A STUDY ON ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES

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Abstract

The silver nanoparticles that have been studied in this research were produced by low cost chemical reduction method. X-ray diffraction was used for estimation of particle size of silver nanoparticles which were found to be 8 nm. The aim of this study was to determine the anti-microbial effects of silver nanoparticles synthesized by low cost chemical reduction method. The antibacterial activities were studied against gram-negative and gram-positive bacteria. The results showed that the small size silver nanoparticles had significant effects on bacteria. These small size nanoparticles rapidly penetrated inside the cell and stopped the growth of bacterial disease. Furthermore, the antifungal activity was studied by inspecting the effects against saprophytic fungi, which conformed that the silver nanoparticles were active against saprophytic fungi.

Introduction

Technology that deals with very small things (less than 100 nanometers) is known as Nanotechnology (Abdi *et al.*, 2008). Presently, the role of this technology is becoming more significant in different fields of science, i.e. research in interdisciplinary regions (Oleg, 2004). Moreover nanotechnology has very considerable commercial applications and very soon it will be a leading technology in economical zone (Singh and Singh *et al*., 2011). Particles having very small size, measured in nanometers, are considered very crucial blocks of this technology. As nanoscale particles have very effective link between physical and atomic or molecular structures of materials so study of these particles has very technical and scientific attraction. The nanoscale particles which are considered the most efficient are from noble metals for example Silver (Ag), Gold (Au), (Pt) and Palladium. The applications of nanoscale particles cover very long range which is from medical to materials-field. Metallic nanoparticles are widely used in different application especially in biological fields due to its exclusive physic-chemical properties (Shrivastava *et al*., 2007).

Since last few decades, plant and plant derived products are getting more consideration as a source of medicine in the medical science. For replacement of extracellularly, fabrication of plants, fruit and vegetables meditation would make the nanoscience compatible with bio (Ncube *et al*., 2008). The good thing is that the time reported for reaction is very short as compare to chemical and microbial techniques of synthesis. Presently at global level the development of ecological and eco-friendly processes is a very crucial need which encourages the nanoscience in biological approaches. Furthermore, study shows that the nanoscale particles of silver are nontoxic to human and act more fruitfully against bacteria and other eukaryotic microbes with low absorption and have no side effects (Mahindra *et al.*, 2009).

It has been accepted worldwide that fabrication of silver nanoscale particles through chemical reduction method is more suitable for antimicrobial activity than its anticancer activity (Sawai *et al*., 2002). Cancer is a growth of abnormal cells which have potential to attack or spread in other parts of body. In the perspective of overall public health, it has been seen that need of anticancer therapy is increasing. In vitro cytotoxicity methods which are used to measure the cell injury help to reduce the use of laboratory. For satisfactory risk evaluation, mechanism derived information is an essential key which is obtained by in vitro systems. From the usage of refined tissue and cells it has been proved analytically that there is an urgent requirement that new active chemotherapeutic agents may be identified from natural sources. White color Cauliflower is a vegetable which is acclaimed a high dietary foodand because of its low calorie, it can also be included in routine diet (Brayner *et al*., 2007). Although the cauliflower is not green however its floret is not only a great source of antioxidants i.e. vitamin C, poly-phenols and phytonutrients but it also has a significant amount of cancer fighting compound such as sulforaphane and Indole-3-carbinol, which not only help to restore the DNA but also prevent growth of cancer cells (Stoimenov *et al.*, 2002).

Methods for synthesis of nanoparticles are grouped as Physical Method and Chemical Method through different techniques including micelles, sol gel process, chemical precipitation, mechanical shaking, hydrothermal method and chemical vapor deposition (CVD) method (White *et al*., 2009). Unluckily, there are

some shortcomings of these techniques such as high cost, exploitation of high energy and geno-toxic effect of carcinogenic chemicals in medical uses.

Keeping above into account, we have explored the fabrication of silver nanoparticles through reducing agent. This study has been focused to form an outline so for it may be helpful in the uses of reducing and stabilizing agent which converts silver ions into nanoscale particles during reaction (Elkins *et al.*, 2011).

EXPERIMENTALS

In the preparation of silver nanoparticles Sodium Dodecyl Sulphate (SDS) and Citric acid ($C_6H_8O_7$) were used as reducing agent along with two stabilizing agent Silver Nitrate ($AgNO_3$) and Hydrazine hydrate (N_2H_4-H_2O). The other laboratory equipment used for this synthesis of nanoparticles were beakers, heater, flasks and distilled water. Solution (1.0 mM per 50 ml) of $AgNO_3$was prepared as metal salt predecessor and 6% (w/w) Hydrazine hydrate (N_2H_4-H_2O) solution was used as stabilizing agent. The reducing agent Dodecyl Sulphate (SDS) and Citric acid ($C_6H_8O_7$) are prepared in ratio of 2.0 mM and (1.0 mM per 50 ml) respectively. When the reducing agent Dodecyl Sulphate (SDS) was mixed in the solution it colored the solution in to the characteristic pale yellow. This formation of colored solution conform the presence of silver nanoparticles, which were purified by distilled water. A centrifuge machine having (21000 rpm at 10^0C) was used to collect the silver nanoparticles from the solution. These particles still have some quantity of water and further sent to furnace at temperature of 400^0 C which gave us final stage of silver (Ag) nanoparticles in the powdered form.

Results and Discussion

Size Calculation: The size of the nanoparticles was the major issue in the study of antibacterial activity. In our study we have found the size of silver nanoparticles by X-ray diffraction. The size was calculated from the most intense peak of XRD graph given in the Fig.1. The most intense peak was found at (111) plane and by using the Sharer's formula the size of silver nanoparticles was about 8 nm (Patterson, 1939).

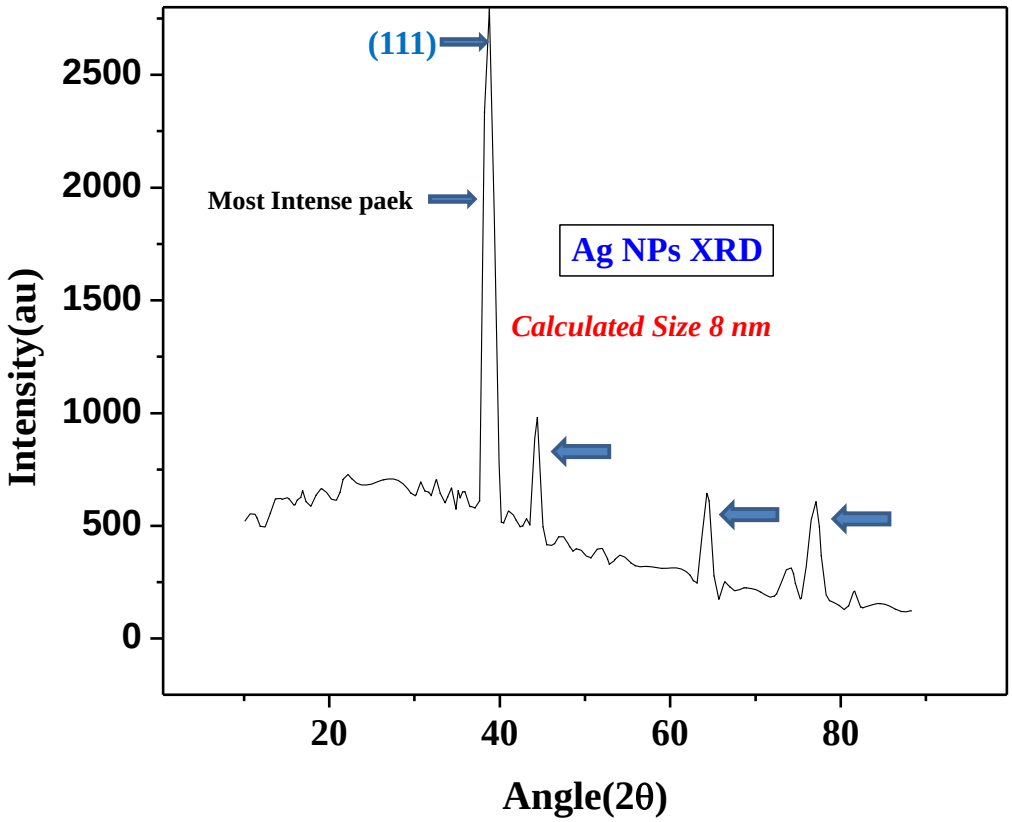

Fig. 1. The X-ray diffraction of silver (Ag) nanoparticles.

ANTIBACTERIAL ACTIVITY AND MINIMUM INHIBITORY CONCENTRATION (MIC)

The least value of anti-microbial concentration which is used to reduce the speed growth of very small scale organism is known as Minimum inhibitory Concentration (MIC), it was also measured by the growth of bacteria in micro titre plate. In distilled water a stock solution of 100 mg/ml was prepared, and keeps it incubating at

37^0C about 24 hours. The increment in absorbance gives the bacterial growth (Jain, *et al.*, 2009), which increases at 600 nm before taking the reading about 30 seconds. We have performed the experiments six times for reading about antibacterial data.

Now days nanoparticles have great interest and attention in the field of medicine and it has wide use in solving problems of emergence of bacteria in multidrug resistance instead of antibiotics. Silver nanoparticles particularly have great attention in this area of medicine; because the use of silver nanoparticles has huge effect for antimicrobial due to its less cytotoxicity. In the past years silver nanoparticles were used against bacterial pathogens and open a new area of research in antimicrobials field, which still not yet fully evaluated it needs more attention of researchers. The different nanoparticles have different interact with microbes and have different impact on the antimicrobials activity depending upon their structure, shape and mode of interaction with bacterial surface. The structure of silver nanoparticles have great importance because the least the size of the particles the greater its surface area to its volume ratio and have greater effects in interactions with bacteria's, this leads the difference in chemical and physical properties of Ag NPs from their bulk material. The silver nanoparticles interact physically with the bacterial surface cell, which has great importance in the case of gram-negative bacteria. The silver nanoparticles having size < 30 nm was considered to best against *Staphylococcus aureus*. The particles having small size have more ability of penetration into bacteria, which interact with membranes and killed the cell, these all dependent upon the size of particles. Smaller size particles with positive zeta potential produce electrostatic force which is more effective for killing of bacteria. If we have comparison of silver nanoparticles with its bulk silver, the interaction found in bulk is lesser than nanoparticles the bulk silver has less antibacterial activity. The nanoparticles of silver if we have careful analysis show that also have more antibacterial activity than its silver ions. Some researchers have also reported that the structure of silver nanoparticles have also great effect on their antibacterial activity, the triangle type size nanoparticles have more anti-bacterial activity than the spherical one. This study suggests the silver nanoparticles interaction with bacterial replication process.

We have collected the antibacterial data of our 8nm sized silver nanoparticles from Microbiology department FUUAST Karachi. In this antibacterial study on 14 Gram Positive and 22 Gram Negative bacteria the main methods used for analyzing the bacteria were Agar well and Micro dilution method in mg/ mL given in Table 1.

Table 1:- Minimum Inhibitory Concentration (MIC) values determined by Micro dilution method.

Gram positive	MIC (mg/mL)	Gram negative	MIC (mg/mL)
Streptococcus fecalis	120	*Acinetobacter baumanii*	82
Streptococcus pyogenes	132	*Serratia marcesens*	92
Staphylococcus saprophyticus	122	*Aeromonas hdrophila*	120
Staphylococcus epidermidis	180	*Shigella dsenteriae*	100

The Table 1 shows the antibacterial data for gram-negative and gram-positive bacteria. The value of MIC is very low (82 mg/mL to 132 mg/mL) that leads us towards the highest value of antibacterial property. If we have a look on bulk of silver as compared to silver nanoparticles we have found that the MIC value of nanosized particles (82 mg/ mL to 132 mg/ mL) is very low that's why nanoparticles are more effective than its respective bulk. The gram positive values for *Streptococcus fecalis*, *Streptococcus pyogenes*, *Streptococcus saprophyticus*, and *Staphylococcus epidermidis* values are given in Table 1 are very low ranging from (82 mg/mL to 132 mg/mL) which shows its high interactivity against bacteria (Libor. *et al.*, 2008). On the same way the gram-negative values for *Acinetobacter baumanii*, *Serratia marcesens*, *Aeromonas hydrophila*, and *Shigella dysenteriae* with their respective values are shown in Table 1. The conformation about effectiveness against bacteria was found after careful analysis of their mg / mL values. The MIC values are very low for above mentioned parameters which conform that silver nanoparticles are of very small size and huge effectiveness for antibacterial activities.

Antifungal activity: The micro broth dilution method is used for the estimation of (MIC) by using 96-well micro titre plate. A solution was prepared by 100 mg/mL in distilled water known as stock solution. A 100 µl broth was used for preparing two fold serial solutions of extracts and in both the wells 10 µl culture matched with 0.5 Mac Farland was added. Both well were performed separate role first one as antifungal and second one as culture control. The used Micro titre plate kept alive at 37^0 for 24 hours.

The type of fungi that cause disease in humans or any other organisms is known as pathogenic fungi. The test organisms in our case were consists of 5 saprophytic fungi which are listed in Table 2, which were used for in this study and fungal isolates acquire at Microbiology department FUUAST Karachi. The resulted fungal isolates were pure and stored in refrigerator at 4^0 on Sabourd Dextrose agar (SDA), which was further tested by

Agar-well method for antifungal activity in the ratio of 1:1.The prepared fungal spore suspension by distilled water was further transferred on SDA plates. A constant temperature (28^0C) maintained for 24-48 hours for incubating the SDA plates, after this process the inhibition zone was measured.

Table 2:- Shows the Zone of inhabitation in (mm) and MIC in (mg/mL).

fungi	Zone of inhibitions in mm(mean ±SD)	FUNGI	MIC (mg/mL)
Microsporum canis	18±2	*Aspergillus flavus*	160
Aspergillus flavus	17±0	*Aspergillus niger*	100
Aspergillus niger	14±0	*Microsporum canis*	100
Penicillium sp	14±0	*Penicillium sp*	50
Rhizopus	22±1	*Rhizopus*	50

Currently a large number of rigorous fungal infections produce harm effects on the immune compromised patient which needs intensive care treatment through antibiotic therapy. Metallic nanoparticles have lot of potential in the field of antifungal activities but we found limited studies on it. In our current research we study the effect of silver nanoparticles on 5 saprophytic fungi. The data of our observations given in Table 2 shows the zone of inhibition (mm) and MIC (mg / mL) values. The data values show that the silver nanoparticles have greater effect on *Rhizopus* with the value (50 mg/ mL), it can kill *Rhizopus* fungi and more suitable against *Rhizopus* fungi. The lowest value conform that silver nanoparticles are of smaller size and very much effective against saprophytic fungi. The nanoparticles required low concentration to destroy the growth of harmful saprophytic fungi; they target the cell membranes and disturb the membrane potential, which decreases the growth and effects of saprophytic fungi.

Conclusion

The silver nanoparticles used in this study were successfully synthesized by using low cost chemical reduction method. The synthesized nanoparticles were in nanometer size as conformed by X-ray diffraction that was 8 nm. The small size synthesized nanoparticles were further studied against bacteria and fungi. The antibacterial activity were studied against different bacteria and found that the small size silver nanoparticles have great effects on bacteria and rapid large interaction with cell to stop their growth. The smaller size silver nanoparticles rapidly penetrate inside the wall of cell membrane and stop the growth of bacterial growth. The low value of MIC leads toward the higher antibacterial activity of silver nanoparticles. The MIC value of these synthesized nanoparticles lies in the range of (88 mg/mL to 132 mg/mL). The effects of silver nanoparticles on 5 saprophytic fungi were also studied, that confirmed the presence of antifungal activity in silver nanoparticles against the saprophytic fungi.

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