Facile synthesis of gold nanoparticles with irregular shapes in polyol and aquatic ecotoxicity effects

Anis Fkiri [a,*], Amine Mezni [a,b], Nesrine Ben Saber [b], Badreddine Sellami [c] and Leila Samia Smiri [a]

[a] *Unite de recherche "Synthese et Structure de Nanomateriaux" UR11ES30, Faculte des Sciences de Bizerte, Universite de Carthage, 7021 Jarzouna, Tunisie*
[b] *Department of Chemistry – Faculty of Science, Taif University, Taif, Saudi Arabia*
[c] *Laboratory of Environment Biomonitoring, Faculty of Sciences of Bizerta, University of Carthage, 7021, Jarzouna,Tunisia*

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Abstract: A simple chemical route, based on a modified polyol process, is used to synthesize mono-disperse triangular gold nanoprisms (Tr-AuNPs) in triethylene glycol (TREG) with polyvinyl-pyrrolidone (PVP) as capping agent. Both the solvent and the surfactant in the solution play important roles in the formation of the Tr-Au NPs. Shape, size and optical properties of the particles were tuned by changing the molar ratio of PVP to metal salts. The formation of such large, single-crystal Tr-Au NPs is explained by the preferential adsorption of some species of molecules from the solution onto the {111} planes of Au nuclei. The anisotropy in the nanoparticles' shape shows strong localized surface plasmon resonance (SPR) in the near infrared region of the electromagnetic spectrum. These nanostructures may be used in areas that include photonics, optoelectronics, optical sensing and also for inducing hyperthermia in tumors. For this reason, the ecotoxicological effects of the Tr-Au NPs and their interactions with marine organisms were also studied in this work. It was found that Tr-Au NPs don't seem to have a large effect on the non-target marine organism R. decussatus. Indeed, no significant modification ($p > 0.05$) was observed after 2 days on the glutathione-S-transferase activity of the clams exposed to Tr-Au NPs when compared to the control, both in the gill and the digestive glands.

Keywords: Gold nanoparticles; Polyol; Triethylene glycol; Surface Plasmon resonance; Aquatic ecotoxicity

INTRODUCTION

Noble metal nanoparticles (NPs) have attracted increasing research attention during the past decades due to their interesting size-dependent optical, magnetic, electronic, and catalytic properties [1-8]. The intrinsic properties of a metal nanostructure can be tailored by controlling its size, shape, composition and crystallinity. Shape-control has been proved to be as effective as size-control in fine-tuning the properties and functions of metal nanostructures. Gold nanoparticles with their tunable Surface Plasmon Resonance (SPR) are a particular class of nanomaterials for their wide practical applications such as catalysis, optics, biomedicine and chemical sensing [9].

The development of simple and versatile synthesis methods of Au NPs in a size- or shape-selected and controlled manner has been a challenging but intellectually satisfying task [10]. Several published works have reported the synthesis of gold nanoparticles with interesting shapes using chemical, biological or physical methods [11-14]. According to the literature, a number of anisotropic gold nanostructures have been successfully synthesized on the basis of a polyol process in various polyol media [15-20]. Cuncheng Li et al. [21] developed a low cost and straightforward PDDA (poly(diallyldimethylammonium) chloride) -mediated polyol route for the controllable synthesis of single-crystalline Au octahedra in

* Corresponding author, e-mail address : fkiri11288@gmail.com

ethylene glycol solution. The synthesis was conducted with a molar ratio of PDDA to AuCl4- ions of 50 with addition of HCl. The same group [17] easily synthesized octahedral Au particles of hundreds of nanometers in size with the reaction conducted in polyethylene glycol 600 (PEG 600) in the presence of PVP as surfactant and NaBH4 as reducing agent. The molar ratio of PVP to Au was found to be 1.73 with no additional shape change of the Au nano-crystals during the growing process. Song et al. [18] prepared gold nanocrystals, with a shape evolution from octahedra to cubes, in 1,5-pentanediol in the presence of PVP as surfactant (R(PVP/Au) =3) and silver species generated from AgNO3 which seemed to determine the final nanocrystal morphology by the selective growth of {111} and/or the restriction of {100}. Triangular and polygonal gold micro-/nano-plates were synthesized by Hamely et al. in 1,2-propanediol as both medium and reducing agent and PVP as a stabilizer (R(PVP/Au) =8) [19]. Despite the high molar ratio of PVP/Au used, the final product consists of particles with micrometer size and polydisperse shape.

Up to now, there have been few reports of a simple method to obtain large triangular gold nanoprisms of low dispersity and high crystallinity through a polyol process under conventional heating conditions and with the minimum amount of surfactant and without addition of any other reagent. In this work, on the basis of the selective adsorption of PVP as a stabilizing layer, a simple polyol assisted route is reported for monodisperse size-tailored triangular gold nanoprisms (Tr-AuNPs) in triethylene glycol as both solvent and reducing agent. The interest of our protocol lies in the use of a small quantity of surfactant in a triethylene glycol medium (R(PVP/Au) << 1).

Different findings regarding gold nanoparticle ecotoxicity have been published in several works; regarding marine organisms and toxic effects, these were assessed in Mytilus edulis, Ruditapes philippinarum and Daphnia magna [28-30]. In addition, stress oxidative enzymes form an important component of the antioxidant response and can provide valuable information regarding the effects of NPs in an organism's biology [31]. Glutathione-S-transferase (GST) has already been associated with the metabolism of NPs in bivalves [32] and identified as a metabolic pathway for NP metabolism [33]. For all these reasons the present study aims to evaluate the effect of Tr-Au NPs on the GST activity of R. decussatus. These can be applied as measures to improve their biomedical applications and risk assessment. However, assessing the safety issues of gold nanoparticles is quite challenging because of the vast physiochemical properties that confound their biomedical and toxicological profiles.

EXPERIMENTAL SECTION

1. Chemicals

All of the chemical reagents were of analytical grade and used without further purification. Triethylene glycol (ACROS Organics, 98%) was used as a solvent, Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) (from Sigma-Aldrich) was used as a precursor of gold and polyvinyl-pyrrolidone (PVP) (K30, Sigma–Aldrich) was used as a surfactant.

2. Synthesis of Au-NPs

The Tr-Au NPs were synthesized by a modified polyol process involving a surface-regulating polymer, the PVP. Briefly, 25 ml of triethylene glycol solution, containing 0.038 mmol of HAuCl4·3H2O and a given amount of PVP were mixed and heated to 150°C. The mixture was kept at this temperature for 30 min under continuous mechanical agitation. The molar ratio of PVP to HAuCl4 (R(PVP/Au)) was kept between 0.045 and 0.3. Gold particles formed within minutes and the final colloidal solution had a blue color. The product was separated by centrifugation, washed several times with ethanol/acetone (2:1) solution and dispersed in ethanol.

3. Characterization techniques

Morphological details of the synthesized gold particles were characterized by transmission electron microscopy (TEM) (JEOL-JFC 1600). An energy-dispersive X-ray spectrograph (EDX) attached to the TEM was used for an elemental analysis. The selected area electron diffraction (SAED) was also conducted on the microscope. The optical absorption spectra of diluted Au NP solution were performed on a Perkin-Elmer Lambda 11 UV/VIS spectrophotometer.

4. Ecotoxicological risk

In order to investigate the effects of the triangular gold nanoprisms in the Mediterranean Clams Ruditapes decussatus, many Tr-Au NP

suspension solutions with different concentrations were prepared. For the preparation of a low-level concentration, the 280 mg L^{-1} triangular gold nanoprism suspension was diluted to a 0.5 mg L^{-1} in standard SW, and in MilliQ-water for comparison purposes. For the preparation of high-level concentrations, the 280 mg L^{-1} Tr-Au NP suspension was diluted to 1 mg L^{-1} according to the required concentration in each particular case. Adult clams (Ruditapes decussatus) of between 2.5 and 3 cm shell length (maximum axis) were purchased from a site in the Bizerte lagoon (37° 13'18.54''N, 9°55'59.61''E). Acclimation took place in free-flow tanks for one week before starting the exposure. For the course of the experiment, 5 individuals were placed in each tank with 3 L of sea water obtained from the sampling site (salinity 37, temperature 18°C, oxygen at 6.5 mg L^{-1}), containing 0.5 and 1 mg L^{-1} of Tr-Au NPs respectively.

A control series without Tr-Au NPs was run in parallel. Each experimental setting was conducted in triplicate. The exposure treatments were labelled as follows: Tr-Au1 for the 0.5 mg $\mathbf{L^{-1}}$ gold concentration and Tr-Au2 for the 1mg $\mathbf{L^{-1}}$ gold concentration.

Unexposed and Tr-Au NP exposed clams were collected after 2 days. Collected clams were dissected and gills and digestive gland separated and immediately frozen in liquid nitrogen and stored at -80 °C until their biochemical analysis.

For the biochemical analysis, glutathione-S-transferase activity was measured according to the method of [34] using 1-chloro-2, 4-dinitrobenzene (CDNB) and GSH as substrates. Absorbance was measured at 340 nm and activity was expressed as nmoles of conjugated product formed/min/mg protein. Protein content was estimated by the method of [35] using bovine serum albumin (BSA) as a standard.

RESULTS AND DISCUSSION

1. Tr-Au NPs characterization

Figure 1 shows typical TEM images of the resulting products at different molar ratios of PVP/Au. In this case, Au particles with different morphologies were obtained (rod-like triangular nanoplates, polygonal, hexagonal, pentagonal plates and others). The size of the 2D gold objects was about 50 to 750 nm. When the molar ratio was decreased to 0.05, equilateral triangular prisms with an average edge length of 150 nm formed

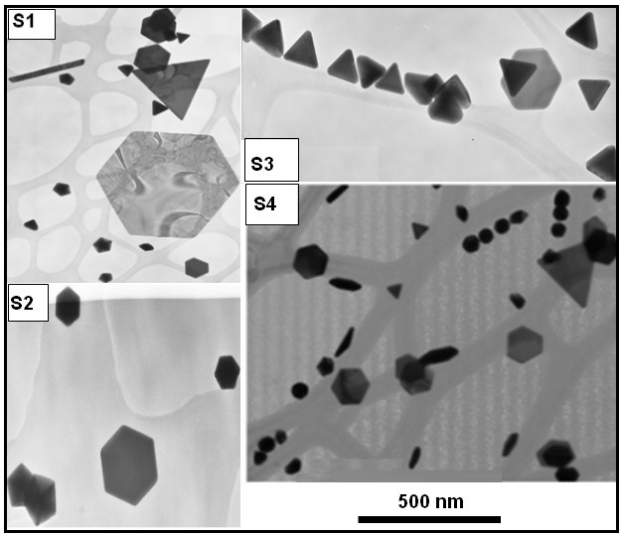

Figure 1: TEM images of the samples synthesized at different molar ratios R(PVP/Au): S1 (R=0.03), S2 (R=0.01), S3 (R=0.05) and S4 (R=0.045).

(Figure 2b). This indicates that the appropriate molar ratio is vital for the formation of Tr-Au NPs. When the molar ratio was lower than 0.05 (R=0.045) the as-prepared Au NPs became much thicker, and a large number of sub-micrometer-sized Au particles (50 to 400 nm) were produced, owing to a lower, or a lack of, adsorption of PVP (Figure S4).

As shown in Figure 2, when the molar ratio R (PVP/Au) was increased to 0.05, rod-shaped micro-particles (~2µm) and spherical-shaped nanoparticles (~100nm) appeared, owing to the excess of PVP adsorbed at the gold surface.

On the basis of previous studies, PVP could act not only as a stabilizer layer to prevent the aggregation of the particles but also as a shape-controller to assist the formation of anisotropic metal nano-structures [17, 18, 22]. At a lower PVP to gold molar ratio, the nucleation and growth of gold nanoparticles were subjected to kinetic control. In

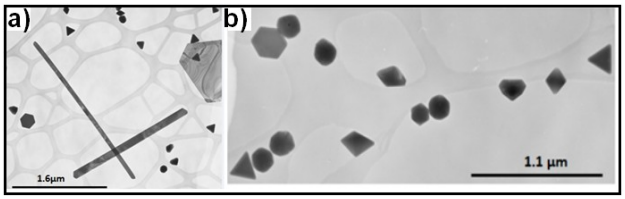

Figure 2 : TEM images of gold nanoparticles prepared at different molar ratios: a (R=0.3) and b (R=0.1).

this case, gold atoms would preferentially add to facets of the seeds with higher surface energy. The PVP/HAuCl4 molar ratio plays a critical role in the formation of gold nanoprisms. Indeed, because of the selective adsorption of PVP molecules on {111} oriented planes, the latter have a minimal growth rate compared to {110} and {100} planes. We believe that PVP preferentially adsorbs on the {111} planes of Au nuclei and consequently the growth rate along the <111> direction is reduced while the growth rate along the <110> and <100> direction is enhanced, leading to the highly anisotropic growth of nuclei into a nanostructure [22, 23]. Note that PVP can play an important role in controlling the shape and monodispersity of gold nanoparticles but it cannot produce such uniform controlled shape gold nanoparticles by itself without the cooperation of a polyol solvent. Indeed, the TREG can act not only as a solvent but also as a capping/stabilizing agent [24]. The TREG molecules are also adsorbed on the {111} oriented planes and thus contribute to slow down their growth, which explains the formation of gold nanoprisms with a small PVP/Au molar ratio, R=0.05. Indeed, the TREG is acting not only as a solvent but also as a capping/stabilizing agent which allows triangular nanoprisms to grow with a minimum amount of surfactant.

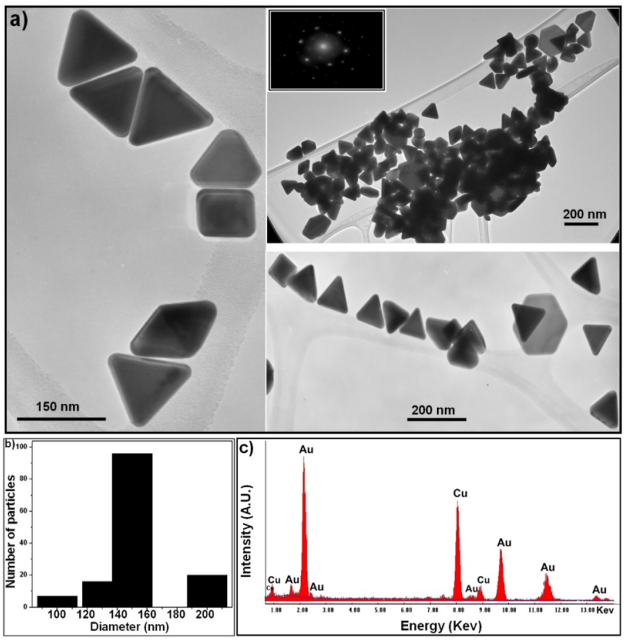

Figure 3 : (a) TEM images, (b) particle size distribution, (c) EDX spectrum of triangular gold nanoprisms. The inset in (a) shows typical selected area electron diffraction (SAED) pattern from a single nanoprism.

In our study, at a lower molar ratio (PVP/ Au=0.05), the concentration of PVP is sufficient to stabilize the gold nanoparticles. Consequently, the gold nanoparticles nucleate more quickly and form monodisperse triangular gold nanoprisms [22, 25]. But at a higher molar ratio (PVP/Au up to 0.05) with increasing PVP concentration, meaning a slowdown of the nucleation and growth of the plates resulting of a mixture of irregular large particles, the fewer the initial nuclei, the larger the resulting nanoplates [26].

The energy dispersive spectrum (EDX) analysis for such as-prepared samples confirms that the Tr-Au NPs consist of only gold (see Fig. 3c, the copper element came from copper grid). The inset of Fig. 1a gives the typical selected area electron diffraction (SAED) pattern obtained by directing the electron beam perpendicular to a single gold nanoplate deposited flat on the TEM grid. The hexagonal symmetrical spots of the SAED pattern clearly reveal that these gold nanoplates are single crystals and the incident electron beam is perpendicular to the {111} facet of the tested plate.

The optical absorbance spectrum of the as-prepared colloidal solution (Fig. 4) shows an extinction band with a maximum intensity at around 720 nm due to the contribution of in-plane dipole SPR absorption of anisotropic products in which the oscillation of free electrons was strongly restricted in the planar structure [27].

It is well-known that a metal nanoprism supports dipolar and quadrupolar surface plasmon modes with resonance wavelengths that depend on the nanoprism edge length, thickness and snip. The

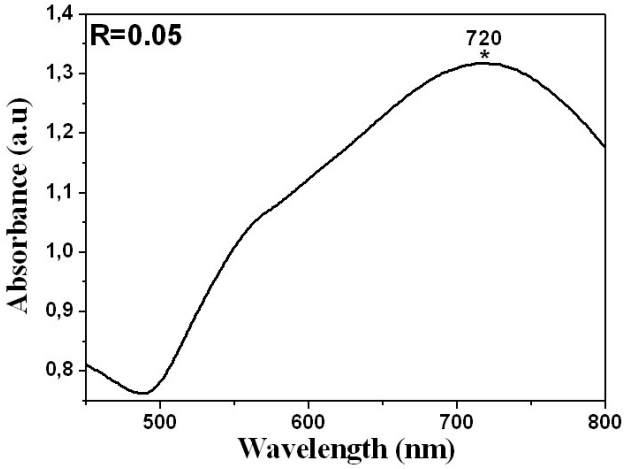

Figure 4 : Plasmonic response of the Tr-Au NPs dispersed in ethanol.

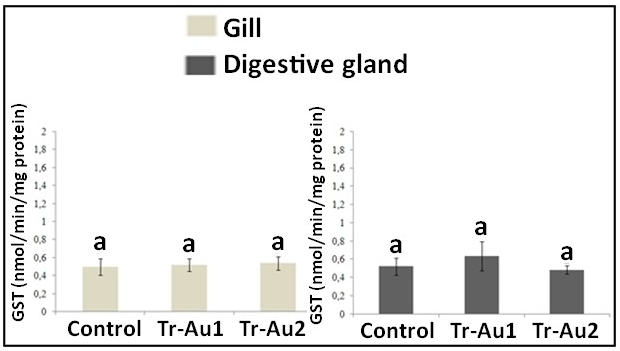

Figure 5: Glutathione-S-transferase (GST) activity in gills and digestive glands of untreated (Control) and treated (Tr-Au1 = 500 ppb and Tr-Au2 = 1000 ppb) clams *Ruditapes decussatus* after 2 days of exposure to Tr-Au nanoparticles. The letters indicate the statistical differences ($p < 0.05$) compared to control. Data are mean ± SD.

TEM images do not give access to the nanoprism thickness. Therefore, in order to fully characterize the nanoprisms and determine their average thickness, the optical response of a single nanoprism was calculated using the discrete dipole approximation (DDA) method and the results were compared to the measured extinction spectrum. Assuming a fixed edge length of 150 nm (from TEM data Figure 3a) the thickness of the nanoprism has been changed and the effect of the nanoprism snip has been considered, as the latter is responsible for a strong blue shift of the surface plasmon resonance. A good agreement between the resonance wavelengths and line-shapes of the calculated and measured optical extinction spectra is obtained for an average nanoprism thickness of 50 nm and with a snip of 27.5 nm (average snip value from TEM data in Figure 3a).

2. Aquatic ecotoxicity of gold nanoparticles

Ecotoxicological effects of gold nanoparticles are still lacking. This review focuses on the impact of Tr-Au NPs on health and particularly on GST activity and addresses potential risks of exposure to this nanoparticle on non-target species *R. decussatus*. GST is known to protect cells against the effects of reactive oxygen species during oxidative processes induced by organic contaminants as part of the phase II biotransformation pathway. The GST has already been associated with the metabolism of NPs in bivalves [32] and identified as a metabolic pathway for NP metabolism [33]. In the present study, GST activity was not affected by Tr-Au NP exposure even at high concentration (1 mg L^{-1}) compared to the control (Fig. 5). This result may explain why Tr-Au NPs have not affected the oxidative status of *R. decussatus*.

CONCLUSION

In summary, gold nanoprisms (Tr-Au NPs) were synthesized using a simple one-pot chemical process on the basis of the selective adsorption of PVP as a stabilizing layer using triethylene glycol (TREG) as solvent. These anisotropic nanoparticles exhibit a strong localized surface plasmon resonance (SPR) in the near infrared region of the electromagnetic spectrum which might be of use in many areas. In the present study, the effect of these Tr-Au NPs on GST activity of *R. decussatus* has also been evaluated. These can be applied as measures to improve their biom dical applications and risk assessment. It has been demonstrated that the exposure to Tr-Au NPs does not have a large effect on the gills and digestive glands of the non-target organism *R. decussatus*. Nevertheless, further studies are required to explore the toxic mechanism and the interactions that occur between these particles and non-target species.

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