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Biological Synthesis of Silver Nanoparticles from Allium Cepa (Onion) & Estimating Its Antibacterial Activity

Benjamin.G
Student, Department of Biotechnology
St. Joseph's college of engineering
Chennai, India
benjaminglbrt@gmail.com

Bharathwaj.S
Student, Department of Biotechnology
St. Joseph's college of engineering
Chennai, India
coolbuddybharath@yahoo.com

***Abstract*— we used onion (Allium cepa) extract as a reducing and capping agent to minimize the serious environmental pollution problems. The onion extract was mixed with Silver nitrate solution by heating to a temperature of 50-60 degree Celsius and the reduction reaction was studied by observing the color change. Nanoparticles were synthesized by this method having 33.6 nm average mean size. The preparation of nanoparticles by using onion extract has desired quality with low cost and convenient methods. These nanoparticles at concentration 50mg/ml were showed complete antibacterial activity against E.coli .The antibacterial activity was studied for 25 hours with varying concentrations of silver nanoparticles. The size of the nanoparticles was confirmed by subjecting the nanoparticle to TEM analysis and the antibacterial activity by UV-VISIBLE SPECTROMETER.**

Keywords-onion, silver nanoparticles, reduction reaction, antibacterial activity, uv-vis spectroscopy, low cost.

I. INTRODUCTIUON

Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research toward a multitude of potential uses for nanomaterials. Metallic nanoparticles exhibit unusual optical, thermal, chemical, and physical properties. [1] The reduction of materials' dimension has pronounced effects on the physical properties that may be significantly different from the corresponding bulk material. Some of the physical properties exhibited by nanomaterials are due to (i) large surface atom, (ii) large surface energy, (iii) spatial confinement, and (iv) reduced imperfections [2]. A lot of interest has been created by the term "green nanotechnology". In a broad sense, this term includes a wide range of possible applications, from nanotechnology-enabled, environmentally friendly manufacturing processes that reduce waste products (ultimately leading to atomically precise molecular manufacturing with zero waste); the use of nanomaterials as catalysts for greater efficiency in current manufacturing processes by minimizing or eliminating the use of toxic materials (green chemistry principles); [3] the use of nanomaterials and nanodevices to reduce pollution (e.g. water and air filters); and the use of nanomaterials for more efficient alternative energy production (e.g. solar and fuel cells). Use of plants in synthesis of nanoparticles is quite novel leading to truly green chemistry which provide advancement over chemical and physical method as it is cost effective and environment friendly easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Now days we are using bacteria, fungi for the synthesis of nanoparticles but use of leaf extract reduce the cost as well as we do not require any special culture preparation and isolation techniques [4].

Here we report synthesis of silver nanoparticles, reducing Ag^+ ions present in the aqueous solution of silver nitrate by the help of onion extract. Through elaborate screening process involving number of plants, we observed that onion (Allium cepa) was potential candidate for synthesis of silver nanoparticles. We also study the antibacterial property of silver nanoparticles toward E.coli. Although, several previous reporter have studied the antibacterial activity of chemically synthesized silver nanoparticles but here we study the biologically (using onion extract) synthesized silver nanoparticles.

II. EXPERIMENTAL DETAILS

A. Materials

For the synthesis of silver nanoparticles, we used onion (Allium cepa) as an extract for reducing and capping agent, which is purchased from market and silver nitrate ($AgNO_3$) from Lakshmi chemicals, Chennai. Lyophilized culture of E.coli, Luria bertani, and Nutrient and Macconky media were used here and supplied by our college laboratories.

B. Synthesis of silver nanoparticles

For the synthesis of silver Nan particles, silver nitrate ($AgNO_3$) and onion extract are taken. We prepared onion extract by taking 50g of fresh onion, which was crushed finely. The extract has to be free from onion debris. The extract was then mixed with 100ml deionized water in a conical flask and the mixture was boiled for 8 min.1ml of onion extract was mixed to 10ml of 0.1M concentration aqueous 99% pure $AgNo_3$ with constant stirring at 60-65 degree Celsius. The color change was observed.

C. CHARACTERIZATION

1) UV-Vis Spectroscopy

Ultraviolet- Visible spectrophotometry (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. [5-7] This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. The above-prepared solution was subjected to UV-vis spectroscopy in our laboratory. Water was used as blank solution.

2) Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device. The TEM analysis was carried out in cancer institute, Chennai.

D. Bacterial Growth Curve

Since ancient times, the silver ion has been known to be effective against a broad range of microorganisms. Today, silver ions are used to control bacterial growth in a variety of medical applications. Silver-based antimicrobials use an ion exchange mechanism that slowly releases silver ions. These ions interact with the microorganisms bonding sites to prevent a wide spectrum of bacteria from reproducing. Data from silver suggest that these ions denature proteins (enzymes) of the target cell or organism by binding to reactive groups resulting in their precipitation and inactivation [8-9]. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfhydryl) groups. These and other findings imply that the interaction of silver ions with thiol groups in enzymes and proteins plays an essential role in its antimicrobial action, although other cellular components, like hydrogen bonding, may also be involved. Silver was also proposed to act by binding to key functional groups of enzymes. Silver ions cause the release of K^+ ions from bacteria; thus, the bacterial plasma or cytoplasmic membrane, which is associated with many important enzymes, is an important target site for silver ions [10]. In addition to their effects on bacterial enzymes, silver ions caused marked inhibition of bacterial growth and were deposited in the vacuole and cell wall as granules. They inhibited cell division and damaged the cell envelope and contents of bacteria. Bacterial cells increased in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibited structural abnormalities. Finally, silver ions interact with nucleic acids; they interact preferentially with the bases in DNA rather than with the phosphate groups [11]. All bacteria use an enzyme as a form of 'chemical lung' in order to metabolize oxygen. Silver ions cripple the enzyme and stop the take up of oxygen. This effectively suffocates any bacteria, killing it within 6 minutes and leaving surrounding tissue or material unaffected [12]. Silver ions denature proteins (enzymes) of the target cell or organism by binding to reactive groups resulting in their precipitation and inactivation. Silver inactivates enzymes by reacting with the sulfhydryl groups to form silver sulfides. Silver also reacts with the amino-, carboxyl-, phosphate-, and imidazole-groups and diminishes the activities of lactate dehydrogenase and glutathione peroxidase. Bacteria (gram+ and gram-) are in general affected by the oligodynamic effect [13].

We have inoculated fresh colonies from agar media into 10ml of broth (Luria Bertani) media. The media is supplemented with 10-50µg/ml silver nanoparticles and bacterial cultures were incubated at 37degree Celsius with continuous shaking at 150rpm. The growth of E.coli in broth media was indexed by measuring the optical density (at λ=600nm) at regular intervals using UV-Vis spectrometer. Whereas control does not contain any exposure of silver nanoparticles synthesized from the onion extract.

III. RESULTS & DISCUSSION

A. UV-Vis Spectrophotometry

The Formation of metal nanoparticles by reduction of the aqueous metal ions during exposure of onion (Allium cepa) extract is easily followed by UV–Vis spectroscopy. UV-Vis absorption spectrum of silver nanoparticles in the presence of onion extract is shown in figure 1. The Surface Plasmon band in the silver nanoparticles solution remains close to 413nm throughout the reaction period, suggesting that the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV-Vis absorption spectrum.

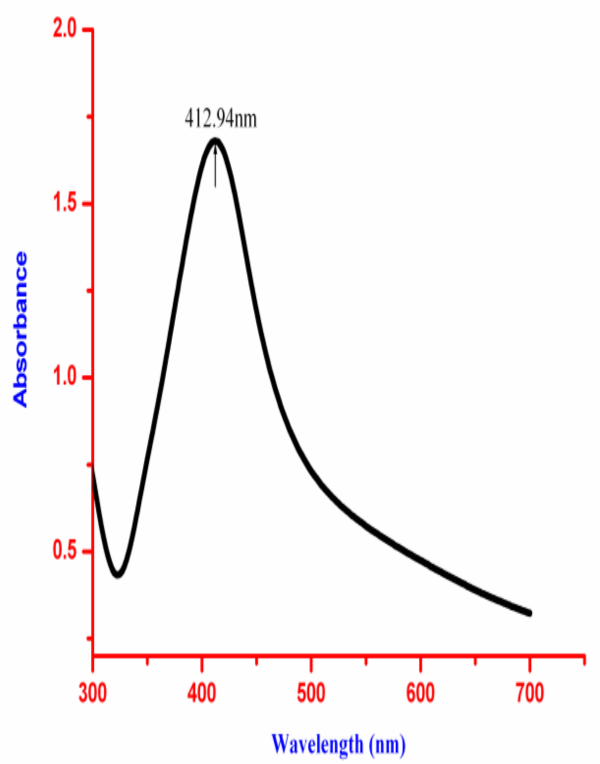

Figure 1. UV-Vis absorption spectra of Silver nanoparticles synthesized by exposure of onion broth with 0.1M silver nitrate.

B. *TEM analysis of Silver nanoparticles*

The silver nanoparticles synthesized by the help of onion extract were scanned using TEM from which we can conclude that the average mean size of silver nanoparticles was 40 nm and seems to be spherical in morphology as shown in figure 2. Thus the transmission electron microscopy gave a detailed descriptive image of the silver nanoparticles synthesized with their structural details and their size.

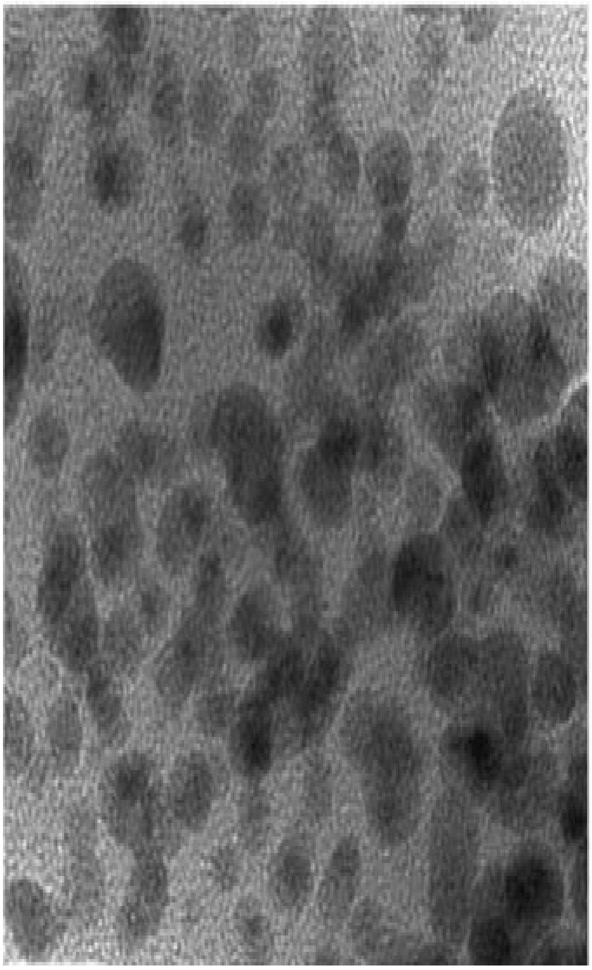

Figure 2. TEM micrographs of silver nanoparticles synthesized from onion extract.

C. *Analysis of Growth Curve*

It was well known that silver nanoparticles exhibits strong antibacterial activity due to their well-developed surface which provides maximum contact with the environment. Here, antibacterial effect of silver nanoparticles were studied by using optical intensity as function of time for 25 hours with varying concentration of silver nanoparticles. From figure 3, we can conclude that in the absence of silver nanoparticles there is increase in optical density showing bacterial growth but as the concentration of silver nanoparticles increases, there is reduction in the bacterial growth of E.coli. An analysis by keeping onion was performed in which growth of *e.coli* was appreciably high compared to the solution containing no traces of silver. This proves that silver has anti-bacterial effect on e-coli thus pertaining to the growth inhibition of the bacterium e-coli.

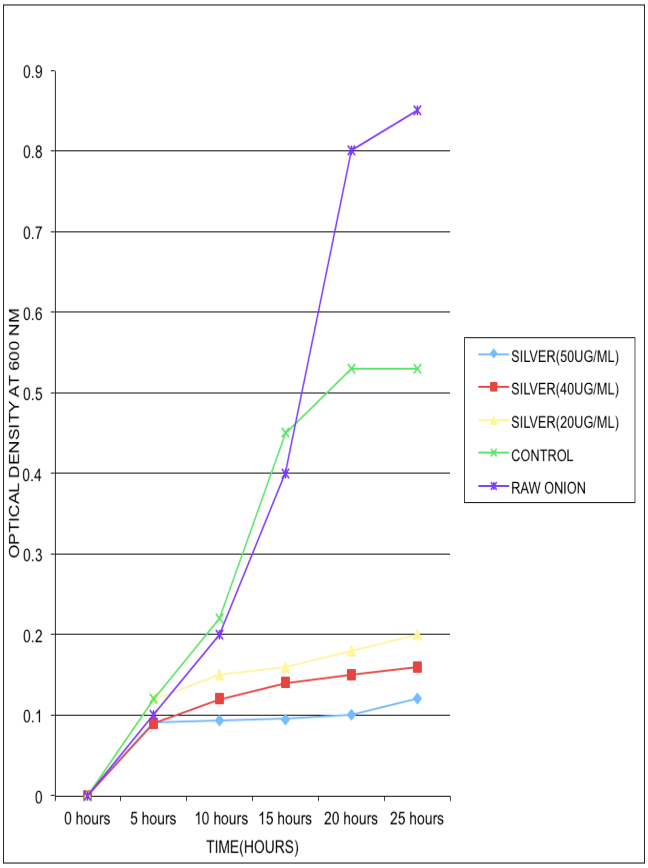

Figure 3. Effect of silver nanoparticles on E.coli growth rate.

IV. CONCLUSIONS

We used onion (Allium cepa) extract as a reducing and capping agent to minimize the serious environmental pollution problems. Silver nanoparticles were synthesized by this method having 40 nm average mean size. The preparation of nanoparticles by using onion extract has desired quality with low cost and convenient methods. These nanoparticles at concentration 50microgram/ml were showed complete antibacterial activity against E.coli.

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