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PROTEIN-CAPPED GOLD NANOPARTICLES OBTAINED BY A GREEN SYNTHESIS METHOD

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ABSTRACT. A green synthesis protocol for the synthesis of colloidal gold nanoparticles is proposed. The synthesis involves the use of garlic (*Allium sativum)* extract as reducing agent. The reduction is carried out by slow addition of the *Allium sativum* extract to the chloroauric acid at boiling and gives spherically shaped nanoparticles, with 10-20 nm size distribution and mean diameters of 15 nm. The gold nanoparticles are characterized by UV-VIS spectroscopy, Fourier Transform Infrared Spectroscopy, Transmission Electron Microscopy, and Surface-Enhanced Raman Scattering. *In vitro* tests are also performed on human fetal lung fibroblast HFL-1 cells and the nanoparticles are found to be non cytotoxic for the cells in the concentration range used in our study. Confocal microscopy shows that the particles are localised in the cell cytoplasm.

Keywords: *gold nanoparticles, FTIR, TEM, HFL-1 cells*

INTRODUCTION

Gold nanoparticles (AuNPs) exhibit unique, size-dependent chemical, optical and electronic properties, which are different from the bulk gold. Their properties are closely related to the shape, size, degree of aggregation and surface functionalizing agents [1]. Nanoparticles are especially attractive for the presence of surface plasmon bands and for their large area to volume ratio

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which allows loading of large amounts of bio-functionalizing agents onto their surface [2] especially important in biomedicine applications. Recently, there is more and more interest directed towards the green synthesis of AuNPs using plant or plant extracts [3-12], bacteria, fungi [13-15], yeasts [16], or eco-friendly reducing agents [17].

In this study we report the synthesis of stable gold nanoparticles with 15 nm diameter using *A. sativum* aqueous extract as a reducing agent for the chloroauric acid ($HAuCl_4$). The reduction process was studied by varying the amounts of *A. sativum* extract. The obtained nanoparticles were characterised by UV-VIS spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Surface Enhanced Raman Scattering (SERS), and Transmission Electron Microscopy (TEM). *In vitro* experiments were also carried out on human fetal lung fibroblast (HFL-1) cells.

RESULTS AND DISCUSSION

The nanoparticle samples will be named according the amount of *A. sativum* extract used in the synthesis process.

The AuNPs formation is confirmed by UV-VIS spectroscopy (Figure 1). The UV-VIS shows absorption maxima at 543-544 nm. Basically at boiling, after the extract is added to the chloroauric acid, the solution changes from almost colourless to purple. The inset in Figure 1 shows the colours of the obtained AuNPs samples, while the UV-VIS spectra indicate that the prepared colloidal solutions absorb visible light at 543-544 nm (Table 1), which coincide with characteristic surface plasmon bands of AuNPs [1]. The presence of sharp, well defined surface plasmon bands indicates the presence of small particles with narrow size distribution. The wavelength of the surface plasmon resonance peak is independent on the amount of *A. Sativum* extract, only the absorption maxima show some dependence.

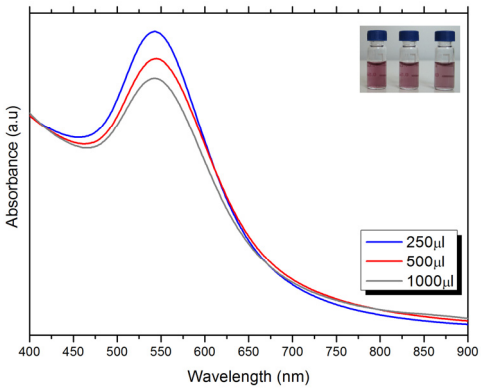
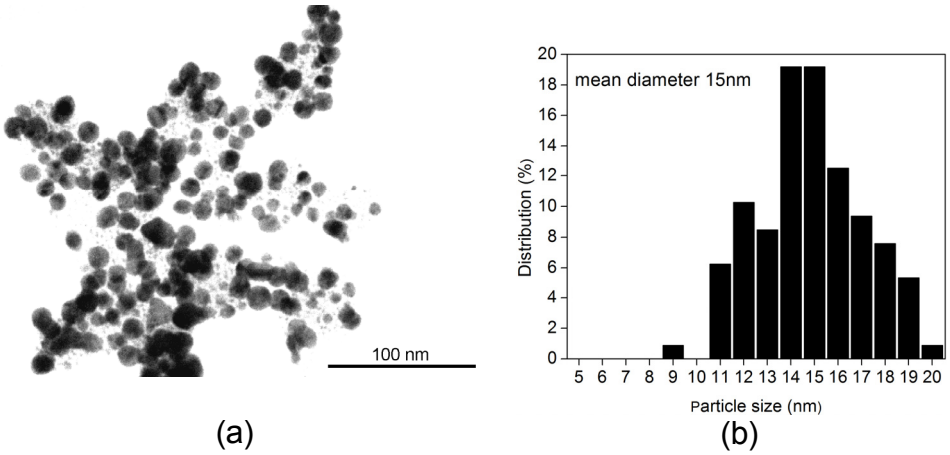


Figure 1. UV-VIS absorption spectra of the AuNP samples prepared by 'dropwise' addition of different amounts of *A. sativum* extract. The colours of the samples can also be observed in the inset.

Table 1. Surface plasmon resonance peak position for the different AuNPs preparations discussed above

Extract amount (µl)	250	500	1000
SPR peak wavelength	543	544	543

The presence of small particles with narrow size distribution is confirmed by TEM experiments. Figure 2 shows a typical TEM image for the 250µl sample, together with the size distribution of the AuNPs. The average size of the AuNPs was estimated using the ImageJ 1.46 software. The other samples, the 500 µl and 1000 µl respectively show very similar behaviour. The vast majority of nanoparticles are spherically shaped. Very few nanotriangles can be observed. The nanoparticles size varies between 10-20 nm, with mean diameters of 15 nm.



(a) (b)

Figure 2. TEM images (a) and particle size distribution (b) the AuNP samples obtained by dropwise addition of 250 µl *A. sativum* extract to the $HAuCl_4$.

Further, FTIR studies were performed for the identification of the nature of the biomolecules involved in the reduction of the chloroauric acid and capping of the nanoparticles (Figure 3). Figure 3 shows the FTIR spectra of the *A. sativum* extract and of the AuNPs obtained by reduction of the chloroauric acid by the same extract. As can be seen, some IR bands (1524, 1651, 2928, 2958, and 3289 cm^{-1}) are common the *A. sativum* extract and the nanoparticles.

The peak at 1524 cm^{-1} corresponds to N-H bending vibrations and C-N stretching vibrations of the amide II band of proteins, while the peak at1651 cm^{-1} corresponds to C=O stretching vibrations of the amide I band. The broad band at around 3289 cm^{-1} is another amide band of N-H stretching vibrations. These findings show that the AuNPs are capped by proteins in the *A. sativum*, implying thus that the extract, apart from being the reducing agent of the chloroauric acid, is also the capping agent of the nanoparticles.

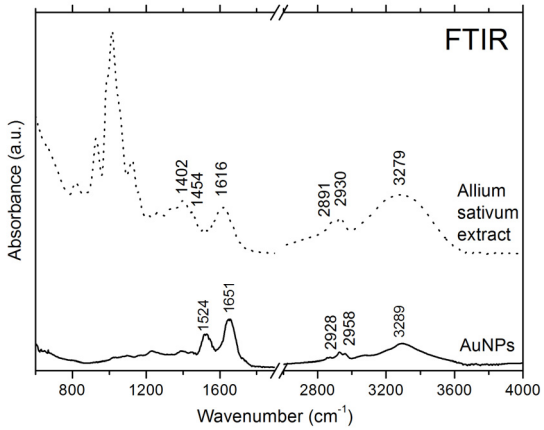


Figure 3. FTIR spectra of the *A. sativum* extract (upper spectrum) and of the AuNPs (bottom spectrum).

The stability of the AuNPs colloidal solutions was assessed by recording the evolution of their characteristic UV-VIS spectra in time (Figure 4). Their stability is not optimum and should be improved. This can be concluded from the slight change in the spectral appearance in time. The same is valid for the other samples obtained by dropwise addition.

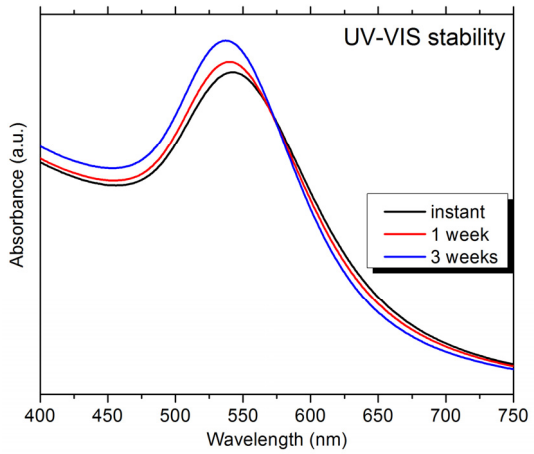
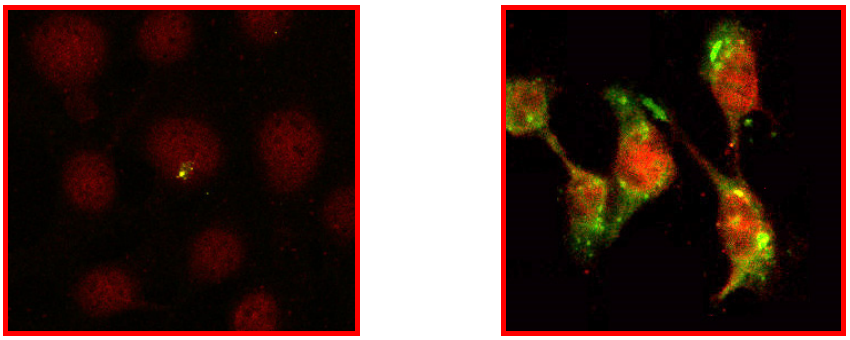


Figure 4. Time evolution of the UV-VIS spectra of the gold nanoparticle samples obtained by dropwise addition of 250µl of *A. sativum* aqueous extract to the $HAuCl_4$.

Some preliminary *in vitro* studies on HFL-1 cells cultures were also carried out for testing the AuNPs cytotoxicity and to check if the nanoparticles are internalized inside the cells. We are able to prove the cellular internalization and localization of R123-labeled AuNPs by confocal miscroscopy (Figure 5). The red areas in the figure represent the cells nuclei, while the green areas are the R123-labeled AuNPs, which, as can be observed, are internalized in the cell cytoplasm.



(a). (b).

Figure 5. Fluorescence **c**onfocal microscopy images of (a) control sample and (b) HFL-1 cells incubated for 24 hours with the 250 µl AuNPs sample labelled with R123. The red areas represent the cell nuclei stained in red with Draq5, while green fluorescence is given by the presence of the R123-AuNPs in the cell cytoplasm.

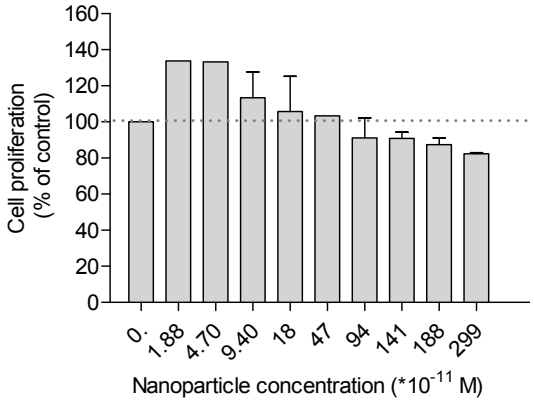


Figure 6. Effect of AuNPs on the proliferation of fetal lung fibroblast HFL-1 cells, treated with different concentrations of AuNPs for 24 h. Cell viability was assessed by the MTT assay. Data are expressed as mean ± SEM (*n* = 3). No statistically significant differences compared with control were identified.

AuNPs did not have any significant anti-proliferation effect on fetal lung fibroblast HFL-1 cells after 24h as shown in Fig. 6. Low concentrations of AuNPs (1.88 - 47×10^{-11} M) stimulated cell proliferation in a dose dependent manner. AuNPs treatment administrated at the highest concentration of 299×10^{-11} M for 24 h decreased the HFL-1 cell proliferation with only 20 %.

The results of the study thus revealed that the nanoparticles developed here would be a suitable delivery system for *Allium sativum* extract in fibroblasts. The fact that the nanoparticles are not cytotoxic is an essential aspect when considering any potential applications of the nanoparticles in the biological field.

CONCLUSIONS

A green synthesis method gold nanoparticles was proposed. The method is based on the reduction of the chloroaurate ions by *A. sativum* extract, which acts both as reducing and capping agent. The obtained nanoparticles are spherical in shape, with 15 nm mean diameters. The nanoparticles show no toxicity towards HFL-1 cells, in the concentration range obtained in this study. As shown by confocal microscopy experiments, the AuNPs are internalized in the cell cytoplasm. The above mentioned aspects indicate potential of using the obtained particles in biomedical applications.

EXPERIMENTAL

Chemicals and materials

The chloroauric acid tetrahydrate ($HAuCl_4{\cdot}4H_2O$) was purchased from Merck. *A. sativum* cloves were obtained from a home grown source. Fetal bovine serum -Lonza, Ham's F12 Nutrient Mixture (Ham's F12), and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Lonza Group Ltd. (Basel, Switzerland). Glutamine, penicillin, streptomicin, and amphotericin were purchased from Sigma. The rhodamine 123 (R123) was purchased from Sigma-Aldrich.

Gold nanoparticles synthesis

The *A. sativum* aqueous extract was obtained by boiling of 20 g of chopped garlic cloves from a home grown source with 150ml distilled water in a 250 ml Erlenmeyer flask. After boiling, the mixture was decanted; the supernatant was filtered and further used as reducing agent for the AuNPs. For the AuNPs synthesis, 25 ml of a 0.2 mM chloroauric acid solution was boiled and different amounts of *A. sativum* extract (250 µl, 500 µl, 1000 µl) were added "drop by drop" under stirring. A purple color formation was rapidly observed in all cases, indicating the formation of AuNPs.

Instrumentation and characterization methods

The UV-VIS spectra were recorded using a Perkin Elmer Lambda 25 UV-VIS spectrometer. The spectral resolution was 1 nm. The FTIR Attenuated Total Reflectance (FTIR-ATR) spectra were recorded on a Schimadzu IR-Prestige FTIR Spectrometer equipped with a diamond PIKE MIRacle single reflection plate unit. The spectra were taken with a resolution of 4 cm^{-1} and by co-adding 64 interferrograms. The TEM images were obtained using a JEOL JEM1010 transmission electron microscope equipped with a MegaViewIII CCD camera. The microscope uses an accelerating voltage of 100 kV. The measurements were performed onto a copper grid. A drop of solution containing the nanoparticles was place onto this copper grid and allowed to dry. Prior to TEM and FTIR experiments, the nanoparticles were centrifugated four times at 13.000 rpm for 15 min, followed by resuspension in distilled water to remove any unreacted compounds. The pellet obtained after the 4^{th} centrifugation cycle was dried prior to measuring the FTIR spectra. This procedure was necessary for achieving good signal to noise ratio in the spectra.

MTT viability assay

For testing the nanoparticles cytotoxicity, MTT viability assays were carried out on the human fetal lung fibroblast (HFL-1) cell line. The HFL-1 cells were grown in a mixture of 1:1 v/v Ham's F12 and DMEM containing 4.5 g/L glucose, supplemented with 10% fetal bovine serum, 2 mM glutamine, 1% penicillin and streptomycin, 0.1% amphotericin. The cells were incubated in 5% CO_2 at 37° C, and 95% relative humidity. The cells were allowed to attach for 24 hours in 96-well plates. A number of 8000 HFl-1 cells were seeded in each plate. After 24 h, the cells were incubated overnight with eight different concentrations of AuNPs. Next day, the cell culture media was removed and MTT reagent in HBSS buffer (0.5 mg/ ml) was added to each well. After 2 h of incubation the removal of MTT solution was carried out and the formazan crystals were dissolved in DMSO. The solubilized formazan formed in viable cells was measured at 550 nm and 630 nm (for sample and background, respectively) using the microplate reader HT BioTek Synergy (BioTek Instruments, USA). The results were expressed as percent survival relative to an untreated control.

Nanoparticles staining for cellular internalization

The nanoparticles cellular internalization was assessed by confocal microscopy. The fluorescence confocal microscopy was performed with a Zeiss LSM 710 confocal microscope, equipped with Zen software for image processing. The AuNPs were labeled with a fluorescent dye prior to experiments. Rhodamine 123 (R123) was used as fluorescent label. The R123-labeled nanoparticles were obtained by simultaneously adding the *A. sativum* extract and appropriate amounts of 1mM R123 to the chloroauric acid at boiling. Cell nuclei were stained using the cell permeable far-red

fluorescent DNA dye Draq5 (1, 5-bis{[2-(di-methylamino)ethyl]amino}-4, 8-dihydroxyanthracene-9, 10-dione). Cell staining was carried out using the non-fixed HFL-1 cells for 5 min, following three washing steps using phosphate buffer (pH 7.4).

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REFERENCES

[1] M.C. Daniel and D. Astruc, Chemical Reviews, 2004, 104, 293.
[2] N.T.K. Thanh and L.A.W. Green, Nano Today, 2010, 5, 213.
[3] K.B. Narayanan and N. Sakthivel, Advances in Colloid and Interface Science, 2011, 169, 59.
[4] S.S. Shankar, A. Ahmad, R. Pasricha and M. Sastry, Journal of Materials Chemistry, 2003, 13, 1822.
[5] S.S. Shankar, A. Rai, A. Ahmad and M. Sastry, Journal of Colloid and Interface Science, 2004, 275, 496.
[6] G.S. Ghodake, N.G. Deshpande, Y. P. Lee and E. S. Jin, Colloids and Surfaces B: Biointerfaces, 2010, 75, 584.
[7] D. Philip, C. Unni, S.A. Aromal and V.K. Vidhu, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2011, 78, 899.
[8] S.L. Smitha, D. Philip and K.G. Gopchandran, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2009, 74, 735.
[9] S.P. Dubey, M. Lahtinen and M. Sillanpã¤Ã¤, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2010, 364, 34.
[10] K.P. Kumar, W. Paul and C.P. Sharma, Process Biochemistry, 2011, 46, 2007.
[11] A.D. Dwivedi and K. Gopal, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2010, 369, 27.
[12] M. Ahamed, M.A. Majeed Khan, M.K.J. Siddiqui, M.S. Alsalhi and S.A. Alrokayan, Physica E: Low-dimensional Systems and Nanostructures, 2011, 43, 1266.
[13] K.B. Narayanan and N. Sakthivel, Advances in Colloid and Interface Science, 2010, 156, 1.
[14] S. Gurunathan, K. Kalishwaralal, R. Vaidyanathan, D. Venkataraman, S.R.K. Pandian, J. Muniyandi, N. Hariharan and S. H. Eom, Colloids and Surfaces B: Biointerfaces, 2009, 74, 328.
[15] L. Du, H. Jiang, X. Liu and E. Wang, Electrochemistry Communications, 2007, 9, 1165.
[16] K.N. Thakkar, S.S. Mhatre and R.Y. Parikh, Nanomedicine: Nanotechnology, Biology and Medicine, 2010, 6, 257.
[17] V.K. Sharma, R.A. Yngard and Y. Lin, Advances in Colloid and Interface Science, 2009, 145, 83.