Journal of
Bionanoscience
Vol. 8, 1–9, 2014

One-Pot Rapid Synthesis of Face-Centered Cubic Silver Nanoparticles Using Fermented Cow Urine, A Nanoweapon Against Fungal and Bacterial Pathogens

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One-pot rapid synthesis of face-centered cubic silver nanoparticles (AgNPs) has been successfully accomplished using fermented cow urine as a reducing medium coupled with ultrasonication at an alkaline pH (9.5) in 1 min. AgNPs formation was monitored using UV-visible spectrophotometer by recording surface plasmon resonance at 365 nm. Transmission electron microscopy (TEM) and particle size analysis has clearly revealed the nano-size of AgNPs (11–20 nm) and structural characterisation was carried out using X-ray diffractometer (XRD). Fourier transformed infra red spectroscopy (FTIR) revealed that AgNPs were surface functionalized with hydroxyl, carboxyl and amide groups. Antibacterial potential of AgNPs was studied using disc diffusion and live/dead assays on *Staphylococcus aureus*, *Streptococcus epidermis*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella abony* and *Klebsiella pneumonia* has evidently revealed that AgNPs significantly inhibit all test bacterial pathogens. AgNPs were also found effective against phytopathogen, *Fusarium oxysporum* NCIM 1008. These studies have confirmed that AgNPs can be employed in cosmetics and wound dressings as a nanoweapon to control bacterial and fungal pathogens.

Keywords: Fermented Cow Urine, AgNPs, Ultrasonication, Antibacterial, Antifungal.

RESEARCH ARTICLE

1. INTRODUCTION

Since the discovery of penicillin by Sir Alexander Fleming, antibiotics have been extensively used worldwide to treat infectious diseases.[1,2] During initial years of antibiotics discovery there was a great success achieved against bacterial diseases, which were very difficult to treat at that time and this era was called as the "golden age" of antibiotics.[3] However after few years due to haphazard and extensive use of antibiotics resulted in significant emergence of drug resistant bacteria, which caused an end to the "golden age" of antibiotics.[4,5] In recent years field of medicine is facing serious challenges of continuous increase in the multidrug-resistant (MDR) human pathogenic microbes with limited treatment options.[6,7]

Mobile genetic elements such as plasmids, integrons and transposons often carry antibiotic resistant genes, thus their transfer confers immediate multidrug resistance in recipient bacterial strains.[1,8,9] As a result of horizontal gene transfer between bacteria, pathogens such as *Pseudomonas aeruginosa*, methicillin resistant *Staphylococcus aureus*, *Mycobacterium tuberculosis*, vancomycin resistant *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Acinetobacter* sp. and *Escherichia coli* are now exist which are resistant to virtually all the known clinically used antibiotics and become more pathogenic, severely complicating treatment strategies and consequently have joined the ranks of 'superbugs.'[1,10–12] Around 37 distinct antibiotic efflux pump encoding genes have been reported in *E. coli* as their arsenal against antibiotic attack.[13] Efflux decreases the intracellular concentration of an antibiotic, thereby allowing bacterial survival in presence of very high concentration of antibiotic.[14] Since 1962–2000 only one antibiotic linezolid has been introduced clinically but in 2009–2011, linezolid resistant *Staphylococcus cohnii, S. aureus* and *Streptococcus pneumonia* were reported.[15,16] Therefore "drug of last resort" is under threat and with advent of multiple drug resistance in bacteria these drugs will no longer remain the arsenal against bacterial pathogens. Therefore there is a pressing need to device new strategies or design novel drugs to combat 'superbugs.'[3,16]

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In the present scenario, nanotechnology is evolved as an art to tailor the essential structures of materials at the nanoscale to achieve specific properties, thus greatly extending the well-used tool kits of materials science.[18] Nanoparticles have diverse biomedical applications in different fields such as cosmetics, drug delivery, biomarkers in imaging technology and treatment of cancer, fungal and bacterial diseases.[5, 18–23] Since ancient times silver in the form of various compounds and bhasmas have been used in Ayurveda to treat several infectious diseases caused by bacterial pathogens.[24] These silver compounds with antimicrobial activity include silver nitrate, silver sulfadiazine, silver zeolite, silver powder, silver oxide, silver chloride and silver-cadmium powder.[24] Using nanotechnology now it is possible to enhance the antimicrobial effects of silver by manipulating their size at nano level and also physical and chemical properties. Nano-size and high surface-to-volume ratio of silver nanoparticles allows them to interact with microbial membranes.[6] AgNPs have been shown to exhibit interesting catalytic, antibacterial and biosensing properties.[6, 25] Due to unique chemical, physical and antimicrobial properties, AgNPs have attracted attention of medical microbiologists worldwide.[5, 6, 25–27] Silver Nanoparticles are capable of penetrating bacterial cells and act as a catalyst, to inactivate enzymes that microorganisms need for their metabolism by interacting with thiol (–SH) groups of proteins and enzymes, disrupt bacterial membranes and also affect DNA replication.[6, 28] Till date there is no conclusive resistance mechanisms reported in bacteria against silver nanoparticles. Majority of researchers employ chemical methods for synthesis of AgNPs, which is very costly and hazardous, therefore ecofriendly and cost effective methods of nanoparticle synthesis should be considered.[29, 30] Green synthesis of metal nanomaterials using plant extracts, milk, saliva and bacteria are referred as "nature's nano factory" as they possess prominent reducing powers to reduce wide range of metals rapidly without generating any hazardous waste.[10, 30–34] These are less expensive bioresources for synthesis of silver/gold nanoparticles and have potential to replace costly chemicals in near future. Animal secretions and waste products are new bioresources for rapid and green synthesis nanomaterial which serves as nanoweapons to control bacterial and fungal infections. Nanoparticle synthesis from animal secretions/excretions is a new field in nanomedicine.[35]

In the present communication we report novel one-pot rapid synthesis of silver nanoparticles using fermented cow urine coupled with ultrasonication at alkaline pH and characterization their using XRD, SEM, TEM, FTIR, particle size analyzer and thermal gravimetric analysis (TGA). Antibacterial activity of these nanoparticles against both Gram positive and Gram negative bacterial pathogens and fungal phytopathogen, *Fusarium oxysporum* NCIM 1008 have also been investigated.

2. EXPERIMENTAL DETAILS

2.1. Biosynthesis of AgNPs Using Fresh and Fermented Cow Urine

Two healthy cows of different breeds (Indian cow and Jersey cow) were selected for urine collection required for silver nanoparticle synthesis. Urine from both cows was freshly collected in clean 1 L polypropylene bottles and analysed for its ammonia content and pH as per standard methods.[36] Urine (100 ml) from both cows was taken in 2 separate glass stoppered bottles (300 ml) and carefully filtered using Whatman filter no. 1 to remove any particles present in it and used for nanoparticle synthesis. Appropriate volume of $AgNO_3$ was added from 1 M stock to cow urine to make the final concentration 1 mM aqueous solution. This reaction mixture was incubated undisturbed at room temperature until the colour of the urine changed to dark brown–black which is an indicator of silver nanoparticle synthesis. The biological reduction of Ag^+ ions to Ag° nanoparticles in solution was monitored by measuring the UV-visible spectrum of the solution using UV-Vis spectrophotometer (Shimadzu, UV-2450, Japan) having operational range of wavelength between 190 nm and 800 nm. Time required for visual change in the colour was recorded for different reaction sets.

In another experiment, fresh cow urine samples (Indian cow and Jersey cow) were dispensed in two stopper bottles (100 ml) and were stored uninterrupted for one week at room temperature (30 °C). During this storage period, the pH of the cow urine was monitored after regular time interval of 12 hrs. After 168 hrs a whitish precipitate formed in both cow urine samples. These fermented cow urine samples were again analysed for their ammonia content. Fermented cow urine samples were mixed with $AgNO_3$ solution to make the final concentration 1 mM. This reaction mixture was incubated undisturbed until dark brown–black colour change was observed and nanoparticle synthesis was confirmed by UV-Visible spectrophotometry. Time duration of silver nanoparticle synthesis was also recorded. In a separate experiment, fermented urine of Indian cow and Jersey cow were mixed separately with $AgNO_3$ solution to make its final concentration 1 mM and reaction mixtures were immediately ultrasonicated at 20 kHz until dark brown–black colour change was observed. Time required for nanoparticle synthesis was also recorded in each case. AgNPs formed in all the reaction sets were separated by centrifugation at 10,000 rpm at 25 °C for 10 min and dried at 50 °C for 4 h to remove moisture. These AgNPs were used for further characterization and biological activity assessment.

2.2. Characterization of Nanomaterial

2.2.1. XRD Analysis

Structural characterisation of the green synthesized silver nanoparticles was carried out by X-ray diffraction (XRD) technique using a Rigaku Miniflex Diffractometer

with CuKα radiation of wavelength 1.5418 Å and filtered through Ni absorber in the range of 10–80°.

2.2.2. TEM and SEM Analysis

TEM images were recorded on a PHILIP CM200 transmission electron microscope operating with an accelerating voltage of 200 kV and providing a resolution of 2.4 Å. Scanning electron microscope (model-JEOL JSM-6360 LV) was used to observe surface morphology of nanoparticles. Surface area of synthesized silver nanoparticles was measured using Quntachrome autosorb (ASiQC0100-4) surface area analyser.

2.2.3. BET, TGA and Particle Size Analysis

The Brunauer–Emmett–Teller (BET) surfaces area was measured by nitrogen adsorption at liquid Nitrogen temperature using a SMART SORB-91 surface area analyzer. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) of precursors were carried on Shimadzu DTG-60 thermal analyzer. Thermal analyses were performed in air at a heating rate of 5 °C/min from 28 °C to 400 °C. Particle size of the silver nanoparticles was determined using a particle size analyzer (Delsa Nano S, Backman Coulter, USA).

2.2.4. FTIR and CHNS Analysis

Synthesized AgNPs (2 mg) was mixed with 200 mg dry KBr followed by pressing the mixture into 16 mm diameter mould. This pellet was used to analyse major functional groups present on AgNPs using IR spectroscopy. FTIR spectrum was recorded in the region 400–4,000 cm^{-1} (SHIMADZU-FTIR 8201PC instrument, Japan). In order to divulge the amount of organic carbon and nitrogen present on the silver nanoparticles, elemental analysis for CHNS was performed using Elementar EAS vario MICRO CHNS analyser, Germany.

2.3. Antibacterial Activity of the Silver Nanoparticles

Antibacterial activity of the AgNPs was determined by disc diffusion method using Muller–Hinton agar. In this technique 5.0 mm diameter discs made of Whatman filter paper no. 41, were impregnated with different concentrations of nanoparticles (5–100 μg/disc) followed by sterilization at 121 °C for 15 min. Overnight grown 0.1 ml cultures (OD value of ~0.5 at 600 nm) of *Staphylococcus aureus* ATCC 6538, *Streptococcus epidermis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Salmonella abony* NCTC 6017 and *Klebsiella pneumoniae* ATCC1003 were then spread-plated on Muller–Hinton agar plates separately and nanoparticle impregnated discs were placed over them followed by incubation at room temperature for 24 h. Zone of inhibition (IZ) formed due to AgNPs was recorded to determine their antimicrobial potential. AgNPs were considered antimicrobial if the IZ was greater than 5 mm. Minimum Inhibition Concentration (MIC) of AgNPs assessed was described as the lowest concentration of the nanoparticles that visibly inhibited the bacterial growth. The MIC test was repeated thrice and results were recorded as mean of the triplicate experiments and standard deviation (SD) was recorded.[5]

2.3.1. SEM Analysis of Silver Nanoparticles Treated Escherichia Coli ATCC 8739

In order to study the effect of silver nanoparticles on bacterial cells, SEM (JEOL JSM-5800LV) was used to examine morphological alteration in *E. coli* ATCC 8739 exposed to 50 μg/ml silver nanoparticles. *E. coli* ATCC 8739 not exposed to silver nanoparticles served as control.

2.4. Live/Dead Assay for Gram Positive and Gram Negative Bacteria

Both Gram positive and Gram negative bacterial strains were grown overnight (to logarithmic phase) in Nutrient broth and cells were harvested by centrifugation at 10,000 rpm at room temperature. Cell suspensions of all six test bacterial strains were prepared separately in 0.85% sterile physiological saline. Optical density (OD) of bacterial suspension was measured at 600 nm and dilution factor needed was then calculated and the dilution was carried out to obtain a bacterial count of 5×10^5 cfu/ml. Two millilitre of each bacterial suspension (5×10^5 cfu/ml) was then taken separately in sterile 10 ml Erlenmeyer flask and amended with 50 μg/ml AgNPs and incubated under agitation for 2 h. Control flask, without any nanoparticles was kept for each bacterial strain. After 2 h incubation with 50 μg/ml nanoparticles, 100 μl of the bacterial suspension from each flask was transferred into a 96-well micro titre plate. A live/dead assay was performed using Live/Dead BacLight, bacterial viability kit, Invitrogen. Briefly, two solutions containing SYTO 9 dye and propidium iodide were mixed and diluted with double distilled water before being added to bacterial suspensions in 96-well micro titre plate. The micro titre plate was incubated at room temperature in the dark for 15 min. Fluorescence intensities for live cells (excitation: 485 nm, emission: 530 nm Green) and dead cells (excitation: 485 nm, emission: 625 nm Yellow) were measured using a fluorescence micro titre plate reader. The live cell intensity of control was considered as 100% and then live cell intensity of test samples were compared with control. Difference in percentage of live cells in experimental well and that of control well was defined as potential antibacterial activity.[5] Average of three different experiments was considered and standard deviation was determined.

2.5. Determination of Anti-Fungal Activity

Antifungal activity of silver nanoparticles was checked using *Fusarium oxysporum* NCIM 1008 as indicator phytopathogen (NCL, Pune, India) by well diffusion method

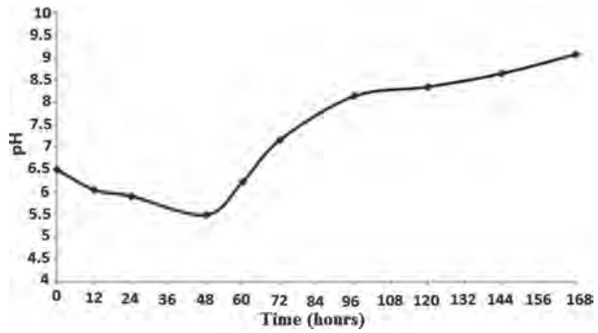

Fig. 1. pH variation in indian cow urine when stored at room temperature for 1 week.

on potato dextrose agar (PDA). The concentration of silver nanoparticle selected was 50 μg/ml as this concentration gave good antibacterial activity as revealed by dead/live assay. PDA inoculated with *Fusarium oxysporum* NCIM 1008 without nanoparticles served as control.

3. RESULTS AND DISCUSSION

3.1. Ammonia Content and pH

Ammonia content was found to be 125 mg/L and 79 mg/L in Indian cow and Jersey cow fresh urines respectively. Ammonia content of fermented cow urine after 7 days was 7802 mg/L in Indian cow and 7016 mg/L in Jersey cow urines. Fresh urine pH of both Indian cow and Jersey cow was slight acidic, (6.5) but during storage of urine pH initially dropped to 5.5 in first 48 hrs but rapidly started increasing as storage time increased and by 168 hrs pH increased to 9.5 (Fig. 1).

3.2. Biosynthesis of AgNPs Using Fresh and Fermented Cow Urine

Dark brown–black colour change of cow urine was considered as an indicator of silver nanoparticle synthesis and was further confirmed by surface plasmon resonance at 365 nm (Figs. 2 and 3). Time required for synthesis of silver nanoparticles from fresh urine of Indian cow was 13 minutes where as from fresh urine of jersey cow it

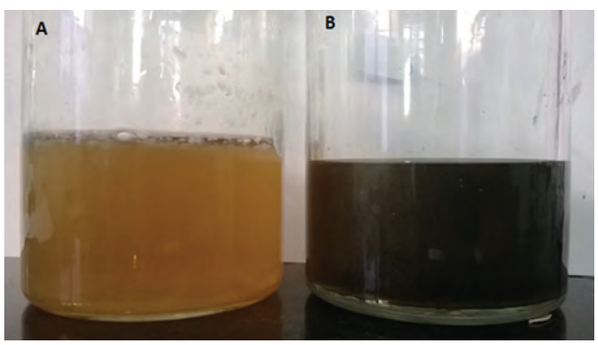

Fig. 2. Matured cow urine: (A) left without addition of Silver nitrate; (B) right, with addition of silver nitrate coupled with ultrasonication.

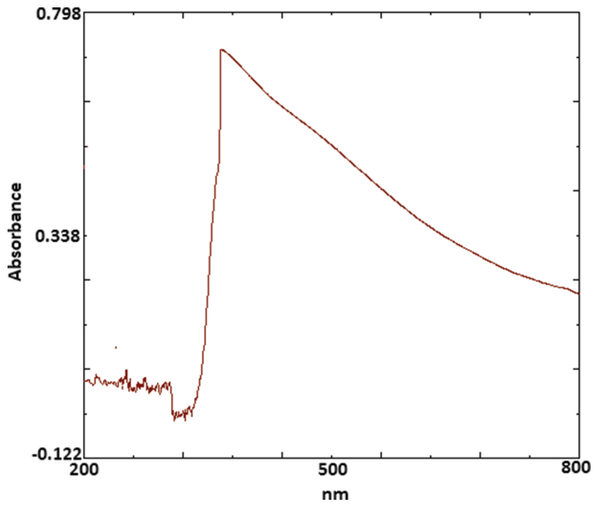

Fig. 3. Surface plasmon resonance of silver nanoparticles ($AgNO_3$ added to matured cow urine coupled with ultrasonication) at 365 nm.

was 18 min. Fermented cow urine (both Indian cow and Jersey cow) took 8 min. for synthesis of AgNPs whereas fermented cow urine coupled with ultrasonication showed rapid reduction of Ag^+ ions to AgNPs within 1 min. This is a fastest time ever recorded to synthesize silver nanoparticles using fermented cow urine. Since time required for synthesis of nanoparticles using fresh urine from Indian cow was 5 min. faster than Jersey cow, these results can be correlated to ammonia content as ammonia content of Indian cow urine was found to be significantly greater than ammonia content of Jersey cow. We have observed that time required for synthesis of nanoparticles using fermented cow urine (both Indian cow and Jersey cow) was 6 and 10 min. faster than fresh cow urine of Indian and Jersey cow, these results can also be correlated with ammonia content and pH of fermented cow urine since ammonia content of fermented cow urine was nearly 70 times more than fresh cow urine. The pH of fermented cow urine was also found to be alkaline (9.5) as compared to pH of fresh cow urine (6.5). Silver Nanoparticles thus obtained were dried at 50 °C and used for further analysis (Fig. 4). These results revealed that increase in pH (alkaline, 9.5) and ammonia concentration in fermented cow urine is responsible for rapid synthesis of AgNPs and

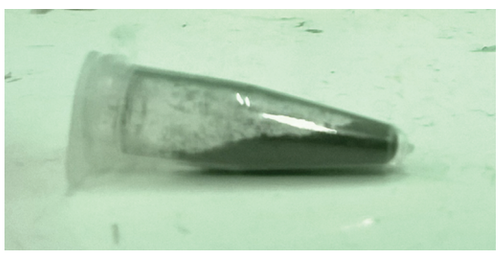

Fig. 4. Silver nanoparticels in powder form synthesized from fermented cow urine coupled with ultrasonication.

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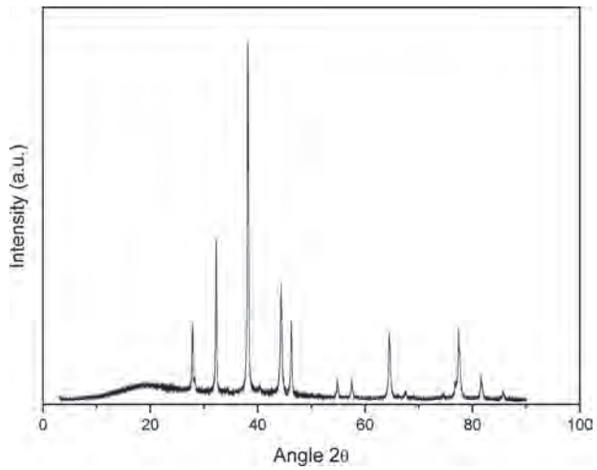

Fig. 5. XRD pattern of AgNPs synthesized using fermented Indian cow urine coupled with ultrasonication.

ultrasonication further enhanced the rate of nanoparticle synthesis.

3.3. Characteristic Properties of Nanoparticles

3.3.1. XRD Analysis

A comparison of our X-ray diffraction results with the standard clearly showed that the silver nanoparticles formed in our experiments by the reduction of Ag^+ ions by cow urine are crystalline in nature (Fig. 5). The presence of peaks at 2θ values 38.15°, 44.38°, 64.53°, 77.43° and 81.58° corresponds to (111), (200), (220), (311), and (222) Braggs reflection planes of silver, respectively. These peaks in XRD pattern can be readily indexed to a face-centered cubic structure of silver as per available literature (Joint Committee on Powder Diffraction Standards (JCPDS), File No. 4-0783). The unassigned peaks at $2\theta =$ 27.87°, 32.29°, and 46.23° are thought to be related to crystalline organic phases. In earlier studies, similar peaks were also observed in silver nanoparticles synthesized from carob leaf extract.[37] These results suggest that silver nanoparticles synthesized using cow urine has organic moieties on its surface.

3.3.2. FTIR, TGA and CHNS Analysis

The FTIR signals of synthesized AgNPs were observed at 3136 cm^{-1}, 2796 cm^{-1}, 1672 cm^{-1}, 1587 cm^{-1}, 1382 cm^{-1} and 1080–1150 cm^{-1} (Fig. 6). The broad stretching at 3136 cm^{-1} was attributed to OH group and band 2796 cm^{-1} was due to the presence C–H stretching in sugars. Broad stretching of C–O–C, C–O at 1,080–1,150 cm^{-1} in FTIR further proved presence of sugars. Bands at 1672 cm^{-1}, 1587 cm^{-1} were due to C═O stretch of amide I and N–H stretch of amide II of proteins responsible for reduction of Ag^+ during nanoparticle synthesis. Bands at 1382 cm^{-1} is attributed to carboxyl group of amino acids. Peaks at 468 cm^{-1} and 487 cm^{-1} in fingerprint region are

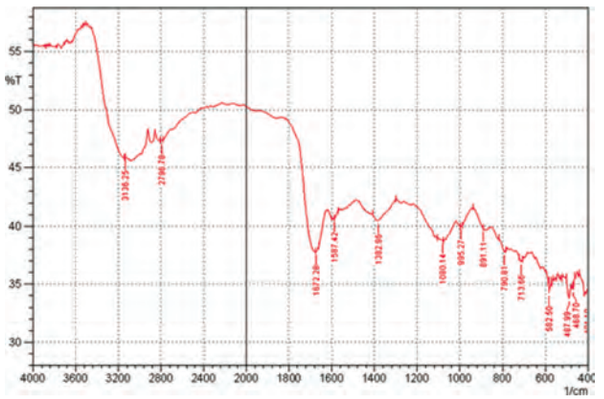

Fig. 6. FTIR spectrum of AgNPs synthesized using fermented Indian cow urine coupled with ultrasonication.

attributed towards AgNPs. FTIR confirmed that these silver nanoparticles are surface bio-functionalised with OH, COOH and amide groups present in sugars and amino acids. FTIR results of silver nanoparticles synthesized from olive leaf extract also support our findings.[26] It is well known that cow urine contain vitamins, sugars, urea, enzymes, proteins and various elements,[38] therefore these compounds present in cow urine certainly have played role in capping and reduction of Ag^+ ions into nanoparticles. By thermal analysis (TG/DTA) plot of nanoparticles (Fig. 7), it was observed that, ~2.8% weight loss occurred in the temperature range 40–100 °C due to elimination of water that may be adsorbed on the surface of the nanoparticles. In the temperature range of 100–350 °C, ~14% of weight loss was observed in TGA and an exothermic peak at 318.9 °C in DTA. This could be assigned to the oxidation of organic molecules present on silver nanoparticles. Heating the sample beyond 350 °C resulted in neither the weight loss in TGA nor formation of any new peak in DTA. Therefore it confirmed that the silver nanoparticles are very stable upto 400 °C. Through CHNS analysis of silver nanoparticles it further confirmed that nanoparticles have around 10% of carbon, 2.32% nitrogen and 3.46%

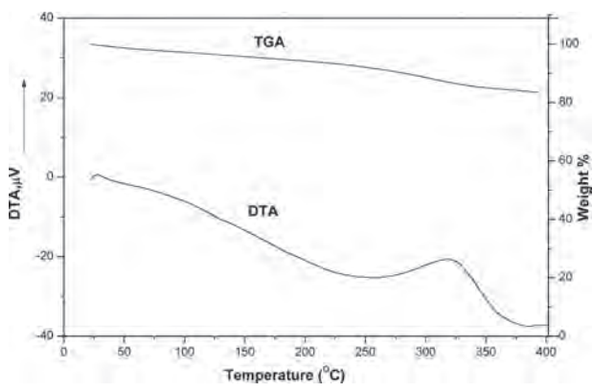

Fig. 7. Thermo gravimetric and differential thermal analysis curves of silver nanoparticles.

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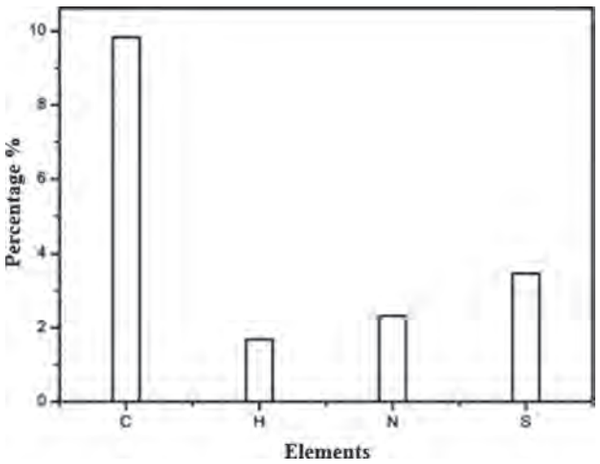

Fig. 8. Elemental analysis for C, H, N and S of AgNPs.

sulphur (Fig. 8 and Table I). This must have come during the synthesis from the cow urine itself as it is very rich in sugars, vitamins and amino acids and elements such as sulphur, copper, zinc etc.[38,39] So XRD, FTIR, TGA and CHNS analysis of silver nanoparticles have confirmed that that these nanoparticles are surface functionalised with sugars and amino acids.

3.3.3. BET Surface Area and Particle Size Analysis of Silver Nanoparticles

Average BET surface area of silver nanoparticles was found to be 53 m^2/g. Particle size analysis of the synthesized particles revealed that average particle diameter of the synthesized silver nanoparticles was 11 nm. The intensity weighted particle size distribution obtained from DLS study at 30 °C is shown in Figure 9, which exhibited cumulative mean diameter of 11.1 nm with polydispersity index 0.085.

3.3.4. SEM and TEM Analysis

The surface topography and morphological features of the synthesized AgNPs were studied using SEM. The SEM image (Fig. 10(A)) revealed agglomeration of silver nanoparticles and a layer of organic moiety around AgNPs. This organic moiety may be responsible for reduction of Ag^+ ions to AgNPs and also agglomeration of nanoparticles. TEM analysis was done to calculate the exact particle size of the nanomaterial synthesized. The Figure 10(B) shows the representative TEM image of silver nanoparticles. The average particle size was confirmed to be

Table I. Elemental analysis for C, H, N and S.

[%]	N	C	H	S
CUNP	2.37	10.05	1.73	3.33
CUNP	2.26	9.61	1.63	3.60
Average	2.32	9.83	1.68	3.46
STDV	0.077782	0.311127	0.068589	0.189505

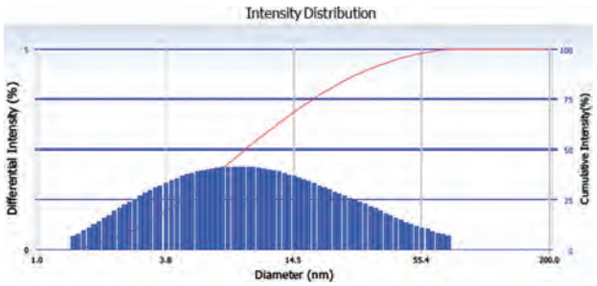

Fig. 9. Particle size distribution of the AgNPs determined using a particle size analyzer.

11–20 nm. By TEM image also it was revealed that silver nanoparticles are covered with organic layers resulting in aggregation of nanoparticles further confirmed our FTIR, XRD, CHNS, TGA and SEM results.

3.4. Antibacterial Activity of the Silver Nanoparticles

Antibacterial activity of the silver nanoparticles has been revealed clearly as zone of inhibition against six test bacterial strains is shown in Figure 11. Out of all the tested concentrations of silver nanoparticles, the MIC observed for *E. coli, S. abony, K. pneumonia, S. aureus*, *S. epidermis* and *B. subtilis* was 20, 10, 10, 20, 20 and 10 μg/disc respectively. Nanoparticles are known to penetrate bacterial cells and act as a catalyst to inactivate enzymes that microorganisms possess for their metabolism by interacting with thiol groups of proteins, disrupting bacterial membranes and also by affecting DNA replication.[28,40] It is interesting to note that *E. coli* ATCC 8739 was significantly inhibited by synthesized AgNPs due to disruption of bacterial cells, as revealed by SEM analysis (Fig. 12). Similar activity of AgNPs has been reported on *Pseudomonas aeruginosa* strain 4 EA.[6] Therefore AgNPs synthesized using green chemistry can be used as nanoweapon to control infectious bacterial pathogens.

3.5. Live/Dead Assay for Gram Positive and Gram Negative Bacteria

Test bacterial strains in 96-well micro titre plate were stained with Syto9 which stained nuclei and propidium iodide detect damaged DNA, causing healthy cells to be stained in green whereas dead cells appeared yellow. In control wells majority of test bacteria showed green fluorescence and only few bacteria showed yellow fluorescence which accounts for normal rate of apoptosis. But when all six test pathogenic bacterial strains were exposed to 50 μg/ml silver nanoparticles, significant decrease in percentage of live cells was recorded in terms of decline in green fluorescence with corresponding increase in yellow fluorescence. These findings indicated that AgNPs possess very good antibacterial activity against both Gram negative and Gram positive bacterial pathogens (Fig. 13).

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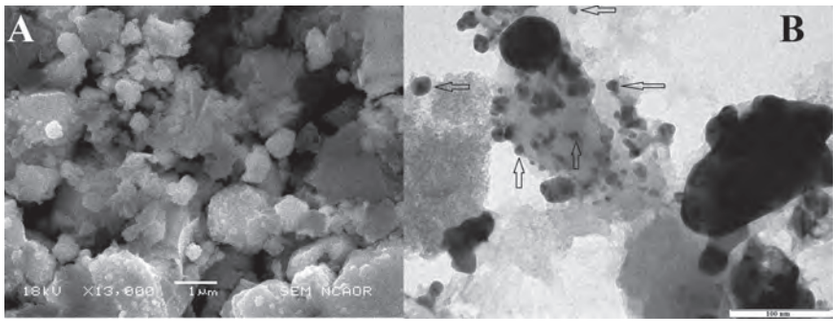

Fig. 10. SEM and TEM images of AgNPs (Arrows are pointing to the AgNPs).

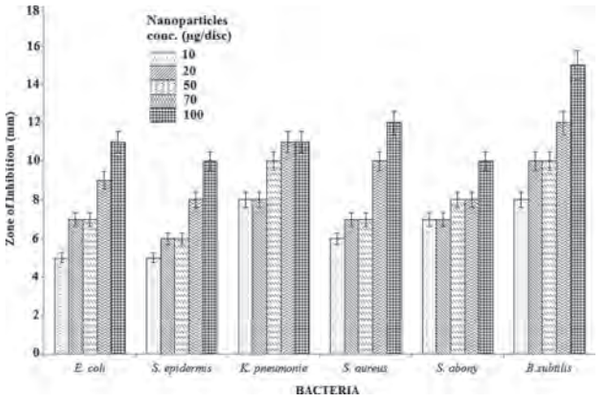

Fig. 11. Antibacterial activity of AgNPs synthesized using fermented indian cow urine using disc diffusion method.

3.6. Anti-Fungal Activity of the Silver Nanoparticles

Silver nanoparticles also showed significant inhibitory zone (2.4 ± 0.6 cm) on PDA agar plates with test phytopathogenic, *Fusarium oxysporum* NCIM 1008 which confirmed their anti-phytopathogenic potential (Fig. 14). *Fusarium oxysporum* is a well known phytopathogen, which can cause significant damage to agro-ecosystem.[41] Therefore these nanoparticles may be used also for seed dressing before sowing them in soil to protect them from phytopathogen, *Fusarium oxysporum* in order to increase the percentage of seed germination.

Antifungal and antibacterial activity of silver nanoparticles synthesized from fermented cow urine may be attributed to its nano size (11–20 nm) and large surface area i.e., 53 m^2/g. Interaction of nanoparticles with bacterial or fungal cell, need a suitable organic molecule which can act as bio-inorganic interface for attachment of nanoparticles to bacterial cell membrane.[32] It is interesting to note that XRD, FTIR, TGA, TEM and CHSN analysis of AgNPs clearly confirmed that they have been functionalised with organic moieties possessing hydroxyl, carboxyl and amide groups which may be responsible for interaction of nanoparticles with bacterial and fungal cells resulting in enhanced antifungal and antibacterial activity. Cow urine has been already granted US Patents (No. 6,896,907) for its medicinal properties, particularly as antibiotic, antifungal and anticancer agent.[42] It is also considered sacred and holy (devine) by Hindus since ancient times and has been used in Ayurvedic medicines to treat various infectious diseases.[24] Therefore fermented cow urine has been used to synthesize AgNPs since it is biologically safe.

Synthesis of gold nanoparticle (AuNPs) using cow urine has been reported earlier,[31] but they did not characterize AuNPs with respect to antibacterial and anti-fungal activity. In the present investigation we are reporting novel method of one-pot rapid synthesis of functionalised face-centered cubic AgNPs using fermented cow urine coupled with ultrasonication in just 1 minute, their detail characterization and evaluated their anti-bacterial and anti-fungal activity for the first time. The enhanced anti-bacterial and anti-fungal activity of silver nanoparticles is attributed to surface functionalization with organic moieties with

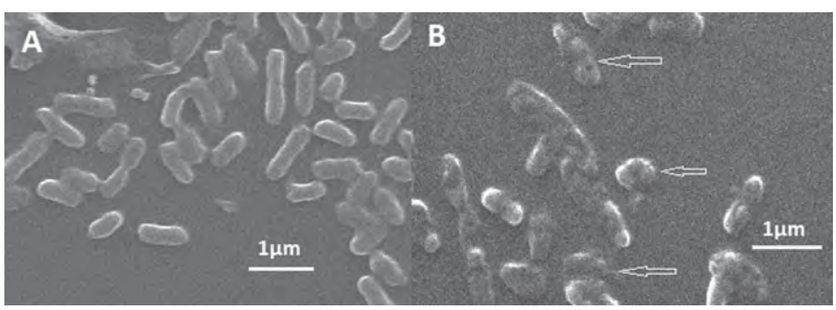

Fig. 12. Scanning electron micrograph (SEM) of *Escherichia coli* ATCC 8739 cells treated with 50 μg/ml silver nanoparticles (b) and control *Escherichia coli* ATCC 8739 (a).

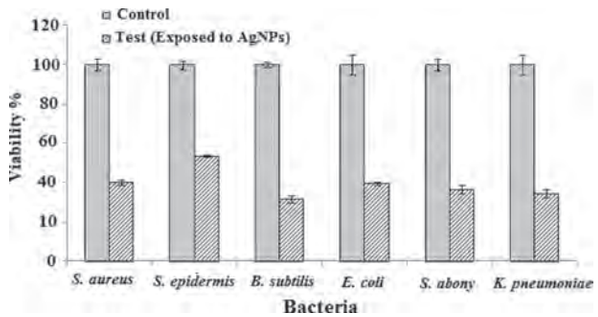

Fig. 13. Viability of Gram negative and Gram positive bacterial strains (in percentage) when exposed to silver nanoparticles (50 μg/ml) using **live/dead assay**. The data sets represent the average of three different experiments. Control bacterial cells (▦), AgNPs treated cells (▨).

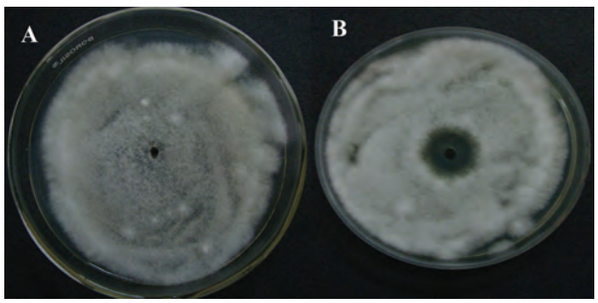

Fig. 14. Anti-fungal activity of the silver nanoparticles on phytopathogen *Fusarium oxysporum* NCIM 1008.

hydroxyl, carboxyl and amide groups. These results go hand in hand with earlier report, where folic acid (having OH and COOH groups) functionalised silver nanoparticles showed enhanced antimicrobial activity as compared to non functionalised nanoparticles.[43] We have also revealed that increase in pH (9.5) and ammonia concentration in fermented cow urine is responsible for rapid synthesis of silver nanoparticles and ultrasonication further enhanced the synthesis rate. These studies on functionalised face-centered cubic AgNPs synthesis using fermented cow urine as a natural bioresource and their application as anti-bacterial and anti-fungal agents in drugs and cosmetics as nanoweapon will prove promising to treat infectious disease caused by multidrug resistant fungal and bacterial pathogens.

4. CONCLUSION

In the present research we have successfully accomplished one-pot rapid synthesis of functionalized face-centered cubic AgNPs using fermented cow urine for the first time. We conclude that fermented cow urine (pH 9.5) acts as reducing and capping agent during synthesis of functionalised AgNPs. Ultrasonication assisted method of nanoparticle synthesis using fermented cow urine found to be much faster than the earlier conventional methods with other animal secretions. AgNPs synthesized using fermented cow urine have demonstrated antibacterial activity against both Gram positive and Gram negative bacterial pathogens and phytopathogenic, *Fusarium oxysporum* NCIM 1008 at very low concentrations for the first time. Therefore in future we can utilize cow urine as a cheap bioresource to synthesize rapid, cost effective and highly stable range of metal nanoparticles and use them in cosmetics and wound dressings as nanoweapon to treat infections caused by bacterial and fungal pathogens.

Acknowledgments: Authors would like to thank vice chancellor Goa University, Dr. Satish Shetye for providing necessary facilities to conduct experiments, SAIF IIT-Bombay for TEM analysis and Dr. N. N. Ghosh, BITS Pilani K. K. Birla Goa Campus for providing facility of TG-DTA, XRD and Particle Size analyser and Ms. Madhavi Naik for SEM analysis.

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Received: xx Xxxxx xxxx. Accepted: xx Xxxxx xxxx.

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