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Extracellular Biosynthesis of Gold Nanoparticles using *Salmonella typhi*

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ABSTRACT

The biosynthesis of gold nanoparticles (GNPs) is demonstrated using Salmonella typhi cell exudates. The optimum parameters for the synthesis of nanoparticles were found to be inherent pH of the extract (7.35), high temperature (100ºC) and 100 ppm aurochlorate. The results were verified by UV-Visible spectroscopy, High Resolution Transmission Electron Microscopy (HRTEM) and X-Ray diffraction (XRD) studies. HRTEM images showed that the nanoparticles were roughly spherical and in the range of 10-15 nm. The crystalline nature of the GNPs was confirmed using XRD. The Nitrate reductase activity was found to be 1.330 µmole/min/ml of bacterial exudates that got reduced to 0.24 µmole/min/ml (in the solution) after the formation of GNPs. This result exhibited the involvement of nitrate reductases in the formation of GNPs.

Keywords: *Salmonella typhi*, Gold nanoparticles, HRTEM, Nitrate reductase

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INTRODUCTION

Use of biological systems for the synthesis of nanometals is rapidly gaining importance due to their anomalous optical [1], chemical [2], photo electrochemical [3] and electronic [4] properties. There is a need for an eco friendly method of nanoparticle synthesis. Apart from physical and chemical methods, biological systems are found to be efficient Nano-factories for gold nanoparticle synthesis since they possess reducing agents such as enzymes, which can reduce metals. The nanoparticles synthesized by various living systems have been shown to be coated with peptides or proteins. This leads to a similar charge distribution all over the surface of nanometal which results in repulsion between them. These inter particle repulsive forces prevent aggregation and so, nano metal solutions synthesized by microbes and algae have been shown to be extremely stable even after a period of six months. Peptide ligands in prokaryotes which are involved in reduction of metal ions include metallothioneins (MTs), small gene-encoded, Cys-rich polypeptides. The reduction potentials of *Adathoda vasica h*as also been studied and exploited for tuning the parameters such as pH and temperature, for GNP formation. Further NADH-dependent Nitrate reductase was also determined which involves GNP biosynthesis [5]. Similar studies were also performed with *M. charantia* [6]**,** *A. racemosus* [7]**,** *A. barbadensis* [8] and *C. limone* [9]. A great effort is been put in the biosynthesis of metal nanoparticles using microorganisms and algae [10], [11]. It has been demonstrated that gold particles of nanoscale dimensions may be readily precipitated within bacterial cells by incubation of the cells with Au^{+3} ions [12], [13], [14]. Many types of micro organisms have been used for the precipitation of gold particles both intracellular and extracellular like *Rhodococcus sp*. and *thermomonospora sp* [15], [16]. A detailed account of living system used for synthesis of plethora of metal nanoparticles can be understood by referring author's exhaustive review [17].

In the present work *Salmonella typhi,* a gram negative rod shaped enterobacteria is exploited for the fabrication of gold nanoparticles. Different parameters such as temperature, pH and aurochlorate concentration at which stable monodispersed nanoparticles are obtained are also studied.

MATERIALS AND METHODS

Preparation of Bacterial sample to be used for synthesis of GNP - A loopful of *Salmonella typhi* culture obtained from NCIM, Pune; was inoculated in 250ml conical flask containing 100ml sterile Nutrient Broth. The inoculated medium was incubated at 37^0C in a rotary shaker at 120 rpm for 24 hours. After 24 hours, the culture was centrifuged to separate bacterial cells. Centrifugation was done at 5000 rpm for 10 minutes. Exudates and pellet were separated. The exudates obtained after centrifugation was used for nanoparticles synthesis.

Chemicals and Glassware - Chemical used for the synthesis of gold nanoparticles were chloroauric acid (HAuCl4) (Sigma-Aldrich). 100mL of 1mM aqueous HAuCl4 solution in 500mL of Erlenmeyer flask was taken for gold nanoparticle synthesis

Fabrication of Gold nanoparticle – The exudates obtained from the above procedure was used for GNP synthesis. The desired pH of the reaction medium was adjusted by adding 1 M NaOH solution or 1 M HCl solution. In order to optimize the nanoparticle formation, the impact of different pH (4, 6, 8 & 9) on synthesis of GNPs was studied at 37^0 and 100º C. The parameters obtained from the above two experiment were kept constant to comprehend the optical as well as morphological features of GNPs. The most influential concentration of aurochlorate was fond to be 100 ppm; hence for all the experiments this concentration was used.

Nitrate Reductase Assay-. The exudate obtained from the above procedure was used as enzyme source. Nitrate Reductase activity was measured by Vega and Cardenas method [18].The standard graph for nitrite was calibrated using 50 µM working standard of Sodium Nitrite. To 0.1ml exudates known amount of KNO_3 was added and incubated for 24 hours. Then 1 ml of diazo coupling reagent (1% sulfanilamide in 3 ml HCl and 0.02% N-(1-naphthyl) ethylenediamine hydrochloride) was added to 3 ml reaction mixture and diluted 10 folds to detect the remainingNO_2.After 30 minutes of incubation in dark at 30°C for development of colour;O.D. was recorded at 540 nm. The result was calculated against the standard graph of nitrite.

Characterization of Nanoparticles – *UV-Vis Measurements:* was carried out on a dual beam spectroscopy Lambda 25 Perkin Elmer, USA using deionized water as the reference. The colloidal solution was then added into a quartz cuvette cell followed by immediate spectral measurements. The SPR peaks were assessed for size and distribution of gold nanoparticles.

Transmission Electron Microscopy- Examination of the nanoparticle morphology by high-resolution analytical transmission electron microscopy (TEM) was performed on a Carl Zeiss Micro imaging, GmbH, Germany with an electron kinetic energy of 200 kV. For sample preparation, 2-3 drops of the colloidal gold solution were dispensed onto a carbon-coated 200-mesh copper grid and dried under ambient condition before examination.

XRD Measurements- Crystallographic information about the samples was obtained from X-ray diffraction (XRD). XRD patterns were recorded by a (PANalytical, Philips PW 1830, The Netherlands) operating at 40 kV and a current of 30 mA with Cu Kα radiation (λ = 1.5404 Å) and the 2θ scanning range was of 30-80° at 2° min^{-1}. The colloidal suspension containing metal nanoparticles was dried on a small glass cover slip.

RESULT AND DISCUSSION

The pale yellow coloured exudates turned pink in colour after addition of aurochlorate which eventually turned wine red indicating the formation of GNPs [19]. This is due to reduction of aurochlorate with the assistance of the biological agents present in the bacterial exudates. However the time required for the change in colour varied with temperature. At higher temperature (100ºC) the change in colour was observed in less than 5 seconds whereas at lower temperature (37ºC), it took almost 24 hours for the formation of GNPs. The impact of aurochlorate concentration was studied on GNP synthesis and 100 ppm was found to be the optimum concentration. Hence 100 ppm aurochlorate was used in all the further optimization of parameters.

Table 1 summarizes the impact of pH on synthesis of GNPs at 37 and 100ºC. The UV-Visible spectral analysis showed that at 37ºC, a broad peak centered at 565 nm was obtained at inherent pH (7.35) (Fig 1.a). The appearance of the broad peak indicates that the nanoparticles are polydispersed in nature. All other pH (4, 6, 8 & 9) flat absorption spectra were observed denoting that no GNP synthesis took place. Also the change in color was not prominent (Table 1). At 100ºC, the surface Plasmon resonance (SPR) band was centered at 533 nm (Fig 1.b) at inherent pH (7.35) and this GNP solution was found to be stable for more than a week. The medium intensity peak of the solution showed less polydispersity as compared to the GNP synthesized at 37ºC. A slight hump was observed at pH 9 centered at 543 nm but the GNP solution was not found to be stable and agglomeration of GNPs was observed [20]. This may be due to the non favorable conditions for the optimum functioning of the stabilizing proteins. Flat absorption spectra were observed at pH 4, 6 and 8. All the above observations indicate that the capping and stabilizing proteins work most efficiently at the inherent pH of the exudates. At other pH values weak reducing properties of the proteins are exhibited but the capping properties are not efficient in their function thus leading to agglomeration of GNPs in the solution.

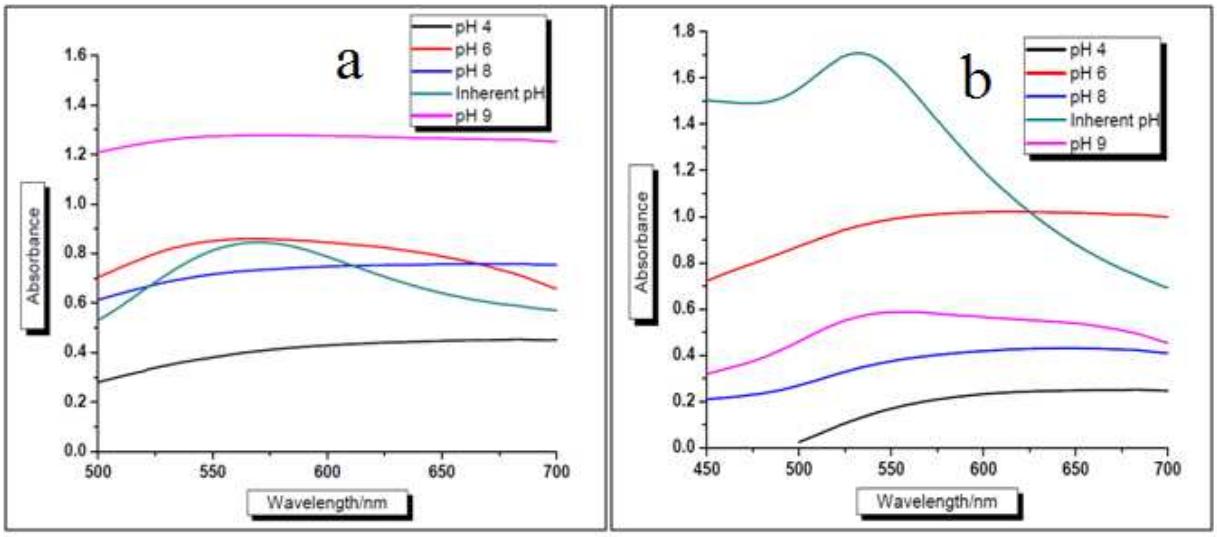


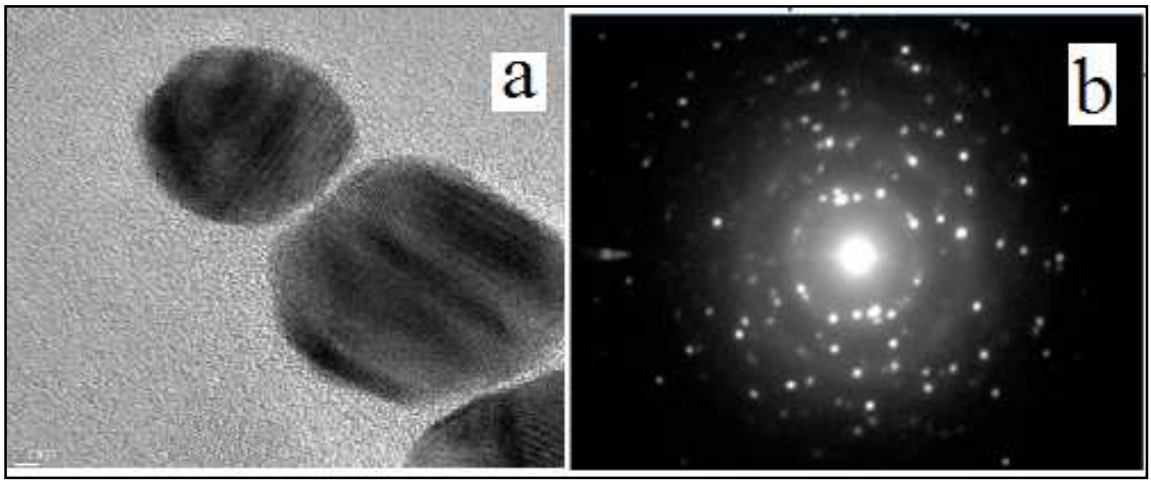
Figure 1: Impact of pH on biosynthesis of GNPs using bacterial exudates at (a) 37º C and (b) 100º C

pH	Observations at	
	37^{0}C	**100^{0}C**
4	No prominent change in color Flat absorption spectra	Change in color in < 5 sec Flat absorption spectra
6	Change in color in 24 h Flat absorption spectra	Change in color in < 5 sec Flat absorption spectra
8	No change in color Flat absorption spectra	Change in color in < 5 sec No prominent peak seen
9	Change in color in 24 h Flat absorption spectra	Change in color in < 5 sec Broad hump seen at 543 nm
Inherent pH (7.35)	Change in color in 24 h broad peak with SPR centered at 565 nm	Change in color in < 5 sec Peak with medium intensity speculated at 533 nm

Table 1: Impact of pH on synthesis of GNPs using bacterial exudates at 37 and 100ºC using 100 ppm aurochlorate

A typical HRTEM image of GNPs synthesized at 100ºC and inherent pH is displayed in Figure 2.a and shows the formation of roughly spherical polydispersed nanoparticles. The nanoparticles are found to be in the size range of 10-15 nm. These results are in accordance with the UV-Visible spectral analysis data. At 37ºC, agglomerated nanoparticles could be seen which may be due to the destabilization of the capping proteins (Data not shown). The nanocrystallinity of the GNPs was confirmed with selective area electron diffraction pattern (SAED). The array of spots corresponds well with a beam direction perpendicular to the [111] plane (Fig 2. b).

Figure 2: (a) HRTEM image of GNPs synthesized at 100ºC, inherent pH and 100 ppm aurochlorate and (b) SAED pattern showing GNPs are crystalline



The confirmation of formation of elemental gold nanoparticles is provided by X-ray diffraction (XRD) analysis of the thin film prepared by coating the gold nanoparticle solution on Si (111) substrate. The colloidal gold nanoparticles on a glass cover slip showed intense peaks at (111), (200) and (220).Bragg reflections in the 2 θ range 30°-80 ° as shown in Figure 3; this is in agreement with the previous data available on gold nanocrystals [21]. The 111 facet is extremely reactive due to high rate of electron transfer.

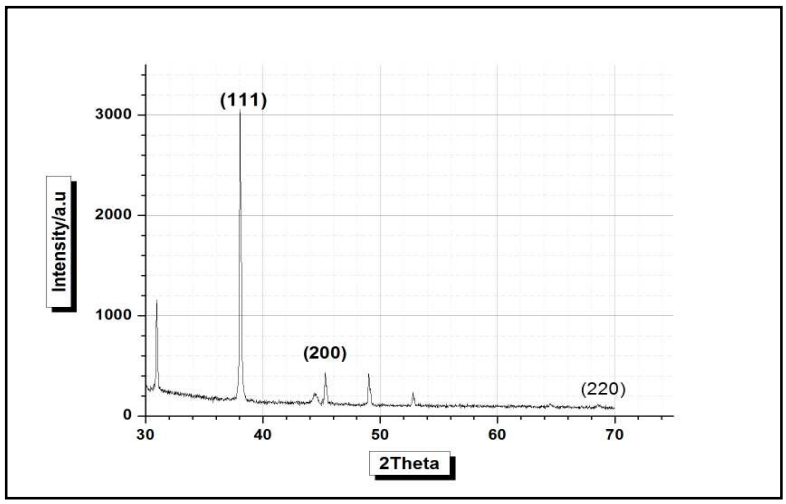


Figure 3: X-Ray diffraction pattern of GNPs biosynthesised using bacterial exudates

The nitrate reductase activity was found to be 1.330 µM/min/ml of the exudates which got reduced to 0.85µM/min/ml when the exudates was subject to 100º C and the activity further got reduced to 0.24µM/min/ml after GNP formation (Fig .4). The substantial decrease in the activity of the nitrate reductase shows that the enzyme might be involved in the reduction of gold ions to form GNPs. Previous studies have indicated that NADH and NADH-dependent nitrate reductase enzyme are important factors in the biosynthesis of metal nanoparticles [22] The reduction may occur by means of the electrons from NADH where the NADH-dependent reductases can act as a carrier [23].

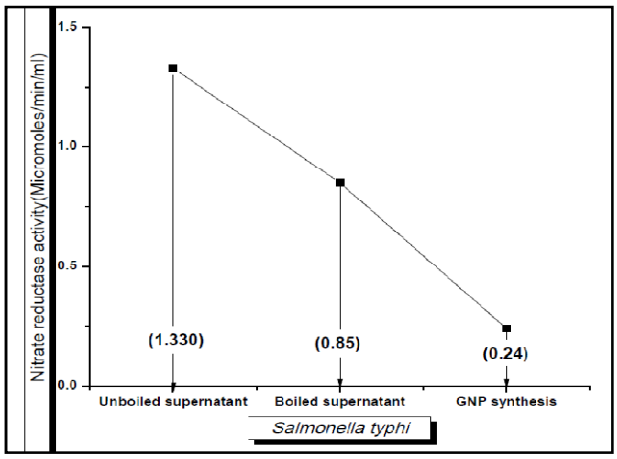


Figure 4: **Nitrate reductases activity of bacterial exudates, boiled exudates and GNPs respectively in µM/min/ml**

CONCLUSION

Salmonella typhi can be used for the extracellular biosynthesis of GNPs. The optimum conditions for the synthesis of nanoparticles were found to be inherent pH and high temperature. HRTEM micrographs depicted the formation of roughly spherical polydispersed nanoparticles. Decrease in the nitrate reductase activity suggests that these enzymes might be involved in the reduction and stabilization of nanoparticles. It presents an ecofriendly and efficient method for the biosynthesis of stable nanoparticles.

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