Preparation and Evaluation of Silver nanoparticles by using Plant Extracts for Antimicrobial Activity

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ABSTRACT

Nanotechnology is the modern research field which deals with design, synthesis and manipulation of particles structure range from 1-100nm in dimension. A nanoparticle is ultra-fine unit with dimension less than 100 nm (1 nm=10-9 meter).Because of their small size they have unique characteristics and their applications in various field such as medicines, engineering and environmental remediation. Nanoparticles can be prepared by various aapproaches like physical, chemical and biological .The biological approach is the emerging approach because it is easier than other methods. In this green synthesis aqueous solution of extract of Azadirathcha indica and AgNo3 were taken for study. The nanoparticles were prepared by using plant extract and silver metal ions. The nanoparticles were characterized by different spectral methods like UV, IR, DLS, SEM, XRD and Zeta analysis.

Key Words: Nanotechnology,Nanoparticles,Green Synthesis.

INTRODUCTION

Nanotechnology is the modern research field which deals with design, synthesis and manipulation of particles structure range from 1-100nm in dimension. A nanoparticle is ultra- fine unit with dimension less than 100 nm (1 nm=10-9 metre).Because of their small size they have unique characteristics and their applications in various field such as medicines, engineering and environmental remediation. International Organization of Standards defined nanoparticle as the discrete nano object with dimensions are less than 100nm.The ISO also defined two dimensional nano objects as nano discs & nano plates, and one dimensional nano objects as nano fibres & nano tubes.[1] The applications of nanotechnology is suitable for biological molecules due to their exclusive properties. They undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis which is found to be reliable and eco-friendly.[2]Sometimes the synthesis of nanoparticles using various plants and their extracts can be advantageous over other biological synthesis processes which involve the very complex procedures of maintaining microbial cultures. [3]

But, synthesis of nanoparticles using plant extracts is the most adopted method of green, eco- friendly production of nanoparticles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites [4]. There has also been several experiments performed on the synthesis of silver nanoparticles using medicinal plants such as Oryza sativa, Helianthus annus, Saccharum officinarum, Sorghum bicolour, Zea mays, Basella alba, Aloe vera Capsicum annuum, Magnolia kobus, Medicago sativa (Alfalfa), Cinamomum camphora and Geranium sp. in the field of pharmaceutical applications and biological industries. Besides, green synthesis of silver nanoparticles using a methanolic extract of Eucalyptus hybrida was also investigated[5].

In the recent days, silver nanoparticles have been synthesized from the naturally occurring sources and their products like green tea (Camellia sinensis), Neem (Azadirachta indica), leguminous shrub

(Sesbania drummondii) , various leaf broth, natural rubber, starch , Aloe vera plant extract , lemongrass leaves extract, etc.[6] With respect to the microbes, the silver nanoparticles get attached to the cell wall, thereby disturbing the permeability of cell wall and cellular respiration. The nanoparticles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorous and sulphur containing compounds such as, DNA and protein, present inside the cell. The bactericidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity [7]. Besides, the potency of the antibacterial effects corresponds to the size of the nanoparticle. The smaller particles have higher antibacterial activities due to the equivalent silver mass content. With respect to the clinical applications of

nanoparticle microorganisms including diatoms, fungi, bacteria and yeast producing inorganic materials through biological synthesis either intra or extracellular made nanoparticles are more biocompatible.

METHODOLOGY

MATERIALS&METHOD OF PREPARATION

Fresh leaves of Azadirachta indica were collected from local area of Muzaffarnagar, and washed several times with water to remove the dust particles and then air dried at room temperature to remove the residual moisture and ground to form powder. Then plant extract was prepared by mixing extract (1%) with deionized water in a 250 ml conical flask. The above solution was incubated at about for 30 minutes. Then the solution was subjected to centrifuge for 30 minutes at 5000 rpm. The obtained supernatant was separated by filtered with the help of vacuum filter. Further the solution was used for the reduction of silver ions Ag+) to silver nanoparticles (Ago).

EVALUATION OF SILVER NANOPARTICLES

UV

The optical property of Silver nanoparticles was determined by UV-Visible spectrophotometer model Perkin- Elmer, Lambda 35, Germany. After the addition of AgNO3 to the plant extract, the spectra were taken in different time intervals up to 24 hours between 350 nm to 500 nm. Then the spectra were taken after 24 hours by addition of AgNO3.

FTIR

The chemical composition and functional groups of the synthesized silver nanoparticles were studied by using FTIR spectrometer (Perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75o C and the dried powders were characterized in the absorbance range 4000– 400 cm-1.The sample was analyzed by using KBr pellet.

XRD

The phase variety and grain size of synthesized silver nanoparticles were determined by X-ray diffraction spectroscopy (Philips PAN analytical). The synthesized silver nanoparticles were studies with CUKα radiation at voltage of 30 kV and current of 20 MA with scan rate of 0.030/s.

Different phases present in the synthesized samples were determined by X′ pert high score software with search and match facility. The particle sizes of the prepared samples were determined by using Scherer's equation as $0.9\lambda D \approx \beta \cos\theta$

Where D is the crystal size, λ is the wavelength of X-ray, Θ is the Braggs angle in radians and B is the full width at half maximum of the peak in radians.

SEM

The morphology of synthesized silver nanoparticles from neem plant extract was studied by Scanning Electron Microscope (SEM) (JSM-6480 LV). On addition of AgNO3 after 24 hours the SEM slides were prepared by using a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an acceleration voltage of 20 KV.

DLS & ZETA

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques were employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in deionized water followed by ultra- sonication. Then solution was filtered and centrifuged for 15 minutes at 25o C with 5000 rpm and the supernatant was collected.

The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer (ZETA Sizer Nanoseries,Malvern instrument Nano Zs).

ASSESSMENT OF ANTIMICROBIAL ACTIVITY

Disc Diffusion method is used to assess antibacterial activity. In this method antibacterial assays were studied by using human pathogenic bacteria such as Escherichia coli and Staphyloccocus aureous ,Mackonkey broth (HiMedia) medium was used to sub culture of bacteria and were incubated at temperature 370C for 24 hours. Fresh overnight cultures were taken and spread on the Mackonkey agar plates for cultivating bacteria. Previously sterilized paper discs of 5 mm diameter saturated with plant extract, silver nanoparticle and double distilled water as control were placed on each plate and were incubated again at temperature 37oC for 24 hours and the antibacterial activity was measured based on the zone of inhibition

around the disc impregnated with silver nanoparticle and plant extracts with time.

RESULTS AND DISCUSSION

UV

After the addition of AgNO3 to the plant extract, the spectra were taken in different time intervals up to 24 hours between 350 nm to 500 nm. Then the spectra were taken after 24 hours by addition of AgNO3.On the behalf of UV-vis data it was cleared that Azadiratcha indica reduced metal ions so further characterizations were carried out with Azadiratcha indica.

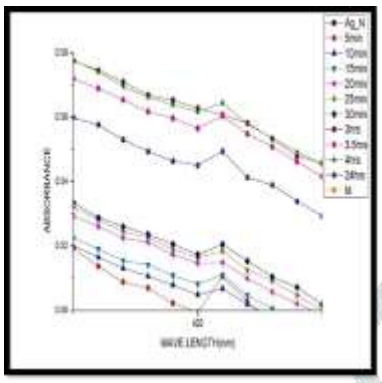
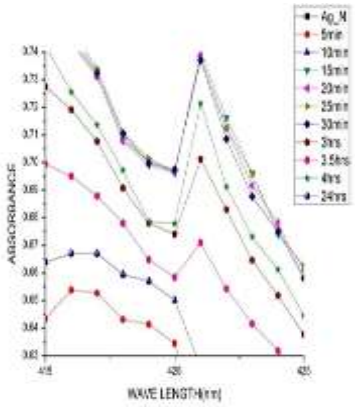


Figure: 1



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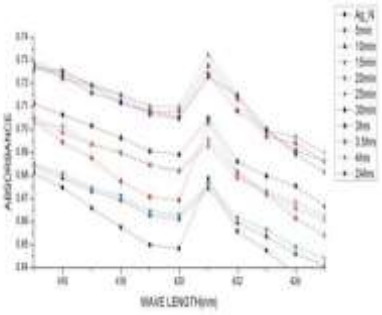


Figure: 3

Figure: 1 UV-visible spectra of Azadirachta indica 60:1 ratio at different time interval

Figure: 2 UV-visible spectra of Azadirachta indica 120:1 ratio at different time interval

Figure: 3 UV-visible spectra of Azadirachta indica 240:1 ratio at different time interval

SEM

SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results shown that the diameters of prepared nanoparticles in the solution have sizes of several µm in case of 30:1, 120:1 & 240:1 ratios where as in 60:1 ratio the size is of several nm. (Figure: 4, Figure: 5, Figure: 6 & Figure: 14). The size of the prepared nanoparticles was more than the size of nanoparticle which should be between 1-100 nm. The size was more than the desired size as a result of the proteins which bound in the surface of the nanoparticles. The result showed that the particles were of spherical shape in case of 30:1, 60:1, and 120:1 ratios but sheet shape in case of 240:1 ratio. The shape varies due to the concentration increased in ratio of 240:1

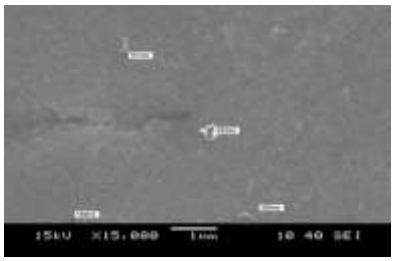


Figure:4

Figure:5

Figure:6

Figure:4 SEM image for 60:1 ratio silver nanoparticles

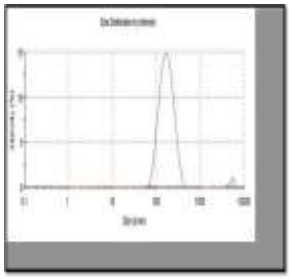
Figure:5 SEM image for 120:1 ratio silver nanoparticles

Figure:6 SEM image for 240:1 ratio silver nanoparticles

DLS

The particle size distribution (PSD) of synthesized silver nanoparticles of different ratios like 60:1, 120:1, and 240:1 are shown in the figures. (Figure: 7, Figure: 8, Figure: 9).According to the figure the colloidal solution of silver nanoparticles of ratio 30:1 contains particles of different sizes some were with average sizes ranging from 5 nm to 180 nm. But in case of 60:1, the solution contains particles of uniform sizes ranging from 68 nm to 396 nm. The average size of nanoparticles is 160 nm.

The particle size in case of 120:1 ratio ranges from 78 nm to 255 nm with mean particle size of 169 nm. Similarly the sizes of nanoparticles in case of 240:1 ratio range from 91 nm to 220 nm with average size of 164 nm. If we compare the above four results we can conclude that the ratios like 60:1, 120:1, 240:1 give uniform distribution of particles but 30:1 ratio does not obey this principle. Among them 60:1 ratio is very appropriate since it gives lowest average size of nanoparticles.



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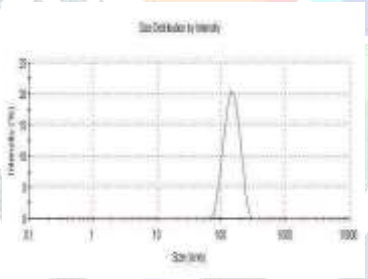

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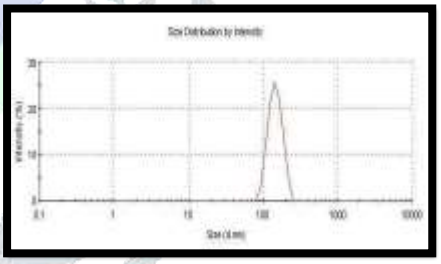


Figure: 9

Figure: 7 DLS result for 60:1 ratio silver nanoparticles

Figure: 8 DLS result for 120:1 ratio silver nanoparticles

Figure: 9 DLS result for 240:1 ratio silver nanoparticles

ZETA POTENTIAL

The Zeta potential measurements of silver nanoparticles synthesized with different ratios like 30:1, 60:1, 120:1, and 240:1 are 15.5 mV, 1.92 mV, 6. 12 mV and 2.45 mV respectively. (Figure: 10 Figure: 11, Figure: 12 shows the result of ZETA analysis of 60:.120:1,240:1 ratio .From the zeta potential analysis the order of stability of nanoparticles synthesized from different ratios is 30:1> 120:1 > 240:1 > 60:1. Nanoparticles are very small in size for which they are energetically very unstable. Therefore the particles undergo agglomeration/aggregation to stabilize themselves. So there were some potential charges on the surface of the nanoparticles which makes them stable. We got the potentials charges from this analysis. Zeta potential (Surface potential) has direct relation with the stability of a form/structure as mentioned below.

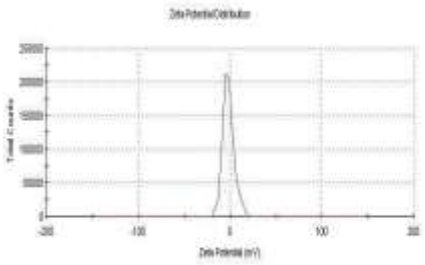
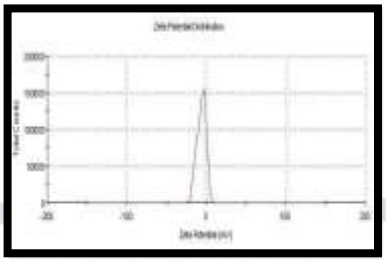


Figure: 10



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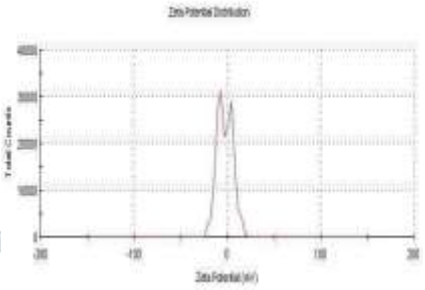

Figure: 12

Figure: 10 Zeta Analysis result for 60:1 ratio silver nanoparticles

Figure: 11 Zeta Analysis result for 120:1 ratio silver nanoparticles

Figure: 12 Zeta Analysis result for 240:1 ratio silver nanoparticles

FTIR

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles (Figure: 23 & Figure: 24) in case both of 60:1 and 120:1 ratios showed the band between 3490-3500 cm-1 corresponds to O-H stretching H- bonded alcohols and phenols. The peak found around 1500-1550 cm-1 showed a stretch for C-H bond, peak around 1450-1500 cm-1 showed the bond stretch for N-H. Whereas the stretch for Ag- NPs were found around 500-550 cm-1. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e.; capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. Carbonyl groups proved that flavones or terpenoids absorbed on the surface of metal nanoparticles.Flavanones or terpenoids could be adsorbed on the surface of metal nanoparticles, possibly by interaction through carbonyl groups or π-electrons in the absence of other strong ligating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles. It is also possible that the terpenoids play a role in reduction of metal ions by oxidation of aldehyde groups in the molecules to carboxylic acids. These issues can be addressed once the various fractions of the neem leaf extract are separated, identified and individually assayed for reduction of the metal ions. This rather elaborate study is currently underway.

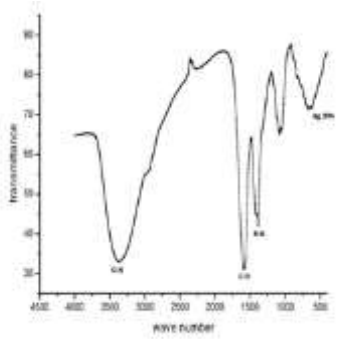

Figure: 13

Figure: 14

Figure: 13 FTIR result for 60:1 ratio silver nanoparticles

Figure: 14 FTIR result for 60:1 ratio silver nanoparticles

XRD

XRD spectrum (Figure: 15) showed distinct diffraction peaks around 38O, which are indexed by the (100) of the cubic face-centered silver. These sharp Bragg peaks might have resulted due to capping agent stabilizing the nanoparticle. Intense Bragg reflections suggest that strong X-ray scattering centres in the crystalline phase and could be due to capping agents. Independent crystallization of the capping agents was ruled out due to the process of centrifugation and re dispersion of the pellet in Millipore water after nanoparticles formation as a part of purification process. Therefore, XRD results also suggested that the crystallization of the bio- organic phase occurs on the surface of the silver nanoparticles or vice versa. Generally, the broadening of peaks in the XRD patterns of solids is attributed to particle size effects. Broader peaks signify smaller particle size and reflect the effects due to experimental conditions on the nucleation and growth of the crystal nuclei.

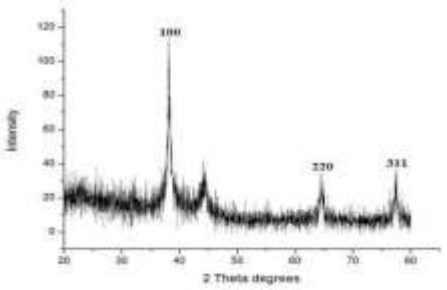

Figure: 15 XRD results of 60:1 ratio nanoparticles

ANTIMICROBIAL ACTIVITY

Silver nanoparticles, due to their antimicrobial properties have been used most widely in the health industry, medicine, textile coatings, food storage, dye reduction, wound dressing, antiseptic creams and a number of environmental application[8]. Since ancient times, elemental silver and its compounds have been used as antimicrobial agents; and was used to preserve water in form of silver coins/silver vessels [9, 10]. We have examined A.indica extract mediated silver nanoparticles as possible antibacterial agents. The plant extract and those mediated silver nanoparticles were immediately tested for respective antimicrobial

activities towards both gram positive (S. aureus) and gram negative (E. coli) bacterial strains showing the zones of inhibition. Based on the zone of inhibition produced, synthesized silver nanoparticles prove to exhibit good antibacterial activity against E. coli and S. aureus. On the other hand, control and plant extract alone did not exhibit any antibacterial activity. Although, it is to be presumed that the leaves extract of the plant used possess the antibacterial activities and must be reflected through greater inhibition zone but it alone shows very low activity due to its medium of extraction as well as lower concentration during experimentation. The results of antibacterial activities of prepared silver nanoparticles evaluated from the disc diffusion method are given in Table: 1.The silver nanoparticles showed efficient antimicrobial property compared to other due to their extremely large surface area providing better contact with cell wall of microorganisms.[11]

Table: 1 Zone of inhibition (mm) obtained by disc diffusion method

Components	Zone of Inhibition	
	E.coli	*S.aureus*
Control	NZ	NZ
Plant Extract	NZ	NZ
Silver nanoparticle	9	9

SUMMARY&CONCLUSION

The rapid biological synthesis of silver nanoparticles using Azadirachta indica leaves extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The synthesized nanoparticles were of spherical and sheet shaped and the estimated sizes were 160-180 nm. The size were bigger as the nanoparticles were surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-vis spectrophotometer, SEM, DLS, Zeta Analyzer, XRD, and FTIR techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The higher concentrated nanoparticles had sheet shaped appearance whereas the lower concentrations showed spherical shaped. The sizes of the nanoparticles in different concentration were also different which depend on the reduction of metal ions. From the data of DLS it was found that the 30:1 ratio solution had sharp nanoparticles of around 5 nm and some has around 180 nm and the had the potential of around 15.5 mV. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

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