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Green Synthesis of Silver Nanoparticles and its Antimicrobial Activity

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Abstract

Silver Nanoparticles (Ag NPs) are being used to reduce as antimicrobials. These particles can be incorporated in materials and cloth exposes them sterile. Aqueous silver ions can be reduced by aqueous extract of plant parts to generate particularly stable Ag NPs in water. Apart from being are environmentally friendly process, use of Saraca asoca leaves extract might add synergistic antibacterial effect of Saraca asoca leaves to the biosynthesized nanoparticles. In this paper the biosynthetic production of Ag NPs by aqueous extract of Saraca asoca leaves and its bactericidal effect against *E. coli* were studied. Ag NPs were synthesized by 3h heating of Saraca asoca leaves extract (10% w/v) and 2 mM $AgNO_3$ solution in 1:3 mixing ratio at room temperature. The synthesized particles were characterized by UV visible spectroscopy, transmission electron microscopy. The effect of temperature, concentration of Saraca asoca extract and $AgNO_3$ solution on the size of silver nanoparticle has been studied. The antibacterial property of the nanoparticles against *E. coli* was also investigated.

Keywords : *Antibacterial and Biosynthesis; E. coli; Saraca asoca leaves; Silver nanoparticles*

1. INTRODUCTION

Metal nanomaterials may provide solutions to environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment (Dahl et al. 2007; Hutchison 2008; Sharavanakumar et al. 2012). Synthesis of noble metal nanoparticles for applications such as catalysis, electronics, optics, environmental, and biotechnology is an area of constant interest (Hussian et al. 2003; Burleson et al. 2005; Cheng 2005; Obare et al. 2005; Yuan 2005; Masciangioli et al. 2003; Albrecht et al. 2006). Metal nanoparticles have a surface plasmon resonance absorption in the UV-Visible region. The surface plasmon band arises from the coherentexistence of free electrons in the conduction band due to the small particle size (Burda et al. 2005). The band shift is dependent on the particle size, chemical surrounding, adsorbed species on the surface, and dielectric constant (Mulvaney 1996). A unique characteristic of these synthesized metal particles is that a change in the absorbance or wavelength gives a measure of the particle size, shape, and interparticle properties (Knoll et al. 1999). The functionalized, biocompatible and inert nanomaterials have potential applications in cancer diagnosis and therapy (Sengupta et al. 2005; Brigger et al. 2004; Alivisatos 2004; Gao et al. 2004; Singh et al. 2008). Silver compounds have also been used in the medical field to treat burns and a variety of infections. Several salts of silver and their derivatives are commercially employed as antimicrobial agents (Murray et al. 1965). The increasing demand must be accompanied by "green" synthesis methods. Metal nanoparticles can be prepared by physical and chemical methods; the chemical approach, such as chemical reduction, electrochemical techniques, and photochemical reduction is most widely used (Chen et al. 2001).

The green synthesis of Ag NPs involves three main steps, which must be evaluated based on green chemistry perspectives, including (1) selection of as antimicrobial agents (Murray et al. 1965). The increasing demand must be accompanied by "green" synthesis

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methods. Metal nanoparticles can be prepared by physical and chemical methods; the chemical approach, such as chemical reduction, electrochemical techniques, and photochemical reduction is most widely used (Chen et al. 2001). The green synthesis of Ag NPs involves three main steps, which must be evaluated based on green chemistry perspectives, including (1) selection of solvent medium, (2) selection of environmentally benign reducing of agent, and (3) selection of nontoxic substances for the Ag NPs stability (Raveendran et al. 2003; Tripathi et al. 2009). The Green synthesis Ag NPs includes Polysaccharide method, Tollens method, Irradiation method, Biological method and Polyoxometalates method.

In this paper we study the synthesis of Ag NPs, reducing Ag+ ions present in the aqueous solution of silver nitrate by the help of Saraca asoca leaves extract. Through elaborate screening process involving number of experiments, we observed that Saraca asoca leaves extract was potential candidate for synthesis of Ag NPs. We also study the antibacterial property of Ag NPs toward E. coli.

2. EXPERIMENTAL

2.1 Collection of plant material

Saraca asoca plant leaves were collected from the small village Chilka situated in Latur district,

Fig 1. : Saraca asoca Plant leaves

Maharashtra (India). The leaves brought were washed thrice with tap water to remove all the dirt & then was washed once with distilled water. These washed leaves were then air dried for about 10 days they were then ground to fine powder using grinder. This fine powder was kept in air tight container to prevent moisture contact. This powder was further used for making plant extract.Saraca asoca is commonly known as Ashoka it is a rain-forest tree. Its original distribution was in the central areas of the Deccan plateau, as well as the middle section of the Western Ghat in the western coastal zone of the Indian Subcontinent.

2.2 Preparation of Plant Extract

5 g of powdered plant leaves was weighed & was added to 50 ml of distilled water in a conical flask. Mixture was then boiled in water bath for 10 min. After removing the flask containing mixture from the water bath, the mixture was cooled. It was then filtered through 2 layers of muslin cloth, supernatant obtained was centrifuged at 2500rpm for 10 min to remove traces if any of plant material. This clear supernatant was then used further as plant extract for synthesis of Ag NPs.

2.3 Synthesis of Ag NPs

1ml of plant extract of pH 6 was taken in a conical flask. 4ml of 2mM silver nitrate was added into it& the mixture was diluted with 5ml of distilled water making the total volume to 10ml. This mixture was kept on magnetic stirrer for 1 hr with medium stirring speed and temperature at 60ºC. Color change was noted. After 1 hr mixture was taken from the stirrer and was cooled to room temperature the mixture was then centrifuged at2500rpm for 5 min, here the filtrate acts as reducing and stabilizing agent for 2mM silver nitrate. Supernatant was obtained and Optical density was taken on UV-VIS spectrophotometer to observe for maximum absorption peak.

3. CHARACTERIZATIONS

3.1 UV-Vis spectral analysis

The reduction of pure Ag+ ions was monitored

by measuring the UV-VIS spectrum of reaction medium after 1h. Spectral analysis was done using UV-VIS spectrophotometer (Double Beam spectrometer systronics 2230) in the spectral range of 400-800nm.

3.2 SEM analysis of Ag NPs

In this research work SEM analysis was done to characterize mean particle size and morphology of synthesized Ag NPs.

4. ANTIBACTERIAL ACTIVITY STUDY

Antibacterial activity of the synthesized Ag NPs was determined using the agar well diffusion assay method. Approximately 20ml of molten and cooled media (Nutrient agar) was poured in 3 sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The bacterial test organisms were grown in Nutrient Broth for 24h. A 100ml Nutrient Broth culture of each bacterial organism was used to prepare bacterial lawns. Agar wells of 5mm diameter were prepared with the help of a stainless steel cork borer.

Two wells were prepared in the agar plates. The wells were labeled as A, B. A well was loaded with 30µl of Ag NPs and B was loaded with 30µl of control (plant extract). In this way all the three plates speeded with different organisms was loaded. The plates containing the bacterial and Ag NPs were incubated at 37ºC. The plates were examined for evidence of zones of inhibition which appear as a clear area around the wells. The diameter ofsuch zones was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

4. RESULTS AND DISCUSSION

Figure 2, shows optical photograph of the color change in the colloidal solution of nanoparticals reduced by *Saraca asoca* leaf extract with 1 hr of incubation. Thus the reduction of silver ion into Ag particles during exposure to the plant extracts was followed by color change. The tube with Silver Nitrate exhibit dark yellowish brown color in aqueous solution due to Surface Plasmon Resonance phenomenon as compared to the tube with only plant extract which shows Green color.

Fig. 2 : Color change of leaf extract after 1h incubation

During the biosynthesis using aqueous extract of *Saraca asoca* leaves the color of the reaction mixture changed rapidly from green to yellowish brown on the formation of the silver nanoparticals. The appearance of typical color was due to excitation of surface Plasmon

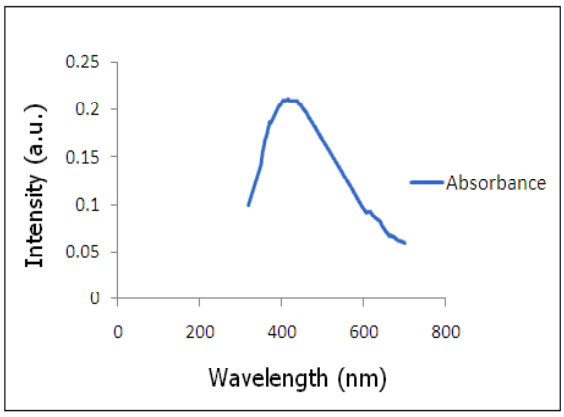

Fig. 3 : UV-Visible Spectrum of Silver Nanoparticals

vibrations, typical of silver nanoparticals. The absorption maxima for the biosynthesized nanoparticals were noted in the visible range of 300-800nm. The typical absorption maxima for Ag NPs synthesized was obtained at 420nm of intensity 0.20 a. u. which is shown in Fig.2.

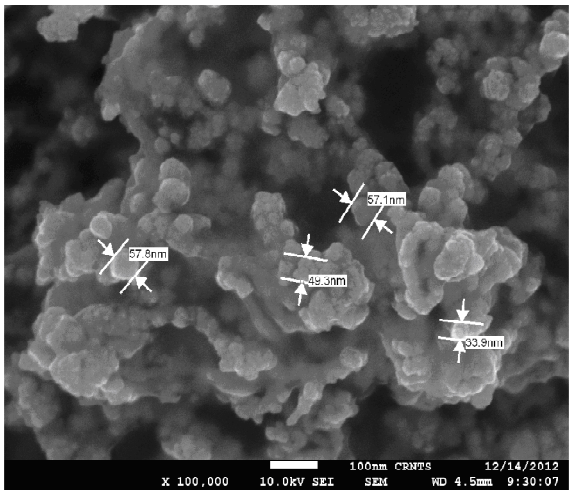

Fig. 4 : SEM images of Ag NPs formed by *Saraca asoca* leaves

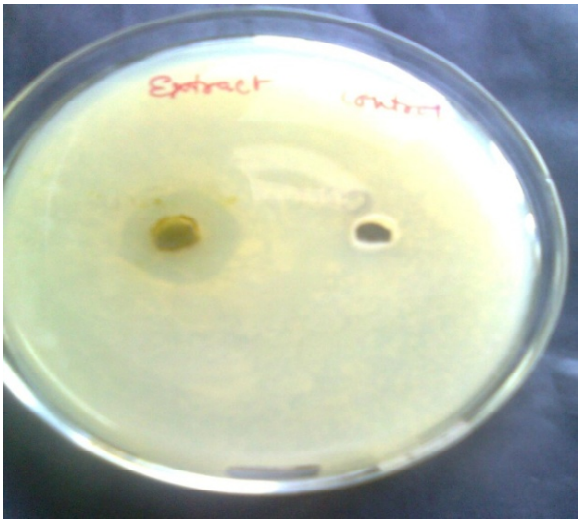

Fig. 5 : Zones of growth inhibition around disks impregnated with Ag NPs against pathogenic *E.coli*

The stability of Ag NPs is observed for 2 months and it shows an absorbance peak at the same wavelength.The SEM image of silver nanoparticals synthesized using *Saraca asoca* leaves and 2mM silver nitrate is shown below. The Ag NPs aremonodispersed with star& cuboids' shaped. The average size of the particles is between 30-60 nm. Some Ag NPs showed size of approximately below 60 nm. A few agglomerated particles are also observed due to the spectral shift.

The antibacterial activity of Ag NPs was studied against pathogenic organism *E. coli*. The zone of inhibition of microorganisms *E. Coli* was found 15 mm. The Fig. 5 shows clear inhibition zone in the well treated with Ag NPs where as in the well treated with only plant extract did not show any inhibition.

5. CONCLUSION

Ag NPs were synthesized successfully at room temperature by green synthesis and eco-friendly method. Two parameters was optimized pH and Silver Nitrate concentration for properly synthesizing Ag NPs. The detailed characterization was carried out with UV-Vis spectroscopy and SEM analysis. Visible spectral peak of Ag NPs was seen at around 420nm and the average size of the nanoparticals by SEM analysis was found to be 30-60 nm. The shape of Ag NPs was found like round and Cubical. Antibacterial potential of synthesized Ag NPs was tested against bacteria *E.coli*. The tests were performed by well diffusion method and a proper antibacterial potential was observed. Plant Extract was used as control; the well loaded with plant extract did not showed any inhibition as compared to the well containing Ag NPs. Synthesis of Ag NPs requires precise Optimization to obtain proper morphology and appropriate size, as Ag NPs agglomerate very fast.

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REFERENCES

Alivisatos P. Nat Biotechnol 22,47(2004).
Burda C, Chen X, Narayanan R, El-Sayed MA. Chem Rev 105,1025(2005).
Burleson DJ, Driessen MD, Penn RL. J Environ Sci Health A 39:2707(2005).
Chen W, Cai W, Zhang L, Wang G, Zhang L. J Colloid Interface Sci. 238,291(2001).
Dahl JA, Maddux BLS, Hutchison JE. Chem Rev 107, 2228(2007).
Gao XH, Cui YY, Levenson RM, Chung LWK, Nie SM. Nat Biotechnol 22,969(2004).
Hussain I, Brust M, Papworth AJ, Cooper AI. Langmuir 19,4831(2003).
Hutchison JE. ACS Nano 2:395 (2008).
Knoll B, Keilmann F. Nature 399,134(1999).
Mulvaney P. Langmuir 12,788(1996).
R. G. E. Murray, P. Steed, H. E. Elson, J.Can Microbiol. 11,547(1965).
Sengupta S, Eavarone D, Capila I, Zhao GL, Watson N, Kiziltepe T, Nature 436:568(2005).
Sharavanakumar, A. P., Vanitha, J., Venkateswaran, K., Srikanth Reddy, Simon, K.T., Jopsy and Karthikeyan, D., Antibacteriaal Activity of Methanalic extrat of thiespesia Populnea (Malvaceae) flower, J. Environ. Nanotechnol., 1(1), 50-52 (2012).
Singh M, Singh S, Prasad S, Gambhir IS. Dig J Nanomater Biostruct 3,115(2008).