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Detection of Pathogenic *Escherichia coli* (*E.c oli*) Using Robust Silver and Gold Nanoparticles

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Abstract

The paper deals with differently synthesized robust metal nanoparticles using chemical synthesis approach for detection of a new pathogenic strain *E. coli* in water sample. The reactions have been completed using aqueous metal salt solution and trisodium citrate as a capping agent to obtain nanoparticles. For different concentration of sodium borohydride, surface plasmon resonance peaks are obtained at 390 nm to 402 nm for silver nanoparticles and 518 nm to 524 nm for gold nanoparticles. It is to be shifted from 395 nm to 408 nm and 520 to 527 nm for gold and silver nanoparticles after the interaction with *E. coli*. These nanoparticles have also been synthesized at 80ºC, exhibited strong surface plasmon resonance peaks respectively at 433 nm and 529 nm. Remarkable shift can be noticed in surface plasma resonance peaks for small amount of *E. coli* added in the solution. The nanoparticles and their interaction against microorganisms were characterized using UV-Vis spectroscopy and Transmission Electron Microscopy (TEM).

Keywords: *E. coli* strain of JB-26; Metal nanoparticles; Silver and gold nanoparticles; Surface plasmon resonance

Introduction

Escherichia coli (*E. coli*) are members of a large group of bacterial germs that inhabit the intestinal microorganism of humans and other warm blooded animals. The presence of *E. coli* in water is strong indication of sewage or animal waste contamination and as few as 10 cells can cause serious human illness and even death. That's why presence of *E. coli* in foodstuffs and drinking water is a chronic worldwide problem. The world wide food production industry is worth about billion and the demand for the biosensors to detect waterborne pathogens and pollutants in food stuffs is growing day by day [1]. Recently, metal nanoparticles as antimicrobial properties have great interest because they have unique optical and electrical properties and perform excellent prospects on biological and chemical sensing [2]. It is estimated that due to bacterial cause thousands of food borne illness and hundreds of hospitalized and deaths each years. It is very important to monitor pathogenic *E. coli* strain and stop their growth by simple methods [3]. Mostly, water quantity standards were set at 103 coliforms per 100 mL and then health goal for total coliforms at zero for drinking water [4]. In addition, human population growth and climate change are expected to increase in the number of species and concentrations of waterborne pathogens in surface water [5].

Bionanotechnology has emerged up as integration between biotechnology and nanotechnology for developing environment friendly and biosynthetic technology for preparation of nanomaterials [6]. The importance of nanomaterials for science and technology is highly increased in past years. The metallic nanoparticles have become more attractive because of their fascinating properties such as optical, physicochemical, electronic and photonic etc. properties due to their high surface area to volume ratio. Mostly the properties are highly affected by their size, morphology and size distribution of nanoparticles. Nanoparticles can be synthesized by growing, shaping or assembling the materials by physical, chemical and biological methods.

Nowadays, silver is currently used to control bacterial growth in a variety of applications. In fact, it is well known that silver ions and silver based compounds are highly toxic to microorganism which shows strong biocidal effects on as many species of bacteria including *E. coli* [7]. The effect of silver ions on bacteria can be observed by the structural and morphological changes. For the last decades, silver ions and silver salts have been used as antimicrobial agents in various fields because of their growth inhibitory capacity against microorganisms. Silver nanoparticles can be synthesized by various methods such as wet chemical method, electrochemical [8], photochemical [9], laser ablations [10], γ- radiations method [11] etc. Silver nanoparticles can be synthesized through reduction of silver nitrate by sodium borohydride along with the stabilizing agents in aqueous solution. Stabilizing agent is used for protecting the growth of nanoparticles by aggregation [12]. Silver nanoparticles were prepared by chemical reduction method in which silver nitrate is reduced by sodium borohydride in aqueous medium at room temperature [13]. Although various approaches have been developed to synthesize stable and monodispersed particles but chemical route method has been found to be most suitable as it is easy, versatile and economical. By controlling the reaction parameters such as temperature, pH, stabilizing agents, reactant concentration, oxidizing or reducing agents etc, variety of particles of different shape and size can be produced. Now a day using wet chemical method, it is possible to produce not only spherical metal nanoparticles and nanoshells but also nanodisks, multipods, triangular nanoprisms etc. When spherical nanoparticles are transformed into one of these shapes the surface plasmon resonance are strongly affected [14].

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Gold nanoparticles have been employed in multiple application involving biological systems, it has remarkable binding properties which makes it attractive for attaching ligands to enhance various bimolecular interactions [15]. It has been well known that inorganic nanoparticles can act as antifungal and antibacterial agents and thus have the ability to interact with microorganisms [16]. Due to its various shapes and sizes, it has been very difficult to predict the positive and negative effects and its mode of action in environment and within living microorganisms. Small size of nanoparticles can modify the physiochemical properties of materials which can lead to adverse biological effects on living cells [17]. In general, the main aspect of the nanoparticles is that the small size of nanoparticles provides for a large surface area for the particle and hence increases the effect. The size of the particles also increase the penetration potential of the silver particles hence again aiding in better utilization of the metal properties [18].

In our present study, simple methods for the detection of *E. coli* and its interaction with gold and silver nanoparticles have been presented. To understand the interaction of *E. coli* with silver and gold nanoparticles various analytical techniques have been used to discuss surface morphology and optical properties of the solution.

Materials and Methods

Materials

Silver nitrate ($AgNO_3$), trisodium citrate ($Na_3C_6H_5O_7$), sodium borohydride ($NaBH_4$) and tetrachloroauric acid ($HAuCl_4$) of Sigma Aldrich make have been used for synthesizing silver and gold nanoparticles. All the solutions have been freshly prepared for the synthesis of nanoparticles especially $NaBH_4$ aqueous solution that was ice bathed before use. Distilled water passed through Millipore system that has been used for all the solution preparation and throughout the experiments. All glassware has been first rinsed with aqua regia solution and then thoroughly with distilled water which is followed by Millipore water.

For identification of *E. coli* stain present in the ground water of near Banasthali area, water sample is sent to IMTECH, Chandigarh, INDIA and finally we have obtained pure culture of *E. coli* for sensing purposes.

Synthesis of robust metal nanoparticles at room temperature

For the synthesis of silver and gold nanoparticles sodium borohydride and trisodium citrate have been used as reducing and capping agent, respectively. $AgNO_3$ (0.2 mM in 10 mL), trisodium citrate (2 mM in 10 mL) have been used as a metal precursor and stabilizing agent, respectively. $NaBH_4$ solution (0.1 M) in 0.1 mL to 1 mL of water (to obtain different samples) has been added drop wise to the above silver nitrate solution and further stirred at 15 min. As soon as the freshly prepared $NaBH_4$ added in the solution, the colour of solution turned light yellow to golden yellow. As we increased the concentrations of $NaBH_4$ solution then the color of silver nanoparticles have been changed. All the reaction has been carried out at room temperature.

Similarly, gold nanoparticles have been synthesized by adding trisodium citrate (10 mM in 10 mL) in $HAuCl_4$ (2 mM in 10 mL) solution and further stirred for 15 min. After 15 min NaBH4 (1 mM) solution in 1 mL to 10 mL of water has been added drop wise in the reaction mixture to obtain gold nanoparticles. The final solution turned from light ruby red to dark ruby red in colour with increased concentration of $NaBH_4$.

Synthesis of robust metal nanoparticles at 80°C

In this method, $AgNO_3$ (2 mM in 50 mL) which has been heated at 80ºC for 15 min then added trisodium citrate (20 mM in 50 mL) to metal precursor solution and resulting mixtures refluxed at 80ºC for 30 min. After 15 min, initially clear solution of $AgNO_3$ turned pale yellow then brownish yellow in colour indicating the formation of silver nanoparticles.

Similarly, the synthesis of gold nanoparticles have been performed using $HAuCl_4$ (2 mM in 50 mL) heated at 80ºC for 15 min then added trisodium citrate (10 mM in 50 mL) to this solution and finally refluxed at 80ºC for 30 min. After 15 min, initially clear solution of $HAuCl_4$ turned light red to ruby red colour indicating the formation of gold nanoparticles.

Characterization techniques

The particle size and surface morphology of the nanoparticles have been examined using TEM of TECNAI make G20 operated at an accelerating voltage of 220 keV. All the samples for TEM have been deposited on carbon coated copper grid by placing the drops of diluted samples of gold and silver. Optical absorption measurements have been performed using a Perkin-Elmer Lambda 750 UV-VIS-NIR Spectrophotometer with pre-aligned tungsten, halogen and deuterium sources. The resolution of the spectrophotometer is 0.17-5.00 nm for UV-VIS and 0.20-20.00 nm for NIR.

Results and Discussions

Silver and gold nanoparticles have been synthesized by wet chemical method using trisodium citrate as well as sodium borohydride at different reaction temperatures. Trisodium citrate merely serves as a capping agent because it is weak reducing agent and cannot perform reduction at room temperature. It is well known from the literature that trisodium citrate merely serves as a capping agent because it is weak reducing agent and cannot perform reduction at the room temperature. $NaBH_4$ being a strong reducing agent, reaction takes place almost instantly and very small particles are obtained [19]. Reduction using sodium borohydride is done at room temperature while trisodium citrate needs higher temperature ~80ºC for the reduction. The sizes and shapes of the nanoparticles were tuned, selecting the reducing and capping agents.

A simple mechanism for the formation of silver nanoparticles using a metal precursor which is given below:

$AgNO_3 \rightarrow Ag^+ + NO^-_3$

$Na_3C_6H_5O_7 \rightarrow 3Na+ + C6H5O_7^{3-}$

$3AgNO_3 + Na_3C_6H_5O_7 \rightarrow 3Ag^+ + C_6H_5O^-_7 + 3NaNO_3$

Similarly, the chemical reactions of the precursor leading to the simply formation of gold nanoparticles which is given below:

$HAuCl_4 \rightarrow Au^{3+} + 4Cl^- + H^+$

$Na_3C_6H_5O_7 \rightarrow 3Na^+ + C_6H_5O_7^{3-}$

$HAuCl_4 + Na_3C_6H_5O_7 \rightarrow 3Au^+ + C_6H_5O_7^- + 6NaCl + 3Cl_2$

Simple mechanism of gold and silver nanoparticles formation is clear from the above equations. When $AgNO_3$ is dissolved in water and it breaks in Ag^+ and NO_3^- ions due to its less hydration energy whereas tri sodium citrate breaks into Na^+ and $C_6H_5O_7^{3-}$. These silver

ions get reduced by $NaBH_4$ by accepting one electron and forms Ag^0 atom. When the number of Ag^0 atoms in the solution approaches to saturation then nucleation starts. After the nucleation, particles grow due to the surface. It may be attributed that some unreduced Ag^+ ions remain on the surface of the particles which attracts negatively charged citrate ions towards them. These negatively charged citrate ions from a layer around the nucleation site and act a capping agent for controlling the shape of the particles. It can be concluded that the formation of double layer is responsible for controlling the size of particles. At lower concentration of $NaBH_4$ formation of silver nuclei will be low so silver nuclei can be combed with other silver ions to form large particles in the solution mixture. On the other hand, at the higher concentration of $NaBH_4$, the rate of formation of silver nuclei will be higher and obtain less nuclei growth which lead to the smaller particles. Similarly this mechanism is valid for gold nanoparticles [20].

Optical properties of silver and gold nanoparticles and its correlation with $NaBH_4$ Concentration

All the spectra show prominent surface plasmon resonance peaks, characteristics of spherical nanoparticles at 529 nm for gold and 433 nm for silver nanoparticles as shown in Figure 1(a) & 1 (b). In silver nanoparticles, the absorption spectrum is showing a hump at 322 nm along with the intense peak at 433 nm. The presence of hump at lower wavelength may owe its presence either due to origin of higher order multipole modes or due to incomplete reduction of silver ions.

Wine red colour of colloidal solution of gold nanoparticles is due to strong absorption of gold nanoparticles. Peak is arising due to absorption of energy via the collective oscillations of free (dipolar plasmon) and interband transitions (HOCO to LOCO). Surface

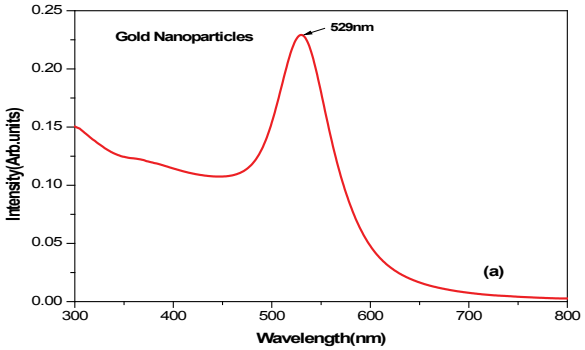

Figure 1a: UV-Vis spectrum of gold nanoparticles synthesized with trisodium citrate at 80°C.

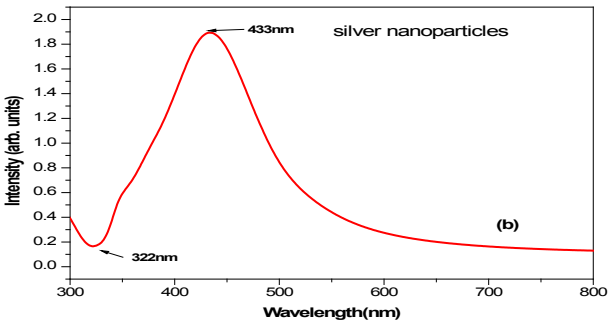

Figure 1b: UV-Vis spectrum of silver nanoparticles synthesized with trisodium citrate at 80°C.

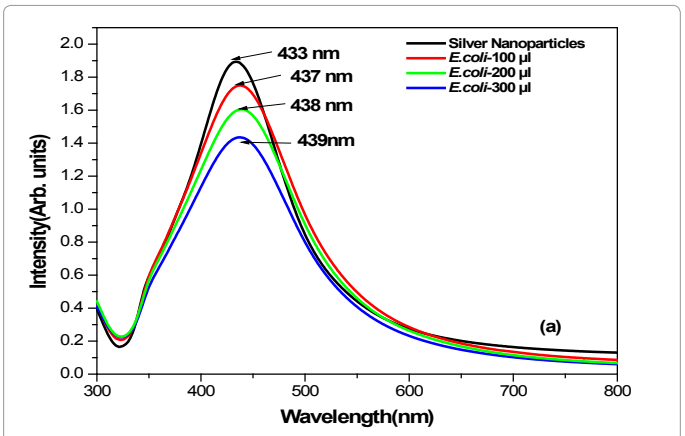

Figure 2a: UV-Vis spectra of silver nanoparticles with interaction of *E. coli* at different concentration e.g. 100 µL, 200 µL, 300 µL synthesized at 80°C.

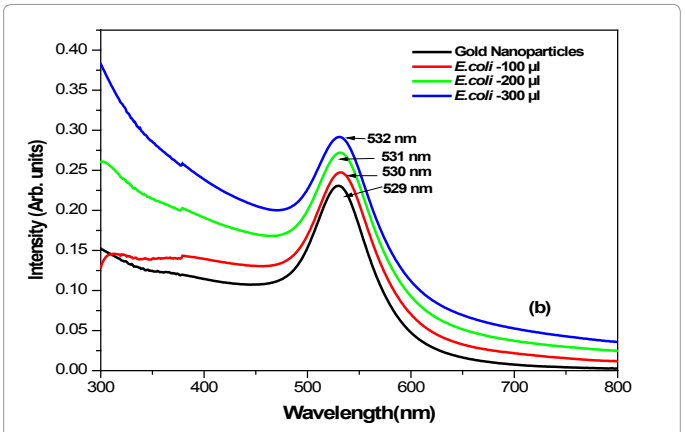

Figure 2b: UV-Vis spectra of gold nanoparticles with interaction of *E. coli* at different concentration e.g. 100 µL, 200 µL, 300 µL synthesized at 80°C.

plasmon resonance gets coupled when the gap between the particles decreases.

The optical absorption spectra have been also recorded for the solution of gold and silver nanoparticles to study the interaction between *E.coli* and metal nanoparticles.

Similarly, red shit is also occurred for the gold nanoparticles as shown in Figure 2b. As the size of the particles increases then the light can be longer polarized the nanoparticles homogeneously and higher modes at lower energy dominate. This causes a red shift, broadening and with less amplitude of the surface plasmon band. Therefore, small gold nanoparticles aggregates will make their surface plasmon combine and results in the color change from red to purple.

Hence we can say that the surface plasmon resonance plays an important role in the determination of optical absorption spectra of metal nanoparticles which shifts to a longer wavelength with increasing the particles size. More and more particles begin to aggregate due to which HUCO-LUCO gap between the particles decreases. Less energy is requires to excite the electron which leads to red shift in surface plasmon resonance wavelength. So red shift and broadening were due

to both surface plasmon coupling and aggregation between closely spaced nanocomposites.

If the naked clusters are in contact with one another aggregation occurs, particle size increases and quantum properties are lost. All the HOCO's merge as do all LOCO's to from band structure.

However, TEM image of silver nanoparticles show the size of particles are less than 10 nm as can be seen from Figure 3(a & b). For this size of particles, higher order of plasmon modes cannot be expected. In Figure 4, a TEM images represent the size of gold nanoparticles before and after the interaction of *E. coli* in the solution. Here we can see that the particles size have been increased after the interaction of *E. coli*.

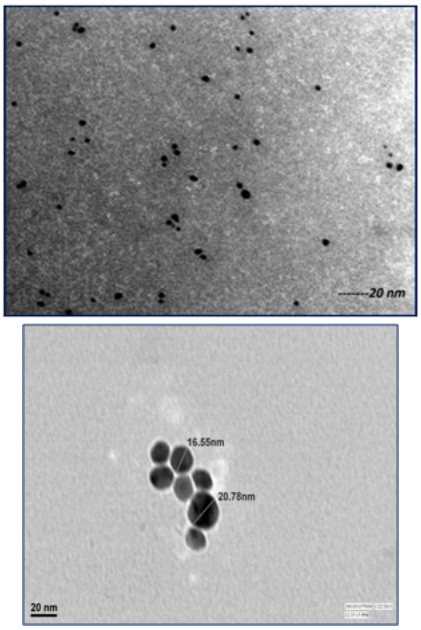

Figure 3: (a) TEM images of silver nanoparticles, (b) after interaction with *E. coli*.

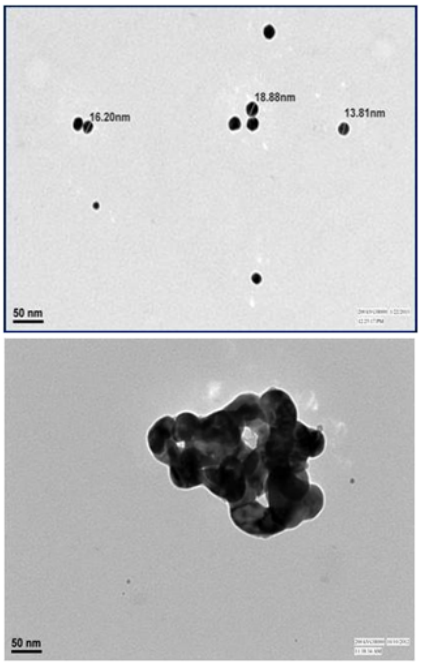

Figure 4: (a) TEM images of gold nanoparticles, (b) after interaction with *E. coli*

All the spectra show prominent surface plasmon resonance peaks, characteristics of spherical nanoparticles in the 518 nm to 524 nm ranges for gold nanoparticles and 390 nm to 402 nm for silver nanoparticles, respectively. $NaBH_4$ solution has been varying from 1 mL to 10 mL for gold nanoparticles and 0.1 mL to 1 mL for silver nanoparticles to examine the effect of $NaBH_4$ on the resulting particle size. We clearly observed a tendency to form a smaller particle size with increasing concentration of $NaBH_4$ in the reaction which can be concluded from a continuous red shift in the absorption spectra with an increasing $NaBH_4$ concentration. The size of these nanoparticles can be tuned by the $NaBH_4$ concentration within certain range.

The absorption spectrum in Figure 5(a) show the prominent surface plasmon resonance peaks, characteristics of nanoparticles in 518 nm to 524 nm ranges for gold nanoparticles with NaBH4 (different concentration of water e.g. 3 mL, 5 mL and 10 mL) and for the same amount of NaBH4, peaks shifted from 520 nm to 529 nm after the interaction of *E. coli* as shown in Figure 5(b).

We also obtained the absorption spectra for silver nanoparticles at 390 nm without and with interaction of *E. coli,* the peak shifted at 395 nm as shown in Figure 6(a). Similarly for another concentration

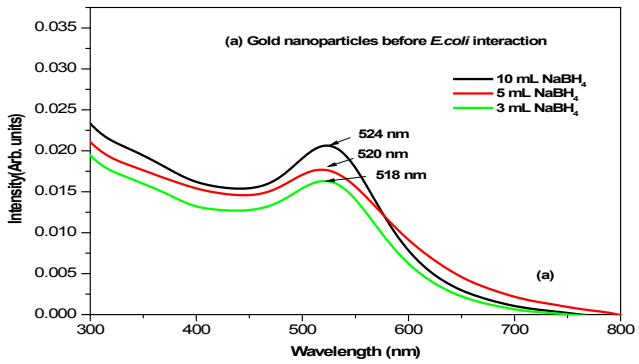

Figure 5a: UV-Vis spectra of gold nanoparticles before *E. coli* interaction of 0.01M $NaBH_4$.

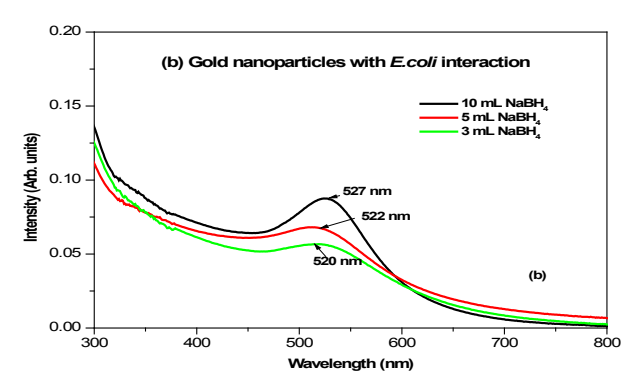

Figure 5b: UV-Vis spectra of gold nanoparticles after interaction with *E. coli* of 0.01M $NaBH_4$.

of $NaBH_4$ we obtained in Figure 6(b), the absorption peak at 402 nm for silver nanoparticles and after the interaction of *E. coli* the peak also shifted at 408 nm. This shows as we increased the concentration of the reducing agent then absorption peaks of bacteria will be increased in the solution, hence the red shift will occurs.

One more interesting result we would like to quote that after interaction with *E. coli* bacteria the surface plasmon resonance peaks shifted at 513 nm to 529 nm for gold nanoparticles, which may be ascribed due to particle size increase with interaction of *E. coli* with nanoparticles and it can be expected that a large number of *E. coli* bacteria coagulate around silver and gold nanoparticles and cluster formation can be expected. To confirm the effect of silver and gold nanoparticles, a comparative study of silver and gold nanoparticles activity against *E. coli* bacteria were performed. In case of silver nanoparticles, surface plasmon resonance peaks shifted at 395 nm to 408 nm after interaction with *E. coli*. TEM images of silver and gold nanoparticles show with and without interaction of *E. coli* in the solution as shown in Figure 7 & 8. In Figure 3(b), 4(b) & 8(b) represent the TEM images of nanoparticles which show the cluster formation after the interaction of *E. coli* bacteria, it may be due the size of the nanoparticles has large surface area to come in contact with the bacterial cell. The smaller nanoparticles interact with bacteria and produce electronic effects which enhance the reactivity of nanoparticles. Hence we can say due to this effect we can be used to determine the presence of the *E. coli* bacteria in the solution.

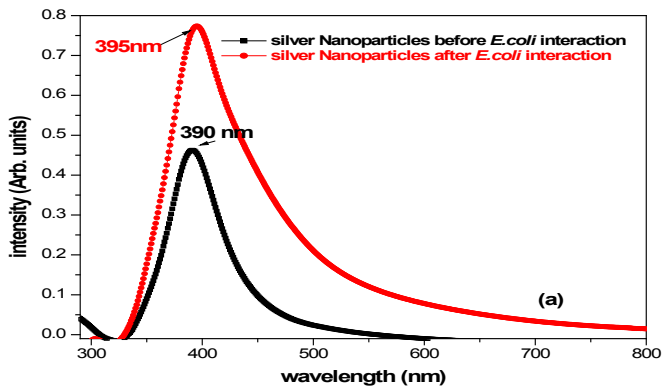

Figure 6a: UV-Vis spectra of silver nanoparticles before and after interaction with *E. coli* of 0.1 M $NaBH_4$.

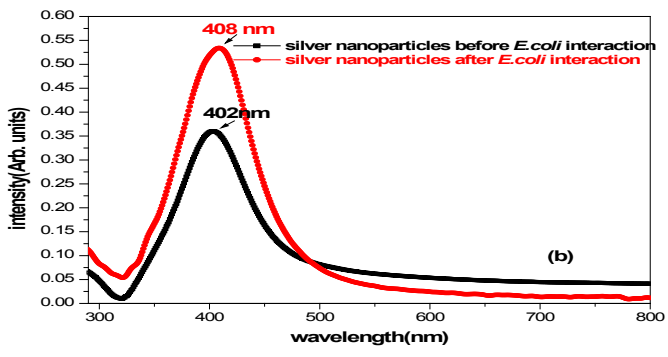

Figure 6b: UV-Vis spectra of silver nanoparticles before and after interaction with *E. coli* of 0.1 M $NaBH_4$.

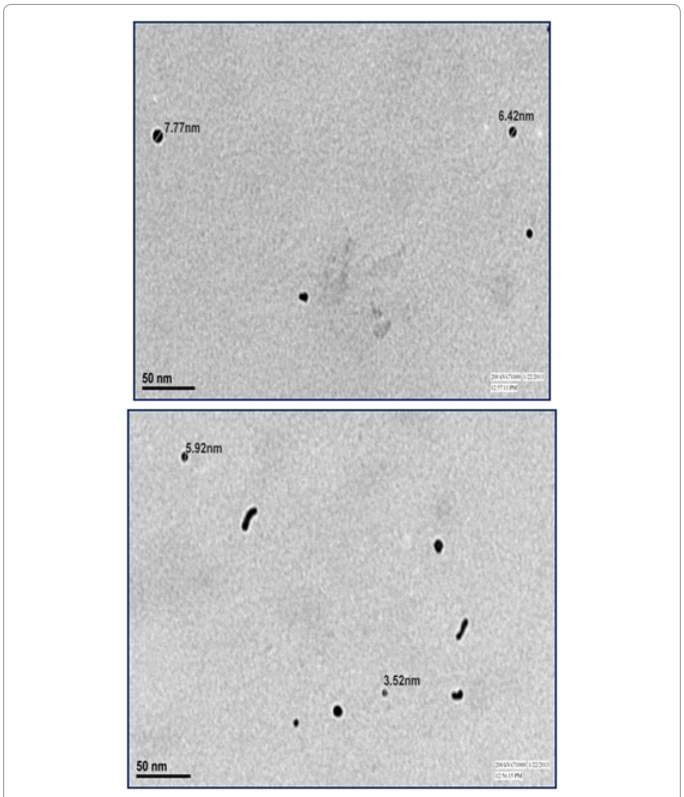

Figure 7: (a) TEM images of silver nanoparticles before interaction of *E. coli*, (b) after interaction of *E. coli* with 0.1 M $NaBH_4$.

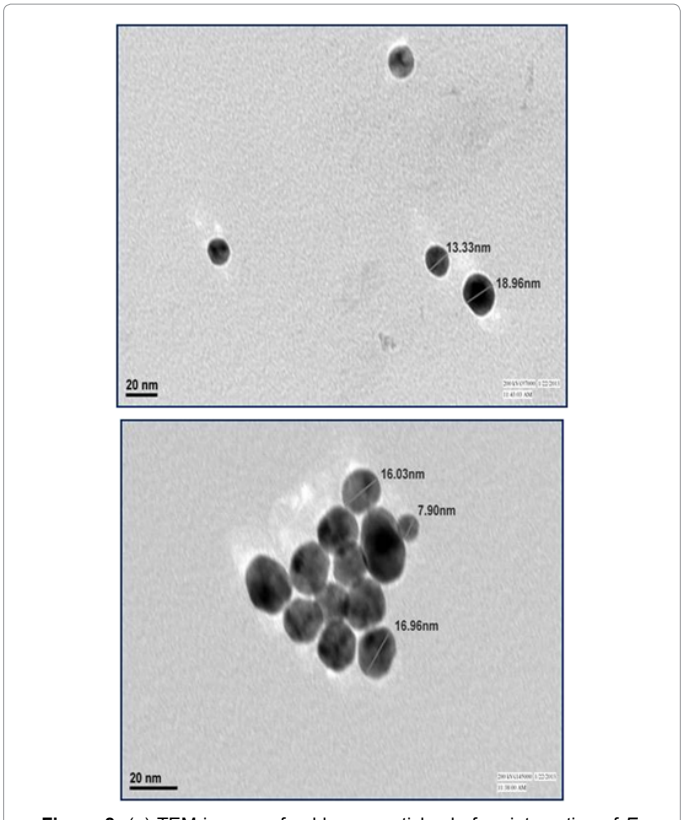

Figure 8: (a) TEM images of gold nanoparticles before interaction of *E. coli*, (b) after interaction of *E. coli* with 0.01 M $NaBH_4$.

Conclusions

Gold and silver nanoparticles were synthesized by wet chemical method using tri sodium citrate and sodium borohydride. As we increase the concentration $NaBH_4$ than a continuous red shift in the absorption spectra is obtained. The size of gold and silver nanoparticles could be tuned by the borohydride concentration within certain range. After the interaction of *E. coli*, the size of particles will increase with increasing the concentration of $NaBH_4$. The size of the nanoparticles implies that it has a large surface area to come in contact with the bacterial cells. Hence, it will have a higher percentage of the interaction than bigger particles. Thus we can say that the bacterial effect of silver nanoparticles and gold nanoparticles are size dependent and can be used to determine the presence of *E.coli* bacteria in the solution.

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