PREPARATION, CHARACTERIZATION AND OLIGODYNAMIC EFFECT OF ROBUST SILVER NANOPARTICLES OVER BACTERIAL PATHOGENS

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Abstract

This paper describes the Oligodynamic effect over to the Gram negative bacteria such as Pseudomonas aeruginosa, Proteus mirabilis, E.coli and Gram positive bacteria like Bacillus cerus, Streptococcus pyogenes and Staphylococcus aureus by the formation of silver nanoparticles. XRD analysis showed crystalline and face centered cubic geometry of synthesized Ag nanoparticles. Nanoparticles growth was evaluated by UV-Vis spectroscopy and it exhibits a characteristic surface Plasmon resonance peak at around 306 nm. The FTIR spectrum analysis evaluated the presence of different functional groups in capping the silver nanoparticles. Elemental Analysis of EDAX spectrum shows the presence of silver nanoparticles. The spherical morphology of silver nanoparticles was confirmed from SEM image. The synthesized Silver nanoparticles showed good antibacterial effect over the standard Amikacins. This reveals that Ag nanoparticles could provide a safer alternative to conventional antibacterial agents.

Key words: *Silver nanoparticles, Trisodium citrate, Aniline, Oligodynamic effect.*

1. Introduction

Nanotechnology is mainly alarmed with the synthesis of nanoparticles and their applications in various fields of physics, medicine, chemistry and materials science [1]. Metal nanoparticles like Silver has renowned because of its distinctive optical, electrical and photothermal properties [2]. The use of silver nanoparticles as antibacterial agent is relatively new and for their sustained anti-fungal, anti-bacterial and anti-viral effects has been practiced for a long time. Such effects are generally referred as oligodynamic action. Recent studies have focused on the synthesis of homogenous silver nanoparticles (NPs) and evaluation of their antimicrobial activities [3]. Silver ion has been known to be effective against a broad range of microorganisms. Silver nanoparticles with higher surface area to volume ratio compared to common metallic silver have shown better antimicrobial activity[4].A variety of methods have been reported for synthesis of metallic NPs. These include thermal decomposition, laser ablation, microwave irradiation, sonochemical, reverse micelles, salt reduction, radiolysis, solvothermal and electrochemical synthesize [5]. However controlling the particle size and production of particles by an industrial scale is an important task of all methods [6]. Chemical reduction of metal salts using various reducing agents in the presence of stabilizer is currently of interest for preparation of metal NPs [7] . The most popular among them is the Chemical reduction of Silver salts by sodium citrate. Silver nanoparticles show optical properties, which are not observed neither in molecular nor in bulk metals [8]. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process [9,10]. The most important application of Silver and Silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds [11]. In the present study the synthesis of silver NPs by chemical reduction method using Trisodium citrate and Aniline as the reducing agents is described.

2. Experimental sections

2.1. Materials

Silver nitrate (AgNO3), trisodium citrate (Na3C6H5O7), Aniline has been used for synthesizing silver nanoparticles. All the solutions have been freshly prepared for the synthesis of nanoparticles.

2.2. Method

2.2.1. Synthesis of robust silver nanoparticles

For the fabrication of silver nanoparticles trisodium citrate and Aniline have been used as reducing and stabilizing agents respectively. In the synthesis of silver nanoparticles, silver nitrate solution (1.69 mg in 10 mL), trisodium citrate (2.94 mg in 10 mL) have been used as a metal precursor and stabilizing agent, respectively. Aniline have been added drop wise to the above silver nitrate solution and further stirred at 15 min. All the reactions have been carried out at room temperature.

2.2.2. Antibacterial assay

The antimicrobial susceptibility of silver nanoparticles was evaluated using the disc diffusion or Kirby-Bauer method. Zone of inhibition was measured after 24 hr of incubation at 35^{0}C. The standard dilution micro method determining the minimum inhibitory concentration (MIC) leading to inhibition of bacterial growth is under way.

3. Results and discussion

Silver nanoparticles have been synthesized by Chemical method using trisodium citrate as well as Aniline. It is well known from the literature that trisodium citrate merely serves as a capping agent. UV-Vis spectroscopy is one of the most widely used techniques for the structural characterization of silver nanoparticles. Fig(1) shows the UV-Vis Absorption spectrum of Ag nanoparticles with a broad spetctrum Plasmon resonance (SPR) peak at 306nm, an indication of silver nanoparticles formation [12].

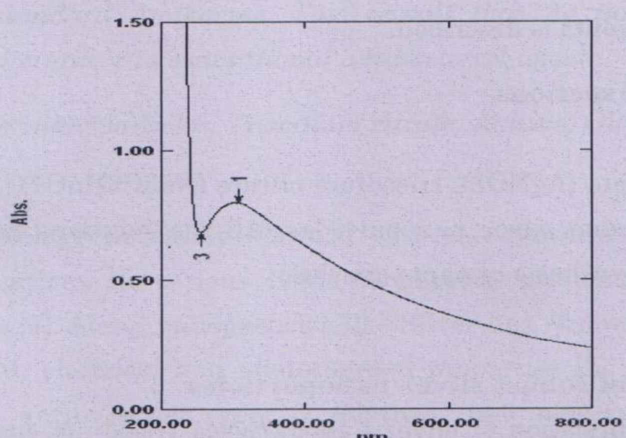

Fig (1) UV-Vis absorption spectrum of silver nanoparticles obtained

Further characterization was done by Scanning Electron Microscope (SEM). A scanning electron microscope is a type of electron microscope that images a sample by scanning it with a beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface, topography and composition. SEM image of the Silver nanoparticle synthesized is shown in Fig (2), which indicates well dispersed particles that are more or less spherical. The elemental analysis of the Silver nanoparticles was performed using the EDX on the SEM. Fig (3) shows the EDX spectrum of the Silver nanoparticles prepared with trisodium citrate as reducing agent. The peaks around 2.9 KeV,0.2 KeV are correspond to the binding energies of Ag respectively. Also, a peak near 0.1KeV corresponding to Carbon is observed. The Carbon and Sodium peaks correspond to the SEM holding grid. Throughout the scanning range of binding energies, no obvious peak belong to impurity is detected. Energy Dispersive X-ray diffraction of AgNPs screening strong signals from silver (97%w) and fragile indicator from carbon [13]. The result indicates that the synthesized product is composed of high purity Ag nanoparticles.

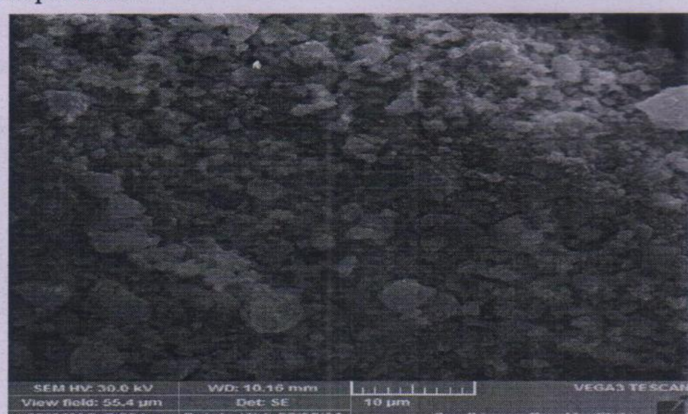

Fig (2) SEM images of synthesized Silver nanoparticles

XRD data indicated the crystalline nature and the estimated size of Silver nanoparticles is 39.21nm Fig (4). The FT-IR spectra of the nanoparticles reveal more information about the local molecular environment of the ligands. Fig (5) shows FT-IR spectra of the Ag nanoparticles. The comparison of these spectra reveals similar features in the wavenumbers range from 4000 to 400 cm^{-1}. The peaks are found, which correspond to the C-H stretching mode 2925 cm^{-1}, C-H bending mode of 893 cm^{-1}. N-H stretching mode with 3432 cm^{-1} and N-H bending mode of 1597 cm^{-1}, C=C stretching mode with 1412 cm^{-1} and C-N stretching mode of 1080 cm^{-1}, respectively.

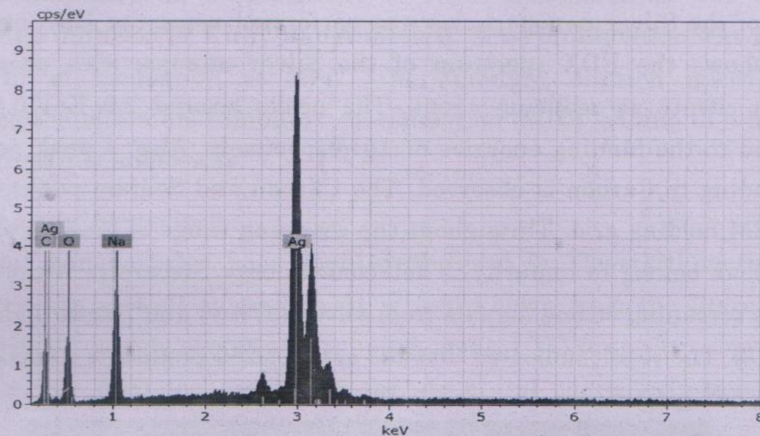

Fig (3) EDX spectrum of synthesized Silver nanoparticles.

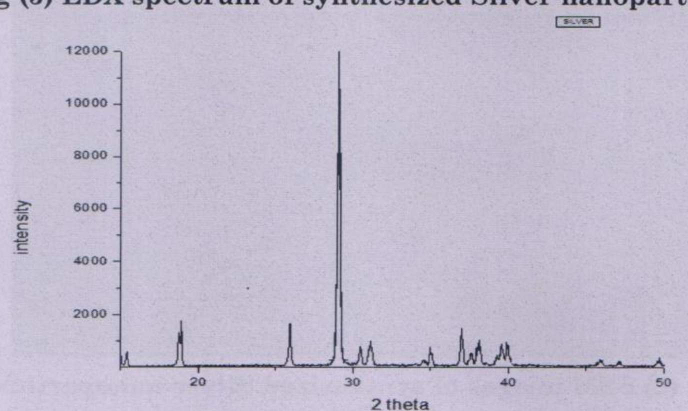

Fig (4) XRD pattern of nanosized silver

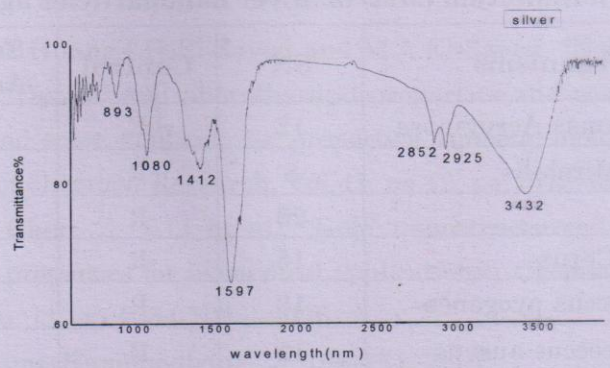

Fig (5) FT-IR spectrum of synthesized Silver nanoparticles

Finally, the antibacterial susceptibility of Silver nanoparticles synthesized was investigated. The Kirby – Bauer diffusion method was used as antibacterial susceptibility testing method. After the disposable plates inoculated with the tested Gram-positive and Gram-negative bacteria, zones of inhibition were measured after 24 hr of incubation at 35C. Fig (6) shows plates to which a bacterial suspension was applied. The presence of nanoparticles at a certain level inhibited bacterial growth by more than 90%. The diameter of inhibition zones (in millimeters) around the different Silver nanoparticles sols with against test strain are shown in Table (1).

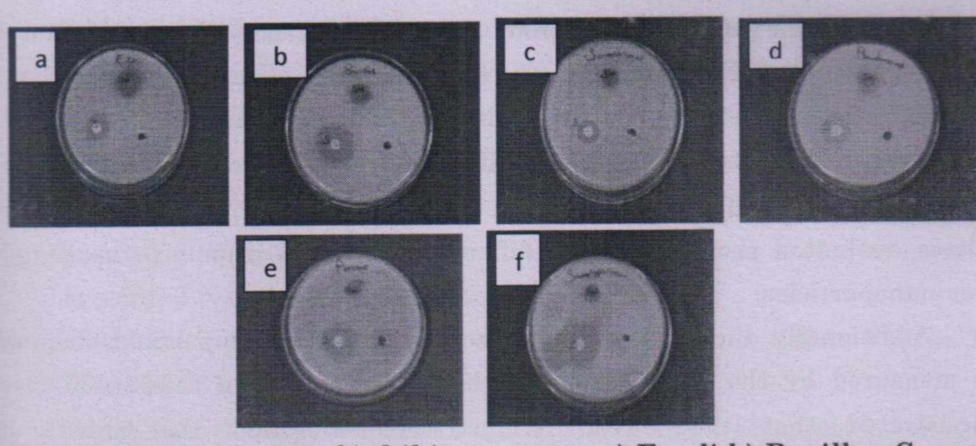

Fig (6) Appearances of inhibitory zones a) E.coli b) Bacillus Cerus c) Streptococcus pyogenes d) Pseudomonas aeruginosa e) Proteus mirabilis f) Staphylococcus aureus.

Table 1. Zone of inhibition (MM) of Silver nanoparticles against test strains

Organisms	SN	Control	Standard Amikacin
Pseudomonas Aeruginosa	12	R	17
Proteus Mirabilis	8	R	17
E.coli	20	R	17
Bacillus Cerus	15	R	20
Streptococcus pyogenes	13	R	18
Staphylococcus aureus	12	R	18

The minimum inhibitory concentration de MIC of each sample was necessarily determined. It reviews the minimum inhibition concentrations of the tested Silver particles samples against Gram-Positive and Gram-Negative bacteria. Similar results have been obtained by Ashish Kumar Singh et al [14].

4. Conclusion

In summary, silver nanoparticles with mean diameter of 39.21nm was synthesized using Aniline and citrate of sodium as a reducing agent. The nanoparticles were characterized by UV/Vis, SEM, EDX, XRD and FTIR.

UV/Vis spectra show the characteristic Plasmon absorption peak for the Silver nanoparticles ranging 306 nm. The SEM images show the spherical morphology of Silver nanoparticles. The energy-dispersive spectroscopy (EDX) of the nanoparticles dispersion confirmed the presence of elemental Silver signal no peaks of other impurity were detected. XRD analysis showed crystalline and face centered cubic geometry of synthesized Ag nanoparticles. The FTIR spectrum analysis evaluated the presence of different functional groups in capping the Silver nanoparticles.

Additionally, the oligodynamic effect of the silver nanoparticles dispersion was measured by the Kirby-Bauer method. The results of this study clearly demonstrated that the Silver nanoparticles inhibited the growth and multiplication of the tested bacteria, including highly multiresistant bacteria such as Staphylococcus aureus, Escheria coli and Pseudomonas aeruginosa.

5. References

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