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Green synthesis route for the size controlled synthesis of biocompatible gold nanoparticles using aqueous extract of garlic (*Allium sativum*)

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ABSTRACT

A green synthetic approach for the highly stable, size controlled synthesis of gold nanoparticles is being described. The study explores the use of aqueous extract of garlic cloves as reducing/stabilizing agent for the synthesis of gold nanoparticles. The synthesis is achieved by heating the mixture of aqueous garlic extract and $HAuCl_4$ at 95ºC in water bath at pH-10 for 2 hrs. The formation of gold nanoparticles was confirmed from the appearance of pink color and an absorption maximum at 530 nm. Further, extract concentration and type of alkali (NH_4OH/NaOH) has been varied to tune the size of nanoparticles. The size of the synthesized gold nanoparticles was found to decrease (56.5 ± 13.6 to 24.7 ± 8.2) with increasing extract concentration (0.5%-1.0%) in the presence of NH_4OH. In the presence of NaOH, the synthesis time was reduced to 20 min, with an average particle size of 5.5 ± 2.7. Transmission electron microscopy analysis indicated that non-aggregated gold nanoparticles of various sizes could be synthesized by simple change in reaction conditions. The synthesized gold nanoparticles were found to be pure face centered cubic crystals as suggested by selected area electron diffraction and X-ray diffraction patterns. Fourier transform infrared spectroscopy revealed possible role of S-allyl-cysteine as the major component responsible for reduction of Au^{3+} to Au^0 and protein/amino acids as stabilizing agents. The gold nanoparticles were found to have remarkable *in vitro* stability: showed no sign of aggregation at room temperature storage for a long time (more than 6 months), could resist aggregation in aqueous media with high concentrations of NaCl, in various buffers including: cysteine, histidine, bovine serum albumin and at host of pH ranges. Further, cytotoxicity evaluations on *S. cerevisiae* have shown non-toxic nature of the synthesized gold nanoparticles up to 100 µM of concentration as assessed by well diffusion and inhibition of colony forming efficiency assay. Ease in size control, high stability and biocompatible nature of garlic extract reduced, stabilized gold nanoparticles suggests that they could be potential candidates for drug delivery applications. Copyright © 2013 VBRI press.

Keywords: Gold nanoparticles; green synthesis; size controlled synthesis; garlic extract; biocompatibility.

Lori Rastogi is working as scientist in National Center for Compositional Characterisation of Materials. Her present research interests are development of facile and eco-friendly methods for the size and shape controlled synthesis of metallic (Ag, Au, Cu) nanoparticles. Use of metallic nanoparticles as effective carriers for drugs (enhanced activity of drugs with nanoparticles) and to study the transport of metal nanoparticles in the mammalian cells lines.

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Introduction

The unique opto-electrical, electronic and catalytic properties of gold nanoparticles (Au NPs) are highly dependent on their size and shapes **[1, 2]**. Also for various biomedical applications like drug delivery, imaging etc., it is highly desirable to have nanoparticles of definite/ pre-chosen sizes **[3-5]**. However, it is a fact that reproducible preparation of stable particles with controlled size is very difficult using popular chemical reduction methods. The most widely used citrate reduction method for the size controlled synthesis of gold nanoparticles suffers from the drawback of agglomeration at lower pH **[6]**. Also the

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synthesized nanoparticles should be isolated from the solution without irreversible aggregation which restricts their biological application. So there is a need to develop/improve the experimental processes for the synthesis of nano-materials that offers ease in size control and stable preparation which could be used in wide range of applications. However, with the development of these new methods, the concern for environmental contamination is also heightened as chemical procedures generates a large amount of hazardous products. Hence, the focus has turned towards green chemistry and bioprocesses to establish clean, non-toxic, environmentally benign methods for nanoparticles production **[7]**, such that the nanoparticles should be water-dispersible and stable in aqueous media for prolonged periods to be useful in biological applications. Micro-organisms **[8-11]**, enzymes **[12, 13]**, plant biomass and plant extracts have shown a great potential as possible eco-friendly alternatives to chemical and physical methods. Using plant biomass/extract is more advantageous as compared to micro-organisms as it eliminates the elaborate time consuming processes of maintaining cultures.

Recently there have been a lot of reports on synthesis of gold nanoparticles using different plant parts e.g. *Azadirachta indica* **[14]** , *Medicago sativa* **[15]**, *Aloe vera* **[16]**, *Pelargonium graveolens* **[17]**, *Cymbopogon flexuosus* **[18]**, *Tamarindus indica* **[19]** , *Coriandrum sativum* **[20]**, *Cinnamomum zeylanicum* **[21]** leaf broths/extracts, *Avena sativa* **[22]**, sundried C*innamomum camphora* **[23]** biomass, purified apiin compounds **[24]** extracted from henna leaves, *Emblica officinalis* **[25]** fruit extract Chinese herbal extract **[26]**, *Camellia sinensis* **[27]**, *Dalbergia sissoo* **[28]** etc.

The main aim of the present study was to develop an eco-friendly and facile method for the size control synthesis of stable Au NPs. Here we have employed the medicinally important herb - garlic (*Allium sativum*) extract for the synthesis/stabilization of Au NPs. Garlic cloves can be used for the synthesis of metallic nanoparticles, since the extract of these colves contains several chemical compounds which potentially can act as reducing agents. Garlic, a common culinary ingredient has been used for medicinal purposes from ancient times **[29]**. It exerts various biological effects such as lowering cholesterol levels **[30]**, inhibiting platelet aggregation **[31]** and tumor growth **[32]**, antiviral **[33]** and antibacterial activity **[34]** and antioxidant activity **[35]**. Garlic contains high levels of organosulphur compounds which can be primarily divided into two groups. One group is lipid-soluble, including diallyl sulphide, diallyl disulphide (DADS) and diallyl trisulphide (DATS). The other group is water-soluble, e.g. S-allylcysteine (SAC) and Sallylmercaptocysteine (SAMC). Apart from the various properties mentioned above one more thing which encouraged us to use extract of garlic as reducing agent is that it is available throughout the year so the there will be not be any problem of availability of biomass as could be the case with extracts of leaves which are generally seasonal. The main objective of the work reported here was not only establish an eco-friendly method for the synthesis of AuNPs but also describes and discusses how various reaction parameters like, extract concentration, type of alkali and heating rate could be varied to easily achieve size controlled , reproducible synthesis of highly stable Au NPs. Synthesis of Au NPs using aqueous garlic extract offers the advantage of fabrication of highly stable, biocompatible and spherical nanoparticles of various sizes in a facile manner with proteins and amino acids as capping agent which should give an added advantage of easy functionalization of the nanoparticles surface with drug and targeting molecules.

Experimental

Materials

Chloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.99%) was purchased from Sigma-Aldrich (USA). NH_4OH (30% solution), NaOH (98%) was purchased from Merck (India). All the chemicals were of analytical grade and used as such without any further purification. Garlic was purchased from local market. Garlic cloves were peeled and washed with 0.22 micron filtered water thoroughly. Aqueous garlic extract was prepared by grinding 10 g of garlic cloves in 100 mL of milliQ H_2O using motor and pestle. The extract was then filtered using Whatman filter 1 and the filtrate was stored at 4 ºC for further use.

Synthesis of AuNPs

The general procedure adopted for the synthesis of gold nanoparticles (AuNPs) using aqueous garlic extract is described as follows: 40 μL of 75 mM $HAuCl_4$ was added to the Schott-Duran bottles containing different amounts aqueous garlic extract under constant magnetic stirring at pH-10. These bottles were then placed in a water bath preset at 95ºC. After 2 hours of reaction colors varying from blue, magenta to pink were obtained with varying reaction conditions indicating formation of different sizes of gold nanoparticles. The amount of the extract (0.5%-1.5%) and type alkali (NaOH / NH_4OH) were varied with the aim to synthesize broad size ranges and reduce the time of AuNPs synthesis.

Characterization of AuNPs

UV-visible spectrophotometer (Elico SL 196) has been used to monitor the reduction of $HAuCl_4$ and formation of AuNPs by recording the spectrum of the reaction mixture from 200-900 nm operated at a resolution of 1 nm. Transmission electron microscope, Philips CM200, The Netherlands operated at an accelerating voltage of 115 kV has been used to characterize the size and morphology of synthesized AuNPs. Samples for transmission electron microscope analysis were prepared by drop-coating colloidal solutions onto carbon-coated copper grids. Crystalline nature of synthesized AuNPs was examined with Rigaku DMAX 2200 diffractometer using monochromatic Cu Kα radiation (λ=0.154056 nm) running at 40 kV and 30 mA. Sample for X-ray diffraction was prepared by depositing a thin film of colloidal solution on a clean glass slide.

The lyophilized powders of washed AuNPs were used for fourier transform infrared spectroscopy spectroscopic analysis in order to get the information regarding capping groups around the particles. The measurements were carried out on a Perkin–Elmer Spectrum-One instrument in

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the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets.

Stability evaluation of synthesized Au NPs

In vitro stability of the synthesized AuNPs in various ionic strengths and buffer solutions were tested in the presence of various biological media including sodium chloride (NaCl), phosphate buffer saline (PBS), cystine, histidine and bovine serum albumin (BSA) solutions. Typically, 1 mL of AuNP solution was added to the tubes containing 1 mL of 10mM,50mM,100mM, 150mM and 200mM of NaCl, 50mM PBS, 1 ml 0.5% BSA, cysteine and histadine solution, respectively, and incubated for 24 h. The stability of the Au colloid was evaluated by measuring the changes in the different environment of AuNPs using UV–vis spectra.

Cytotoxicity evaluation

The cytotoxicity of the synthesized Au NPs was evaluated by well diffusion and inhibition of colony formation efficiency assays using *Saccharomyces cerevisiae* (YPH-500) as an eukaryotic cell model. Yeast cell suspension was prepared by growing a single colony overnight in yeast extract peptone dextrose (YEPD) broth and then adjusting the turbidity to 0.250 this was further used for following experiments:

(a)Well diffusion assay

100 µl aliquot of this cell suspension (1x 10^7cells/mL) was then plated on solidified YEPD agar plates. After plating the cells on YEPD agar plates, wells were punched at required places. AuNPs ((5.0µM to 100 µM)) were then loaded into the wells followed by incubation at 37ºC over night. Plates were then observed for the appearance of zone of inhibition (ZOI).

(b)Inhibition of colony formation efficiency assay

Cells (1 x 10^7cells/mL) were incubated with different concentrations of AuNPs (5.0 µM to 100 µM) at 37ºC, 150 rpm for 18hrs. Following incubation absorbance (O.D.) of the cells were measured and also the treated cells were serial diluted and plated on YEPD agar plates which were further incubated at 37ºC, for 24 hrs, number of colonies formed were then counted.

Results and discussion

Synthesis of AuNPs by water bath heating

As shown in **Fig.1** (inset) that the heating of $HAuCl_4$ in the presence of 1% garlic extract has led to change in the color from yellow ($HAuCl_4$) to dark pink. It is well established in the literature that colloidal solutions of AuNPs show a very intense color **[36]**; this is taken as the primary indication of the formation of AuNPs. Presence of a strong absorbance band at 530 nm in the recorded UV-visible spectrum (**Fig. 1a**) further assured the synthesis of AuNPs because gold colloids are known to exhibit a distinct absorption band in visible region between 500-600 nm. This absorption band arises due to surface plasmon resonance (SPR), the frequency at which conduction electrons oscillate in response to the alternating electric field of incident electromagnetic radiation is highly responsive of any change in size shape and dielectric constant of the medium. Presence of SPR λ_{max} between 518-580 nm (**Fig., curve a-f**) at various conditions (described later) of synthesis thus indicated the synthesis of spherical nanoparticles of various sizes as absorption peak at longer wavelengths relating to various other morphologies (triangles, hexagons and rods) were absent.

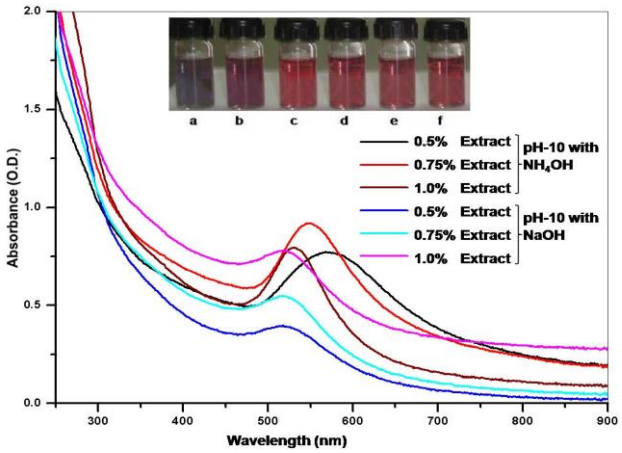

Fig. 1. UV-Vis spectra of gold nanoparticles synthesized at different extract concentrations. A-Spectra a-c when NH_4OH was used to increase the pH to 10, B- d-f when NaOH was used to increase the pH to 10 for the synthesis. Inset- digital photogarph of the gold nano-colloid synthesized at various extract concentrations.

Effect of extract concentration

Keeping $HAuCl_4$ concentration (0.15 mM) constant, the concentration of aqueous garlic extract in the synthetic mixture has been varied to investigate its effect on the bio-reduction process (pH-10 with NH_4OH). It is evident from the series of UV-visible spectra shown in fig. 1 that the SPR observes a blue shift with increasing extract concentration (0.5-1.0%) and various colors at different extract concentrations as shown in **Fig. 1**, blue (0.5%) (1c), magenta (0.75%) (1b) and pink (1.0%) (1a) having the λ_{max} at 580 nm, 548 nm and 530 nm respectively. It is well reported in the literature that SPR is highly sensitive to particle properties (size/shape), the environment in which the metal particles are dispersed, the refractive index of the surrounding medium, as well as the average distance between neighboring metal nanoparticles. Here the blue shift was indicative of formation of smaller particles with increasing extract concentration as confirmed by transmission electron microscopy (TEM) results of the synthesized nanoparticles. TEM analysis of the synthesized AuNPs was carried out to get the accurate size and shape information. The TEM results were in good agreement with UV-visible observations, the average size of the AuNPs decreased from 56.5 ± 13.6 nm (AuNP1) (**Fig. 2a**) at 0.5% to 34.2 ± 10.4 nm (AuNP2) (**Fig. 2b**) at 0.75% and 24.7 ± 8.2 nm (AuNP3) (**Fig. 2c**) at 1.0% extract concentration. The possible explanation for this kind of effect could be the presence of more nucleation site for $AuCl_4^-$ complexation with increasing extract concentration which led to the formation of smaller particles. Since at lower extract concentration less number of nucleation sites would be

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present so more reduction has taken place at one nuclei leading to formation of bigger particle. However, it is also possible that at higher concentrations the garlic extract components had effectively protected the synthesized nanoparticles thus preventing their aggregation, which probably leads to the formation of bigger particles at lower extract concentration. These results indicated that the size of AuNPs can be easily controlled by varying the amount of extract using this method. These results were in good agreement with the earlier reported studies where controlled synthesis of metallic nanoparticles was achieved by varying extract concentration **[37-39]**. The synthesis obtained was highly reproducible in terms of λ_{max} obtained at particular extract concentration (see supplementary **Fig. 1** and **Table 1**).

Table 1. Reaction conditions vs. SPR

S. no.	Reaction conditions	SPR obtained
1	0.5 % extract + 0.25mMH$AuCl_4^{3-}$+ pH-10 with NH_4OH	580 ± 5nm
2	0.75 % extract +0.25mM $HAuCl_4^{3-}$ + pH-10 with NH_4OH	548 ± 3nm
3	1.0 % extract + 0.25mM $HAuCl_4^{3-}$ + pH-10 with NH_4OH	530 ± 2nm
4	0.5 -1.0 % extract +0.25mM $HAuCl_4^{3-}$ + pH-10 with NaOH	518 ± 2nm

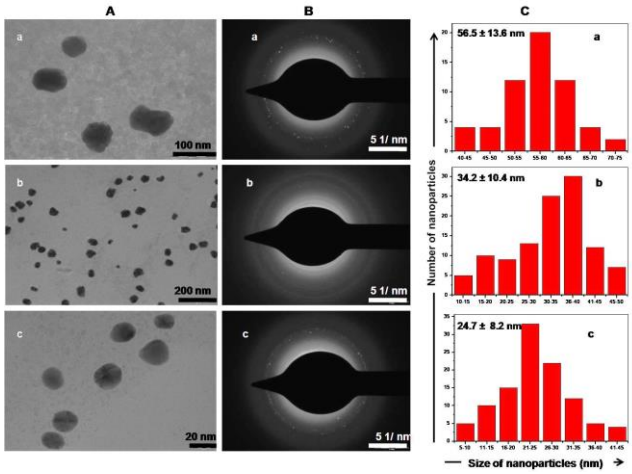

Fig. 2. (A) Transmission electron micrographs, (B) SAED pattern and (C) particle size distribution of the gold nanoparticles synthesized at increasing extract concentrations (a) 0.5%, (b) 0.75%, (c)1.0%. (NH_4OH was used to increase the pH to 10).

Effect of type of alkali

It is must mentioning here that the mixing of 0.15 mM $AuCl_4^-$ to the 1.0% extract results into the solution mixture with pH-2.8. Heating of this reaction mixture though leads to the reduction of gold as indicated by dark pink color but the colloidal solution was not homogeneous, instead it was in the form of aggregates. Addition of NH_4OH before heating the reaction mixture was found to overcome this problem of aggregation (results have already been discussed in the previous section). This further inspired us to investigate NaOH as an alkali to increase the pH of the reaction mixture. As shown in **Fig. 1d** that addition of NaOH also leads to the synthesis of clear gold colloid without aggregates with absorption maxima centered at 518 nm. Most interestingly the reduction time decreased to 20 min as opposed 2 hrs when NH_4OH was used to raise the pH of the reaction mixture. Moreover in this case the variation of extract concentration did not lead to any shift of SPR maxima, instead intensity of absorption increased with increasing extract concentration (**Fig. 1 d-f**). This suggests that the concentration of synthesized nanoparticles increased with increasing extract concentration without a significant change in the size of the nanoparticles. **Fig. 3 a, b** shows TEM results where it is clearly seen that AuNPs of average size of 5.7 ± 2.7 nm (AuNP4) and 8.7 ± 3.5 nm (AuNP5) are being synthesized at two different concentration of garlic extract. As the particles sizes in both the cases are confined to a specific range at both the extract concentration the SPR maxima remains same (**Fig. 1 d-f**).

Decrease in the time of synthesis indicates that NaOH has an accelerating effect on the synthesis process. Very recently Min Zhou *et. al*. **[40]** have also reported similar accelerating effect of NaOH and it was reasoned that structure or chemical property of PVP is changed by introducing NaOH. By comparing the results of gold nanoparticle synthesized in the presence of NH_4OH and NaOH where concentrations of $HAuCl_4$, extract and pH of the reaction mixture remains same, it can be seen that the gold nanoparticles synthesized in the presence of NH_4OH are much bigger as compared to the particles synthesized in the presence of NaOH. This means that the size of gold nanoparticles also depends on the ionic strength of the solution **[41]**. NaOH being a stronger base will favor better hydrolysis of chloroauric species and extract components thus favoring the synthesis of smaller particles.

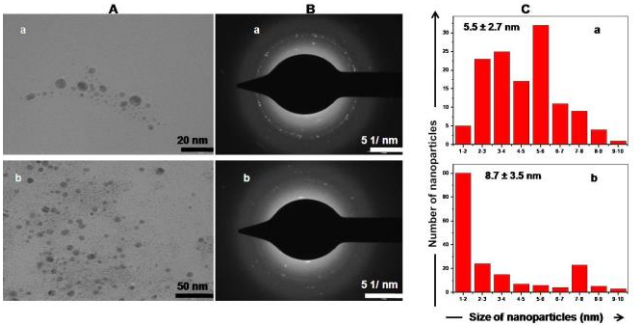

Fig. 3. (A) Transmission electron micrographs, (B) SAED pattern and (C) particle size distribution of the gold nanoparticles synthesized at increasing extract concentrations (a) 0.5%, (b) 0.75%. (NaOH was used to increase the pH to 10).

It should be noted that though the rate of reduction was increased significantly by addition of NaOH and as a result there was a burst of smaller nanoparticles but stabilization of Au NPs by garlic extract component was highly efficient as it did not allow sintering of the spherical particles (prone to aggregation) into anisotropic structures. Thus only spherical nanoparticles of various sizes were obtained at various reaction conditions (different concentration of extract and alkali) which is main advantage of present

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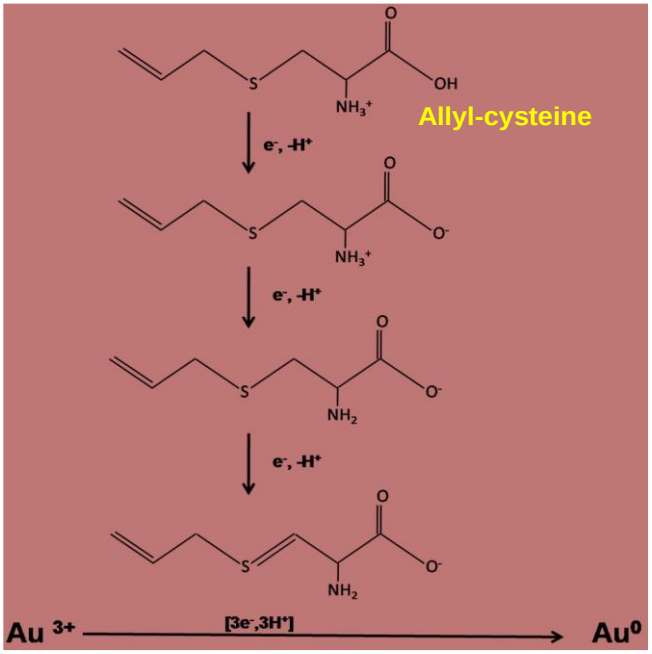
method whereas most of the biosynthesized nanoparticles are generally the mixture of spherical and anisotropic nanoparticles. It has been previously reported that spherical nanoparticles are taken up more efficiently than rods or triangles. Thus present method could be highly useful in synthesizing the spherical nanoparticles of various sizes free of anisotropic structures for biomedical applications like drug delivery.

FTIR analysis

FTIR analysis was carried out in order to investigate the possible mechanism of the gold nanoparticles synthesis by aqueous garlic extract as shown in **Fig. 4**. The IR spectrum of pure garlic extract (a) is compared with as synthesized gold nanoparticles in NH_4OH (b) and NaOH (c) medium. Almost identical IR spectrum of gold nanoparticles in NH_4OH and NaOH medium indicates similar reduction mechanism in both the cases. **Fig. 4** shows increased band intensity at 3300 cm^{-1} in as synthesized nanoparticles when compared to pure extract which may be due to binding of –OH groups with the $AuCl_4^-$ ions. Further a single band at 1630cm^{-1} in (a) found to show clear overlap of two bands 1560 cm^{-1} and 1660 cm^{-1} which can be assigned to amide-II band/ N-H bend of 1° amines and carboxylic C=O stretching of peptides respectively in the (**Fig. b and c**). This fact indicates involvement of these groups in the reduction process. The spectrum of the extract (a) also shows a strong absorption band at 1400 cm^{-1} which can be attributed to C-O-H bending of carboxylic group is found to shift at 1380 cm^{-1} in (**Fig. b and c**) which again indicates the involvement of O-H group in the reduction process. A lower frequency band at 819 cm^{-1} which may be assigned to S-C absorption **[42]** of organo-sulfur compounds present in garlic extract is found red shifted at 843 cm^{-1} this may be due to possible changes in the chemical environment and hence symmetry around the S-C bond.

Possible mechanism of gold nanoparticles synthesis

The reduction reaction can be written as follows:



It is thus evident that a compound containing –COO, -N-H and S-C have a major role in the reduction process. Garlic contains high levels of organosulphur compounds which can be primarily divided into two groups. One group is lipid-soluble, including diallyl sulphide, diallyl disulphide (DADS) and diallyl trisulphide (DATS). The other group is water-soluble, e.g. S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC). As, we have used aqueous extract for the synthesis reaction so SAC and SAMC are the two possible compounds which may be involved in the synthesis reaction. Since there were no band indicating the presence of –SH groups were found in the spectra so we strongly think that SAC has played major role in the synthesis process. Based on above results and discussion we propose SAC plays a crucial role in the reduction of $AuCl_4^-$. The Au^{3+} ions are reduced to Au^0 by three successive electron donation reactions between Au^{3+} and allyl-cysteine leading to the formation of Au NPs. Thus synthesized Au NPs are then effectively capped by protein /amino acids present in the extract (as suggested by FTIR analysis).

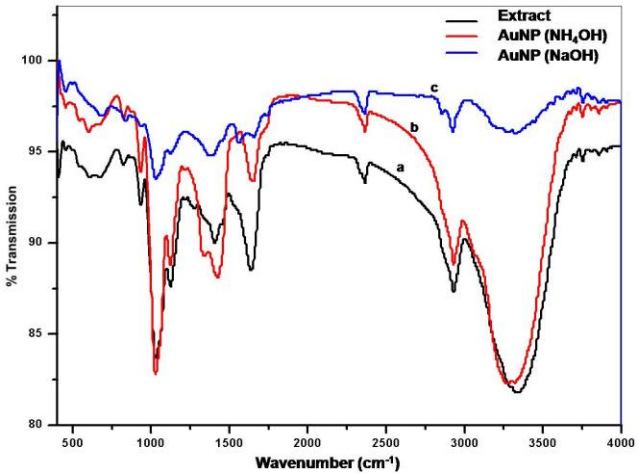


Fig. 4. FT-IR spectra of the (a) pure garlic extract and gold nanoparticles synthesized at 1% extract concentration in the presence of (b) NH_4OH and (c) NaOH.

Crystallinity analysis of synthesized gold nanoparticles

X-ray diffraction (XRD) was used to establish metallic nature of the particles. **Fig. 5** shows X-ray diffractogram of gold nanoparticles synthesized using aqueous garlic extract. The XRD pattern of gold nanoparticles synthesized at all conditions aligned well with that of Au (0) face centered cubic crystal structure (JCPDS file no.-00-004-0783). A number of Bragg's reflection with 2θ values-38.2°,44.3°,64.6°,77.6° sets of lattice planes were observed which may be indexed to (111), (200), (220), (311) facets of Au(0). The XRD patterns did not show any micro-distortion from cubic to other phase structure thus clearly confirming that the gold nanoparticles were essentially crystalline. The XRD results were consistent with selected area electron diffraction (SAED) patterns recorded from group of nanoparticles at various conditions (**Fig. 2** and **Fig. 3 C**). The SAED pattern with a single set of diffraction spots indicated that an entire nanoparticle is crystalline in nature with same lattice orientation running around whole

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particle. The patterns are indexed according to (111), (200), (220), and (311) reflections of gold.

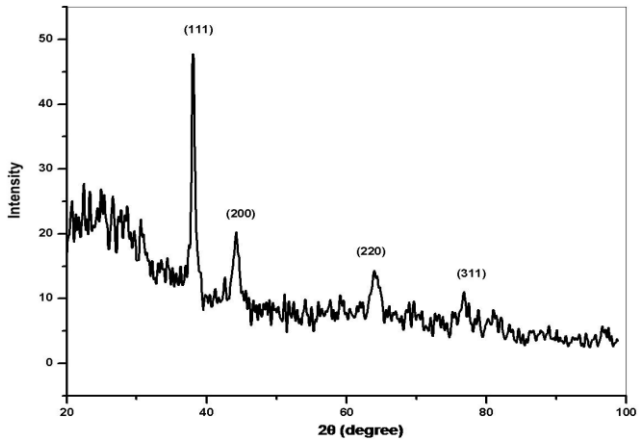

Fig. 5. XRD spectrum of the gold nanoparticles synthesized at 1% extract concentration in the presence of NH_4OH.

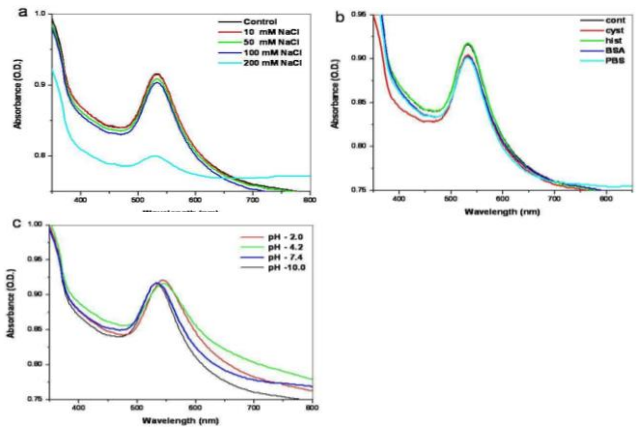

Fig. 6. UV-visible spectra of garlic extract synthesized AuNP2 (a) in the absence and presence of different concentrations of NaCl, (b) different additives and (c) at pH range of 2.0-10.0.

Stability of the garlic extract synthesized AuNPs

Stability constitutes the very important aspect of the AuNPs if they have to be employed for various biomedical applications **[6, 43-45]**. For such applications the AuNPs should be able to resist aggregation under a wide range of environmental conditions such as: high salts, wide range of pH, biological buffer additives (PBS, BSA, histadine, cysteine) and storage time. In the present study we have investigated the effect of various environmental conditions on the satiability of as synthesized AuNP2 by monitoring their UV-vis spectra. As shown in **Fig.6 (a)** that no significant change in the SPR of the synthesized AuNPs was observed in the presence of NaCl up to 150 mM concentration. Aggregation of AuNPs was observed when the NaCl concentration was increased to 200mM. Since, the *in vivo* concentration of NaCl is ~154 mM, thus the synthesized AuNPs should be suitable for biomedical applications. **Fig. 6 (b)** compares UV–vis spectra of the synthesized AuNPs in the absence and presence of, 0.5% cysteine, 0.5% histidine, 0.5% BSA and 100 mM PBS. No significant shifts in the UV–vis absorption spectra are observed within at least 24 h, indicating that the resulting AuNPs are stable at these conditions. Moreover, all of these AuNPs were found to be stable over the pH range of 2.0–10.0 (**Fig. 6(c)**) with no discernible change in intensity or peak position was observed. The synthesized nanoparticles remained stable at room temperature for more than a year (see supplementary **Fig. 2**). These results confirmed that the AuNPs synthesized using garlic extract were highly stable at various employed environmental conditions thus making them highly suitable for various biomedical applications.

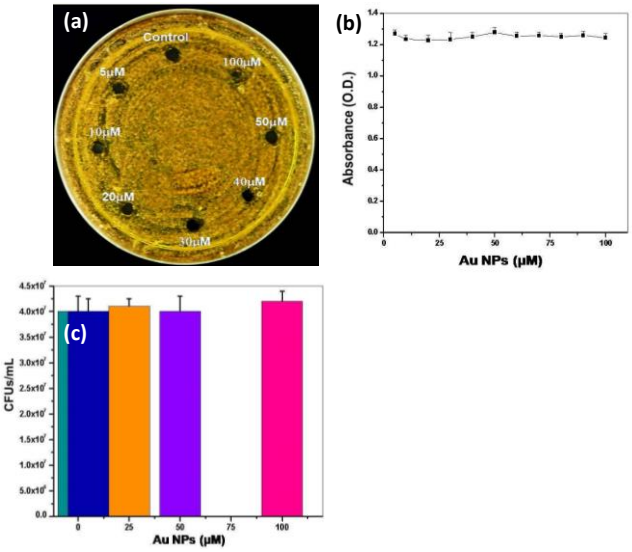

Fig. 7. Cytotoxicity evaluation of the garlic extract synthesized AuNP2: (a) well diffusion assay, (b) graph showing optical density of yeast cells and (c) bar graph showing CFUs at different concentration of AuNPs.

Cytotoxicity evaluation

Scientist worldwide have shown numerous nanomaterials based applications which hold lots of potential in various fields, but there is a serious concern about the increased toxicity of nanoparticles due to their tiny physical dimensions **[46]**. The practical use of nanomaterials in the field of nanomedicine is being limited by the lack of sufficient literature relating to their toxicity and hazards. Gold nanoparticles have been recognized as promising candidates in the field of bio-imaging, diagnostics and therapy were variously described as nontoxic **[47]** or toxic **[48]**. Since we hope that the garlic extract reduced stabilized Au NPs synthesized by our method are highly suitable (because of their stable nature) as drug delivery systems, the cytotoxicity of synthesized AuNP2 was evaluated by assessment of ZOI and colony forming units (CFUs) on yeast (YPH-500) cells as eukaryotic model cell system. As shown in the digital photograph of the well diffusion assay plate that there was no inhibition of cell growth around the wells loaded with different concentration of AuNPs (0-100 μM) suggesting that Au NPs were not toxic to the yeast cells at the tested concentrations (**Fig 7a**). In another assay where the yeast cells were incubated with increasing concentrations of Au NPs (0- 100 μM) in broth, the optical density (measure of cell growth) of the control and AuNPs treated cells were found to be same at all the concentrations after 18 hrs (**Fig 7b**), moreover when these

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cells serially diluted and plated on YEPD agar plates, no significant difference in CFUs were observed after 24hrs of incubation as shown by the bar graph between CFUs vs. Au NPs concentration (**Fig 7c**), thus suggesting that the synthesized Au NPs have no effect on cell viability and its multiplication efficiency. The results thus show garlic extract synthesized Au NPs were non-toxic in nature though more number of experiments are required to establish this point using various cell lines.

Conclusion

In this paper we report a highly reproducible green chemical approach for the synthesis of AuNPs by the reduction of aqueous $AuCl_4^-$ ions using aqueous garlic extract. This approach provides a simple and efficient way for the size controlled synthesis of gold nanoparticles. Since, the amount of extract/type of alkali is playing important role for controlling the size of the nanoparticles. From the point of view of nanotechnology this is a significant advancement to synthesize gold nanoparticles economically. These nanoparticles are found to be stable in water for more than a year that can be attributed to surface binding of garlic proteins with the reduced materials. From the FTIR results we concluded that S-allyl cysteine could be the most probable molecules responsible for the reduction. Though, further experimental investigations are required to establish this fact. It is must to mention here that the method is highly reproducible, as the size of the nanoparticles obtained with different batch of garlic extract was found to be significantly same at a given extract and $HAuCl_4$ concentration. The synthesized AuNPs have shown extremely good *in vitro* stability under various environmental conditions hence it may find applications in the field of nanoparticles based diagnostic and drug delivery systems.

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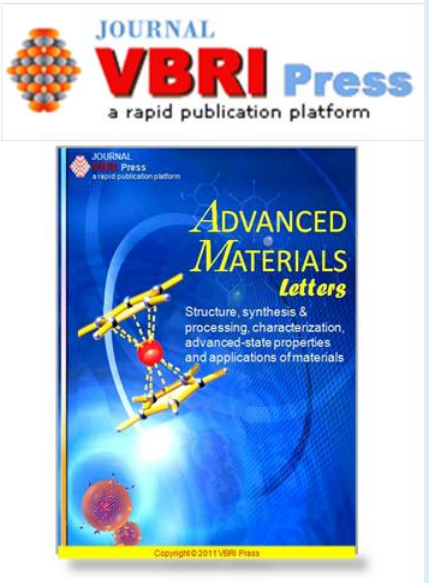

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