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Biosynthesis of Silver Nanoparticles using Lemon Extract and its Antibacterial Activity

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Abstract

The physical and chemical methods employed in production of silver nanoparticles are expensive and the reagents used are harmful. This project deals with the production of silver nanoparticles using biological methods. Silver nitrate is reduced by biological extracts. Thus, the production is mainly dependent on the reducing ability of the plant used. Lemon extract has been used for the synthesis of Silver nanoparticles. The characterization of the silver nanoparticles was done using UV-Spectra, AFM and SEM. SEM analysis showed that the size of nanoparticles obtained by lemon extract and silver nitrate solution was around 75nm. Further analysis was carried out by varying parameters- pH, temperature, concentration of $AgNO_3$ and concentration of stabilizer. The nanoparticles obtained were then used to find the antibacterial activity. The silver nanoparticles were effective in inhibition of bacteria like E.coli and Bacillus subtilis. The diameter of inhibition zones formed by using silver nanoparticles was around 3mm.

Keywords: *Silver nanoparticles, Lemon extract, Biosynthesis, Antibacterial activity*

Introduction

Nanoparticles are those whose size ranges 1 nm to 100 nm. They come in various sizes and shapes, such as triangular, spherical, irregular, etc. Oftentimes, silver oxide is present, since the surface to bulk ratios of these is large. Nanoparticles have large surface area to volume ratio, which makes their properties different from bulk materials. This ratio is what is responsible for several of the properties that are unique to nanoparticles. Also, at that size, quantum effects pre-dominate. Their electrical, optical properties differ widely from those of bulk materials of the same substance. Silver too shows the same difference in several properties at the nano – level. These properties make nanoparticles very suitable for several applications across various fields, including the field of Biology. For example, it is used for antimicrobial activity, cell tagging, drug delivery, etc (Jun Sung Kim *et al*., 2007).

In the traditional methods, synthetic reducing agents are used, such as ethanol, hydrazine hydrate, sodium borohydrate, formaldehyde and ethylene glycol (Sahoo *et al*., 2009). Similarly, biological reducing agents such as flavonoids, tannins and vitamin C can be used instead of chemical ones, so as to solve problems of price and pollution, among others. Silver nanoparticles are produced by the reduction of silver ions to neutral silver atoms. This is done by the reduction of silver ions by a reducing agent (Kaushik *et al*., 2010).

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The microbial enzymes or the plant phytochemicals with anti-oxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles (Balprasad *et al*., 2005). Methods employed biosynthesis of metal nanoparticles are eco-friendly, biocompatible, nontoxic and clean (Sharma & Yangard, 2009).

The stability of silver nanoparticles depend on the kind of water it is suspended in. if the water is saline, that is, rich in calcium and magnesium ions, agglomerates are formed faster. However the critical coagulation concentration (CCC) in case of organic solution is high, which means chances of coagulation is lower (Delay *et al*., 2011). In the study Sodium Dodecyl Sulfate (SDS) was added to stabilize the silver nanoparticles formed in $AgNO_3$ solution.

Silver has long been used as an antimicrobial agent, though the mechanism is understood recently. Silver forms stable bonds with sulfur with sulfur in proteins that have –SH group (thiol) in the cell. This in turn affects ion transport and trans-membrane energy generation (Klueh *et al*., 2000). In the study silver nanoparticles were used to check the antibacterial activity.

Experimental Method

Fresh fruits of lemon were taken, washed, cut, and squeezed and the extract was taken. This extract was then filtered using Whatman filter paper. The mixture was boiled for 10 minutes and decanted. 10ml of the above prepared extract was taken in 5 separate flasks. 50 mL of aqueous $AgNO_3$ solution (1mM, 2mM, 3mM, 4mM, and 5mM) was added to the respective flasks drop by drop with continuous stirring for reduction of Ag+ ions at room temperature under dark conditions. 3mL of 8%w/v SDS was added to the above solution. The conical flasks were sealed using cotton plugs and observed for color change (Prathna *et al*., 2011)].

Addition of stabilizing agent (Sodium-Do-DecylSulphate)

10ml of the filtered lemon extract of was taken in separate flasks. 50ml of aqueous $AgNO_3$ solution (5mM) was added to the respective flasks drop by drop with continuous stirring for reduction of Ag+ ions at room temperature under dark conditions.1 to 5ml of 8%w/v SDS was added to the silver nanoparticle solution prepared using 5mM $AgNO_3$. Solutions were observed for aggregate formation and the optimum volume of SDS was determined.

Effect of temperature: 5ml of silver nanoparticle solution prepared at room temperature and 40°C in separate conical flasks. 3mL of 8%w/v SDS was added to each flask. The conical flasks were sealed using cotton plugs and observed for color change.

Effect of pH: 10ml of the filtered lemon extract was taken 8 separate conical flasks. pH of the extract was set from 3 to 10. To the above, 50mL of 1mM silver nitrate solution was added drop by drop with constant stirring at 40°C. 3mL of 8%w/v SDS was added. The flasks were sealed using cotton plugs and observed for color change.

Effect of time: 5ml of silver nanoparticle solution prepared at room temperature and 40°C in separate conical flasks. 3mL of 8%w/v SDS was added to each flask. The conical flasks were sealed using cotton plugs and observed for color change after one hour, after one day and after 3days.

Characterization

Characterization of silver nanoparticles is done using UV-Visible spectrometer, Atomic force microscope and Scanning electron microscope. The procedure followed for each characterization is described below.

UV-Visible spectroscopy analysis: Change in color was visually observed in the silver nitrate solution added to the biological extracts. The bio-reduction of precursor silver ions was monitored by sampling of aliquots (1 ml) at different time intervals. Absorption measurements were carried out on UV-Visible Spectrophotometer at a resolution of 1 nm. UV Visible analysis of several weeks old samples was also carried out to check the stability of silver nanoparticles.

Atomic force microscope: Small volume of sample was spread on a well-cleaned glass cover slip surface mounted on the AFM stub, and was dried with nitrogen flow at room temperature. Images were obtained in tapping mode using a silicon probe cantilever of 125µm length, resonance frequency 209-286 kHz, spring constant 20-80 nm-1 minimum of five images for each sample were obtained with AFM and analyzed to ensure reproducible results.

Scanning electron microscope: Scanning Electron Microscopic (SEM) analysis was done by the following method. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper. Then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min.

Results and discussions

Effect of SDS dosage, temperature and pH on the production of silver nanoparticles using lemon extracts is described below.

It was observed that minimum 3ml of (8% w/v) SDS solution needed to the 5mM $AgNO_3$ solution to avoid clumping of silver nanoparticles. Production was tried at room temperature and 40^{o}C using lemon extract. Lemon extract showed best results at 40^{o}C. The reason behind this maybe that lemon is a rich source of ascorbic acid. pH was varied with constant volume of $AgNO_3$ with lemon as the plant extract. It was observed that below the pH of 4 and at pH 7, there was no clumping of particles. Production stages were observed at various time intervals. Lemon extract was very fast, and showed results within a day. The production of silver nanoparticles is indicated by a color change to a golden yellow color.

UV-VIS Spectroscopy Results

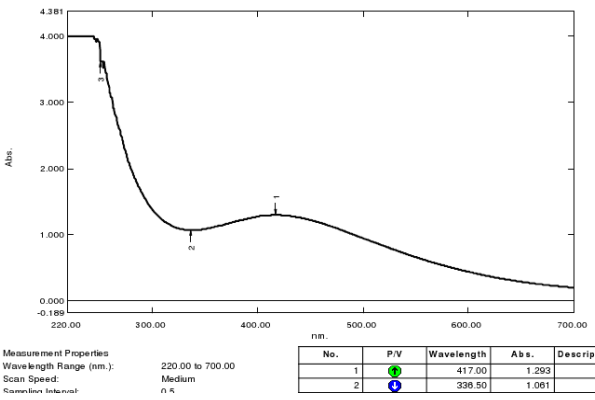


Fig. 1: UV spectrum of Ag nanoparticles obtained by 1mM AgNO3 and Lemon extract

Stock solution of $AgNO_3$ with different concentrations (1mM to 5mM) was prepared and 10ml of lemon extract added to the solution. UV Results are obtained for these samples and shown in figures from 1 to 5.

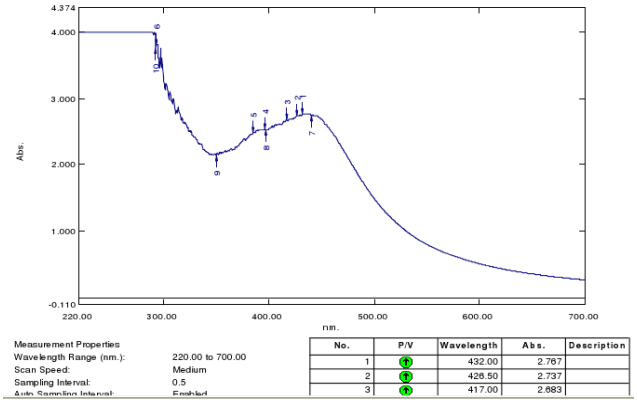


Fig. 2: UV spectrum of Ag nanoparticles obtained by 2mM AgNO3 and Lemon extract

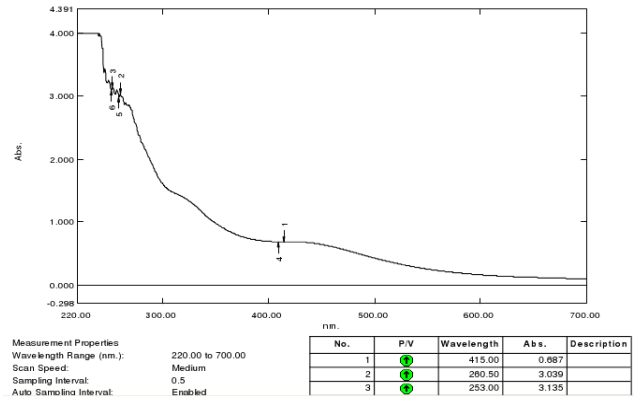


Fig. 3: UV spectrum of Ag nanoparticles obtained by 3mM AgNO3 and Lemon extract

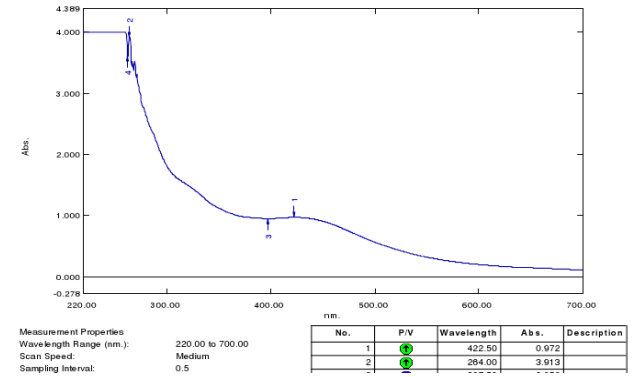


Fig. 4: UV spectrum of Ag nanoparticles obtained by 4mM AgNO3 and Lemon extract

The absorption peaks obtained for these samples are in the range of 400-430nm which is prescribed for Ag nanoparticles. Hence the results obtained ensure the existence of Ag nanoparticles in the solution.

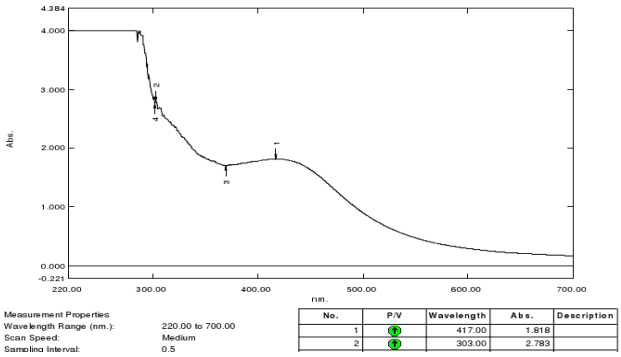


Fig. 5: UV spectrum of Ag nanoparticles obtained by 4mM AgNO3 and Lemon extract

AFM Images

The solution containing Ag nanoparticles obtained from 5mM AgNO3 and lemon extract was tested under AFM. Results are shown in figure 6 to 8.

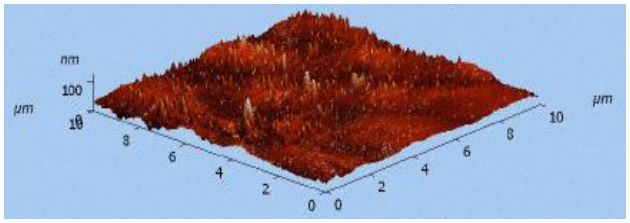


Fig. 6: AFM image of Ag nanoparticles obtained by 5mM AgNO3 and Lemon extract

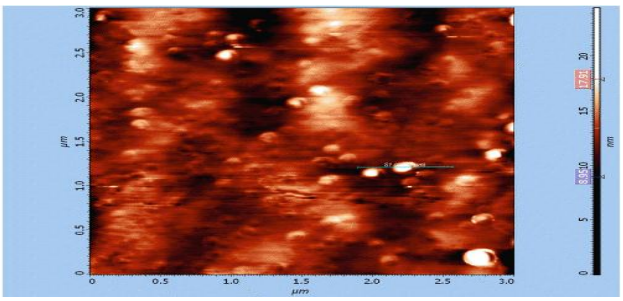


Fig. 7: AFM image of Ag nanoparticles obtained by 5mM AgNO3 and Lemon extract

Fig. 8: Line profile of Ag nanoparticles obtained by 5mM AgNO3 and Lemon extract

The white peaks seen in figure 6 and 7 indicated the Silver nanoparticles. Line profile showed that the particle in the image is approximately 12 nm height and 100 nm in width.

SEM Images

The solution containing was tested under Scanning Electron Microscope image of Ag nanoparticles obtained from 5mM AgNO3 and lemon extract is shown in the figure 9.

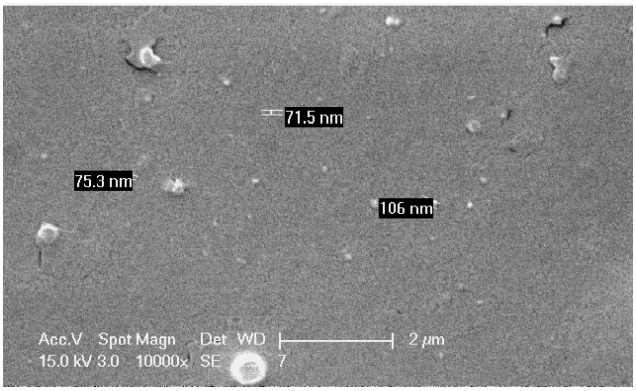
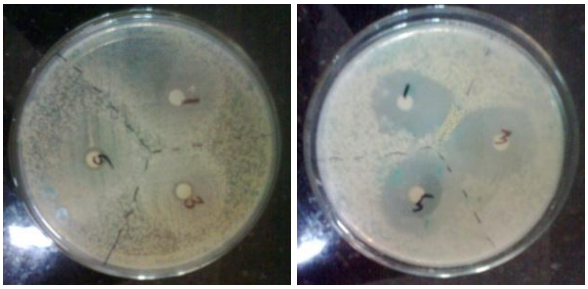


Fig. 10: SEM image of Ag nanoparticles obtained by 5mM AgNO3 and Lemon extract

The results by SEM indicate that the nanoparticles consist of agglomerates of small grains with diameter approximately 75nm.

Antibacterial Activity

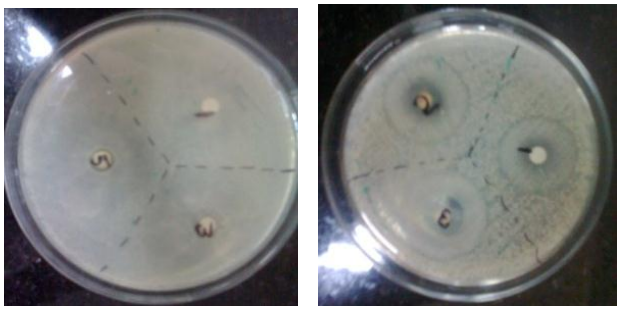
Among inorganic antibacterial agents, Silver has been employed most extensively since ancient times to fight infections and control spoilage. The antibacterial activity of biologically produced was tested on different bacteria *E.coli* and *Bacillus subtilis.*The inhibition tests were carried out by disc diffusion method. Growth inhibition of bacteria by Ag nanoparticles was compared with $AgNO_3$ solution and found that Ag nanoparticles were competent enough with $AgNO_3$ solution.



(A) (B)

Fig.11 : (A) - *E.coli* treated with 1mM, 3mM and 5mM (A) AgNO3 discs(1mM, 3mM and 5mM), (B) - *E.coli* with Ag nanoparticles discs(1mM, 3mM and 5mM)

Figure 11 shows the inhibition zones for *E. Coli* bacteria treated with $AgNO_3$ solution and Ag nanoparticles. E.Coli was the gram negative bacterium is equally sensitive to 1mM (3.5mm) and 3mM (3.5mm) and less sensitive to 5mM (3mm) $AgNO_3$. The result obtained for Ag nanoparticles (around 3mm) was similar to inhibition zones shown by $AgNO_3$ solution.



(A) (B)

Fig.11 : (A) - *Bacillus subtilis* treated with 1mM, 3mM and 5mM (A) AgNO3 discs(1mM, 3mM and 5mM), (B) - *Bacillus subtilis* with Ag nanoparticles discs(1mM, 3mM and 5mM)

Figure 12 shows the inhibition zones for *Bacillus subtilis* bacteria treated with $AgNO_3$ solution and Ag nanoparticles. *Bacillus subtilis* was the gram positive bacterium is equally sensitive to 1mM (3mm) and 5mM (3mm) and more sensitive to 3mM (3.7mm) $AgNO_3$. The result obtained for Ag nanoparticles (around 3mm) was similar to inhibition zones shown by $AgNO_3$ solution.

Conclusions

Silver nanoparticles are produced by the reduction of silver ions to colloidal silver. In this study lemon extract was used as reduced agent which is rich in ascorbic acid. UV Spectrum, SEM and AFM results confirmed the formation of silver nanoparticles. The UV spectral peaks for silver nanoparticles range from 400 to 430nm. Two bacteria were used to test the antibacterial activity by disc diffusion method. It was found that effective for reducing the growth of bacteria.

References

[1]. Kaushik N. Thakkar, Snehit S. Mhatre, Rasesh Y. Parikh (2010). "Biological Synthesis of Metallic Nanoparticles" Nanomedicine: Nanotechnology, Biology and Medicine **6** (2): 257-262

[2]. P.K. Sahoo, S.S. Kalyan Kamal, T. Jagadeesh Kumar, B. Sreedhar, A.K. Singh & S.K. Srivastava(2009). "Synthesis of Silver Nanoparticles using Facile Wet Chemical Route". *Defence Science Journal*. **59**(4): 447-455

[3]. Delay M, Dolt T, Woellhaf A, Sembritzki R, Frimmel F H(2011). " Interaction and Stability of Silver Nanoparticles in the Aqueous Phase: Influence of Natural Organic Matter

(NOM) and Ionic Strength " J Chromatogr A.1218(27): 4206-12.

[4]. Prathna TC, Chandrasekaran N, Raichur AM, Mukherjee A. (2011). "Biomimetic Synthesis of Silver Nanoparticles by *Citrus limon*(lemon) Aqueous Extract and Theoretical Prediction of Particle Size". *Colloids Surf B Biointerfaces*. **82**(1): 152-159.

[5]. Balaprasad Ankamwar, Chinmay Damle, Absar Ahmad, MuraliSastry (2005). "Biosynthesis of Gold and Silver Nanoparticles Using *Emblica officinalis* Fruit Extract, Their Phase Transfer and Transmetallization in an Organic Solution"

[6]. Klueh U, Wagner V, Kelly S, Johnson A, and Bryer J D (2000) " Efficacy of silver-coated fabric to prevent bacteril colonization and subsequent device-based biofilm formation" J Biomed Mater Res 53: 621-631.

[7]. Sharma VK and Yangard RA (2009) "Green Synthesis and their antimicrobial activities" J Colloid Interface Sci. 9, 83-96.

[8]. Jun Sung Kim, EunyeKuk, Kyeong Nam Yu, Jong-Ho Kim, Sung Jin Park, BS, Hu Jang Lee,So Hyun Kim, Young Kyung Park, Yong Ho Park,Cheol-Yong Hwang,Yong-Kwon Kim, Yoon-Sik Lee, Dae Hong Jeong, Myung-Haing Cho (2007). "Antimicrobial Effects of Silver Nnaoparticles" *Nanomedicine*. **3**: 95-101.