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SIZE-CONTROLLED SILVER NANOPARTICLES SYNTHESIZED OVER THE RANGE 5–50 NM AND THEIR ANTIBACTERIAL EFFICACY STUDY ON BACTERIA ISOLATED FROM UTI INFECTION

Medical Science

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ABSTRACT

A systematic and detailed study for size-specific antibacterial efficacy of silver nanoparticles (AgNPs) synthesized using Tamarind kernel powder and gum acacia is presented here. Nucleation and growth kinetics during the synthesis process was precisely controlled and AgNPs of average size 5, 7, 10, 15, 20, 30 and 50 nm were synthesized with good yield and monodispersity. We found the bacteriostatic/bactericidal effect of AgNPs to be size and dose-dependent as determined by the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against four bacterial strains. Out of the tested strains, Escherichia coli 256 and Staphylococcus aureus 2039 were found to be the most and least sensitive strains regardless of AgNP size. For AgNPs with less than 10 nm size, the antibacterial efficacy was significantly enhanced as revealed through delayed bacterial growth kinetics, corresponding MIC/MBC values and well diffusion tests. AgNPs of the smallest size, i.e., 5 nm demonstrated the best results and mediated the fastest bactericidal activity against all the tested strains compared to AgNPs having 7 nm and 10 nm sizes at similar bacterial concentrations.

KEYWORDS

Urinary Tract Infection, Antimicrobial Efficacy, Bactericidal Effect, Minimum Inhibitory Concentration, Monodispersity

INTRODUCTION

Urinary tract infection (UTI) is a very common disease in both men and women. It occur when the bacteria is able to invade the host defence mechanism adhere in a portion of the urinary tract , multiple there and form colonies in urinary tract. If it is detected in the initial state means when the parts of lower urinary tract like bladder, vagina and urethra get infected then it can be easily curable. But if it causes upper urinary tract infection i.e kidneys and ureters then prolonged medication is necessary to treat the disease[1,2]. It is mostly causes by bacteria but there are some viruses and fungi also that causes this disease. Among bacteria both gram –positive and both gram-negative causes UTI infection. For our experiment we are taken in gram-negative bacteria E.coli and S.aureus in gram-positive bacteria [3,4]. In UTI infection the pathogen migrate from genital tract, urethra to the bladder, ureter and then affect the kidney. It is most common in women than men. Near about one women among five men are infected. One women yet repeated infection among two women. Sometimes they get infected for years. This is because in the urinary tract of women most of the bacteria get aborted and they have sorter urethra[5,6]. There are some symptoms which indicate that you have UTI infection. Before treatment it should be clear whether the person have infection for the first time or for the sixth time. Repeated infection means the bacteria form colony in the urinary tract so prolonged treatment is necessary. It also necessary to observe the potency and medicine during the previous infection period. May be the patients resistant towards the particular antibiotics. The resistant may be fully or partially as the person get infected several times. Pain during urination, pain in lower abdomen or in back, dark, bloody, cloudy and strain smelling urine, have in fever for long time, feeling tired are the normal symptoms for UTI infection.

The patients present in the UTI infection become resistant towards the antibiotics normally used to treat UTI infection. Normally ampicillin is used to kill *Escherichia coli* and cefoxitim is used to kill *Staphylococcus aureus* [7,8]. Nowadays antibiotic resistant becoming a world wise problem. Due to the resistance towards normal antibiotic drugs used to treat UTI infection new combination of drugs should be design to treat the disease. Technical each composition of the drugs should be different from the normal antibiotics used to treat the disease. Their mode of killing bacteria should also be different so that the genetic changes develop in the bacteria must be overcome [9,10]. Silver nanoparticle can be used as an alternative drug resistant bacteria causes UTI infection. Several nanoparticle have been used as antimicrobial agent for about it also above a large scale of biomedical application [11]. Metallic nanoparticles in recent years, have occupied a large place of interest due to their small size, physicochemical properties, and surface plasmon behaviour [12]. Among all the nanoparticles, silver nanoparticles (AgNPs) show the commercialization of highest level [13] and occupied 55.4% among all the nanomaterial based products [14,15]. Indiverse domains like optoelectronics, surface enhanced Raman scattering (SERS), biosensors, catalysis silver nanoparticles have been used [16,17,18]. The antimicrobial nature of silver nanoparticles explored for its ability of killing bacteria in broad spectrum range,[19] and microbial resistance development against it decreases[20,21].In different type of consumer products such as textiles, cosmetics, surgical coatings, food packaging, dietary supplements,water disinfection and medical implants silver nanoparticles have been used due to its extensive antimicrobial property[22,23,24,25,26]. In different literature it is reported that antimicrobial property depend on both shape and size of the particle, where antimicrobial activity is more for smaller size nanoparticle [24,25]. When it come to shape among all the shape, spherical nanoparticles show best antimicrobial in both immobilized and colloidal state. For smaller size silver nanoparticle sodium borohydride ($NaBH_4$) is used as it is a strong reducing agent result in instant nuclei generation [26]. Sometimes dual reducing agent sodium borohydride and trisodium citrate ($NaBH_4$and TSC) used to control the nucleation and growth of silver nanoparticle[27].Both $NaBH_4$and TSC are hazardous for environment and the nanoparticle synthesised by this process is also hazardous for environment.So in this article we have preferred green method for the synthesis of nanoparticle. Here we use acacia and ladies finger as reducing cum stabilizing agent. *Acacia nilotica* we have collected from the rural area of west bengal and *Abelmoschusesculentus* is collected from the local market. Both E.coli and S. aureus these two bacteria were isolated from urine of UTI infected person and used for antibacterial test.The severe present in silver nanoparticle can bind with both sulphur containing protein in bacteria or with the protein present in DNA. These E.coli is resistant towards ampicillin and S.aureus is resistant towards cefoxitim. In some literature size dependent antimicrobial study is done but this antibacterial study depending on the size of different nanoparticle on drug resistant bacteria is lacking. This study also focus on size selective comparative study of nanoparticle over various Gram-positive and Gram-negative bacterial strains. A comprehensive study of antimicrobial activity was made on the basis of bacterial growth kinetics using different amounts of silver nanoparticles, along with MIC values determined based on liquid cultures using different sizes of silver nanoparticles.

2. Materials

Silver nitrate (>99.9% pure) were purchased from Merck (India). Ethyl alcohol and acetone were obtained from Merck (Germany) and were used at the desired dilutions. All reagents supplied were of analytical grade and were used without further purification. Throughout the procedures, double deionized (DI) water was used. The antibacterial experiments were carried out using various bacterial strains. Escherichia coli U 256, Escherichia coli U 364 and Staphylococcus aureus U 2039 and U 873 were obtained from urine by serial dilution method.

3. RESULTS AND DISCUSSION:

3.1. Antimicrobial Activity

Different sizes of silver nanoparticle are synthesised by using acacia and TKP both as the reducing agent. Different experimental condition in which the nanoparticle is synthesised is listed in Table1.The images obtained from TEM analysis was presented in Figure1. Three different sizes

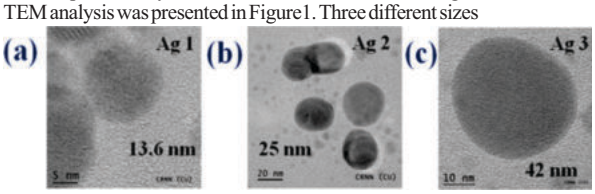

Figure 1: TEM images of three different type of silver nanoparticle (a) $AgNP_{0.05/1/7.3}$ (Ag1) (b) $AgNP_{0.5/1/9.1}$ (Ag2) (c) AgNP0.5/1/10.1 (Ag3)

of nanoparticle obtained are 13.6 nm (A1), 25 nm (A2) and 42 nm (A3) respectively. Growth kinetics of bacterial strain were done using dose dependent kinetics method. Figure2 represent the dose dependent plot for the four different strains of bacteria. From this plot we can get the MIC value

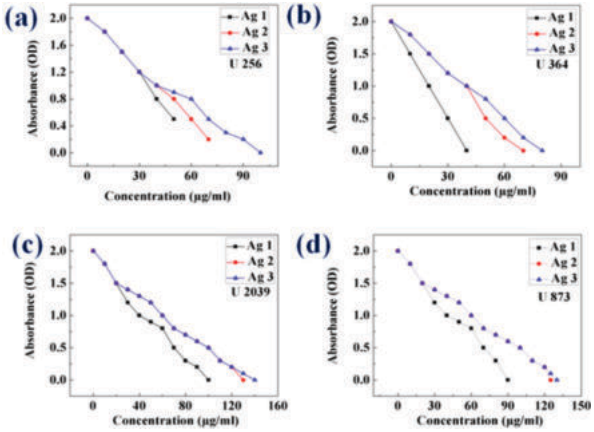

Figure 2: Comparison of effect of AgNPs (Ag1,Ag2,Ag3) on four different strain of bacteria (a) U256 (b) U364 (c) U2039 (d) U873

(Table1) for three AgNPs in four different strain of bacteria.The comparison of MIC values are also shown in Figure 2a and Figure 2b. Figure 2a for U256 and 2b for U364 strains whereas U2039 and U873 were represented in Figure 2c and 2d.The MIC value for three different nanoparticle (A1,A2 and A3) in case of U256 are 60,80 and 100 µg/ml respectively, for U364 40,70 and http://refhub.elsevier.com/S0167-732, for U2039 100,130 and 140µg/ml

Table 1: Different type of silver nanoparticle and their zone of inhibition (ZOI) and Minimum Inhibitory Concentration calculation (MIC) on both E.coli and S. Aureus

Types of Bacteria	Zone Of Inhibition (ZOI) (mm)	Types of silver nanoparticle (AgNP)	Minimum Inhibitory Concentration MIC (µg/ml)	
E.Coli (U364)	11	Ag1(13.6 nm)	40	
	9	Ag2(25 nm)	70	
	8	Ag3(42 nm)	80	
E.Coli (U256)	10	Ag1(13.6 nm)	50	
	8	Ag2(25 nm)	80	
	7	Ag3(42 nm)	100	
S.Aureus (U2039)	Partial Inibition	Ag1(13.6 nm)	100	
		Ag2(25 nm)	130	
		Ag3(42 nm)	140	
S.Aureus (U873)	20	Ag1(13.6 nm)	90	
	16	Ag2(25 nm)	125	
	15	Ag3(42 nm)	135	

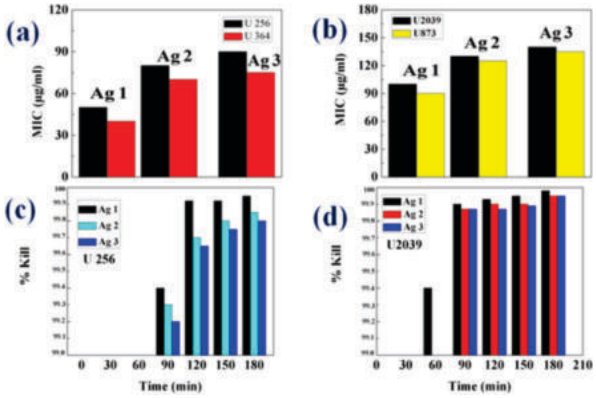

Figure 3: Comparison of MIC of Ag1, Ag2 and Ag3 in (a) E.coli (U256 and U364) and (b) S.aureus (U2039 and U873) and killing kinetics study in (c) E.coli (U256) and (d) S.aureus (U2039) and for U873 90,125 and 135µg/ml respectively. The MIC value is least in case of U256 then U364, U873 and U2039.The zone of inhibition of three different sizes of nanoparticle in four drug resistant strain of bacteria was reported in Table1 and Figure 3. The clear zone of inhibition indicate the bactericidal activity of the nanoparticle and the activity increases with decrease in size. The bactericidal activity is also different for different bacteria. For E.coli the effect is more whereas for S. aureus the effect is low. The reason is that E.coli is a gram-negative bacteria whereas S.aureus is a gram-positive bacteria. This difference in bactericidal effect arises due to the structure present in the peptidoglycan layer. The peptidoglycan layer is thinner for E.coli whereas it is thicker for S.aureus. The nanoparticle can invade gram-negative cell easily than gram-positive cell. As nanoparticle can invade cell easily, disrupt the membrane and cell death is more.For U2039 partial ZOI observed which indicate partial inhibition occur. It may be due to the lesser sensitivity towards this bacteria.

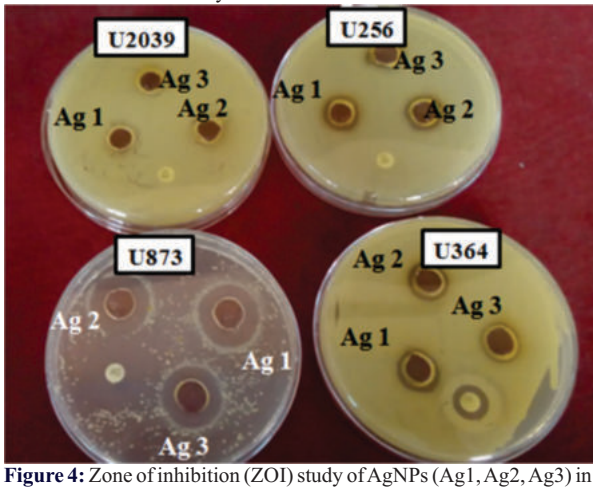

Figure 4: Zone of inhibition (ZOI) study of AgNPs (Ag1, Ag2, Ag3) in four (U256, U364, U2039, U873) different strain of bacteria

For U873 the sensitivity is more as the initial bacterial concentration was low also it may be more sensitive towards this particular strain. From the image in Figure 4 it was clear that the initial growth of the bacteria is lesser in comparison to other three strain. Figure 2 demonstrate the growth profiles of the four different bacterial strain treated with 3 different sample of different concentration and Figure 3 (a and b) represent the growth profile of bacteria for U256 and U2039 within 3 hours with three different type of nanoparticle. From the growth profile it is clear that all the cultures are affected by silver nanoparticles. In all the four strain the bactericidal growth is reduced with the treatment of silver nanoparticle of different concentration. Here the bactericidal growth is less for gram-negative E.coli than gram positive S. aureus.Figure 3 (c and d) represent bacterial killing kinetics with respect to time in different bacterial strain. More than 99.9 % bacteria are died within 3 hours . Not the 100% death was observed as within 3 hour its not possible to kill all the bacteria. With respect to time the death of bacteria also increases. As the time is more, silver can invade cell more, cell damage more result in a larger number of cell death. This bactericidal effect indicate that the nanoparticle with lowest size invade cell more. It causes cell death within 60 min. The

bactericidal activity is best in case of U 256 strain of E.coli. In 3 hour the full bactericidal effect can' t be study properly. Many researchers like El-Kheshen and El-Rabin in their research article reported a MIC of nanoparticle 125 µg/ ml[28]. This is the bactericidal activity of citrate stabilized silver nanoparticle studied on E. coli ATCC 8739 strain were 10^5 to 10^6 CFU/ml initial bacterial concentration was maintained. Silver nanoparticle varies in size from 20-65 nm. Also the AgNPs are of different shape. The AgNPs are spherical, rod, triangular and irregular in shape. So in referred article the bactericidal activity is not explained only on the basis of size. Both size and shape determined the bactericidal activity of AgNPs [29]. The MIC value of more than 150 µg/ml was reported against a DH5α strain of E.coli were initial cell concentration is10^8 CFU /ml. The colloidal solution of AgNP used for the bactericidal activity study[30].In some multidrug resistant strain collected from the hospital the MIC/MBC value varies from 60/160 µ/ml and 80/160µ/ml. The initial concentration of bacteria was10^3 to 10^4 CFU/ ml and the size of AgNPs varies from 120 to160 nm[31]

Over all the MIC values reported in different research report are not comparable because reaction condition, number of initial cell, bacterial strain, media for culture, size, shape, nature of the capping agent taken and depending on that the surface chemistry of silver nanoparticle determine the bactericidal activity of AgNPs [32].In different literature it was reported that the antimicrobial activity of AgNPs varies on different size. As the size decreases from 100 to 60,40 ,20 nm the antimicrobial activity also increases. The 10 nm sized particle have more pronounced antimicrobial activity is also reported [33,34].The antibacterial activity is also strain specific as for nanoparticle of same size show different range antimicrobial activity in different strain. In some previous study the zone of inhibition for E.coli was near about 2.3 mm [35,36].Though it was different strain but our sample show promising antibacterial activity. The bactericidal potential was indicated by zone of inhibition (ZOI). ZOI indicate that there was no bacterial route in this region. Due to release of silver nanoparticle bacteria dye in this region, Beyond this region the bacteria remain alive as diffusion rate is low through the agar. For larger size nanoparticle the ZOI obtained was lower which indicate its lesser antibacterial activity. It was reported that for larger size nanoparticle the antibacterial activity is lower[37]. Due to large size the mobility of larger size nanoparticle through the semi-solid agar was retarded as its mobility was decreased and inhibition zone also decreased.

3.2. FESEM analysis

The antibacterial activity discussed previously was further confirmed by FESEM images. Figure 6b and 6c represent the cell treated with silver nanoparticle where as Figure 6a represent the untreated one. Only one E.coli (U256) strain was taken for SEM analysis. There was no change in the appearance of the untreated cell. It appear in its normal characteristic shape where as the cells treated with AgNPs show cell deformation, irregular shape, cell shrinkage. There are three broad explanation

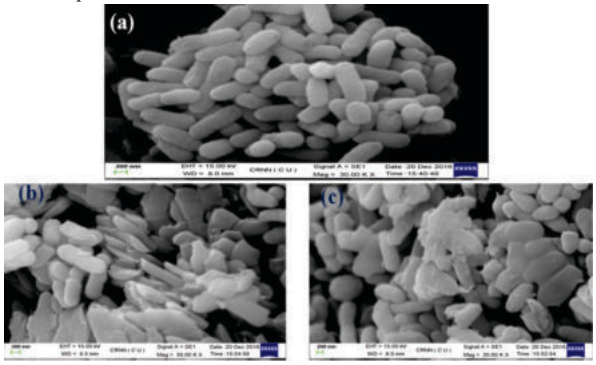

Figure 5: FESEM images of (a) untreated bacteria (E.coli) and treated with silver nanoparticle (b) (Ag2) and (c) (Ag1)

can be presented for the silver nanoparticle-bacterial interaction. The first reason can be explained by the attraction between the positively careed internal membrane protein present on the surface of the bacteria and tentatively care AgNP. [38] The structural interity of the cell membrane may be altered or there may be any pysiochemical changes was the another reason for cell death. The alteration in the osmoregulation of the bacterial cell wall was result in extrusion of intracellular material out of the cell and causes cell death ultimately. The third approach was the internalization of AgNP inside the cell The silver nanoparticle can penetrate the cell and attack the neucleus result in cell death. The disruption of cell prove the internalization of AgNP. After internalization AgNP inside the cell it can act in various way. It can bind wit the DNA and can stop DNA replication [39], can also affect the cellur respiration or can causes intracellular ROS generation [40,41,42].

3.3. Estimation of viability of E.coli and S. aureus

Bacterial viability was measured on the basis of membrane integrity by flow cytometry. Cells with intact membrane exclude the Nucleic acid binding dye propidium Iodide. Propidiumiodide (sigma) intercalates into DNA and exhibits very high fluorescence and this can be detected by flow cytometry.In nature under stress condition bacteria show heterogenetity depending on their metabolic

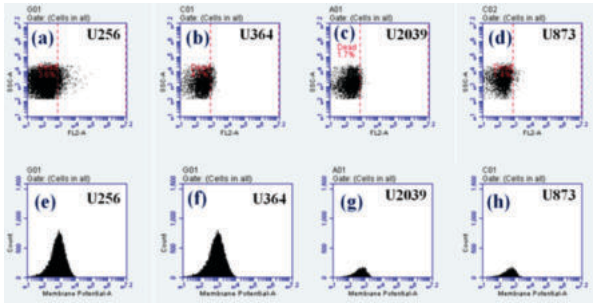

Figure 6: Live-dead (a-d) cell calculation and membrane integrity (e-h) study in both E.coli (U256 and U364) and S.aureus (U2039 and U873)

activity [43]. To difference the bacteria that whether they are dead, alive or injured can be determined on the basis of flow cytometric analysis. In quasi-continuous scale light scattering and fluorescence are measured it is possible to separate the cell in live and dead category. In a literature it was reported how live and dead cell calculation done in gram-positive non-spore forming bacterium [44]. In our experiment we have studied percentage of dead cell in both E.coli and S.aureus. In the four different strain the bacterial cell viability was calculated where it was clear that number of dead cell was more in case of E.coli where as number of dead cell was least in S. aureus. In Figure 6 (a-d) the percentage of dead cell was reported for U256, U364, U2039 and U873 respectively. The cell membrane potential was represented in Figure 6 (e-h) for the four bacterial strain. When there was more propidium iodide erclusion, fluorescence intensity was more indicate less intact cell membrane indicate more injured or dead cell [45].

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