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In Vitro biosynthesis of gold nanotriangles for Surface-Enhanced Raman spectroscopy

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Gold nanoparticles of triangular shape were bio-synthesized by reduction of aqueous chloroaurate ions in pelargonium plant extract. Optical properties and morphology of gold nanotriangles were analyzed by UV-VIS absorption spectroscopy, X-ray diffraction, Electron Diffraction and Transmission Electron Microscopy (TEM). The possibility of using as bio-synthesized gold nanotriangles in surface enhanced Raman scattering (SERS) applications was demonstrated with p-aminothiophenol as probe molecule. The biosynthesis method can be considered safe and ecological for nanomaterials fabrication.

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1. Introduction

An important area of research in nanotechnology deals with the synthesis of noble metal nanoparticles of different sizes, shapes and controlled monodispersity as well as multilayers [1] and biomolecule coatings [2] for applications as waveguides for electromagnetic radiation [3], substrates for surface enhanced Raman spectroscopy (SERS) [4], surface-enhanced infrared absorption (SEIRA) [5], surface-enhanced fluorescence [6,7] and surface plasmons (SP) biological sensors [8], as well .

Among different shapes of noble metal nanoparticles, triangular plates exhibit the most interesting optical properties when compared to spherical particles. For example, triangular plates or nanotriangles exhibit two (or more) prominent absorption bands; a low wavelength transverse SP absorption band (out of plane SP vibration) situated in the visible and an often more intense longitudinal absorption band (in-plane SP vibration component) situated in near-infrared (NIR), similar to that observed for nanorods [9,10]. The NIR region is of significant biological importance, as it represents the only spectral window for both therapeutic and imaging applications in tissue because the components of tissue do not have significant absorption in this range of wavelengths. Therefore, gold nanotriangles with strong NIR absorption are suitable for therapy of cancer cells inducing cell hyperthermia within the excitation laser spot. They are expected to have application not only in cancer treatment and cell imaging but also as infrared absorbing optical coatings and multifunctional substrate for SERS-SEIRA [5,11].

The main route to the production of noble metal nanostructures is reduction of the metal salt with a suitable reducing agent, usually in the presence of a stabilizer. However, the synthesis of nanoparticles of anisotropic shape, like nanorods and nanotriangles, in high yield remains a challenge for nanochemists, often requiring complex and time-consuming steps such as morphology transformation from the nanospheres or the seeded growth process. On the other hand, it has been discovered that many biological organisms, both unicellular and multicellular, are able to produce inorganic materials, either intra or extra-cellulary, with anisotropic morphology and hierarchical assembly, often to nanoscale dimensions. Examples include the formation of iron oxides by bacteria, silica deposition in diatoms, calcification in cyanobacteria, magnetic nanoparticles in magnetotactic bacteria, and the formation of various other biogenic minerals by bacteria, algae, and fungi [12,13]. As regard to noble metal nanoparticles, it has been known for a long time that certain microorganisms have the capacity to reduce metallic ions into elemental metals. As a result nanoparticles of different shapes, sizes, and compositions can be synthesized using different microorganisms bacteria, fungus, yeast, actinomycetes [14]. Besides, certain plants are also able to produce metal colloids without the need for an external reducing reagent [15,16]. However, the exact mechanism of the reduction process has not been elucidated yet.

In the current study, we exploit the ability of plant extract to reduce gold salts and produce single crystalline triangular gold nanoparticles at high yield. In addition to previous studies, in this paper, gas chromatography-mass spectrometry (GC-MS) analysis was used for the first time to monitor the reduction process and identify the chemical composition of extract plant, before and after reaction with gold ions. Moreover, in view of future application as intracellular spectroscopic probes, we test the efficiency of synthesized gold nanotriangles as SERS substrates. Their morphology and optical properties have been analyzed by transmission electron microscopy (TEM), X-ray diffraction (XRD), electron diffraction and UV–VIS absorption spectroscopy.

2. Experimental

In our experimental protocol, the following reagents and chemicals were used: tetrachloroauric acid ($HAuCl_4$) and p-aminothiophenol (p-ATP) from Merck, Germany.

The first step was to prepare the extract of plant leaf. A 3 g portion of thoroughly washed plant leaves were finely cut into small pieces and boiled in 1 L of ultrapure water. The second step was the synthesis of gold nanotriangles by following procedure: 1 mL leaf extract were added to 100 mL solutions of 10^{-3} M aqueous $HauCl_4$ for reduction of $AuCl_4^-$ ions to Au^0.

The GC-MS study was performed on a Perkin Elmer gas cromatograph (GC) model 990 equiped with a packed column 1% EGA on Chromosorb WAW 80-100 mesh, 2m x 4mm i.d. was used. The GC was coupled with a double focusing mass spectrometer MAT 311 EI. The gold nanoparticles optical absorbance was followed with a Jasco V-530 UV-Vis Spectrophotometer. To image and determine the crystalline structure by electron diffraction of nanotriangles a JOEL JEM 1010 Transmission Electron Microscope was used. The crystallinity of the gold nanotriangles was also checked with a BRUKER D8 Advance Difractometer using graphite monochromated Cu KR radiation (λ= 1.5418 Å). The Raman and SERS spectra of p-ATP molecules were recorded with a confocal Raman microscope (CRM 200 Witec) setup equipped with a 20x microscope objective (NA=0.4). The 632.8 nm line of He-Ne laser with a spot of 5 microns diameter and a power of about 1.5 mW was employed. For SERS measurements, the p-ATP molecules were adsorbed on the surface of triangular particles deposited on glass substrate.

3. Results and discussion

Addition of extract plant to aqueous $HAuCl_4$ solution led a brownish color in solution after about 4 h of reaction, signaling the formation of gold nanotriangles.

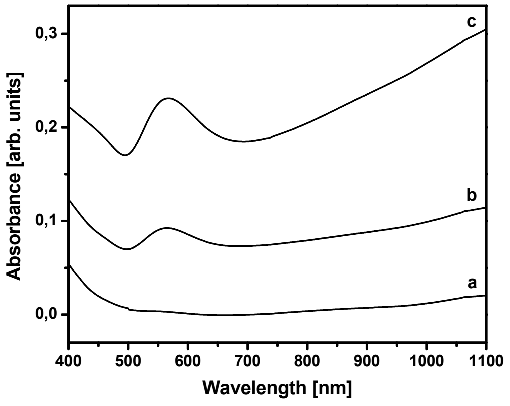

Fig. 1. UV-Vis absorption spectra of gold nanotriangles recorded function of reaction time 30 min (a), 4 h (b) and 24 h (c).

Fig. 1 shows the UV-VIS absorption spectra recorded as a function of time of reaction of aqueous solution of $HAuCl_4$ with plant extract. It is well-known that triangular nanoparticles of gold exhibit two characteristic absorption bands referred to as the transverse (out of plane) and longitudinal (in plane) surface plasmon resonance bands. While the out of plane transverse absorbance is situated in visible at 565 nm (and coincides more or less with the surface plasmon resonance of spherical gold nanoparticles), the in plane surface plasmon band is a strong function of the edge length of the triangle [8]. In our experiment, this second peak is developed at longer wavelength that the spectral limit (1100 nm) of our UV-VIS spectrometer (see Fig. 1).

As it can be seen the plasmon resonance bands of gold solution steadily increases intensity as a function of time of reaction. The broadness and asymmetry of the absorption band reflects the polydispersivity in the shape and size distributions of the anisotropic gold nanoparticles as can be seen in TEM pictures (Fig. 2).

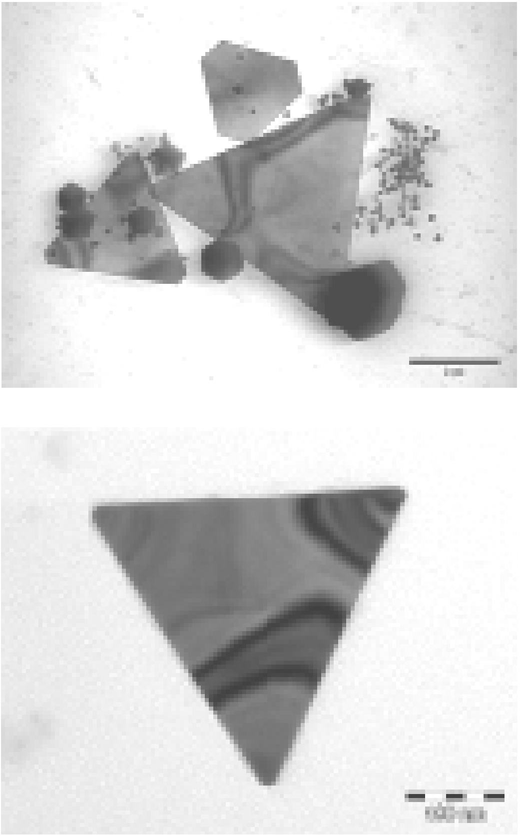

Fig. 2. Representative TEM images of biosynthesized gold nanoparticles.

In order to determine the organic compounds which are responsible for reduction of gold ions a GC-MS analysis was used by comparing the chemical composition of extract plant before and after reaction with gold ions. It was found that the possible reducing and stabilizing agent could be citronellol molecules, which is present in large concentration (23,95%) in the extract plant while an important decreasing (0.86%) is noticed after mixing the plant extract and gold salt solution. Moreover, after addition of extract plant to gold salt solution, some specific absorption bands in UV assigned to organic compounds almost disappear as a result of reaction between extract and gold ions. Additionally, the specific band in UV assigned to $AuCl_4^-$ ions is decreasing in intensity (not shown) due to the reduction of Au ions in solution and formation of triangular nanoparticles. TEM pictures presented in Fig. 2 reveal the formation of triangular plates together with a small amount of hexagonal plates and spherical nanoparticles. From electron transmission contrast in TEM pictures we can estimate that the plates are about 25-30 nm in thickness while the edge length can ranges from tens to hundreds of nanometers. As the mechanism of gold salt reduction is not yet elucidated it is very hard to point out the exact chemical components in extract which is more relevant for building triangular or spherical nanoparticles.

The crystalline nature of as bio-synthesized gold nanotriangles was investigated by using the X-ray diffraction (XRD) and electron diffraction (fig. 3) methods. From X-ray diffraction it can be seen (Fig. 3) two Bragg reflections centered at $2\theta = 38.37°$ and $2\theta = 81.91°$ and which can be indexed to a face centred cubic (fcc) structure of gold nanocrystal. The peaks corresponds to (111) and respectively (311) lattice plane of the fcc lattice of gold. The intensity of diffraction maximum (111) is much higher compared to (311) and demonstrates that during the nanocrystal growth the gold atoms preferentially attach to the (111) faces of the gold.

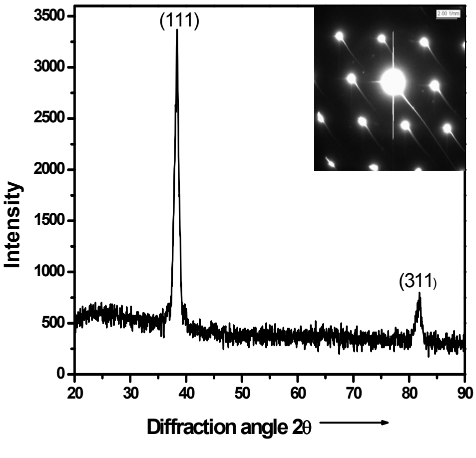

Fig. 3. XRD patterns recorded from drop-coated film of gold nanotriangles onto the glass slide. The inset shows the electron diffraction on a single gold nanotriangle

The inset in figure 3 shows the selected area electron diffraction pattern recorded from the gold nanotriangle. The electron diffraction, which was obtained by aligning the electron beam perpendicular to triangular face of a single gold triangle, shows hexagonally arranged diffraction spots with 6-fold symmetry characteristic of (111) oriented single-crystal gold nanotriangle. The hexagonal symmetry of diffracted spots suggests that gold nanoparticle is a face centered cubic single crystal bounded by (111) facets. The crystal shape is determined by the relative growth rates in different crystallographic directions.

Spherical gold nanoparticles have been extensively reported as the substrate in SERS-based molecule detection. In this case the plasmon coupling between adjacent nanospheres with the formation of so-called “hot-spots” are considered as the key and necessary conditions for local field enhancing. Recently, several groups demonstrated significant field enhancing effect by nano-tip and nano-edge, both in their numerical simulations and experiments [17,18]. Because our nanotriangles have geometrical features of both nano-tip and nano-edge, we can expect an excellent local field enhancement from that area. It is the reason why we have explored the use of as bio-synthesized triangular nanoparticle plates as SERS substrate. To better interpretate the SERS spectra of *p*-ATP on substrates, the normal Raman (NR) spectra of *p*-ATP have been determined at 633 nm, as shown in curves of Figure 4. Four strong and medium-strong bands can be observed at 1587, 1174, 1079, and 405 cm-1, which are assigned to the vibrations of νCC, δCH, νCS and γCCC, respectively. The frequencies and assignments for *p*-ATP vibrations are given in literature [19, 4]. Good SERS spectra we collected from nanotriangles dispersed on glass substrate which confirm the idea that the apexes and edges of triangular plate behave as efficient hot-spots. More study is underway on the precise quantification of SERS enhancement factors.

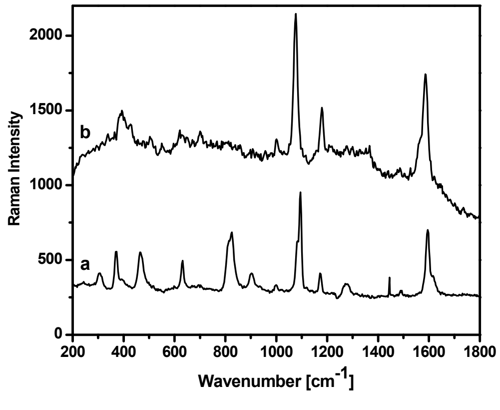

Fig. 4. The Raman spectrum of solid p-ATP recorded with 633 nm laser line (a) and the SERS spectrum of p- ATP adsorbed on gold nanotriangles film (b)

4. Conclusions

The biological synthesis of gold nanotriangles using pelargonium extract provides a simple and efficient route for the synthesis of noble metal nanomaterials with tunable optical properties directed by particle shape. The advantage of this method over the standard chemical methods is the nontoxicity, biosynthesis methods can be considered safe and ecologically for the nanomaterials fabrication. The formation of gold nanoparticles was confirmed by UV–Vis spectra of the resulting gold solution and TEM image provides evidence for the formation of gold nanoparticles. GC-MS spectrometry gave information about the possibility that the citronellol molecule is mainly responsible for the reduction of chloroaurate ions and the stabilization of the nanoparticles. The crystalline nature of the gold nanoparticles was confirmed by corresponding XRD and electron diffraction of nanotriangles. Further, we have demonstrated the potential of these nanotriangles for surface enhanced Raman spectroscopy.

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References

[1] F. Toderas, S. Boca, M. Baia, L. Baia, D. Maniu,, S. Astilean, S. simion, J. Optoelectron. Adv. Mater. **9**(3), 625 (2007).
[2] D. Maniu, V. Chis, M. Baia, F. Toderas, S. Astilean, J. Optoelectron. Adv. Mater. **9**(3), 733 (2007).
[3] Nanotechnology, Gregory Timp Ed., AIP Press, (1999).
[4] M. Baia, F. Toderas, L. Baia, J. Popp, S. Astilean, Chem. Phys. Letters **422**, 127 (2006).
[5] F. Toderas, S. Boca, M.Baia, D. Maniu, S. Astilean, S. Simon, J. Optoel. and Adv. Mat., **9**, 625, (2007)
[6] K. Aslan, S. N. Malyn, C. D. Geddes, J Fluoresc, **17**, 7, (2007).
[7] M. Iosin, F. Toderas, P. Baldeck and S. Astilean (in preparation)
[8] Weiqing Xua, Shuping Xua, Xiaohui Jib, Bo Songa, Hang Yuanc, Lan Mac, Yubai Baib, Colloids and Surfaces B: Biointerfaces **40**, 169 (2005).
[9] K. L. Kelly, E. Coronado, L. L. Zhan and G. C. Schatz, J. Phys. Chem. B, **107**, 668, (2003).
[10] L. J. Sherry, R. Jin, C. A. Mirkin, G. C. Schatz, R. P. Van Duyne, Nano Letters, **6**, 2060, (2006).
[11] S. P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry, Biotechnol. Prog, **22**, 577, (2006).
[12] P. Mukherjee, S. Senapati, D. Mandal, A. Ahmad, M. I. Khan, R. Kumar, M. Sastry, Chem. Bio. Chem, **3**, 461, (2002).
[13] S. P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry, Biotechnol. Prog, **22**, 577, (2006).
[14] J. R. Lloyd, FEMS Microbiol. Rev. **50**, 411, (2003).
[15] S. S. Shankar, A. Ahmad, R. Pasrisha, M. Sastry, J. Mater. Chem., **13**, 1822, (2003).
[15] J. L. Gardea-Torresdey, JG Parson, E Gomez, J Peralta-Videa, HE Troiani, P Santiago, M Jose-Yacaman, Nano Lett **2,** 397 (2002).
[16] M.Baia, L. Baia, S. Astilean, Applied Physics Letters, **88,** 143121 (2006).
[17] S. Zou, G. C. Schatz, Chem. Phys. Lett. **403**, 62 (2005).
[18] F. Toderas, M. Baia, L. Baia, S. Astilean, Nanotechnology **18,** 255702 (2007).

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