

Size-Controlled Facile Synthesis of Silver Nanoparticles by Chemical Reduction Method and Their Antibacterial Performance Against Staphylococcus Aureus and Escherichia Coli

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Abstract

This article describes the size-controlled one-pot facile synthesis of silver nanoparticles (AgNPs) and their antibacterial response against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) bacteria. AgNPs coated with and without trisodium citrate (TSC) were synthesized using silver nitrate aqueous precursor and hydrazine as a reducing agent. The appearance of yellowish color with surface plasmon resonance (SPR) with maximum absorbance (λ_{max}) at 416 nm from the ultraviolet-visible (UV-vis) spectrum of the product indicated primarily the formation of the desired product. X-ray diffraction (XRD) pattern confirmed the crystallinity of those products. Field emission scanning microscopy (FESEM) and transmission electron microscopy (TEM) images indicated only spherical AgNPs formation. The existence of a functional group of chemically bonded surface capping agents is confirmed by Fourier transform infrared (FTIR) and the thermogravimetric analysis (TGA) weight loss assessment proved the attachment of good amount of capping agents. Energy dispersive X-ray (EDX) spectroscopic analysis of products revealed the existence of high percentage (96.36%) of silver. Exposing the synthesized AgNPs to Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria, the zone inhibition was found to be 8 mm and 6 mm at a concentration of 50 mgL^{-1} , respectively. These results imply that TSC capped AgNPs can be considered as effective human pathogens for *S. aureus* and *E. coli* which is very inspiring.

1. Introduction

The impact of bacterial disease and the promotion of effective antibacterial agents to combat pathogenic diseases have appeared as a serious global healthcare challenge[1]. To overcome such a challenge, some antibacterial agents are invented, and efforts are being given to invent more new antibacterial agents. Researches are trying to be confirming about the effectiveness of existing agents in parallel to their development. Nanomaterials, such as silver nanoparticles (AgNPs—sizes between 1 and 100 nm), have been used for the last few decades for a remedial of antibacterial diseases because of their inhibitory and bactericidal effects[2]. It is because of the encouraging antibacterial activity and surface properties of that AgNPs.

Scientists have been extensively researching on preparation and antibacterial/disinfectants tests of AgNPs which are enormously reported in the literature[3][4][5][6]. If the dimensions of AgNPs are compared with a bacterium, AgNPs are comparatively large because of having high surface areas to volume ratio with a higher percentage of surface atoms. Consequently, the properties of AgNPs are mostly governed by the surface areas that are ultimately determined by the size of AgNPs. The smaller the particle size, the larger the numbers of surface atoms are, therefore, so are the surface electrons which increases the possibility of contribution in chemical activity to pathogens. Therefore, synthesizing AgNPs by keeping the particle size small is a key technique for obtaining better performance in many areas especially in antibacterial activity.

AgNPs can be obtained by several methods such as physical, chemical, and biological[7][8][9]. Among these, the chemical method is widely used because of its advantages such as it requires simple

instruments, economically cheap, short reaction time, and high yield percentage¹⁰. To synthesize AgNPs, most chemical reduction methods use silver nitrate, hydrazine/sodium borohydride, tri-sodium citrate, and water as precursor (P), reducing agent (RA), capping agent (CA), and reaction media, respectively. One-pot facile synthesis is possible by mixing an aqueous precursor solution, reducing agent, and capping agent. AgNPs are produced by the reduction process of silver nitrate (AgNO_3) which involves several stages such as atomization (from silver ion), aggregation, nucleation, and particle growth and formation. Silver particle formation only by the reduction (without CA) is possible, however, products are very unstable and aggregation occurs i.e. particle size becomes larger very fast due to the high surface energy of nanoparticles[10].

To increase the stability of the product, the surface capping agent is added with metal salt precursor and reducing agent[5]. Although there are many attempts, still the preparation of AgNPs with well-defined size is difficult because of particle aggregation/agglomeration. To prevent the aggregation/agglomeration, the synthesis of AgNPs using capping agents have been reviewed and reported[5]. Capping agents attached chemically to the surface of the AgNPs allow the AgNPs to be dispersed stably in the solvent. Additionally, attachment of CAs to the surface of the AgNPs suppresses the particle growth, consequently small particles are obtained[11]. In this way, surface capping of AgNPs enables them to be produced in real nanometer sizes. Some researches focused on the preparation AgNPs by chemical reduction method, but the sizes and size distribution obtained were large[12]. Another study implied that the growth of silver nanowires was directing by polyvinylpyrrolidone (PVP) molecules used for synthesis[13]. A higher molar ratio of TSC stabilizer reduces the sizes of AgNPs[14]. According to a report, AgNPs synthesized by chemical reduction showed good dispersion in poly(ethylene glycol)[15]. Comparison of the dispersion between the oleic acid and poly(acrylic acid) surface capped AgNPs were also performed[16]. AgNPs were synthesized with cellulose fiber and lignin as a multifunctional agent[17]. Triangular and spherical AgNPs were synthesized by chemical method and antibacterial activity was performed. Results revealed that the AgNPs with triangular shape shows more antibacterial activity than that of spherical ones[18].

To the best of our survey, the comparative study on the change in the particle size of silver with and without surface capping agents including their antibacterial activity has not been done yet. In the present study, we synthesized AgNPs from AgNO_3 and with and without TSC and tested them for antibacterial activity to Gram-positive *S. aureus* (pathogenic microorganism, colonizes in the groin, axillae, and gastrointestinal tract)[19] and Gram-negative *E. coli* (one of the best indicators for drinking water that is extensively used in bacteria studies because of its abundance on mammal's body surfaces and often create infection)[20] bacteria. Finally, a summary discussion was made.

2. Experimental

2.1 Materials

Silver nitrate (AgNO_3 , 99.5%,) and hydrazine hydrate ($\text{H}_4\text{N}_2\text{XXX}\cdot\text{H}_2\text{O}$, 80%) were purchased from Sigma-Aldrich, Germany, and were used as precursor and reducing agent, respectively. Trisodium citrate (TSC), a

capping agent, was purchased from Merck, India. De-ionized water (DW) was used as a solvent for all solution preparation and other purposes like washing etc. Tryptone Soya Agar and Tryptone Soya Broth were purchased from Oxion, UK, and Mueller Hinton Agar from Himedia, India.

2.2. Synthesis of TSC coated and uncoated AgNPs

AgNPs were synthesized by a chemical reduction method according to the procedure described below. Two types of samples were prepared—‘TSC uncoated AgNPs’ and ‘TSC coated AgNPs’. The earlier type is obtained by using only the precursor and the reducing agent, described in our previous research²². The latter type is synthesized by using TSC as a capping agent in addition to precursor and reducing agent. The preparation method is the same for both types of samples. To prepare TSC coated AgNPs, typically 100 mL of 0.01 M AgNO₃ aqueous solution was prepared by dissolving the required amounts of AgNO₃ salt into DW. Separately, hydrazine solution was prepared in DW. Both the solutions were mixed followed by the drop-wise addition of TSC with vigorous magnetic stirring. The molar ratio of 1:3:6 for the precursor, reducing agent, and capping agent are selected after assessing the effects of the capping agent's ratio. Next, the solution mixture was vigorously stirred for 4 h. Black coloration could be marked in the beginning. After completion of the reaction, the solution turned into yellowish/brown color which indicates the formation of AgNPs.

AgNPs are obtained as colloidal dispersions in a mixture of water, unreacted hydrazine, and the precursor solution (in case of without capping agents, the product is obtained as precipitates. For analysis purposes, the solid products were collected, by removing the unreacted hydrazine and silver ions through centrifugation at 8000 rpm for 15 min. Next, the particles were re-dispersed in water for washing and collected after centrifugation at 8000 rpm for 15 min. To purify the products properly, the process was repeated, and then dried well in the oven. Finally, the synthesized products were stored in desiccators until further characterization.

2.3 Characterizations of synthesized AgNPs

To get the primary idea of whether the AgNPs were formed or not, an UV–vis spectrum was taken using a spectrophotometer (UV-1800, Shimadzu, Japan) with the variable wavelength ranging from 200 nm to 800 nm in quartz cuvettes having a path length of 10 mm. The crystallinity of the synthesized products was examined using the X-ray diffractometer (SmartLab 9MTP; Rigaku Corporation) with the CuK α radiation in a 2θ – θ setup. The 2θ angle was scanned between 10° and 90° at a scanning rate of 0.02°/0.6 s. The measured data were compared with the data from the Joint Committee for Powder Diffraction Studies (JCPDS) file for silver (No. 00-004-0783). The average crystallite size was calculated based on the Debye-Shreerer's equation with the two vital peak patterns. SEM was taken to see the particle morphology using an electron microscope (FESEM, 7610F, JEOL, USA), and EDX was taken to know the elemental configuration using an energy dispersive X-ray spectrophotometer section of the same instrument. Particle size, shape, and morphology were examined using a transmission electron microscope (TEM, HD 2700, Hitachi Corporation, Japan) with 200 kV acceleration voltage. The mean particle size was obtained by measuring the size of 100 particles in the TEM images. Fourier transform

infrared (FTIR) spectra were acquired using a JASCO FT/IR–680 spectrometer to investigate the existence of chemical bonding between silver and TSC, and functional groups of the citrate anion on the surface of the AgNPs. Absorbance IR spectra were collected from 400 to 4000 cm^{-1} . The samples were prepared in KBr pellets. The percentage amount of TSC attached to the surface of AgNPs were measured using thermogravimetric analysis (TG, DTG60AH; Shimadzu Corporation, Japan) under an N_2 gas flow atmosphere (100 ml/min) at a temperature from room temperature to 800 °C at a heating rate of 10 °C/min. Also, TGA was used to evaluate the stability and content of the residue particle of AgNPs.

2.4 Procedure adopted for assessing the antibacterial performance of AgNPs

The antibacterial susceptibility testing of the synthesized TSC coated AgNPs was assessed following the disk agar diffusion method[21]. Gram-positive *S. aureus* and Gram-negative *E. coli* were taken to accomplish the antibacterial potential test of AgNPs. The bacterial strains were sub-cultured on tryptone soya agar (TSA) media. From freshly cultured plate, one loopful colony was picked and used to inoculate 9 mL of tryptone soya broth (TSB) and incubated at 37 °C overnight and match the turbidity of 0.5 McFarland Standard (Cell density = 1.5×10^8 CFU/ml). Amoxicillin disc was used as the control.

3. Results And Discussion

3.1 Nanostructure and morphology (XDR, TEM, FESEM & EDS)

The XRD patterns of synthesized TSC coated and uncoated AgNPs are displayed in Fig. 1. The XRD patterns clearly show five diffraction peaks at (2θ) values of 38.09°, 44.27°, 64.43°, 77.37° and 81.51° corresponding to the (Ag)₁₁₁, (Ag)₂₀₀, (Ag)₂₂₀, (Ag)₃₁₁ and (Ag)₂₂₂ planes (h/k values), respectively which indicates the crystalline nature of the synthesized products of both types. Comparing with JCPDS (File No. 00-004-0783), the patterns of the chemically synthesized AgNPs indicates a face-centered cubic (fcc) structure with space group as seen in Fig. 1 (a), (b) and (c).

The average crystallite size of the AgNPs was estimated using Debye–Scherer’s equation (Eq. 1).[22]

$$D = 0.9\lambda/\beta\cos\theta. \quad (1)$$

Where D is the mean dimension of the crystallite, β is the full width at half maximum (FWHM) of the diffraction peak, θ is the diffraction angle, λ is the wavelength of CuK radiation (0.15406 nm), and k was a constant (0.9). By determining the FWHM of the different planes of Bragg’s reflection, the estimated average crystallite sizes of uncoated and TSC coated AgNPs are found to be 33 nm and 19 nm, respectively as shown in Table 1. The values are close to the obtained values from the TEM study.

Table 1. Type of AgNPs, peak position, orientation, FWHM, crystallite size, and average crystallite size of chemically synthesized AgNPs obtained from XRD analysis.

AgNPs type	Peak position (deg)	Orientation (<i>h/k</i>)	FWHM (deg)	Crystallite size (nm)	Average size (nm) of first 2 peak
uncoated	38.1193	(111)	0.3048	33	33
	44.2953	(200)	0.3355	33	
	64.4395	(220)	0.3235	57	
	77.3806	(311)	0.3420	106	
	81.5184	(222)	0.3376	160	
TSC coated	38.0913	(111)	0.4899	21	19
	44.2660	(200)	0.7131	16	
	64.4345	(220)	0.5339	34	
	77.372	(311)	0.5701	64	
	81.507	(222)	0.6174	87	

The FESEM images of the uncoated and TSC coated AgNPs are presented in Fig. 2. AgNPs exhibit a smooth surface, almost uniform spherical shape. Comparing the FESEM sizes with that of obtained from XRD, it can be said that the uncoated AgNPs aggregates. It can be noticed from the FESEM images (Fig. 2(b)) that the sizes of coated AgNPs decreased compared to that of uncoated ones (Fig. 2(a)) considerably probably because of using the surface capping agent. The size of the particles/aggregates was \approx 100 nm as seen from Fig. 2(a,b). Similar results were reported in literature²¹. To get a clearer picture, TEM analysis may be worthy.

Figure 3 depicts the TEM images of the TSC uncoated and TSC coated AgNPs. TSC uncoated AgNPs aggregates fast as seen from the Fig. 3(a). However, TSC coated AgNPs shows small sizes with spherical shapes (Fig. 3(b)). The average particle size obtained from TEM calculation is 35 nm for TSC coated AgNPs which is comparable with the value that of obtained from XRD calculation. Attachment of CAs to the surface of AgNPs suppresses the particle's growth. Consequently, smaller particles are obtained. In this way, the surface coating of AgNPs enables them to be produced in real nanometer sizes.

Figure 4 shows the EDX spectrums of the prepared AgNPs which suggests the existence of silver since it shows a typical strong signal peak at 3keV²³. Peaks in Fig. 4 also reveals that mostly Ag element exist including a single weak peak of elemental oxygen (O) peak which might be coming from the chamber of EDX. Moreover, XRD analysis didn't suggest any existence of AgO/Ag₂O. Few other unlabeled small peaks are due to the Pt used for the coating of the sample. This result indicates high percentage and purity of AgNPs.

Quantitative elemental compositions obtained from EDX analysis are given in Table 2.

Table 2. Elemental composition of chemically synthesized AgNPs obtained from EDX.

Types of AgNPs	Element	Mass%	Atom%
TSC uncoated	Ag	88.63	53.61
	O	11.37	46.39
TSC coated	Ag	96.36	79.69
	O	3.64	20.31

3.2 Nano surface analysis (FTIR, TG)

Figure 5 indicates the FTIR spectroscopic analysis of TSC coated AgNPs which ascertains the interaction of TSC on the surface of AgNPs. The figure confirms the existence of the TSC is attached to the surface of the AgNPs. Peaks at 1381 cm^{-1} indicate symmetric stressing and 1624 cm^{-1} indicates asymmetric stretching of carboxylate anion of TSC, the existence of chemically attached tri-citrate anion on the surface of AgNPs as shown in Fig. 5. However, no peaks around 1300 cm^{-1} indicate no free TSC meaning the particles were washed perfectly and all the excess unreacted CA molecules were removed properly.

Figure 6 depicts the TGA plot obtained from the synthesized AgNPs with and without capping. It was observed that the weight loss of uncoated AgNPs was $<0.5\%$ including the weight loss for moisture at $100\text{ }^{\circ}\text{C}$ whereas for TSC coated AgNPs it was about 1.5% at $100\text{ }^{\circ}\text{C}$. It was calculated that the weight loss of the sample was about 1.5% at $100\text{ }^{\circ}\text{C}$ is probably due to the moisture content present in the sample. Above $100\text{ }^{\circ}\text{C}$, a sharp weight loss started which continued up to around $550\text{ }^{\circ}\text{C}$. At that stage the weight loss was about 3% which might be due to the loss of TSC existing with the AgNPs. This weight loss indicates the chemically attached TSC on the surface of AgNPs. A negligible weight loss was observed above $550\text{ }^{\circ}\text{C}$. Also, the constant weight of silver after $550\text{ }^{\circ}\text{C}$ indicates the stability of the produced AgNPs.

3.3 Dispersion of AgNPs in water (UV-vis)

The obtained absorbance spectra of synthesized samples prepared from AgNO_3 aqueous solution are given in Fig. 7. Characteristic strong absorption peak maxima (λ_{max}) at 416 nm of wavelength indicates primarily the particle formation of the colloidal product of surface capping whereas no peak is observed for uncapped particles as these sediments immediately after production as is seen in Fig. 7(a).

Previously, the absorption peaks were obtained in the visible range at 410 and 430 nm for different samples[23]. Three molar ratios (P: RA: CA= $1:3:1$, P: RA: CA= $1:3:3$, and P: RA: CA= $1:3:6$) of TSC was used. Fig. 7(b) also indicates that the P: RA: CA= $1:3:6$ gives the most intense peak (Fig. S1. Supplemental Data). In the same way, the stability of AgNPs in dispersions could be monitored ¹⁷ since the UV light absorption by the AgNPs, peak intensity and peak shifting depends mainly on the particle size which is related to stability. Observation of this peak, assigned to an SPR, is well documented not only for AgNPs

but also for some other metal NPs with sizes ranging from 2 to 100 nm. Also, small spherical NPs (<20 nm) exhibit a single surface plasmon band. Present data indicates good dispersion of TSC capped AgNPs in water.

3.4 Antibacterial performance

The antibacterial performance of the AgNPs synthesized with and without TSC capping agent was investigated against Gram-negative *E. coli* using the agar well diffusion assay. Fig. 8 shows the zone inhibition of the bacteria. In both cases, the zone inhibition was observed.

By comparing images 8 (a) and (b), it can be said that the zone inhibition was larger for TSC coated AgNPs (6 mm) compared to that of TSC uncoated AgNPs (5 mm). TSC coated AgNPs were also applied against *S. aureus* and compared that of with *E. coli*. The zone inhibition was quite large (8 mm) for *S. aureus* with TSC coated AgNPs. The zones of inhibition for *E. coli* and *S. aureus* bacteria were found to be around 6 mm and 8 mm, respectively. These results suggest that the smaller AgNPs are more reactive against human pathogens, especially on *S. aureus*.

Conclusion

Production of controlled size spherical AgNPs is possible by using TSC surface capping agents through a simple chemical reduction method. XRD analysis indicates the crystalline structure of AgNPs with an fcc structure. TEM images revealed TSC coated spherical AgNPs with an average particle size of 35 nm. EDX elemental analysis restated the existence of AgNPs with a high percentage (96.36 %) of silver. The existence of chemically attached TSC on the surface of AgNPs is confirmed by FTIR and TGA. TSC coatings suppress the particle growth which enables the AgNPs to be small in size which is impossible without coatings. UV–vis absorbance peak maximum at 416 nm restates the formation of AgNPs and their good dispersion in water. TSC coated AgNPs showed potential antibacterial performance against both the pathogens tested especially on Gram-positive *S. aureus* with 8 mm of zone inhibition at a concentration of 50 mgL⁻¹. These results suggest that smaller-sized AgNPs are useful for pharmaceutical, biomedical, cosmetic, therapeutic, and industrial applications including many others.

Declarations

Acknowledgments

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Figures

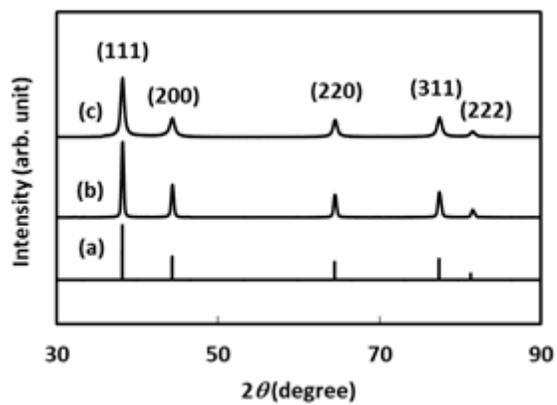


Figure 1

XRD patterns of the (a) JCPDS file No. 00-004-0783 (b) synthesized uncoated AgNPs and (c) TSC coated AgNPs.

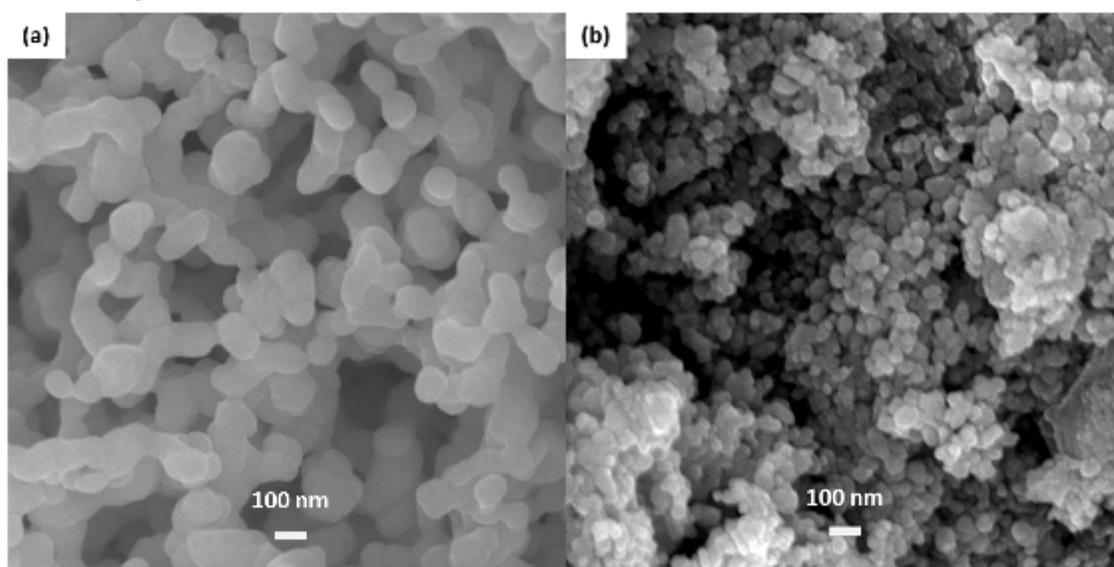


Figure 2

FESEM images of synthesized AgNPs at $\times 50000$ magnifications: (a) uncoated and (b) TSC coated.

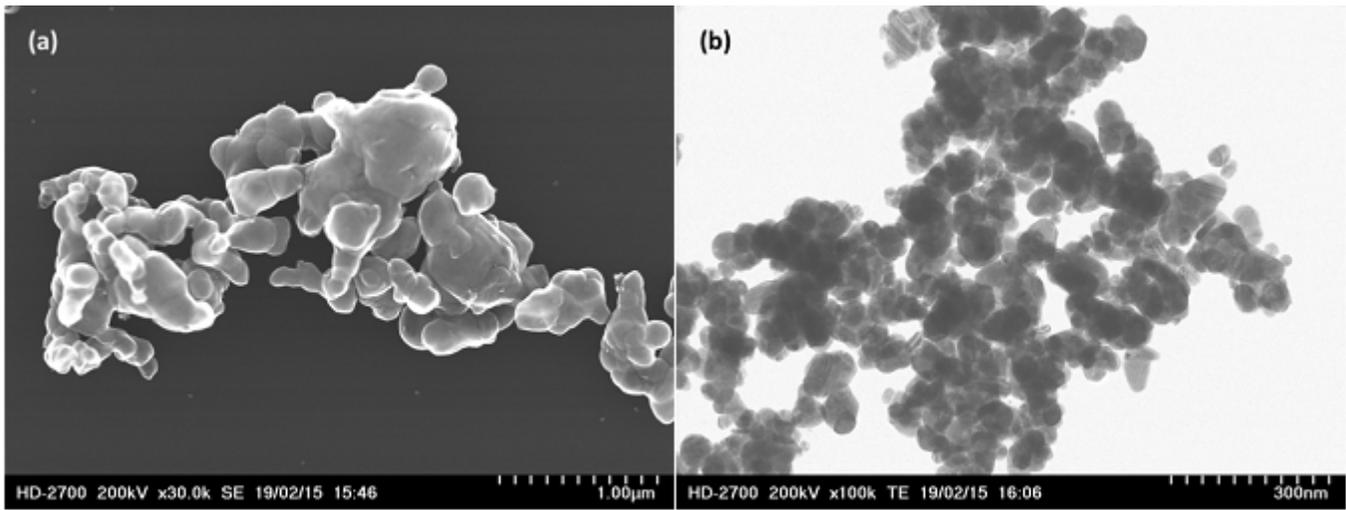


Figure 3

TEM images of synthesized AgNPs: (a) TSC uncoated AgNPs × 1 μm scale bar and (b) TSC coated AgNPs × 300 nm scale bar.

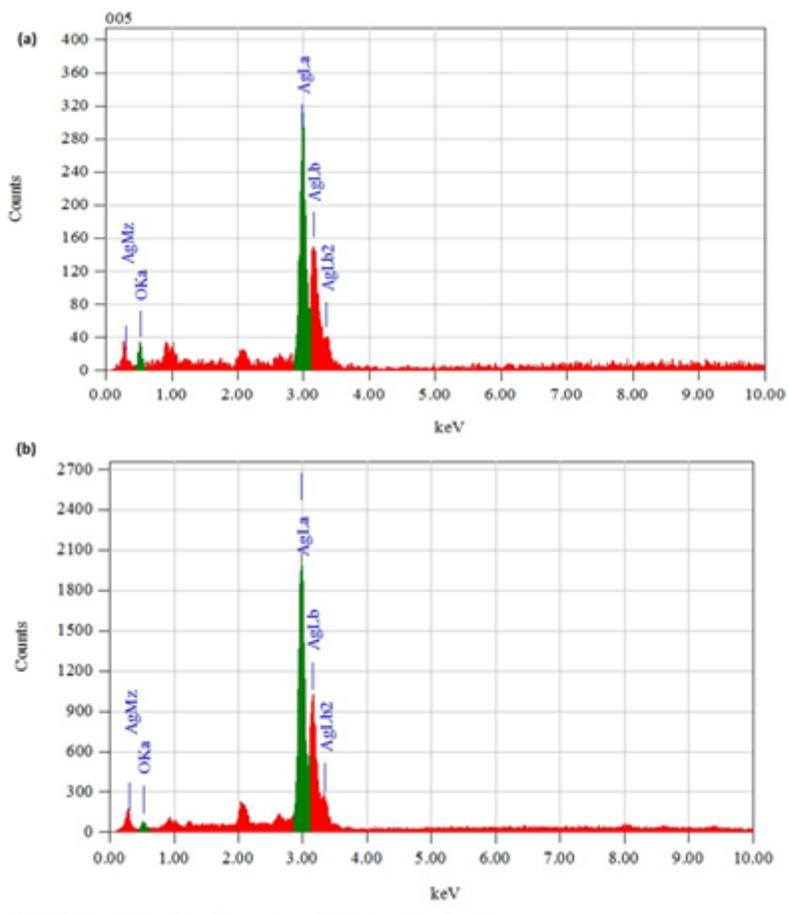


Figure 4

Energy dispersive X-ray spectrum of the synthesized AgNPs; (a) TSC uncoated AgNPs and (b) TSC coated AgNPs.

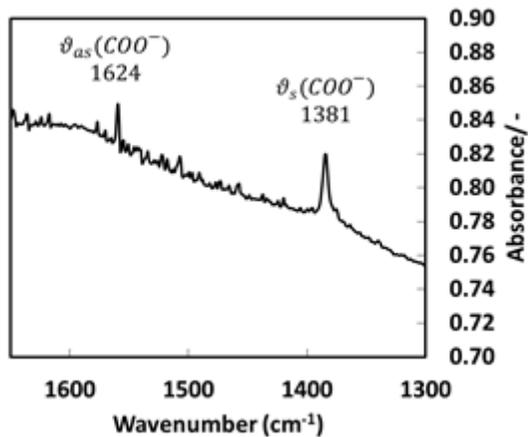


Figure 5

FTIR spectrum of the synthesized AgNPs.

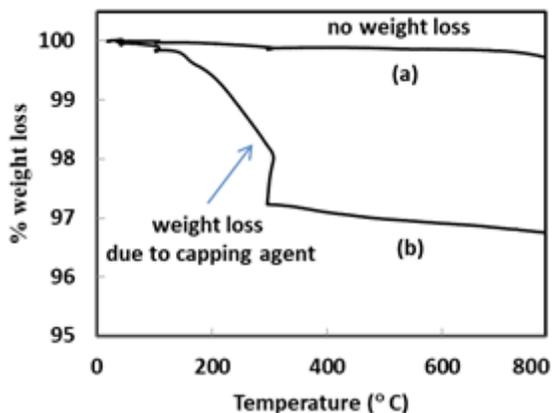


Figure 6

TGA plot of synthesized AgNPs (a) without capping agent and (b) with capping agent.

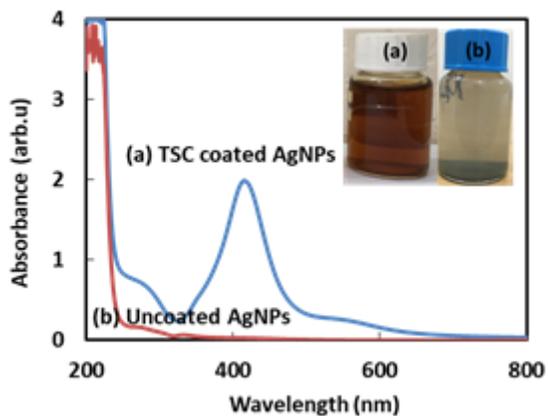


Figure 7

UV–vis absorption spectra of the colloidal dispersion [Inset: photo of products just after the synthesis];
(a) TSC coated AgNPs and (b) uncoated AgNPs.

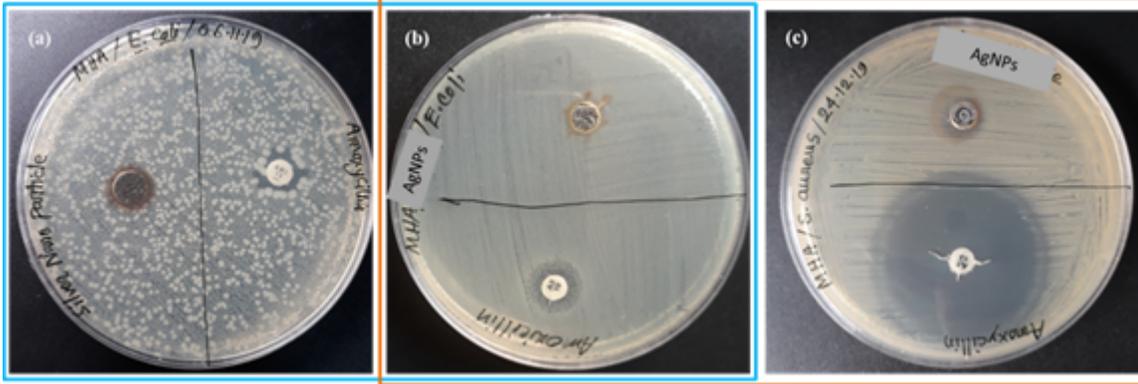


Figure 8

Antibacterial performance of (a) TSC uncoated AgNPs against Gram-positive *E. coli*, (b) TSC coated AgNPs against Gram-positive *E. coli* and (c) TSC coated AgNPs against Gram-negative *S. aureus* bacteria.

Supplementary Files

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