Binding Properties And Anti-Microbial Activity Studies Of Ag Nanoparticles

Priyadharsini K and Vasanthan T

Department of Physics and Nanotechnology, SRM University, Kattangulathur, Kancheepuram, India.

Work done at: Centre for Bioscience and Nanoscience Research (CBNR), Coimbatore-21, India.

Abstract:

Microbial contamination is a major issue everywhere ranging from industries to consumables including water which makes antimicrobial agents more important. The binding of Ag nanoparticles on cellulose nitrate membrane and its antimicrobial activity synthesised by different methods against the bacteria – *E.Coli, Bacillus, Pseudomonas, Candida* and *Staphylococcus* were studied. Ag nanoparticles were initially synthesised by chemical method using Sodium citrate. The solution containing Ag nanoparticles were then passed by syringe filters through two cellulose nitrate filter membrane of pore size – 0.2 µm and 0.45 µm. The two filtrates and the two discs (filter membranes) were checked for anti-microbial activity against *E.Coli, Bacillus* and *Pseudomonas* by disc and well method. Inhibition zone was seen only in E.coli culture. In the next step, the *E.coli* culture in liquid medium was centrifuged and the supernatant was taken as the biological medium to grow silver nanoparticles to which the discs were added to initiate the Ag nanoparticles synthesis. Antibacterial activity of biologically synthesised Ag nanoparticles was studied against *Bacillus, Pseudomonas, Candida* and *Staphylococcus*. The inhibition zone was seen for *Bacillus* and *Staphylococcus* culture with increase in size of inhibition zone. 0.45 µm membrane filter was more effective due to large no. of nanoparticles. The effect of biosynthesis of Ag nanoparticles on anti-microbial activity, particle size and binding of Ag nanoparticles were also studied.

Keywords: Silver Nanoparticles, Cellulose membrane, anti-bacterial activity, inhibition zone

Introduction:

Microbes are seen everywhere which can be useful or harmful to us. The problem is with the harmful microbes which contaminate and damage a wide range of products ranging from food industries, drinking water, textiles to consumables. *E.Coli*, a gram-negative bacterium, is the major contaminant in drinking water that makes water unfit for drinking. Here, the anti-microbial / anti-bacterial agents play an important role to overcome the contamination/damage caused by the microbes.

Silver is generally a good anti-bacterial agent but it is observed that silver as silver nanoparticles exhibit enhanced anti-bacterial activity. This makes silver, a possible material to act as a better anti-bacterial agent in crucial areas like water filtration. From previous studies, it has been shown that silver nanoparticles are able to reduce/eliminate the *E.Coli* bacteria from impure water well below EPA Standards but the problem seen is that the silver nanoparticles are eluted along with purified water. Here, it is important to study the binding properties of Silver Nanoparticles to various substrates.

In this paper, we have studied the binding properties of Silver nanoparticles to two different types of membranes, the anti-microbial activity of chemically synthesised and biologically synthesised silver nanoparticles and a comparison between the two methods is presented.

II. Experimental Section:

A. Synthesis Of Silver Nanoparticles (Chemical Method):

Silver Nitrate was used as the precursor and Sodium Citrate as the precursor. The medium in which the silver nanoparticles were synthesised is water. 5 mM concentration of Silver Nitrate solution in excess water and 1% Sodium Citrate solution in water were prepared. The Silver Nitrate solution (mixed with water) was heated in a boiling water bath and the Sodium Citrate solution was added to the boiling mixture. The solution was initially pale yellow and then turned into brown

after few minutes of boiling at approx. 100 ˚C. Brown colour of the solution indicates the presence of silver nanoparticles. The boiling process was stopped and the solution was allowed to cool at room temperature. The conical flask containing the solution was covered by light-tight black paper to prevent the reaction of silver with light. The presence of Silver nanoparticles had been confirmed through characterization by UV Visible Spectrophotometer. This solution would be referred as 'Crude' in the following text.

B.Growth of Bacteria:

E.Coli, *Bacillus* and *Pseudomonas* were obtained from CBNR and maintained in Nutrient agar slants. The culture of each bacterium was grown in LB (Luria – Bertani). The bacteria in the liquid medium was added to this LB Broth medium in small quantities (about 50 µl) and incubated at 37^{o}c for 24 hrs.

C. Binding:

To study the binding properties of silver nanoparticles, two membranes made of cellulose nitrate of different pore size – 0.45µm and 0.2µm were used. The previous studies showed that a simple blotting paper coated with silver nanoparticles can act as water filters to effectively reduce *E.Coli* concentration but the discharge of silver nanoparticles along with the filtered water was estimated to be about 20% more than the EPA standards. It is because of the ineffective binding of silver nanoparticles to the pores in the blotting paper.

The Cellulose Nitrate membranes were cut into proper size to fit the syringe filter. The 'Crude' solution was divided into three parts. One part was kept as such and the other two parts were passed through the two different filter membranes. Some nanoparticles bound to the membrane pores and the other smaller nanoparticles were present in the elute/filtrate obtained from the two membranes

D. Anti-Bacterial Studies:

The anti-bacterial activity of silver nanoparticles was studied using the membranes, filtrates and the crude. The medium used was Mueller Hinton Agar No.2. Two wells were made in the solid medium using gel puncher. Using a micropipette, 100 µl of each bacterium was added in two plates and was made to get spread evenly by using an L-shaped rod. The rod was previously heated in a dry flame and cooled to destroy organisms in it, if any.

The crude and filtrate were poured in the two wells and the disc was kept in the plates. The 0.25µm membrane and the filtrate obtained from it were kept in one plate and the other membrane and filtrate, in the other plate. All the six plates were kept in the incubator for 6- 8 hours. The whole process was carried out under ideal atmospheric conditions in a laminar horizontal flow chamber.

Results – I:

Bacteria	Inhibition zone				
	Crude	**0.45 µm disc**	**0.45 µm elute**	**0.2µm disc**	**0.2µm elute**
E.Coli	3 mm	5 mm	7 mm	8 mm	7 mm

The inhibition zone was observed only in *E.Coli* plate. Silver nanoparticles proved inefficient against *Bacillus* and *Pseudomonas*. It was observed that the 0.45µm membrane's elute and 0.2 µm membrane showed better inhibition because of better binding of silver nanoparticles to that membrane and that the pore size is small.

Fig . (1)

Fig. (2)

Figures 1 & 2: *Anti-bacterial activity seen against* E.Coli *– Visible inhibition zones*

Synthesis of Silver nanoparticles from E.Coli:

The *E.Coli* culture grown in the Luria Bertani Broth medium (liquid) was centrifuged at 10000 RPM for 5 minutes at 4˚C and the supernatant free from bacteria was taken. This supernatant is used as the biological medium to synthesis silver nanoparticles. This medium contains a protein called 'Tyrosine' which acts with the chemically synthesised silver nanoparticles and possibly a small amount of the precursor to synthesise more nanoparticles with increased anti-microbial activity.

The supernatant solution was taken in three 5 ml test tubes. One was kept as such which is referred to as the "control". To the second test tube containing the supernatant, 0.2µm disc was added. To the third test tube, the 0.45µm filter disc was added. These mixtures were stirred every half-an-hour to ensure the uniform distribution of synthesised silver nanoparticles.

Confirmation of Silver Nanoparticles:

The presence of silver nanoparticles was confirmed by the colour change of the medium from pale yellow to brownish yellow indicating the presence of silver nanoparticles. This solution was then tested for anti-bacterial activity against 4 bacteria and it produced inhibition zones. This further confirms the presence of Ag nanoparticles.

A plausible reaction, here , is that the Protein here acts with the silver ions to reduce it and then all the nanoparticles are covered by a protein layer which acts to prevent agglomeration of nanoparticles which leads the particles to remain stable.

Petri Plate Preparation from 4 Bacteria:

Muller Hinton Agar plates were prepared, as done earlier for 4 bacteria - *Bacillus, Staphylococcus, Candida, and Pseudomonas.* 4 plates were prepared, each containing one bacterium. Three wells were made using gel puncher for silver nanoparticles grown in supernatant+ 0.2µm membrane, supernatant + 0.45µm membrane and control. The plates were kept in the incubator for 6-8 hours.

Results – II:

The zone of inhibition was observed in *Bacillus* and *Staphylococcus* whereas *Candida* and *Pseudomonas* did not show any inhibition zone.

Bacterium	**Inhibition zone**	
	0.2µm membrane + supernatant	0.45µm membrane+ supernatant
Staphylococcus	8 mm	11mm
Bacillus		11mm

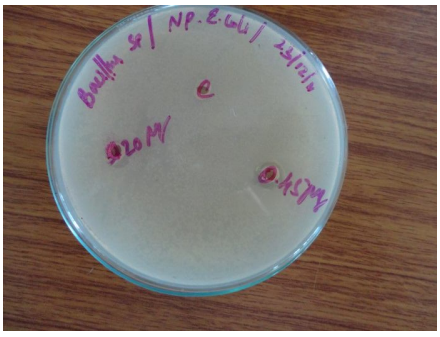
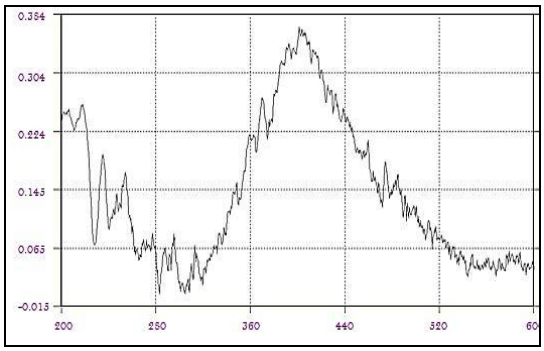
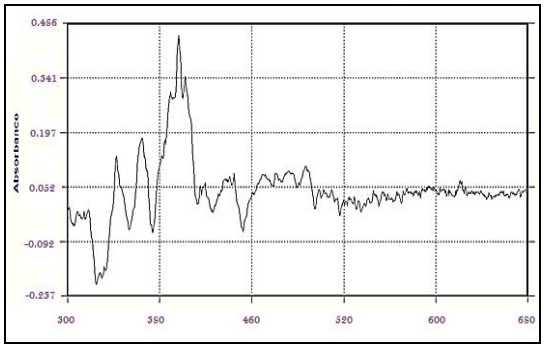


Fig. (1)

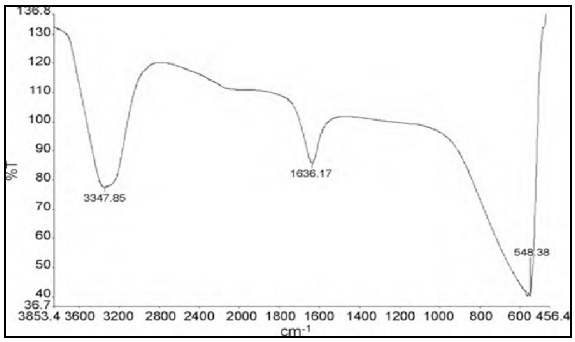
Characterization:



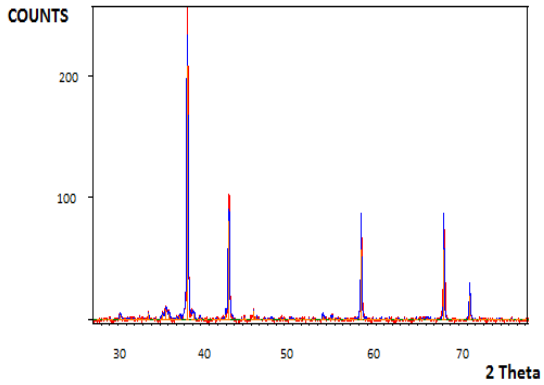
UV-Visible spectra showing peak at 410nm indicating the presence of silver nanoparticle



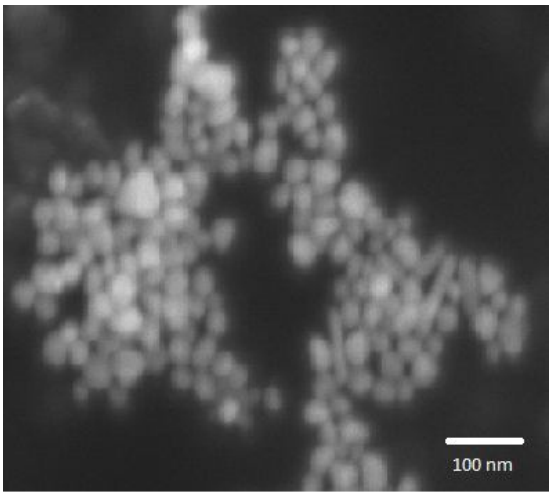
UV-Visible Spectra of biologically synthesised nanoparticles showing a blue-shift at 396 nm.



FTIR spectra showing peaks for appropriate bonds - confirmation of protein.



XRD Spectra of silver nanoparticles. The average crystallite size was calculated by the software Xpert Highscore Plus to be 22 nm.



SEM image showing the morphology of silver nanoparticles. They were spherical with least particle size dispersion. ImageJ software was used. The average particle size was 20 nm.

Conclusion & Future Studies:

This protocol followed here to synthesize silver nanoparticles enables the production of Ag particles of greater anti-bacterial activity, lower particle size (indicated by blue-shift). This is more efficient because the nanoparticles smaller than the pore size of the materials get eluted out which can be used for other applications and the biological synthesis that utilises the particles and solution on the membrane produces more silver nanoparticles. It is seen that the anti-microbial activity had increased for Silver nanoparticles synthesised from the extract of *E.Coli*. The inhibition zone increased and the bacillus which was insusceptible to chemically synthesised silver nanoparticles then

became susceptible. As it is inferred that silver nanoparticles bind strong because of nitrogen group, Binding properties of various natural or synthetic polymers/biomaterials ending with nitrogen group can be studied for their binding properties with silver nanoparticles. These binding property studies and anti-microbial activity studies can be exploited for construction/ improvement of Nano filters, Nano fabrics etc.

References:

1) N. Saifuddin, C. W. Wong and A. A. Nur Yasumira, "Rapid Biosynthesis of Silver Nanoparticles
Using Culture Supernatant of Bacteria with Microwave Irradiation", *E-Journal of Chemistry*, 2009, **6**(1), 61-70

2) Virender K. Sharma , Ria A. Yngard, Yekaterina Lin, "Silver nanoparticles: Green synthesis and their antimicrobial activities", *Advances in Colloid and Interface Science*, 145 (2009) 83–96

3) D. Jain, H. Kumar Daima[2], S. Kachhwaha, S. L. Kothari, "Synthesis of Plant-Mediated Silver Nanoparticles using Papaya fruit extract and evaluation of their Anti-Microbial Activities", *Digest journal of nanomaterial and biostructures*, Vol. 4 No. , September 2009, p.557 – 563.

4) N. Saifuddin, C.Y. Nian, L.W. Zhan and K.X. Ning. "Chitosan-silver Nanoparticles Composite as Point-of-use Drinking Water Filtration System for Household to Remove Pesticides in Water", *Asian Journal of Biochemistry*, Year: 2011 ,Volume: 6,| Issue: 2 ,Page No.: 142-159

5) F. Heidarpour *, W. A.W. A. K. Ghani, A. Fakru'l-Razi, S. Sobri, A. Torabiana, V.Heidarpour, M. Zargar, "New Trends on Microbiological Water Treatment", *Digest journal of nanomaterial and biostructures*, Vol. 6, No 2, April - June 2011, p. 791 – 802.

6) Katherine Wallington, North Carolina State University. "Emerging Nanotechnologies for Site Remediation and Wastewater Treatment" ,Prepared for US EPA.. Accessed from : http://www.cluin.org/download/studentpapers/K_Watlington_Nanotech.pdf

7) WebsiteURL: http://www.environmentalhealthnews.org/ehs/newscience/simple-cheap-filter-with-silver-nanoparticles-cleans-water