**Fig. 3.** (a) Extinction spectra of gold nanoparticles obtained by direct reduction by L/D cysteine with different concentration ratios (shades of blue for L-cysteine and red for D-cysteine) are shown. (b) g-values of the same samples are shown. The color code is identical for the figures. The darker the shade of blue, the higher the concentration of L-cysteine relative to Au salt in the solution.

Turning to g-value spectra of these samples, it is readily evident that as long as SPR peaks are observable with any intensity i.e. in the case of samples with 1:05 and 1:1 ratios, no optical activity is observed. For samples in which cysteine dominates (Au:Cys = 1:2 and 1:4), optical activity is observed at wavelengths below 410 nm, which is enhanced with increasing cysteine concentration. The disappearance of the weak SPR peaks that is observed for samples with 1:05, and 1:1 concentration ratio, coincides with the appearance of optical activity, indicating that the formed CNPs are not plasmonic. Previous reports on Au-cysteine NPs suggest the formation of polymeric structures, held together by bridging thiol groups [38, 40-41]. The data shown here are in partial agreement with the available literature. While helical gold-sulphur motifs complexed by cysteine also show optical activity in the range of 225 nm and 410 nm [38], there are also discrepancies between the data. A minor difference is in the shape of the bands between the g-value spectra reported in this manuscript and the circular dichroism (CD) spectra reported in the literature. This is a general problem when comparing optical activity data in the literature and can be mostly circumvented by reporting gvalues. The major difference is, however, in the sign of the observed optical activity for NPs formed by L/D cysteine. Moving towards lower wavelengths from 410 nm, our data show negative values for optical activity changing sign at ~365 nm. This is opposite in sign compared to the data reported in some of the reported data. Based on these observations we cannot be fully confident with regards to the real nature of the formed Au CNPs.



#### 4. Conclusions

In summary, we have presented a simple green and bio-compatible approach to fabricate Au CNPs formed in aqueous solution, which show a great potential for improvement by further tuning the properties of the products by simply adjusting the concentrations of the components. Further improvement can also be achieved by including additional reducing agents such as citrate or borohydrides to promote reduction to metallic gold NPs and forming plasmonic Au CNPs. Our findings further demonstrate that fabricating gold CNPs cannot be achieved with the same simplicity of Ag CNPs. This highlights the lack of universality in wet chemical synthesis and indeed provides one of the major driving forces for the continued search for alternative methods for NP formation. Given the polymeric nature of the Au CNPs formed through direct reduction by L/D cysteine, it would be interesting to study the relation between the enantiomeric excess of the chiral agents and the obtained optical activity in the samples.

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