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Indian
Journal of Advances in
Chemical Science

Indian Journal of Advances in Chemical Science 2 (3) (2014)171-177

Green synthesis of Silver Nanoparticles from *Achyranthes Aspera* Plant Extract in Chitosan Matrix and Evaluation of their Antimicrobial Activities

V. Durga Praveena[1*], K. Vijaya Kumar[2]

[1]*Dept. Of Chemistry, GDC, Palakollu-Wg.Dst., Andhra University, Andhra Pradesh, India.*
[2]*Centre for Nano Technology, Dept. Of Physics, K L University, Guntur-522502, Andhra Pradesh-India.*

Received 20th January 2014; Revised 30th May 2014, Accepted 20th June 2014.

ABSTRACT
The exploitation of various plant materials for the biosynthesis of nanoparticles is considered a green technology because it does not involve any harmful chemicals. The present study reports the synthesis of silver nanoparticles using Achyranthes aspera plant extract. These AgNPs were embedded in chitosan (CS) biopolymer to protect against nanoparticle aggregation. The resulting CS-AgNPs were characterized by UV-Visible and FT-IR spectroscopy. The formation of AgNPs whose relative absorbance was found to be in between 425 nm to 435 nm was justified by UV-VIS spectroscopy. Further these biologically synthesized nanoparticles exhibited a tremendous antibacterial activity against E.coli and Staphylococcus aureus.

Key words: *Antimicrobal activity, Green synthesis, Achyranthes aspera, silver nano particles.*

1. INTRODUCTION

There is an increasing commercial demand for nano particles due to their wide applicability in various areas such as electronics, catalysis, photonics, optics, chemistry, energy, antibacterial activity and medicine [1-6] since they possess defined chemical, optical and mechanical properties [7-8].The most important and distinct property of nanoparticles is that they exhibit increased chemical activity due to their large surface area to volume ratios and crystallographic surface structure [9].

In nanotechnology, silver nano particles are the most prominent one. Silver nano particles are nano particles of silver, i.e. silver particles of between 1 nm and 100 nm in size and have attracted intensive research interest. Ag nano particles can be synthesized using various methods: chemical, electrochemical [10], γ- radiation [11], photochemical [12], laser ablation [13], bio-based[14] protocol etc. The bio-based protocol is the most important and eco-friendly production method [15].

Therefore, there is a growing need to develop eco-friendly processes for nanoparticles synthesis without using toxic chemicals. Biosynthetic methods employing plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods. The use of plant materials for the synthesis of nanoparticles could be more advantageous, because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures. Several plants have been used for efficient and rapid extracellular synthesis of silver and gold nanoparticles such as Alfalfa [16,17], Aloe Vera [18], Cinnamomum camphorra [19], Emblica officianalis [20], Carica papaya [21], Parthenium hysterophorus [22], Diopyros kaki, Eucalyptus hybrid [23], Hibiscus rosasinensis [24], Capsicum annum [25], Cissus quadrangularis, Pelargonium graveolens [26], Medicagosativa [27], Lemongrass [28], Capsicum annuum [29], Caricapapaya [30], Coriandrum sp [31], Boswellia ovalifoliolata [32], Tridax procumbens, Jatropha curcas, Solanum melongena, Datura metel, Citrus aurantium [33], and many weeds [34,35], Ocimum sanctum (Tulsi) and Tamarind [36] have been reported, the potential plants as biological materials for the synthesis of nanoparticles is yet to be fully explored.

Achyranthes aspera (Amaranthaceae) a wild herbaceous plant is widespread in the world as a weed, in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. In the northern part of India it is known as a medicinal plant in different systems like antibacterial, antifungal, anti-oedematic, anti-ulcerogenic, anti-inflammatory, and anti-diarrhoeic ones. The plant has been widely acknowledged for the treatment of diarrhoea, snake bites, dog bites and rheumatic pain in traditional medicine [37-39]. In this study the antibacterial

*Corresponding Author:
Email: veena.nano@gmail.com,
kvk64nano@kluniversity.in

activity of Ag nanoparticles synthesized using the plants of *Achyranthes aspera* were assessed. According to previous investigations water-oluble components play significant role in the reduction of silver ions. These components also proved to be an effective capping and stabilizing agents for silver nanoparticles [40].

Inorder to enhance the stability and to avoid the aggregation of silver nano particles requires the use of stabilizers in the form of surfactants, nano particles are also stabilized by polymers either synthetic polymers (i.e. poly acrylo nitrile, PVP, PVA) or bio polymers (i.e. starch [41] , chitosan [42], cyclodextrins [43] and bacterial biomass [44]). The use of synthetic polymer lead to environmental toxicity, biological hazards. This includes the use of biopolymers. Chitosan, a positively charged polysaccharide biopolymer derived from chitin , exhibits a no. of interesting biological properties such as antimicrobial activity , chelating and poly cationic properties, biodegradability, biocompatibility, the ability to induce disease resistance in plants ,and display of stimulating or inhibiting activities toward human cells [45,46] for these reasons, chitosan has received great attention in the fields of medicine ,food, chemicals, pharmaceuticals ,and agriculture [47-50].

Hence in this context, the present work is reported for the first time synthesis of films consisting of chitosan matrices containing dispersions of Ag nano particles produced from medicinal plant extract of Achyranthes aspera. The films exhibit interesting structural, chemical and optical properties. Here we report that CS-AgNPs exhibit potent antibacterial activity against E.Coli and Staphylococcus aureus.

The nano particles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorus and sulphur containing compounds, such as DNA and protein [47], present inside the cell. The bacteriocidal properties of silver nano particles are due to the release of silver ions from the particles, which confers the antimicrobial activity [49]. Besides, the potency of the antibacterial effects corresponds to the size of the nano particle. The smaller particles have higher antibacterial activities due to the equivalent silver mass content.

2. EXPERIMENTAL

Chitosan (degree of deacetylation: 79%, molecular mass: 500,000 g/mol) was purchased from seafoods (Cochin), India. The other chemicals were analytical grade from Fischer Scientific without further treatment.

For the preparation of plant extract, the fresh leaves of achyranthes aspera (Figure 1.) were washed several times with millipole water to remove dust then it was cut into small pieces. 25g of thoroughly washed leaves were heated in 250ml of milli pole water for 15min in an Erlenmeyer flask using a water bath then the solution is filtered using whatman no.4 filter paper. The filtered leaf extract was stored in a cooled atmosphere for further use.

Figure 1. Leaves of *Achyranthes aspera*.

The silver nitrate solution was reduced using plant extract at room temperature, resulting in a dark brown solution indicating the formation silver nano particles (Table 1).

2.1. Deposition of Silver Nano particles in Chitosan matrix

The CS was prepared by the method described below: 2 g CS was dissolved in 200 ml 2% (V/V) acetic acid solution under magnetic stirring. When the solution became clear, silver nano particles, CS solutions were mixed in 2:3 ratio. Finally, films were made by casting the solution on the glass slides, dried at room temperature. A range of CS-AgNP concentrations were used to treat the bacteria and for other experiments in this study.

Table 1. Concentrations of the prepared samples.

$AgNO_3$ concentration		Plant extract
Sample solutions		
4mM	20ml	10ml
10mM	20ml	10ml
Distilled water (control)	20ml	10ml

These developed Chitosan based silver nano composites are characterized by using FTIR and UV-VIS Spectrophotometer. FTIR spectra were recorded in a Perkin Elmer Version 10.03.06. UV-VIS spectra were measured on a Systronics double beam spectrophotometer 2203 with a range of 200-700 nm.

To examine the bactericidal effect of silver nanoparticles on Gram-negative bacteria and Gram-positive bacteria , approximately 10^5 colony forming units (CFU) of E.Coli strain B and *Staphylococcus aureus* were cultured on LB agar plates supplemented with nano sized silver particles in concentrations of 10 to $100\mu g/cm^3$.

3. RESULTS AND DISCUSSION

In FTIR spectra, the spectral band for chitosan appear at 3,526 cm^{-1} (axial OH group), 3,337 cm^{-1} (N-H stretching) , 2,364 cm^{-1} (CN asymmetric band stretching) , 1,754 cm^{-1} (amide linkage), 1,673 cm^{-1} (CO band stretching), 1,523 cm^{-1} (NH angular deformation in CONH plane), 1,320 cm^{-1} (CN band stretching ,axial deformation of amino group) and 1,140-1,026 cm^{-1}(ether linkage , C-O-C band stretching).

When CS-Ag NPs(4mM) characteristic bands are observed at 3339 cm^{-1}(overlap of O−H and N−H stretching vibrations), 2885.28 cm^{-1},2154 cm^{-1} (C-H stretching), 1744.89 cm^{-1}, 1728.74 cm^{-1} ($-NH_2$ bending ,amide linkage), 1565 cm^{-1}, 1414.18 cm^{-1},1324.56 cm^{-1},1064 cm^{-1},616.42 cm^{-1} [C-C, C-O(esters and ethers) and C-O (polyols)] [26], more pronounced shift in the FTIR spectrum could be observed in the complexes (Fig. 3). The major differences are: the peak at 3526 cm^{-1} corresponding to the stretching vibration of amino group ($-NH_2$) and hydroxyl group (-OH), shifted to lower frequency (3339 cm^{-1}), and the peak of 3339 cm^{-1} becomes wider, which indicates hydrogen bonding is enhanced and may be explained as that the additive effect of water absorbed on the surface of Ag nano particles and the -OH group of CS. This suggests that NPs were capped by the polymer.

In CS-AgNPs (10 mM),(Fig. 4,) shows bands are shifted to higher frequencies i.e, 3353.51 cm^{-1} (overlap of O-H and N-H stretching vibrations), 2922.34 cm^{-1}(C-H stretching), 1744.89 cm^{-1}, 1728.74 cm^{-1}($-NH_2$ bending ,amide linkage), 1569.07 cm^{-1}, 1411.41 cm^{-1}, 1070.14 cm^{-1}, 649.17 cm^{-1}[C-C, C-O (esters and ethers) and C-O (polyols)] in comparision to CS-AgNPs (4 mM) indicates the enhanced strength of AgNPs.

UV-visible spectroscopic data of Each sample was analyzed by UV-visible spectrophotometer in the range 250-750 nm and the wavelength corresponding to maximum absorption (λ_{max}) was recorded which are identical to the characteristics of UV-visible spectrum of metallic silver nano particles. chitosan in 1% (v/v) acetic acid solutions is used as blank sample. The evolution of UV-VIS spectra is shown in Fig. 5. It is a well-known fact, that silver ions and nanoparticles are highly toxic and hazardous to microorganisms. It is found

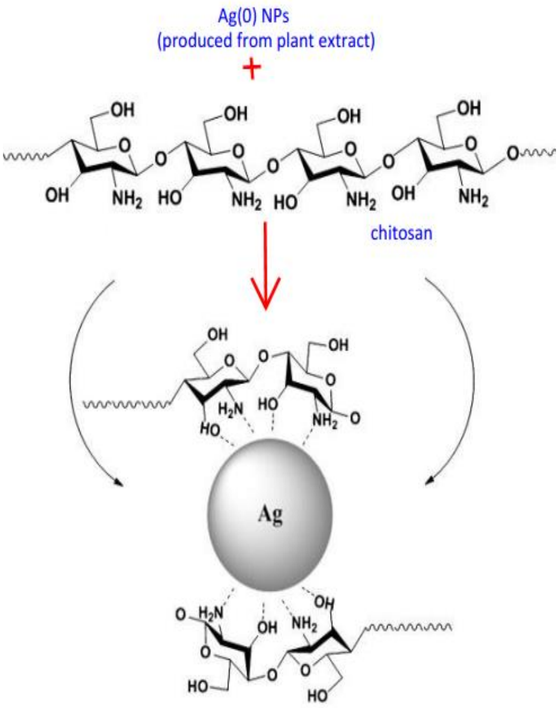

Figure 2. Schematic diagram of showing absorption of AgNPs in chitosan matrix.

out that the silver nanoparticles have many inhibitory and bactericidal effects and so its application is extended as an antibacterial agent. The antibacterial activity of silver nanoparticles is estimated by the zone of inhibition. Many different studies have shown that silver nanoparticles can affect the membrane permeability and respiratory function by attaching to cell surface. Another possibility is that silver nanoparticles not only interact with the surface of the membrane, but can also penetrate deep inside the bacteria. Another observation explains that the silver nanoparticles have relatively higher anti-bacterial activity against gram negative bacteria than gram positive bacteria, which may be due to the thinner peptidoglycan layer and presence of beta barrel proteins called porins.

For the nanoparticles produced from plant extract embedded in chitosan biopolymer exhibited a characteristic inhibitory zone 18mm, 27mm diameter for Staphylococcus aureus, where as E.Coli exhibit 17mm, 24mm diameter of zone of inhibition for 4 mM,10 mM AgNPs respectively.

The silver nanoparticles synthesized via green route are highly toxic to multidrug resistant bacteria hence has a great potential in biomedical applications. The present study showed a simple, rapid and economical route to synthesized silver nanoparticles.

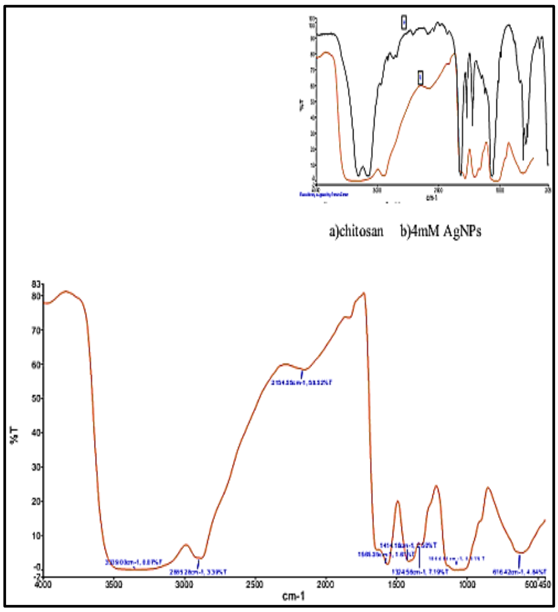

Figure 3. FT-IR Spectra of 4mM CS- AgNPs.

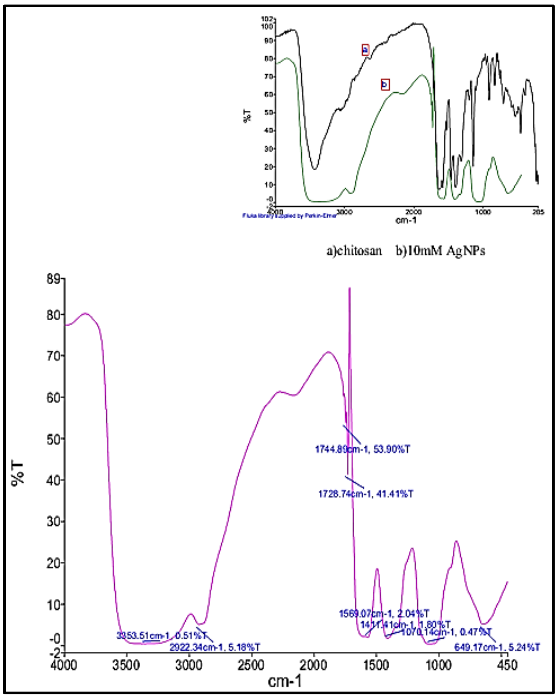

Figure 4. FT-IR Spectra of 10 mM CS- AgNPs.

4. CONCLUSION

The silver nanoparticles were synthesised using the aqueous extract of Achyranthes aspera. The green chemistry approach used in the present work on the synthesis of Ag nanoparticles is simple, cost effective and the resultant nano particles are highly stable and reproducible. Our results demonstrate the possibility of synthesizing Ag nanoparticles from the aqueous extract of Achyranthes aspera. Their antibacterial activity represents a significant advancement in the nano materials field, with realistic applications. Chitosan coated AgNPs were

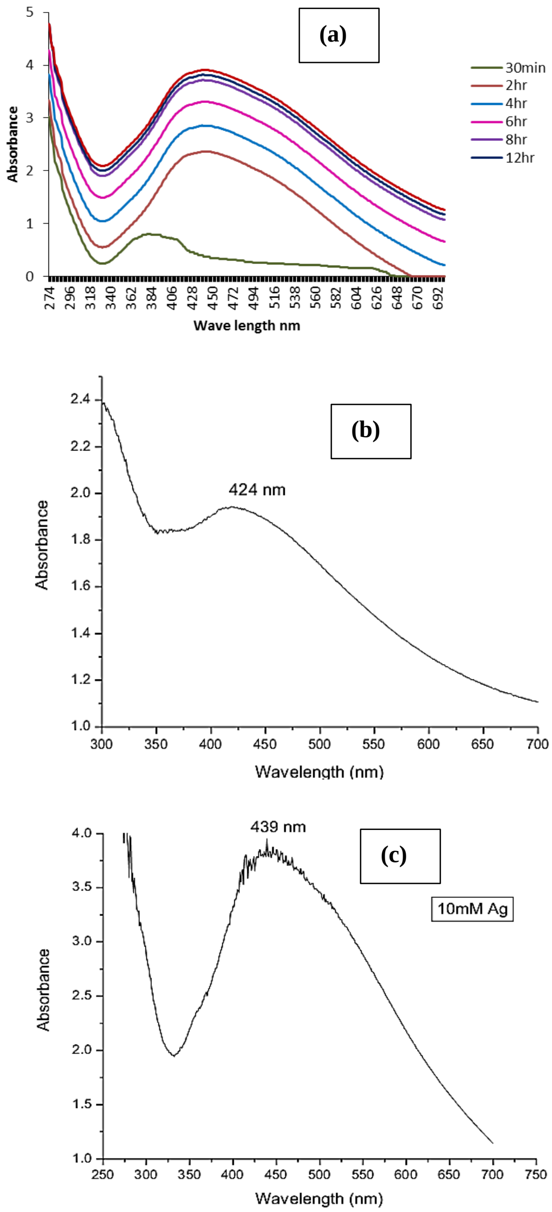

Figure 5. Evolution of UV-VIS absorption spectra (a) at different time intervals (b) 4 mM CS-AgNPs (c)10 mM CS-AgNPs.

Table 2, Values of zones of inhibition obtained by disc diffusion method.

Components	Diameter of zone of inhibition in mm	
	E. coli	S.aureus
Distilled water (control)	NZ	NZ
Silver nitrate solution	NZ	NZ
Achyranthes aspera extract	08	08
4 mM CS-AgNPs	17	18
10 mM CS-AgNPs	24	27

successfully prepared by an in situ polymerization method. These films of two different compositions were characterised using UV-VIS spectroscopy and FTIR spectroscopy and assessed for its antibacterial activity. The Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus were isolated and were characterized using appropriate tests. The AgNPs were highly efficient bactericidal agent against Escherichia coli and Staphylococcus aureus.10mM concentrated $AgNO_3$ used to produce AgNPs have higher antibacteial effect than 4mM $AgNO_3$.

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