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INTRACELLULAR SYNTHESIS OF GOLD NANOPARTICES BY *E. COLI*

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ABSTRACT

The main focus of the present study was on reliable, ecofriendly and economic synthesis of gold nanoparticles. The synthesis of AuNPs by *E. coli* cells was confirmed by UV-Visible absorption spectrums and electron microscopy. The Transmission Electron Microscopy analysis exhibited intracellular synthesis of AuNPs of 10 to 20 nm sizes. The presence of functional groups amine, carbonyl and amide responsible for reduction and synthesis of AuNPs was confirmed by Fourier Transform Infra red spectroscopy.

Keywords: Gold nanoparticles, characterization, Transmission Electron Microscopy, Biogenic

1. INTRODUCTION

Nanotechnology has been recognized as continuously emerging field from the last few decades. It deals with the fabrication and engineering of materials and systems with nano-scale size at least in one dimension of the order of 100nm or less **[1]**. They are known to have various types of shapes such as nanorods/nanowires, nanoplates, nanocubes, nanodumbells, nanodiscs, nanotapes/nanobelts, nanotetrapods/arrows and many other shapes **[2]**. Nanoparticles have attained popularity due to their unique physico-chemical and biological properties compared to bulk counterpart and also due to their high surface-to-volume ratio **[3]**. Owing to unique physiochemical properties, nanoparticles may play a significant role in various fields like medicine, diagnostic,

therapeutic, electrical and sensor based applications **[4]**. Among metal nanoparticles, AuNPs are more explored because of their excellent optoelectronic properties and ability for easy surface functionalization **[5]**. Various synthesis methods have been reported for the synthesis of different types of metal nanoparticles **[6]**. Physical and chemical methods are mainly used in the synthesis of nanoparticles due to their ability to produce large quantities of nanoparticles with a desired size and shape in less time. These methods require high temperature and pressure and harmful chemicals that are toxic, not only to the environment but also to human. Existing methods are complex, outdated and require high operational costs. Limitations associated with these conventional methods restrict their application for synthesis of nanoparticles. Therefore, there is a great need to develop efficient, reliable and eco-friendly methods for the synthesis of nanoparticles. For this, Green nanotechnology; utilize the biological systems like bacteria, fungi, actinomycetes and plants which are known for the benign nanoparticles synthesis **[7]**. Keeping in view the limitations associated with physical and chemical synthesis method, the aim of this study was biological synthesis of gold nanoparticles.

2. MATERIALS AND METHODS

Chloroauric acid was purchased from Souvenier chemicals, Mumbai. All chemicals and solvents were of analytical grade and purchased from Hi media and Sigma Aldrich. The AuNPs (mean size: 20 nm) were purchased from Autus laboratory, Ahemdabad. Purchased nanoparticles were characterized by UV-Visible spectroscopic analysis and Scanning electron Microscopy ananlysis.

2.1 Synthesis of gold nanoparticles by Turkevich method

In this experiment, 10 ml of solution of 10^{-3} M $HAuCl_4$ was diluted to 90 ml. The diluted solution was heated with vigorous stirring and quickly added 10 ml of 10^{-2} M aqueous citric acid solution. The solution was boiled for 15 min after mixing. The synthesized nanoparticles were further characterized by UV–Vis spectrophotometric analysis after 15 min and after 3 days to check the stability of peak.

2.2 Gold nanoparticles synthesis from *E. coli* cells

Two bacterial strains *E. coli* MTCC 40 and *E. coli* MTCC1585 were utilized in this study. The strain was inoculated in 50 ml nutrient broth and incubated at 30 °C. The cells collected after centrifugation, were washed with sterile distilled water and

suspended into 10 ml sterile water. The 40 ml chloroauric acid was added into the suspension containing cells, leading 1 mM concentration of gold ions in the reaction mixture. The resulting mixtures were incubated at different temperature conditions (mainly at room temperature and in case of incubator shaker at 30°C and 37 °C) as shown in Table 1. The control (without the gold ions only pellet in distilled water) was also incubated under same conditions. The UV-Visible spectrum of the samples was monitored at different time intervals. The synthesized nanoparticles were further purified by using ethanol and collected by the repeated centrifugation. After ultrasonication of synthesized nanoparticles, purified nanoparticles were analyzed by Transmission electron Microscopy (SEM). The gold nanoparticles were further characterized by Fourier Transform infrared spectroscopy and Transmission electron Microscopy.

3. RESULTS

UV –Vis spectra of purchased AuNPs with ruby red color appearance, exhibited peak at 523nm as shown in **Figure 1(a).** On the basis of SEM image, the average estimated size was 20 nm with spherical and hexagonal shape shown in **Figure 1(b)**.

3.1 Synthesis of gold nanoparticle by Turkevich method

After mixing of $HAuCl^{4-}$ aqueous solution with citric acid and incubation for 15 min, color of solution changes to blue light color which was considered as a primary indicator of gold nanoparticles synthesis. The UV-Viible spectrum of gold nanoparticles synthesized by Turkevich method shows sharp absorption peak at 544 nm with light blue colored nanoparticle solution and after 3 days, peak was shifted to 541nm as shown in **Figure (2)**.

3.2 Intracellular AuNPs synthesis by *E. coli* cells

Out of various temperature and incubation conditions, only the batch 3 and batch 1 of *E. coli* K12or MTCC1585 strain showed promising results for the synthesis of intracellular gold nanoparticles. On the basis of intensity of colour, Only Batch 3 was selected for the further characterization as shown in **Figure 3**.

After addition of $HAuCl^{4-}$ aqueous solution to *E. coli* cells K12 or MTCC1585 suspended in deionized water, pale yellow color appears. After incubation of 5 days, the light purple precipitates were observed, which indicates the formation of AuNPs and a peak at 551 nm was observed in UV-Vis absorption spectroscopy analysis. After 7 days of incubation, peak was approximately at 552nm as shown in **Figure 4**.

The Fourier transform infrared spectroscopy of intracellular synthesized AuNPs is shown in **Figure 5**.The spectral data of synthesized nanoparticles for batch 3 revealed two types of vibrations (i.e. stretching and bending) .The absorption peaks are located at about 3390 cm^{-1}, 2135 cm^{-1}, 1644 cm^{-1}, 1077 cm^{-1}, 1037 cm^{-1} and 741 cm^{-1}.The presence of an amine vibration band was observed at 3,390 cm^{-1} representing a primary amine (N-H) stretching, and amide (N-H) bending vibration band was seen at 1,644 cm^{-1}. The peak observed at 1,037 cm^{-1} and 1077 cm^{-1} corresponds to the carbonyl group. The presence of the intense peak at C=O stretching mode suggests the presence of carboxylic groups in the material bound to gold nanoparticles. The small band at 2,135 cm^{-1} arises from the -C=C stretching vibrations corresponding to the -C=O stretching vibrations may be due to carboxylic acids and carbonyl groups and peak at 741cm^{-1} represent out of the plane bending of –C-H- group. The AuNPs synthesized with the help of *E. coli* cells (Batch 3) was scanned using TEM as shown in **Figure 6**. It shows, *E. coli* cell with nanoparticles inside the cytoplasmic membrane and average size range between 10-20 nm was checked by Image J free public domain software by national institutes of health for size calculation. The Transmission electron micrograph clearly shows *E. coli* cells with pilli and black spots inside the cytoplasmic membrane which are AuNPs.

Table1: The intracellular synthesis of AuNPs by the *E.coli* cells

Batch name	Description
EK1P2	***E.coli*K12(2 pellets), Room temperature**
EK2P1	***E.coli*K12 (1 pellet), Incubator (37 °C)**
EP1S	***E.coli* (1 pellet), Incubator shaker (37 °C)**
EP1I	***E.coli* (1 pellet),Incubator (37°C)**
Batch 1	***E.coli* K12(1 pellet), Incubator (30°C)**
Batch 3	***E.coli* K12(1 pellet), Incubator (30°C)**
Batch 4	***E.coli* pellet (2pellets), Incubator (30°C)**
Batch 5	***E.coli*K12 (2pellet), Incubator (30°C)**
Batch 6	***E.coli* (2pellet),Incubation (37 °C)**

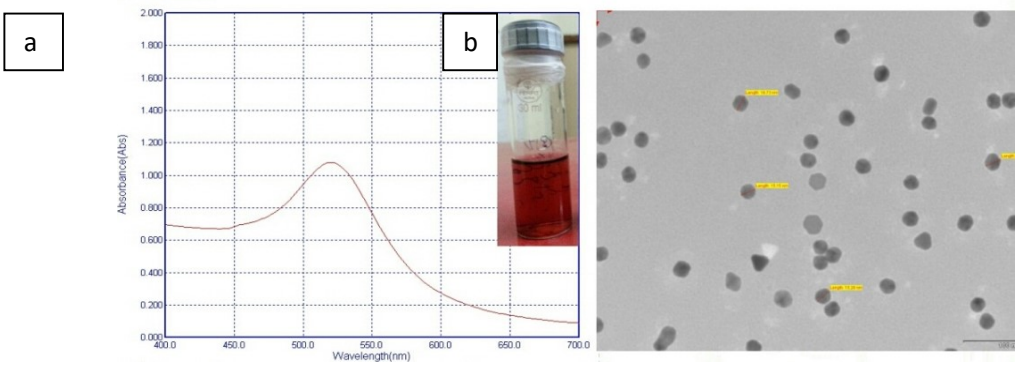

Figure 1: (a) The UV-Vis Spectra of the citrate capped AuNPs(b) SEM image showing nanoparticles of 20nm size with different shapes (right)

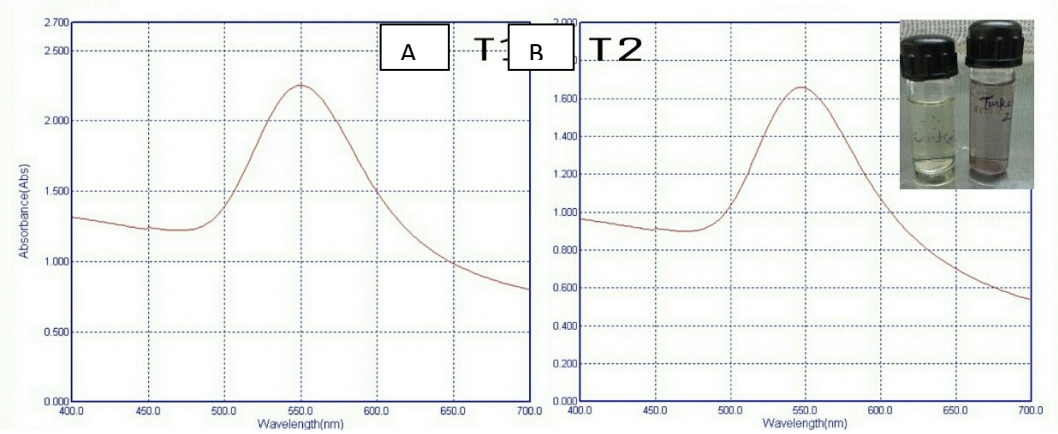

Figure 2: The UV- Visible spectroscopy analysis of the AuNPs synthesized by Turkevich method (A) after 15 min B) after 3 days

Figure 3: Visual color appearance of the intracellular AuNPs synthesized by the *E. coli* cells (Batch 3). (A). Gold ion solution as the control (right) *E. coli* cells mixed with gold ions before incubation (left), (B). After 5 days incubation, Pale yellow color turned into the light purple color (right) with the control (left)

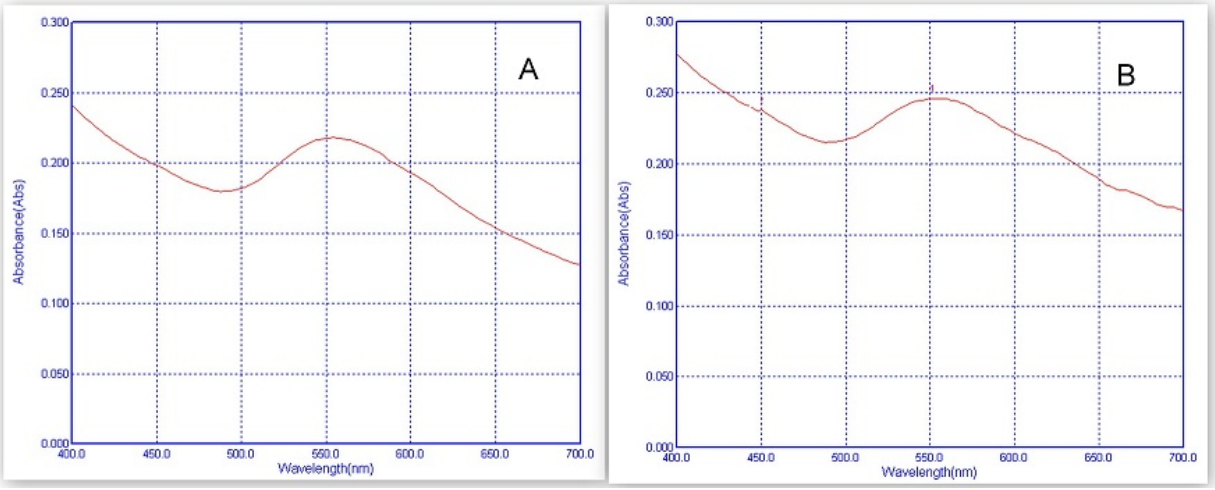

Figure 4: The UV-Visible spectroscopy analysis for the intracellular AuNPs synthesized by the *E.coli* cells (batch 3) (A) after 5 days and (B) after 7 days of Incubation

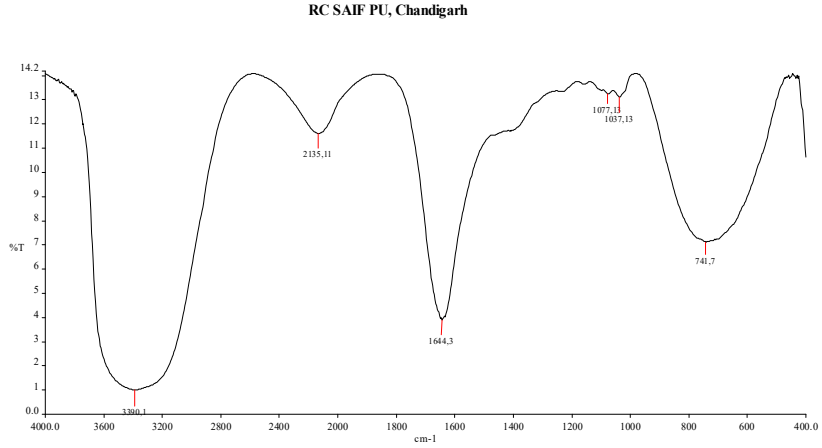

Figure 5; The FTIR spectra for the intracellular AuNPs synthesized by the *E.coli* cells

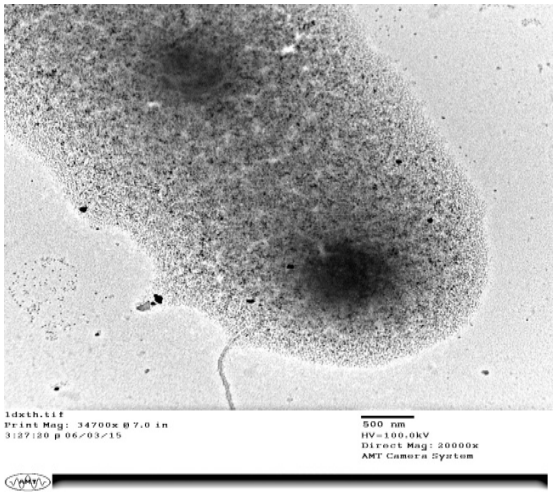

Figure 6: The TEM image of the intracellular AuNPs synthesized by the *E.coli* cells

3. DISCUSSION

In the present study, biogenic gold nanoparticles were found to be synthesized by bacterial strain *E. coli* K12 MTCC1585. This characteristic color generation suggests the formation of AuNPs in the medium due to the surface plasmon resonance (SPR) effect described by the Mie theory **[8]**. The SPR was analysed by UV–visible absorption spectra in order to check the formation and stability of the synthesized AuNPs. The absorption spectrum for AuNPs usually ranges from 510 to 560 nm in aqueous solutions depending on the shape and size of nanoparticles. In this study AuNPs clearly shows one absorption peak at 551 nm respectively. Mie theory also concluded that the sharp peaks indicate the monodispersity whereas broad peaks are indication of the polydispersity nature of AuNPs. Further theory suggests that spherical AuNPs exhibit only one SPR band, usually in the region of 500-600 nm, whereas anisotropic particles show two or three bands. In our study absorption peak confirms spherical shape of nanoparticles but contradicts monodispersity as a broad peak was seen but their TEM images indicates that the majority of nanoparticles were of monodispersed nature.

The absorption spectrums of AuNPs synthesized by *E. coli* suspension (batch 3), and Turkevich method (TK1) were compared. It was noted that SPR peak of biosynthesized nanoparticles was broader and had decreased intensity as compared to TK1. This is already reported that broad resonance peak may be due to the scattering from the rough bio-nanocomposites surface not because of polydispersity nature **[9]**. However, different factors such as dielectric constant of the medium, size and shape of the particles, type of capping agents, as well as the refractive index of the surrounding medium may affect on the exact position peak **[10]**. SPR peak broadening and associated decreased intensity may arise due to interaction between the membrane fraction and AuNPs **[11]**. AuNPs in the vicinity of bacterial cells may adhere to membrane fraction or lipopolysaccharides, which may result in reduced peak intensity (adding scattering background) as compared to otherwise observed SPR of AuNPs alone. This finding gives satisfactory justification of surface Plasmon peak observed in both the cases.

4. CONCLUSION

Both the Biogenic and chemical methods adopted for the synthesis of gold nonaoparticles were equally efficient in

synthesis of nanoparticles. Biogenic synthesis being ecofriendly has advantages over chemical method of synthesis. Biogenic synthesis methods can be explored for future synthesis and industrial applications.

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