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ANTIMICROBIC, CYTOTOXICITY AND DNA CLEAVAGE EVALUATION OF SILVER NANOPARTICLES

Asma.M. Tatagar[1,2*], Javed.I. Moodi[3], M.A. Phaniband[4]

*[1] Research and Development Centre, Bharathiar University, Coimbatore, Tamilnadu, India

[2] Department of Chemistry, SDMCET, Dharwad, India

[3]Department of Chemistry, Kittel Science College, Dharwad—580002, Karnataka, India

[4]Department of Chemistry, Al-Ameen Arts, Science and Commerce College, Bangalore, Karnataka, India

Abstract: The present set-up of bio-medical applications stresses on the cost effectiveness and eco-friendly nature of Nanoparticles which are very well answered by silver nanoparticles making it a front runner towards the biomedical investigation and assay. For this purpose, in this study, we aimed to synthesize the silver nanoparticles (AgNps) using chemical reduction method, a Partial Green Synthesis involving Ascorbic acid a form of Vitamin-C. The Synthesized AgNps were characterized using UV-Visible Spectroscopy, X-ray diffraction (XRD) and TEM. The antimicrobic studies included the bactericidal and fungistatic evaluation of AgNps on bacterial strains S. aureus, S. mutans and the fungal strains of A. niger, C. albicans. Norfloxacin a standard antibacterial drug and Griseofulvin a well- known antifungal drug was used as standards for the study. Cytotoxic evaluation was conceded using protocol of Meyer et al (brine shrimp bioassay). The DNA dissociation activity of synthesized AgNPs was studied. The AgNPs were found to be more susceptible towards the bacterial strains as compared to the fungal strains.

Keywords: Silver nanoparticles (AgNps), Cytoxicity, DNA cleavage, Norfloxacin, Griseofulvin.

INTRODUCTION

Metal-based nanoparticles are the most acknowledged inorganic nanoparticles that match up to a hopeful answer against the resistance to conventional antibiotics. The mechanisms of action are categorically diverse when compared to the traditional antibiotics, exhibiting activity against the bacteria that have already developed resistance, nevertheless it also targets multiple bio molecules negotiating the development of resistant strains[1]. The surface/volume ratios of NPs are extremely high and NPs gain different properties and functions due to their volumetric ratio. This property accounts to selection of NPs in both in vitro and in vivo studies. Among nanostructured noble metals AgNPs have attracted considerable attention for their application in many fields as catalysts, as optical sensors, in textile engineering, in electronics, in optics, and most importantly in the medical field as biosensors, a bactericidal, a biomedicine and as a therapeutic agent [2]. Contrasting to the standard antimicrobial agents, low doses of silver nanoparticles are needed in the treatment of diseases [3]. Silver nanoparticles anchor to the bacterial cell wall and later penetrate it, thus triggering structural changes in the cell membrane. AgNps create 'pits' on the cell surface which leads to accretion of the nanoparticles on the cell surface [4]. The interaction of silver nanoparticles with microorganisms gives rise to the release of silver ions which are prospective destroyers of microbial cells due to their ability to deactivate enzymes in microbial cell and disrupt membrane permeability leading to lysis and apoptosis [5]. The anti-inflammatory and anti-angiogenesis abilities of AgNps make their application in medical field paramount. The chemical and physical properties of the metal nanoparticles are reliant on their structure, shape as well as size distribution. Consequently, control over the size and size distribution is crucial and is often achieved by varying the synthesis methods, reducing agents and stabilizers [6]. In the synthesis of nanoparticle by chemical reduction method, the stabilizing agent can also perform as a reducing agent in the same reaction. Pertinent average size, polydispersity, and shape of AgNPs can be attained by controlling the nucleation stage by monitoring the experimental parameters, such as the precursor used in the reaction, reducing agents, reagent concentration, pH, and temperature. A critical step in the synthesis of AgNPs is their stabilization, especially in order to prevent agglomeration and oxidation processes. Therefore, one of the most common strategies is the use of stabilizing agents that can protect AgNPs. The stabilization can be achieved by electrostatic repulsion by incorporating a negative charge on the surface of these NPs [7]. Moreover, the choice of the surfactant is vital as it determines the stability, solubility, reactivity, dispersibility and even the size and shape of the nanoparticles during the synthesis [8]. The present study describes a simple, low-cost, and partially green method, to synthesize silver nanoparticles. This synthesis method is considered partially green as it uses ascorbic acid which is one form of vitamin C. It has also been widely used as a reducing agent in synthesis processes. Whereas, in the present study, ascorbic acid has been used as a surfactant, rather

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than as a reducing agent, in order to prevent the aggregation of metal particles. The combined effects of trisodium citrate and ascorbic acid on the AgNPs were characterized by XRD, whilst the particle morphology and size were observed by TEM and UV-visible (UV–vis) absorption spectroscopy. The present investigation comprised of the antimicrobic studies including the bactericidal and fungistatic evaluation of AgNps on bacterial strains S. aureus, S. mutans and the fungal strains of A. niger, C. albicans. A Cytotoxic evaluation was conceded using protocol of Meyer *et al*. (brine shrimp bioassay) and the DNA dissociation activity of synthesized AgNPs was deliberated.

EXPERIMENTAL

Materials and Methods

Synthesis of Silver nanoparticles (Ag Nps):

Chemical reduction method was employed to synthesize AgNPs. Silver nitrate ($AgNO_3$) is used as a starting material, aqueous solution of trisodium citrate ($C_6H_5O_7Na_3$) and ascorbic acid ($C_6H_8O_6$) were used as the reducing agent and surfactant, respectively. The experimental technique employed is a slightly modified version of the procedure reported by Dadosh *et al.* [9]. 100 ml, 1M silver nitrate ($AgNO_3$) solution was heated an oil bath at 60 ºC for 30 min, 25 ml solution of trisodium citrate ($C_6H_5O_7Na_3$) and ascorbic acid ($C_6H_8O_6$) was added to the hot solution $AgNO_3$ with vigorous stirring. A colloidal solution of Ag NPs was accomplished when the subsequent mixture was stirred at the rate of 150 rpm in an orbital shaker for 30 minutes till the solution attained the room temperature.

Characterization of Silver nanoparticles (Ag Nps):

AgNPs were characterized by UV, TEM and XRD analyses. The synthesized AgNPs colloid was characterized by XRD (model D5000 Siemens Diffractometer) with 0.15405 nm Cu Kα radiation. The optical absorption spectrum was analyzed by UV–Vis spectrophotometer on UV-1800 (200–700 nm). The size and morphology of the Ag nanoparticles produced were studied by TEM (model Philips CM12 equipped with Docu Version 3.2 image analysis system).

Biological Assay

Antibacterial and antifungal studies:

The antimicrobial studies were performed using disc diffusion methods. The MIC is described as the lowest concentration of a substance that inhibits a microbial growth [10]. The bacterial strains of Staphylococcus.aureus, Streptococcus.mutans and the fungal strains of Aspergillus.niger, Candida. Albicans were selected for the study of bactericidal and fungistatic activities of synthesized AgNps respectively. Norfloxacin a standard antibacterial drug and Griseofulvin a well- known antifungal drug was used as standards for the study. The microorganism cultures were inoculated in a nutrient broth (1.3 g in 100 ml D/W10) The inoculum is regulated to McFarland 0.5 turbidity standard, a sterile cotton mop is dipped into the inoculum and swiveled alongside the walls of the container to remove excess inoculum. After inoculation, discs were kept in an incubator for 24 h at 37 ºC for bacteria and 48 h at 25 ºC for fungi. The MIC was determined as the lowest concentration of Ag NPs that inhibited of microbial growth.

Cytotoxicity:

Cytotoxicity studies were conceded using protocol of Meyer *et al.*[11] (brine shrimp bioassay). Test samples were prepared by using 20 mg compound dissolved in 2 ml DMSO. A total of 9 vials were prepared by taking three for each dilution viz., 100, 50 and 10 µg ml^{-1} and were used individually to test the $LD_{50.}$ The solvent from the sample was allowed to evaporate overnight. The shrimp larvae were ready to be used after 48 hours. 10 shrimps were added to each vial of 1mL and the volume was adjusted to 5 ml using seawater. The number of survivors were counted after a time interval of 24 hours and then using Finney computer program the data was analyzed LD_{50} values were determined [12].

DNA Cleavage Studies:

DNA cleavage capability of AgNPs was inspected using agarose gel electrophoresis using E. coli (ATCC 25922) extracted genomic DNA as a target [13]. The cleavage performance of the synthesized of Ag NPs compared to that of the control is because of its competent DNA cleavage ability [14]. The nanoparticles (100 mM) were mixed with the target plasmid (1:1) and this mixture was then incubated at 37 °C for 2 h. After incubation, the DNA and the compound mixtures were loaded in wells, and electrophoresis was carried out at a voltage of 70 V for 30 min, untreated genomic DNA was used as a negative control. The plasmid DNA (pUC19) was used as target DNA molecules. The efficiency of cleavage of the molecule was probed using agarose gel electrophoresis. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be detected for the super coiled form (Form I). If one strand is cleaved, the super coil will relax to produce circular form (Form II) with a slower-moving AgNPs. If both strands are cleaved, a linear form (Form III) will be generated that migrates in between. The Silver nanoparticle complex was found to promote the cleavage of pUC19 plasmid DNA from supercoiled (Form I) to the circular form (Form II) by varying the concentration of AgNPs (100–400 μM).

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RESULTS AND DISCUSSION

Characterization of Synthesized AgNps

The TEM analysis (Fig.1) results reveal that the synthesized nanoparticles are quasi-spherical at all concentrations of the reductant trisodium citrate dihydrate, the existence of other shapes such as polygons is also noted, the particles are found to be well-dispersed. TEM images indicate a particle size of 80-100 nm. From the representative X-ray diffractogram (Fig.2) it can be observed that the peaks are considerably widened, indicating that the material is composed of very small silver crystallites. Many distinct diffraction peaks at approximately 38.14°, 46.9°, 64.25°, 77.32° and 81.56° were allocated to reflections from the 2θ region for AgNPs. Preceding works by Oliveira *et al.*[15] indicates that nano-sized metal particles are expected to present a lattice contraction due to high surface pressure, and a characteristic non-crystalline atomic arrangement. UV–Vis spectrophotometer was also used to authorize the formation of AgNPs in the aqueous colloidal dispersion. Samples showed a sharp SPR band at 422 nm, which is characteristic peak of NPs (Fig.3)

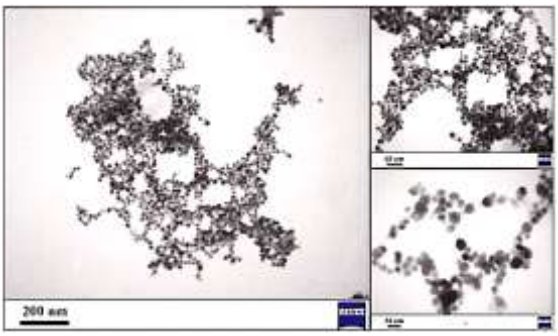

Fig 1. TEM images of synthesized AgNPs

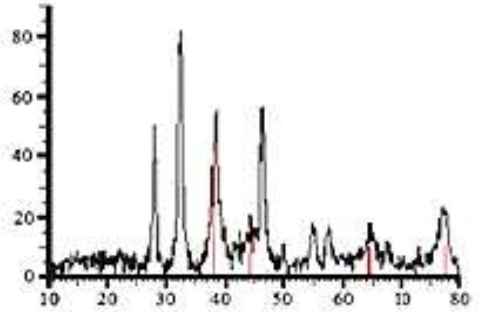

Fig 2. The X-ray diffractogram of the synthesized AgNPs.

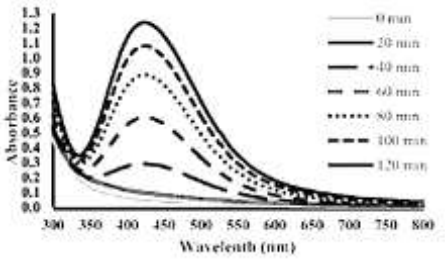

Fig 3. UV–Vis absorption spectra of resulted solution of AgNps

Biological Assay:

In the present inspection, the AgNPs were tested for their antimicrobial activity against test bacterial and fungal strains. Results show that synthesized of AgNPs had potent antimicrobial activity. The synthesized Ag nanoparticles showed prominent susceptibility towards the bacterial strains than the fungal strains. The zone of inhibition depicting the bactericidal and fungi static activities of synthesized Ag nanoparticles is represented in Fig. 4 (a-d). The antibacterial and antifungal activities are shown in Table 1. The MIC results determined as µg / mL active compounds are shown in Table 2.

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Fig 4. Zone of inhibition depicting antimicrobial activity of synthesized Ag nanoparticles

Table 1. Antimicrobial assay of synthesized Ag nanoparticles (size ≤ 100 nm) (zone of inhibition in mm for 100 µg/mL)

Compound Code	Anti-Bacterial		Anti-fungal	
	S. aureus	*S. mutans*	*A.niger*	*C. Albicans*
AgNps	22	20	15	17
Norfloxacin	28	26	--	--
Griseofulvin	--	--	27	28

Table 2. MICs* values of the some AgNPs in µg / mL

Compound Code	Anti-Bacterial		Anti-fungal	
	S. aureus	*S. mutans*	*A.niger*	*C. Albicans*
AgNps	6.25	7.40	12.50	12.50
Norfloxacin	1.5	1.5	--	--
Griseofulvin	--	--	1.6	1.6

Cytotoxicity

Cytotoxicity studies were approved using protocol of brine shrimp bioassay. A total of 9 vials were prepared by taking three for each dilution viz., 100, 50 and 10 µg ml^{-1} and were used individually to test the LD_{50}. 10 shrimps were added to each vial of 1mL and the volume was adjusted to 5 ml using seawater. The number of survivors were counted after a time interval of 24 h and then using Finney computer program the data was analyzed. The recorded LD_{50} value was the mean of the values recorded and these are represented in Table 3.

Table 3. Cytotoxicity studies, the mean of the LD_{50} values recorded using Finney computer program

Concentration of AgNps (size ≤ 100 nm)	LD_{50} values
100 µg ml^{-1}	2.748 x 10^{-2}
50 µg ml^{-1}	2.064 x 10^{-2}
10 µg ml^{-1}	1.689 x 10^{-2}

DNA Cleavage studies

The cleavage studies were accomplished by gel electrophoresis. The electrophoresis reveals that AgNPs acted on plasmid DNA molecules. The Results are shown in Figure 5. Indicated the differences in the bands of Lanes 2–4 when compared with control DNA. One of the most stimulating electrophoretic results of the AgNPs was noted when the experiment was carried out in presence of H_2O_2, the cleavage of the super coiled DNA Form (I) into DNA Form (II) was observed to be greater than AgNPs complex alone on using H_2O_2. The effect of reactive oxygen species on this development was verified with standard hydroxyl radical scavenger (DMSO) and singlet oxygen scavenger (NaN_3). DMSO remarkably inhibited the DNA splintering (36–40%) persuaded by the AgNPs (100 μM) (lane 5 in Fig. 5). Interestingly, the singlet oxygen scavenger NaN_3 failed to protect the AgNPs induced DNA cleavage (lanes 6 in Fig. 5), which suggests that singlet oxygen does not play an important role in the cleavage mechanism pathway.

Fig 5. DNA Cleavage studies using synthesized AgNPs

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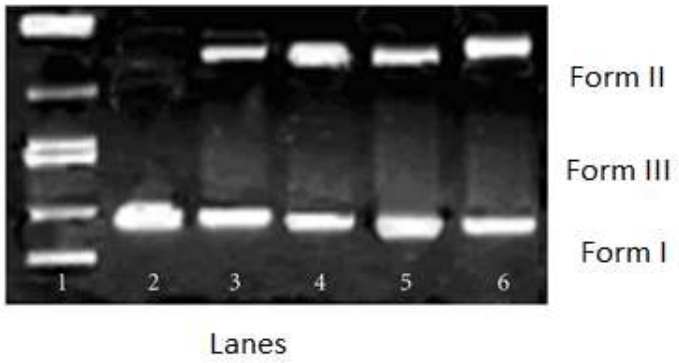

CONCLUSIONS

In the present study the AgNps were synthesised by Chemical reduction method. and characterized using XRD with 0.15405 nm Cu Kα radiation. The size and morphology of the Ag nanoparticles produced were studied by TEM. The size distribution of AgNPs was found to be monomodal, with an average particle size of >100 nm. A Biological assay of synthesised AgNps were carried out to unveil the antibacterial, antifungal, cytotoxic and DNA cleavage properties. The results are promising and the DNA cleavage and antimicrobial activities of AgNPs are very projecting. The AgNPs were found to be more vulnerable towards the bacterial strains as compared to the fungal strains. The synthesised AgNps sow low cytotoxicity at lower concentrations. The DNA cleavage study of the synthesized nanoparticles was determined by agarose gel electrophoresis using E. coli (pUC19) extracted genomic DNA as a target. One of the most interesting electrophoretic results of the AgNP complex takes place when experiment done in presence of H_2O_2. The results indicate that the cleavage reaction involves hydroxyl radicals, that is, a Fenton type reaction may lead to the formation of the oxygen active species which finally cleave the DNA. Such research may be advantageous in the preparation of nano-drugs and targeted drug delivery in the near future.

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