Silver Nanoparticle and their synthesis, characterization and study of anti-bacterial property

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1. Introduction

In literature in the recent past has demonstrated a potential role of metal nanoparticles as antibacterial agents. However, functional properties of metal nanoparticles can be improved through the green synthesis approach. Biological synthesis of nanoparticles is seeking an extraordinary consideration due to the fact that it is eco-friendly as compared to other routes of nanoparticle synthesis [7]. Despite the ability of physical and chemical methods to synthesize nanoparticles of particular size and shape, use of hazardous material and economically lesser feasibility make their application limited [8]. Commonly used chemical and physical methods are chemical reduction, ion sputtering, sol gel, etc., which have higher energy requirements and include improvident purifications [9, 10]. Stability of synthesized nanomaterials and reproducibility make green synthesis a preferred technique over other methods [11].

Among inorganic NPs, silver nanoparticles (Ag-NPs or nanosilver), due to its novel chemical, physical, and biological properties as compared to their bulk form, have attracted the attention of researchers from various academic laboratories [5]. AgNPs have distinctive physical and chemical properties, for example, high thermal and electrical conductivity, surface enhanced Raman scattering, chemical stability, catalytic activity, and nonlinear optical behavior [6]. These properties take Ag-NPs to the top of the priority list, to be used in inks, in electronics, and for medical purpose [7]. Furthermore, Ag-NPs are widely known for its antimicrobial properties against microbes such as bacteria, fungi, and virus [8]. Due to their proven antimicrobial properties, AgNPs are widely used in the daily used commercial products, such as plastics, food packaging, soaps, pastes, food, and textiles, which has increased their market value to a great extent [9]. Its importance can be judged from the fact that AgNPs can be used in various form, such as colloidal (enamel, coating, and in paints), in liquid form (shampoo), or in solid form (blending Ag-NPs with a solid material such as polymer scaffolds) and even can be found suspended in materials like soap and nonwoven

fabrics. Ag-NPs importance cannot be neglected even in textile industry, where Ag-NPs are used in the water filtration membranes. The idea behind the use of Ag-NPs in the water filtration membrane is based on the utilization of their proven antimicrobial properties and slow release rate of Ag-NPs from the membrane. The slow release rate prolongs capability of the membrane to be used as a protective barrier against various bacterial and other pathogenic microbes present in the water [9, 10]

2. Experimental Section

In this work, Silver Compounds Commercially manufactured 30-50 nm spherical silver nanoparticles surface-coated with 0.2 wt% PVP (PVP coated AgNPs) were used for these studies (Nano Amor, Lara et al. Journal of Nanobiotechnology 2010, 8:15 http://www.jnanobiotechnology.com/content/8/1/15 Page 8 of 11 Houston, TX). Stock solutions of PVP-coated AgNPs were prepared in cell culture media with 10% FCS. Serial dilutions of the stock solution were made using RPMI + 10% FCS media. The solution was filtered and washed several times with water to remove the remaining impurities. The washing process was carried out using a simple decantation of the supernatant with centrifugation technique at 5000 rpm for 30 minutes which results in the formation of Silver nanoparticle.

2.1 Characterization

X-ray diffraction (XRD) scans of graphite flakes, and graphene oxide were performed with Bruker's D8 advanced X-ray diffractometer using CuKα radiation (λ=1.5418 A). Dynamic Light Scattering (Model no: HORIBA Nano particle analyzer SZ-100) was used to measure the size of the particle. The surface morphology of the prepared GO was examined by a Carl Zeiss ultra 55 Field Emission Scanning Electron Microscope (FESEM).Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify types of chemical bonds, i.e. functional groups in a molecule (Model no: Perkin Elmer precisely FT-IR spectrometer) over the wave number range of 4000-500cm^{-1}.

2.2 BIOMEDICAL APPLICATION

Sources required

Materials used for antimicrobial activity of Graphene oxide was Nutrient broth 1.3 g, Nutrient agar 5.6 g, Agar-agar 0.5 g, petriplates, cotton abs, *Klebseilla*, *Staphylococus*. Well diffusion method was used for antibacterial activity of Silver nanoparticle.

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Preparation of inoculums

Nutrient broth (1.25g in 100 ml D/W10) was prepared in two conical flasks and sterilized. In one conical flask clinically isolated strain of *Klebseilla*, was inoculated and in the other conical flask clinically isolated strain of Staphylococus was added. The bacterial cultures inoculated nutrient broth was kept on rotary shaker for 24 hours at 100r.p.m.

Inoculation of test plate

Nutrient agar is prepared (8g nutrient agar 0.5g Agar in100ml distilled water) [12] and sterilized. The agar suspension is poured into sterile petri-plates and allowed to solidify. Then the two pathogenic strains *Klebseilla* was taken and spreaded evenly over the entire surface of the plate by swabbing in three directions. Plates were allowed to dry before applying the sample.

Preparation of AgNPs sample

Antimicrobial activity enhancement of Silver nanoparticle was obtained by using Silver nanoparticle. Silver nanoparticle of two different concentrations are used to know the effective concentration for its activity. 0.01grams of Silver nanoparticle were suspended in distilled water. Two different concentrations of Silver nanoparticle i.e., 0.01grams and 0.05grams were suspended in distilled water separately.

Well diffusion method

The wells were casted by porer on the test plates. The samples were loaded with equal volume (50µl) on the plates. Control plate does not contain any antibiotic. The test plates were incubated at room temperature. The activity was clearly visible from19-24 hrs on the plates. The zone of inhibition was measured & the sample of the Graphene oxide showing maximum antibacterial activity was noted.

3. Result and discussion

3.1 X-ray diffraction analysis

Figure 1 shows the XRD patterns of Silver nanoparticle. It is exhibits a strong and sharp peak at 26.4^{o} in Fig.1(a), indicating a higher ordered structure, that corresponds to a basal spacing d_{002} = 0.334nm. The pattern of Silver nanoparticle, on the other hand, exhibits a 001 reflection at 9.09^{o} corresponding to a basal spacing of d_{001} = 0.961nm. The interlayer spacing of GO was calculated to be 0.961 nm according to the diffraction peak at 2Θ =90.9º. This

value is higher than interlayer spacing of graphite flakes (d-spacing= 0.334nm, 2Θ =26.4°), due to the presence of oxygenated functional groups and intercalated water molecules.

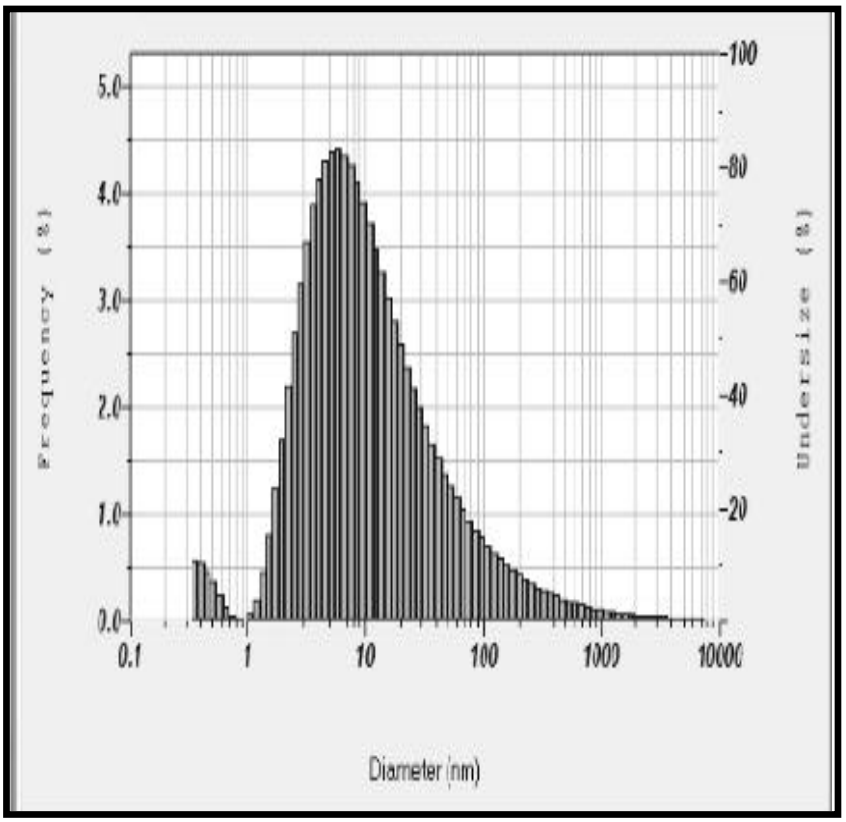

Fig: 1. X-ray diffraction patterns of Silver nanoparticle

3.2 Dynamic Light Scattering (DLS)

The measurement of particle size distribution of Silver nanoparticle is done by Dynamic Light Scattering (via Laser input energy of 532 nm). In the prepared sample it was observed that, particle have a wide size distribution, but the majority of them were dispersed within a narrow range, as shown in Fig (2). The average particle size from the histogram was found to be 30 nm.

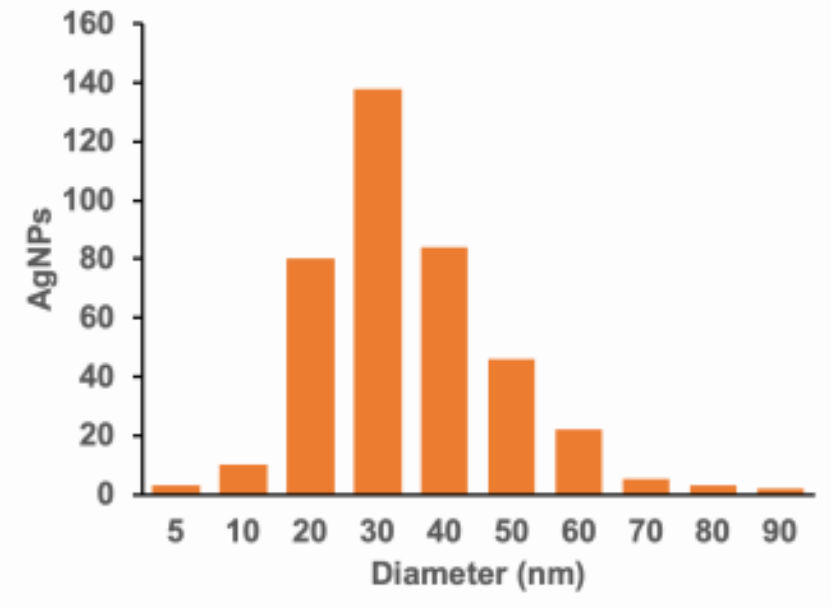

Fig: 2 Particle distributions in the Dynamic Light Scattering

3.3 Field Emission Scanning Electron Microscope (FESEM)

The grain size and surface morphology were observed by the field emission scanning electron microscope (FESEM). FESEM images of the Silver nanoparticle have well defined and interlinked three-dimensional Graphene sheets, forming a porous network that resembles a loose sponge like structure as shown in Fig (3). It was synthesized using graphite flakes which resemble the layers of an onion as shown in Fig 3, on other hand graphene nano rods have been observed as when fine powder was used.

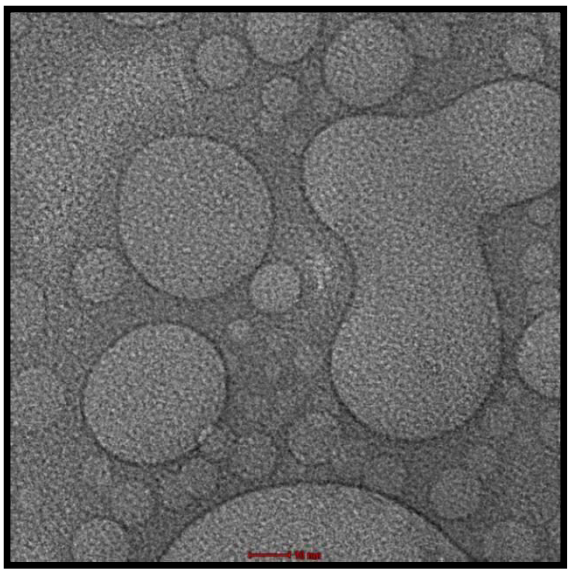

Fig: 3 FESEM image of represents the Silver nanoparticle.

3.4 Fourier-Transform Infrared Spectroscopy (FTIR)

FT-IR spectrum of the Silver nanoparticle obtained in these steps confirms the successful oxidation of the graphite as shown in Fig (4). The presence of different types of oxygen functionalities in Silver nanoparticle were confirmed at broad and wide peak at 3447 cm^{-1}can be attributed to the O-H stretching vibrations of the C-OH groups and water [14, 15].The absorption bands at 1560 cm^{-1} can be ascribed to benzene rings [16]. The sharp intense peak at 1419 cm^{-1} can be attributed to CO- carboxylic.

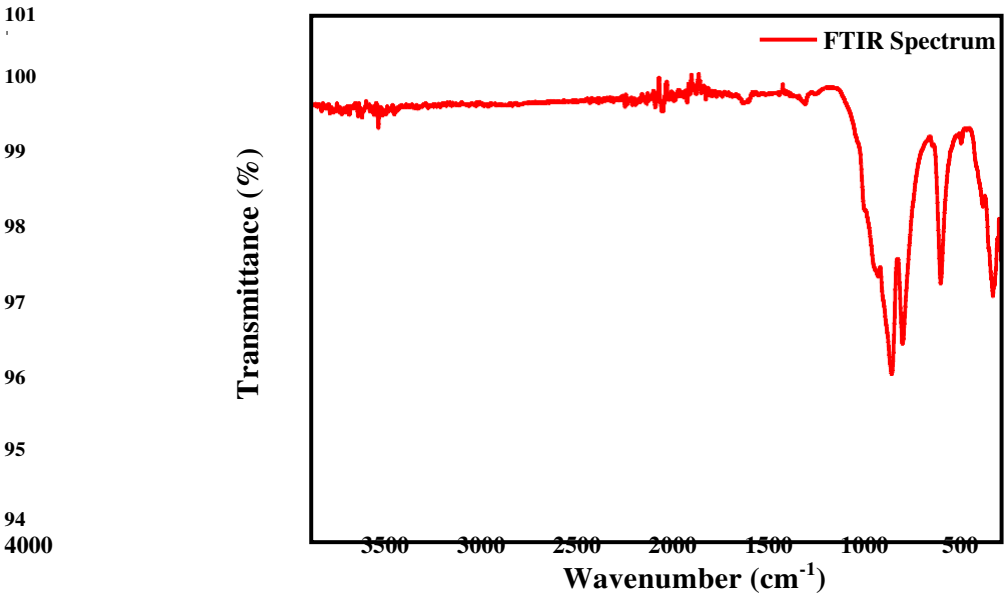

Fig: 4.FT-IR Spectra for the Silver nanoparticle

3.5 Zeta Potential

Recent studies have demonstrated that the zeta potential of NPs has a strong influence on bacterial adhesion. Because of the electrostatic attraction between positively charged NPs and the bacterial cell membrane, which is negatively charged, and AgNPs, which have a positive surface charge, are prone to being adsorbed on the bacterial surface and are closely connected with bacteria, in contrast to their negatively charged counterparts. The potential of NPs to selectively gather at sites of bacterial infection increases vascular permeability

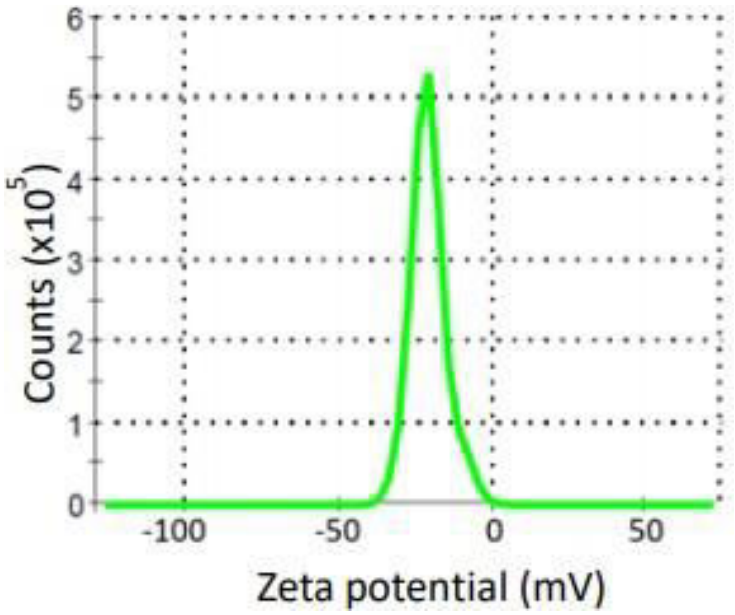

Fig: 5. Zeta potential analysis for the Silver nanoparticle

4. Antibacterial activity

Well diffusion method was used for the assessment of antibacterial activity. The antibacterial activity of the sample was identified by the formation of Zone of Inhibition. Zone of Inhibition is the area on an agar plate where growth of a control organism is prevented by an antibiotic usually placed on the agar surface. If the test organism is susceptible to the antibiotic, it will not grow where the antibiotic is present. The size of the zone of inhibition is a measure of the Compound's effectiveness, the larger the clear area around the antibiotic, the more effective the compound.

The activity of the sample was observed by the formation of Zone of inhibition after 24 hours. Presence of zone of inhibition confirmed inhibitory activity of Silver nanoparticle. The Zone of Inhibitions of different bacteria is given in the figure. The control plates show the growth of bacteria in the absence of antibacterial agents. The clear zone surrounding the sample in the remaining plates shows the activity of the sample. Figure shows the petridishes with samples of Silver nanoparticle, 0.01gms of Silver nanoparticle and 0.05gms of Silver nanoparticle. The zone surrounding the sample is clear that shows complete zone of inhibition. The space surrounding the complete zone of inhibition is partial zone of inhibition where the activity decreases than complete zone of inhibition. The Zone of inhibition is more for the high concentration of Silver nanoparticle. The results showed that the zone of inhibition increases within the concentration of Silver nanoparticle in both the bacteria.

Comparing the two, inhibitory activity of Silver nanoparticle on *Klebseilla* was higher.

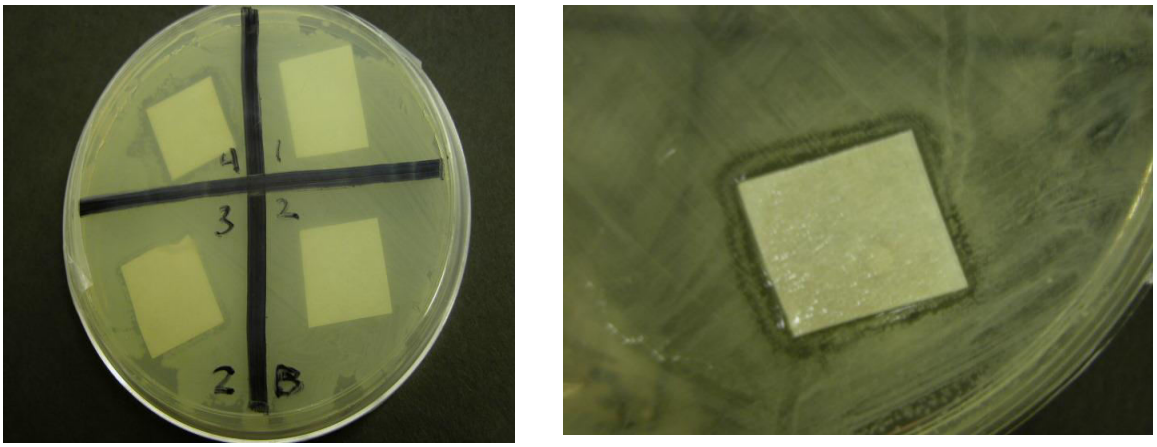

Fig. 2.6 Silver nanoparticle Shows antibacterial activity.

5. Conclusion

Modified Hummers method has been synthesized completely to produce large area Silver nanoparticle. This method was carried out with the highest conversion level to Silver nanoparticle and shows that pure Silver nanoparticle is formed. XRD conform its Silver nanoparticle to hexagonal structure .The average particle size obtained from particle analyzer (DLS) was 8nm. The particle sizes were in the range of 35 nm to 65 nm from FESEM. FTIR shows formation of Silver nanoparticle. The antibacterial activity of Silver nanoparticle was confirmed by Zone of inhibition. As the diameter of the zone of inhibition is high, we can conclude that Silver nanoparticle is a very effective antibacterial agent.

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