

An Investigation on Effect of Capping Agent on Silver Nanoparticles Antibacterial Activity

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Abstract

Silver nanoparticles (Ag NPs) are receiving much attention due to their various physical, chemical and antimicrobial properties. The aim of this study is to synthesize the Ag NPs using various capping agents ("B-cyclodextrin, starch, sodium citrate and chitosan") and investigate the effective capped Ag NP, based on their particle size, stability and antibacterial properties against a range of gram positive and gram negative bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus mesentericus*). This will contribute to further understanding the effects of capping agents on how they can improve stability and properties of Ag NPs as well as help determine their potential for future application as an alternative treatment to antibiotics against multidrug resistant bacteria. The capped Ag NPs were characterized by UV-Vis spectroscopy, FTIR and DLS. The UV-Vis spectra exhibited an absorption band between 400-430 nm for all capped Ag NPs, indicating colloidal spherical shaped Ag NPs. Amongst all the synthesized capped Ag NPs, greater stability was achieved by the sodium citrate capped Ag NPs (SC-Ag NPs) until six weeks. The smallest particle size was observed for chitosan capped silver nanoparticle (CH-Ag NPs) and both chitosan capped Ag NPs and sodium citrate capped Ag NPs (SC-Ag NPs) proved to be the most effective capping agents in terms of antibacterial activities with CH-Ag NPs consistently displaying the strongest and broadest antibacterial activity against all tested bacteria followed by SC-Ag NPs. Conclusively, the influence of capping agents on Ag NPs was seen to better stabilize Ag NPs to varying degrees and enhance their antibacterial activity when compared to uncapped Ag NPs.

Keywords

Antibacterial, β -Cyclodextrin, Chitosan, Capping agent, Sodium citrate, Starch, Silver nanoparticles, Multidrug resistant

Introduction

In recent times, silver nanoparticles (Ag NPs) are receiving much of the attention due to their various physical and chemical properties (e.g. high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity, and non-linear optical behavior to name a few), broad spectrum of antimicrobial activity and low toxicity to humans [1]. Furthermore, increasing hospital and community-acquired infections due to bacterial multidrug-resistant (MDR) pathogens for which current antibiotic therapies are not effective, represents a growing problem. The emergence of such resistance against newly developed antibiotics brings about a major need for innovative treatments. Ag NPs are seen as potential viable innovative alternative treatment to antibiotics for the fight against infection due to their various known antimicrobial properties.

Ag NPs are also widely used in the food industry (food packaging materials, sensors etc.) and the medical sector (applications in disinfecting and biocidal sprays for treatment of burn wounds, coating in medical devices, wound dressings, bond prostheses and heart valves) [2]. In addition, Ag NPs have found further applications in textile coatings, filtration membranes, soaps, paint and in water purification systems where they serve as effective disinfectants [3-5]. The properties of Ag NPs are related to their shape and size [6]. Specifically, in relation to their antimicrobial capability, the cytotoxicity of Ag NPs has been shown to increase as the particle size decreases which is believed to be attributed to easier uptake, easy dissolution and smooth release of silver ions along with increased surface area [7]. Small particle sized Ag NPs have a larger surface area to come in contact with microbial cells and hence will have a higher interaction with microbial cells. Their shape is also correlated with their antibacterial potential. In a study by Pal et al. [8, 9] whereby different shaped Ag NPs were investigated for their antibacterial activity, it was concluded that truncated triangular Ag NPs demonstrated bacterial inhibition with a silver content of 1 µg, whilst spherical ones inhibited bacteria with a silver content of 12.5 µg and rod-shaped NPs inhibited bacteria with a silver content of 50-100 µg. Similarly, Hong et al. [10] found that cubed Ag NPs and spherical shaped Ag NPs displayed stronger antibacterial activity than wired ones due to the larger surface area of the cubes and spheres which is also in agreement with the findings of Gao et al. [11] whereby sphere shaped Ag NPs exhibited the greatest antibacterial activity due to the larger surface area.

Apart from their unique properties, one limitation of Ag NPs is that they are unstable under most environmental conditions with evidence emerging of them coalescing, agglomerating, oxidizing and forming large clusters which results in losing such properties. It also influences their bioavailability and thus effectiveness as an antibiotic alternative. They are intrinsically hydrophobic and as a result tend to aggregate in aqueous solutions also. To combat this problem, a surface coating or functionalization to avoid NPs agglomeration is suggested will provide stability to the NPs. Stabilization of Ag NPs can be achieved utilizing capping agents which bind to the surface providing electrostatic, steric or electro-steric repulsive forces between particles which results in improved stability and water solubility of the nanoparticles whilst also helping prevent coalescing/ agglomeration and enhancing their antimicrobial effect [12].

There are many different coatings used to stabilize Ag NPs such as carboxylic acids, polymers, polysaccharides and surfactants. Various types of polymers have previously been explored as potential coatings including polyvinylpyrrolidone (PVP), polyacrylate, polyvinylalcohol (PVA), polyacrylamide, and thiol-modified oligonucleotides whilst polysaccharides such as gum arabic, sophorolipids and other sugars are also common coatings [13-15]. Moreover, biological agents such as bovine serum albumin (BSA) fatty acids and several fungi, including *Lactobacillus*, *Fusarium oxysporum* and *Aspergillus flavus* have further been investigated and proven to be effective capping agents for Ag NPs [16, 17].

However, with the best of author knowledge limited studies have been conducted which compare a range of capped Ag NPs and their influence on the Ag NPs stability and performance as an antibacterial agent. Therefore, the aim of this study is to synthesize Ag NPs using various different capping agents (β -cyclodextrin, starch, sodium citrate and chitosan) and investigate the effective capped Ag NPs, based on their particle size, stability and antibacterial properties against a range of gram positive and gram negative bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus mesentericus*) in order to determine the most effective capping agents and the potential application for capped Ag NPs as a future antibiotic alternative for multidrug resistant bacteria.

Materials and Methods

Synthesis of capped silver nanoparticles

All capped silver nanoparticles (sodium citrate, β cyclodextrin (β -CD), starch and chitosan) were synthesized according to the published articles with some slight modification. In brief, the precursor silver nitrate (1 mM, 50 mL) was heated until boiling and then 5 mL of 1% sodium citrate was added dropwise. The solution was mixed vigorously until a colour change to pale yellow occurred which indicated the formation of sodium citrate capped Ag NPs [18]. The β -CD capped Ag NPs were prepared by mixing the aqueous 10 mM β -CD solution into equal volumes of 2 mM silver nitrate for 15 minutes. Equal volumes of ice-cold 20 mM sodium borohydride were then slowly added to the solution which were then left for a period of time to stabilize [19]. The starch capped Ag NPs were synthesized by mixing an aqueous solution of 1 mM silver nitrate with 1% (w/v) starch solution until a homogenous solution was achieved. The resulting solution was then autoclaved at 15 psi pressure at 121 °C for 5 minutes. This resulted in a color change of the solution to yellow, which aforementioned above indicated the formation of Ag NPs [20]. The chitosan capped Ag NPs were prepared by mixing 2 mL of silver nitrate (10 mM) with 47 mL of chitosan solution (0.2% (w/v)) for a duration of 30 minutes. 0.008 g of sodium borohydride was then added quickly to the silver nitrate/chitosan solution from which a bright yellow colour was observed [21]. The control Ag NPs were synthesized with sodium borohydride without using any capping agents [22]. All chemicals utilized were purchased from Sigma Aldrich, Ireland and were used without any further purification. All solutions were prepared with distilled water.

Characterisation

All capped and uncapped Ag NPs were characterized using a UV-Visible spectrophotometer and wavelength ranges of between 300-700 nm (UV-1800 Shimadzu UV Spectrophotometer) operating at a resolution of 2 nm. The stability of all prepared Ag NPs was monitored weekly by observing with the UV-Visible spectrophotometer for a duration of 3 weeks in order to find the change in the characteristic peak. The average particle size (hydrodynamic diameter) was determined at 25 °C by Dynamic Light

Scattering (DLS) using a Malvern Zetasizer Nano instrument (Malvern Instruments) equipped with a 4 mW He-Ne laser and measured using an automatic mode with a scattering angle of 90°. The sample was loaded into a quartz microcuvette and five measurements were performed from which the mean result was recorded.

Bacterial strains and growth conditions

Gram-negative bacteria *E. coli* and *P. aeruginosa* and Gram-positive bacteria *S. aureus* and *B. mesentericus* were prepared, sub-cultured and maintained on Nutrient agar and stored at 4 °C. A single colony of each microbe was inoculated into 10 mL of Nutrient broth and incubated overnight at 37 °C whilst shaking at 200 rpm. The optical density of the overnight culture was adjusted to 0.5 McFarland turbidity standard (equivalent to 1.5×10^8 colony-forming units (CFU)/ mL) using a Densimat photometer (BioMérieux, France). The adjusted bacterial suspension was then further diluted with Nutrient broth to give a final concentration of 1×10^6 CFU/ mL.

Antibacterial activity

The antibacterial activity of the different capped and uncapped (control) Ag NPs was determined by the microtitre well broth dilution method [23]. Sodium citrate, β -CD, starch and chitosan capped samples were diluted with sterile water (1:1) and starch and uncapped Ag NPs were used as an undiluted sample. 200 μ l of the nanoparticles (NPs) samples were added to the first row of the microtitre plate. The remaining rows were filled with 100 μ l of Nutrient broth. Two-fold serial dilutions were then performed along each row by taking 100 μ l from the first row of NPs samples and diluting them into the next row and so on. 100 μ l of the prepared bacterial suspension (1×10^6 CFU/ 100 μ l) were then added into all the wells. Negative (Ag NPs 100 μ l with sterile nutrient broth 100 μ l), positive (sterile nutrient broth 100 μ l with bacterial suspension 100 μ l) and blank controls (200 μ l Nutrient broth) were included in each microtitre plate. The plates were incubated for 18 hours in a microtitre plate reader (PowerWave™ Microplate spectrophotometer and BioTek Synergy HT Multidetection Microplate Reader) at 37 °C. Samples were tested in duplicate on each plate and each plate was analysed in triplicate.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

From the microtitre well broth dilution method, the minimum inhibitory concentration of the capped and uncapped Ag NPs was determined by observing which was the lowest concentration of each of the capped nanoparticle (NPs) samples required to suppress the growth of each bacterial strain. The lowest concentration which suppressed bacterial growth was recorded as the MIC of the sample.

The MBC was determined from the microtitre well plate by taking an inoculum from a well in each row and plating it onto Nutrient agar. The plates were incubated overnight at 37 °C and then observed for bacterial growth. No growth indicated the sample is bactericidal at that concentration whilst growth following incubation is indicative the sample is

bacteriostatic at that concentration. The lowest concentration at which no growth occurred was recorded as the MBC.

Percentage inhibition

The antibacterial activities of all synthesized uncapped and capped Ag NPs against the gram-positive and gram-negative bacterial strains were determined by calculating the percentage of inhibition of growth using the below formula [19].

$$I\% = \frac{(C18 - C0) - (T18 - T0)}{(C18 - C0)} \times 100$$

where I is the percentage inhibition of growth, C18 is the optical density (OD) at 600 nm of the blank compensated positive control of the organism at 18 h, C0 is the blank-compensated OD at 600 nm of the positive control of the organism at 0 h, T18 is the OD at 600nm of the blank compensated negative control of the organism in the presence of test sample at 18 h and T0 is the negative control-compensated OD at 600 nm of the organism in the presence of test sample at 0 h.

Kinetic measurement of bacterial growth

The effect of uncapped and capped Ag NPs on all tested bacterial strains were determined over time by measuring the OD₆₀₀ every hour following 30 seconds of agitation. Antibacterial kinetics were analyzed graphically as a plot of contact time versus OD₆₀₀.

Statistical analysis

Statistical differences between multiple comparisons were evaluated using analysis of variance (ANOVA) followed by least significant difference testing. All data was statistically analyzed using STATGRAPHICS centurion XV.

Results and Discussion

Characterisation of synthesized silver nanoparticles

Characterisation of Ag NPs is important in order to evaluate the functional physicochemical properties for their behavior, bio-distribution, safety and efficacy. Characterisation of the synthesized Ag NPs was investigated using UV-Visible Spectroscopy and DLS. Figure 1 shows the synthesized capped and uncapped Ag NPs surface plasmon resonance (SPR) absorption band between 375-417 nm which indicates the Ag NPs were spherical in shape as an SPR around 400 nm is correlated with this shape. If the particles were not spherical, the absorption band would of appeared at longer wavelengths [24]. In Ag NPs, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a SPR absorption band, occurring due to the oscillation of electrons of Ag NPs in resonance with the light wave [25]. Since the intensity of the plasmon resonance band depends on particle size, shape, metallic material and its surrounding environment, the number of particles cannot be related linearly to the absorbance intensities.

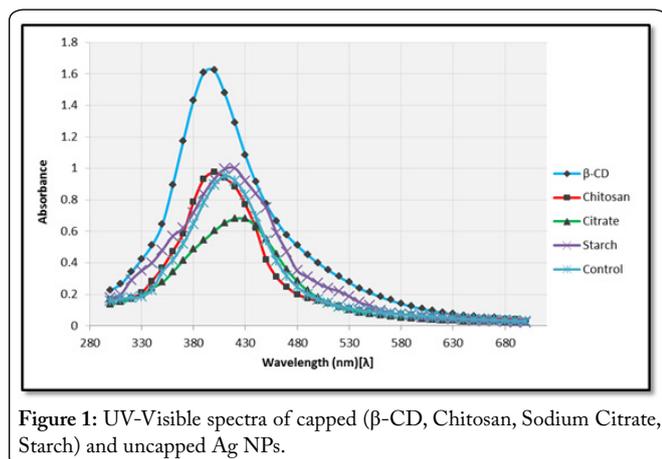


Figure 1: UV-Visible spectra of capped (β -CD, Chitosan, Sodium Citrate, Starch) and uncapped Ag NPs.

The particle size distribution of synthesized capped and uncapped Ag NPs colloidal solution were studied by DLS (Table 1) which measures a hydrodynamic size of the particles and size distribution in solutions/suspensions [26]. Majority of peaks fall between 10-100 nm with some observed above/below this range. It was noted that among all the samples, the control and chitosan Ag NPs were mono dispersed. Results further indicated that in descending order, the size of the capped and uncapped Ag NPs were as follows: starch-Ag NPs (122.42 nm) > uncapped-Ag NPs (68 nm) > β -CD Ag NPs (58.7 nm) > sodium citrate-Ag NPs (42 nm) > chitosan-Ag NPs (37 nm) (Table 1). Chitosan capping agents provide a large number of hydroxyl groups that can co-ordinate with the metal ions. In addition, they provide a means of controlling the size, shape and dispersion of the NPs and subsequent release of the active ionic silver. Capping Ag NPs with chitosan polymeric compounds has also been shown to modify their biodegradability and may be attributed to forming smaller nanoparticles without aggregation [27].

Table 1: Particle size distribution of synthesized capped and uncapped Ag NPs.

Capped /Uncapped Ag NPs	Particle Size (nm)
β -CD-Ag NPs	58.7 \pm 3.2
Sodium Citrate-Ag NPs	42 \pm 5.2
Chitosan-Ag NPs	37 \pm 1.2
Starch-Ag NPs	122.42 \pm 2.3
Uncapped-Ag NPs	68 \pm 4.6

The difference in the silver nanoparticles morphology & size noted in this study between the capped and uncapped samples is due to the organic compounds used for capping agents as it is known that the control of such characteristics of nanoparticles is largely influenced by choice of capping agent, capping agent concentration and reaction temperature [28].

Stability of Ag NPs

UV-Visible spectroscopy was employed to monitor the stability of Ag NPs overtime. Measurements were taken on day 1, week 3 and week 6 of testing (Figure 2). Uncapped Ag

NPs exhibited an absorption peak at 403 nm in comparison to absorption peaks of between 400 nm – 430 nm which were noted for the capped samples.

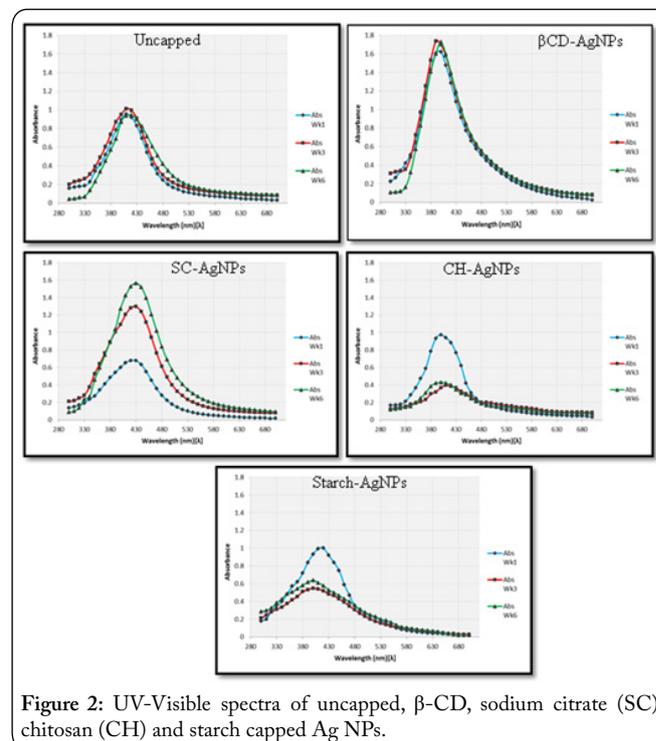


Figure 2: UV-Visible spectra of uncapped, β -CD, sodium citrate (SC), chitosan (CH) and starch capped Ag NPs.

Overall, β -CD capped Ag NPs maintained the greatest stability over time with no significant changes observed over the duration of the testing. The β -CD capped Ag NPs solution remained stable for the 6 weeks with no color change observed demonstrating the superior ability of β -CD as a capping agent to stabilize silver nanoparticles to retain and enhance their properties. Unlike the β -CD capped Ag NPs, a slight color change in the uncapped sample was observed in week 6 indicating the particles started to aggregate due to the absence of a capping agent.

In comparison, for the sodium citrate, chitosan and starch capped silver nanoparticles there was a clear shift in absorption peaks after one week with the absorbance seen to increase each week for sodium citrate capped Ag NPs. Furthermore, there was a color change observed in the sample when monitored for 6 weeks from dark yellow to cloudy orange yellow thus suggesting the beginning of the agglomeration process. Contrastingly for CH-Ag NPs and starch-Ag NPs, a major decrease in absorbance after week 1 was evident for both samples. The increasing integrated peak area of the band designates decreased inter particle spacing, which is also evidence of aggregation. Moreover, there was a slight color change of both samples from light yellow to almost transparent and a slight change in viscosity, signifying agglomeration also.

Antibacterial Activity

Percentage Inhibition

With the rise in demand for new treatments to develop

drug resistant microbes to fight against disease, the various capped Ag NPs were tested for their activity against different bacterial strains which was determined based on percent of inhibition, minimum inhibitory concentration and minimum bactericidal concentration and comparing the results to that of the uncapped equivalents. All tested Ag NPs inhibited bacterial growth to different extents with antibacterial activity found to be dependent on the silver concentration with increased inhibition, which is in agreement with the findings of Hong et al. [10] and Ugwoke et al. [1].

The bacterial percentage inhibition (>99%) was achieved by all capped and uncapped Ag NPs against all tested bacterial strains at different silver concentrations (Table 2). β -CD Ag NPs and chitosan capped Ag NPs (CH) showed the strongest

This was followed by sodium citrate (SC) capped Ag NPs where >99% inhibition was achieved against *P. aeruginosa* and *B. mesentericus* and 89% and 88% inhibition of *S. aureus* and *E. coli* respectively at a silver concentration of 9.6 ppm. In comparison, 63% bacterial inhibition was achieved against *B. mesentericus* and >91%, 94% and 98% for *P. aeruginosa*, *S. aureus* and *E. coli* respectively in the presence of the uncapped sample (control) at a silver concentration of 10.6 ppm, which is notably a higher silver concentration than sodium citrate and chitosan capped Ag NPs. In addition, the bacterial inhibition in the presence of uncapped Ag NPs was seen to be greater than that of starch Ag NPs against the tested bacterial strains which was expected since the uncapped Ag NPs maintained greater stability than the starch capped Ag NPs over the 6 weeks (Figure 2).

Table 2: Percentage growth inhibition of various bacteria in the presence of uncapped and capped Ag NPs (β -CD, starch, sodium citrate (SC), chitosan (CH) containing different concentrations.

β-CD capped Ag NPs					
	56.3 ppm	28.2 ppm	14.1 ppm	7.0 ppm	3.5 ppm
<i>E. coli</i>	100 \pm 0.2	100 \pm 0.5	100 \pm 0.4	100 \pm 0.4	15 \pm 8.0
<i>S. aureus</i>	100 \pm 14	100 \pm 10.0	100 \pm 15	26 \pm 14.0	10 \pm 12.0
<i>P. aeruginosa</i>	100 \pm 0.5	100 \pm 1.1	94 \pm 1.3	77 \pm 4.8	71 \pm 4.3
<i>B. mesentericus</i>	100 \pm 12	100 \pm 10.0	100 \pm 15	26 \pm 14.0	12 \pm 10.0
Starch capped Ag NPs					
	169 ppm	84.2 ppm	42.3 ppm	21.1 ppm	10.6 ppm
<i>E. coli</i>	100 \pm 0.6	99 \pm 0.2	99 \pm 0.3	99 \pm 0.6	86 \pm 21.5
<i>S. aureus</i>	100 \pm 0.8	99 \pm 0.3	81 \pm 14.8	49 \pm 6.1	17 \pm 2.4
<i>P. aeruginosa</i>	99 \pm 0.3	99 \pm 0.3	81 \pm 29.1	61 \pm 34.1	31 \pm 9.3
<i>B. mesentericus</i>	99 \pm 0.1	99 \pm 0.2	99 \pm 0.7	88 \pm 3.9	23 \pm 6.4
Sodium citrate capped Ag NPs					
	76.8 ppm	38.4 ppm	19.2 ppm	9.6 ppm	4.8 ppm
<i>E. coli</i>	99 \pm 0.1	99 \pm 0.2	99 \pm 0.7	88 \pm 3.9	23 \pm 6.4
<i>S. aureus</i>	100 \pm 0.3	99 \pm 0.8	99 \pm 1.1	89 \pm 15.4	22 \pm 10.9
<i>P. aeruginosa</i>	100 \pm 0.3	99 \pm 0.3	99 \pm 0.8	99 \pm 0.2	21 \pm 5.6
<i>B. mesentericus</i>	100 \pm 1.0	99 \pm 0.1	99 \pm 0.2	99 \pm 0.1	68 \pm 22.0
Chitosan capped Ag NPs					
	33.8 ppm	16.8 ppm	8.5 ppm	4.2 ppm	2.1 ppm
<i>E. coli</i>	100 \pm 0.5	99 \pm 1.0	99 \pm 1.5	99 \pm 7.5	13 \pm 14.0
<i>S. aureus</i>	100 \pm 1.2	99 \pm 0.7	99 \pm 0.7	99 \pm 0.5	52 \pm 11.8
<i>P. aeruginosa</i>	100 \pm 0.5	100 \pm 0.3	99 \pm 0.6	98 \pm 1.8	37 \pm 8.5
<i>B. mesentericus</i>	100 \pm 0.6	99 \pm 0.6	99 \pm 0.6	99 \pm 0.5	86 \pm 13.0
Control					
	42.3 ppm	21.2 ppm	10.6 ppm	5.3 ppm	2.6 ppm
<i>E. coli</i>	100 \pm 0.5	100 \pm 1.7	98 \pm 0.2	89 \pm 25	12 \pm 18.6
<i>S. aureus</i>	100 \pm 0.4	98 \pm 0.9	94 \pm 4.8	33 \pm 8.3	12 \pm 10.8
<i>P. aeruginosa</i>	100 \pm 0.3	100 \pm 0.8	91 \pm 1.6	29 \pm 6.7	13 \pm 10.8
<i>B. mesentericus</i>	100 \pm 0.9	100 \pm 1.3	63 \pm 3.5	40 \pm 9.5	11 \pm 16.8

antimicrobial activity and broadest action against *E. coli*, *S. aureus* and *B. mesentericus* with >98% inhibition at silver concentrations of 14.1 and 4.2 ppm respectively.

MIC and MBC

The MIC and MBC values of the capped and uncapped (control) Ag NPs are shown in table 3. All tested capped

Table 3: MIC and MBC of capped (β -CD, starch, sodium citrate, chitosan) and uncapped Ag NPs against tested bacteria.

Microorganisms	β -CD		Starch		Sodium citrate		Chitosan		Uncapped	
	MIC*	MBC*	MIC*	MBC*	MIC*	MBC*	MIC*	MBC*	MIC*	MBC*
<i>E. coli</i>	7	7	21	21	19	19	4	4	21	21
<i>S. aureus</i>	14	14	84	84	10	10	4	4	42	42
<i>P. aeruginosa</i>	28	28	84	84	10	10	8	8	21	21
<i>B. mesentericus</i>	14	14	42	42	10	10	4	4	21	21

*concentration in ppm

Ag NPs displayed bactericidal properties against the tested bacteria at different silver concentrations.

Among all the tested capped Ag NPs, chitosan capped Ag NPs showed the highest antibacterial activity against *E. coli*, *S. aureus*, and *B. mesentericus* with MIC and MBC values of 4 ppm. This was followed by sodium citrate capped Ag NPs with an MIC and MBC value of 10 ppm against *S. aureus*, *P. aeruginosa* and *B. mesentericus*.

Uncapped Ag NPs exhibited bacterial inhibition of *E. coli*, *P. aeruginosa* and *B. mesentericus* at silver concentrations of 21 ppm and a similar concentration was required to kill these bacteria, however approximately double this concentration was required to kill *S. aureus*. In addition, uncapped Ag NPs showed 4 times higher MIC and MBC values against *S. aureus* and *P. aeruginosa* and 2 times higher MIC and MBC values against *B. mesentericus*, than the starch capped Ag NPs due to the smaller size (Table 1). Among all the capped Ag NPs, the increasing antibacterial activity was as follows: chitosan capped Ag NPs > sodium citrate capped Ag NPs > β -CD capped Ag NPs > uncapped Ag NPs > starch capped Ag NPs.

Growth Kinetics

The bacterial growth kinetics in the presence of capped and uncapped Ag NPs at various concentrations were determined in which it was observed that the capped Ag NPs displayed good antibacterial properties against all tested strains over an 18 hrs incubation period.

Figure 3a, shows the delaying of *E. coli* bacterial growth until 10 hrs of incubation in the presence of most of the synthesized capped Ag NPs at different concentrations. The slowest bacterial growth was noted for starch-Ag NPs which may be due to the fact a higher concentration of starch was used in comparison to the rest of the capping agents. β -CD, SC and CH Ag NPs delayed bacterial growth upto between 7-8 h after which rapid growth occurred but signs of deceleration towards the last few hours (≥ 11 h) was observed interestingly. Contrastingly, the control Ag NPs proved to delay bacterial growth of *E. coli* only up to 7 hrs which then rapidly continued growing for the remainder of the incubation period with no sign of any more inhibition.

Figure 3b shows the bacterial growth of *S. aureus*. Bacterial growth was delayed up until 13 hrs and 9 hrs in the presence of sodium citrate capped Ag NPs (SC) at [4.8 ppm] and chitosan (CH) capped Ag NPs at [2.1 ppm] respectively thus indicating the superior antimicrobial activity of these capped

Ag NPs against *S. aureus*. In comparison, for the uncapped Ag NPs [at 2.6 ppm] rapid bacterial growth was evident after 8 hrs of incubation with no signs of deceleration. β -CD capped Ag NPs appeared to have weak activity against this particular bacterial strain as growth was only delayed for 4 hrs of incubation after which an increase in growth was evident.

Figure 3c, shows the growth kinetics of *P. aeruginosa* from which it is seen that sodium citrate capped Ag NPs at [4.8 ppm] and chitosan (CH) capped Ag NPs at [2.1 ppm] displayed strong antibacterial influence on this strain delaying growth until 12 hrs of the 18 hrs incubation period after which accelerated growth occurred. Among all synthesized capped Ag NPs, again due to the higher concentration of starch Ag NPs [10.6 ppm] it displayed the greatest antibacterial activity against this bacterial strain followed by CH-capped Ag NPs at the lowest concentration of 2.1 ppm thus indicating the superior activity of this sample.

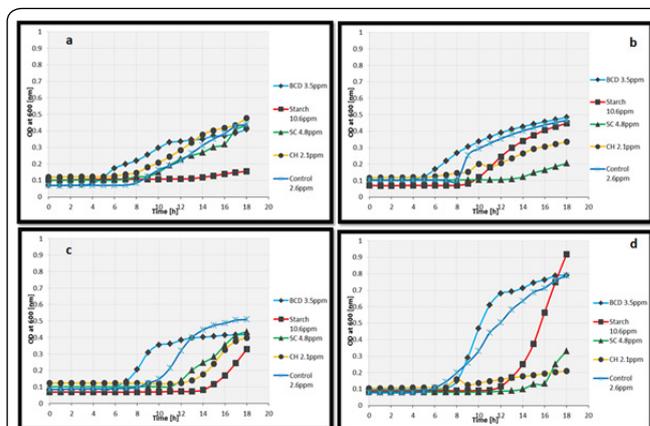


Figure 3: Bacterial growth curves of bacteria (a) *E. coli* (b) *S. aureus* (c) *P. aeruginosa* and (d) *B. mesentericus* in the presence of synthesised capped (β -CD, sodium citrate, chitosan, starch) Ag NPs at different concentrations (ppm).

Figure 3d, shows the bacterial growth curve of *B. mesentericus* in the presence of capped and uncapped Ag NPs in which it was observed the strongest antibacterial activity was exerted by sodium citrate capped Ag NPs at [4.8 ppm] which growth was suppressed until 13 hrs, followed by starch Ag NPs [10.6 ppm] up to 11 hrs. Again β -CD capped Ag NPs had little affect against this bacterial strain as bacterial growth was evident after just 6 hrs. To compare, control Ag NPs delayed growth only until 5 hrs. In the presence of chitosan capped Ag NPs at 2.1 ppm, very little growth was observed, indicating the superior antimicrobial activity of this sample against *B. mesentericus*.

Overall it can be said depending on the bacterial strain the different capped samples had varying degrees of antibacterial activity. In summary, the strongest antibacterial activity was exerted predominantly by chitosan capped Ag NPs followed by sodium citrate capped Ag NPs against all strains which is presumed to be due to the fact both of these samples had the smallest particle sizes of 37 ± 1.2 nm and 42 ± 5.2 nm respectively among all the samples. Aforementioned, the smaller size of the capped Ag NPs means they have a larger surface area to come in contact with microbial cells and thus have a higher interaction with microbial cells to inhibit their growth [7, 11]. The lag phase of the four bacterial strains appeared to be prolonged with a sharp deceleration of the log phase and maintenance at the stationary phase in capped Ag NPs in comparison to their uncapped equivalents where the bacteria were seen to continue to grow over the 18 h incubation period. The findings of this study of the antibacterial activity of Ag NPs are in line with other work done by several other researchers who have also previously demonstrated Ag NPs to be effective against various gram-negative and gram-positive bacteria but the mechanism of how Ag NPs kill pathogens is not fully understood [8, 29]. The mechanisms of antibacterial action of the capped Ag NPs may be due to the releasing Ag⁺ ions into bacteria and accumulating on the cell membrane, further disrupting the integrity of the cell wall and membrane causing the leakage of cell constituents and inducing the bacterial cell death [10, 11, 30].

The capped Ag NPs enhanced the antibacterial activities, which may be attributed to a biodegradable capping agent forming smaller nanoparticles without aggregation. These have a high surface-to-volume ratio and undergo a higher level of interaction with the bacterial cell surface than the larger uncapped Ag NPs, resulting in higher antibacterial activity. It is proposed that intimate contact between Ag NPs and organisms may enhance the transfer of Ag ions to the bacterial cell, whilst bacterial degradation of the chitosan polysaccharides promotes the release of silver ions. These results are in accordance with a previous report in which Ag NPs synthesized by disaccharides had higher antibacterial activities than those synthesized by monosaccharide [31]. However, further investigations need to be done to further investigate how Ag NPs interact with bacteria and the mechanism by which they inactivate the cells.

Conclusion

In summary, capping Ag NPs can improve their stability and enhance their antibacterial properties. Among all synthesized capped Ag NPs chitosan capped Ag NPs were the smallest in size followed by sodium citrate capped Ag NPs. The varying sizes between the capped and uncapped Ag NPs is not fully understood but there is evidence from scientific literature this may be linked to the choice of capping agent, capping agent concentration and experimental conditions which are deemed important for the control of Ag NP morphology and size. With respect to the stability of synthesized Ag NPs, CH-AgNPs remained stable up to one week, whereas sodium citrate Ag NPs and β -CD capped Ag NPs were stable until

six weeks. In addition, chitosan capped Ag NPs and sodium citrate capped Ag NPs both proved to be the most effective capping agents in terms of antibacterial activity as CH-Ag NPs consistently displayed the strongest and broadest antibacterial activity against all tested gram-positive and gram-negative bacteria followed by SC-Ag NPs. The antibacterial properties of Ag NPs was also seen to be dependent on particle size with smaller particle sizes allowing for greater activity due to the larger surface area allowing close contact with the microbial cells. Further investigations need to be conducted to better understand the mechanism of action of all synthesized capped Ag NPs and their interaction with gram-positive and gram-negative bacteria to inhibit their growth.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Ugwoke E, Aisida SO, Mirbahar AA, Arshad M, Ahmad I, et al. 2020. Concentration induced properties of silver nanoparticles and their antibacterial study. *Surf Interfaces* 18: 100419. <https://doi.org/10.1016/j.surfint.2019.100419>
2. Guan Q, Xia C, Li W. 2019. Bio-friendly controllable synthesis of silver nanoparticles and their enhanced antibacterial property. *Catal Today* 327: 196-202. <https://doi.org/10.1016/j.cattod.2018.05.004>
3. Samberg ME, Orndorff PE, Monteiro-Riviere NA. 2011. Antibacterial Efficacy of silver nanoparticles of different sizes, surface conditions and synthesis methods. *Nanotoxicology* 5(2): 244-253. <https://doi.org/10.3109/17435390.2010.525669>
4. Chudasama B, Vala AK, Andhariya N, Upadhyay R, Mehta R. 2009. Enhanced antibacterial activity of bifunctional Fe₃O₄-Ag core-shell nanostructures. *Nano Res* 2(12): 955-965. <https://doi.org/10.1007/s12274-009-9098-4>
5. Kumar A, Vemula PK, Ajayan MP, John G. 2008. Silver nanoparticle embedded antimicrobial paints based on vegetable oil. *Nat Mater* 7(3): 236-241. <https://doi.org/10.1038/nmat2099>
6. Krutyakov YA, Kudrinskiy AA, Olenin AY, Lisichkin GV. 2008. Synthesis and properties of silver nanoparticles: advances and prospects. *Russian Chemical Reviews* 77(3): 233-257. <https://doi.org/10.1070/RC2008v077n03ABEH003751>
7. Powers CM, Badireddy AR, Ryde IT, Seidler FJ, Slotkin TA. 2011. Silver nanoparticles compromise neurodevelopment in PC12 cells: critical contributions of silver ion, particle size, coating and composition. *Environ Health Perspect* 119(1): 37-44. <https://doi.org/10.1289/ehp.1002337>
8. Pal S, Tak YK, Song JM. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Appl Environ Microbiol* 73(6): 1712-1720. <https://doi.org/10.1128/AEM.02218-06>

9. Morones J, Elechiguerra J, Camacho A, Ramirez J. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology* 16(10): 2346-2353. <https://doi.org/10.1088/0957-4484/16/10/059>
10. Hong X, Wen J, Xiong X, Hu Y. 2016. Shape effect on the antibacterial activity of silver nanoparticles synthesized via a microwave-assisted method. *Environ Sci Pollut Res Int* 23(5): 4489-4497. <https://doi.org/10.1007/s11356-015-5668-z>
11. Gao M, Sun L, Wang Z, Zhao Y. 2013. Controlled synthesis of Ag nanoparticles with different morphologies and their antibacterial properties. *Mater Sci Eng C Mater Biol Appl* 33(1): 397-404. <https://doi.org/10.1016/j.msec.2012.09.005>
12. Hotze EM, Phenrat T, Lowry GV. 2010. Nanoparticle aggregation: challenges to understanding transport and reactivity in the environment. *J Environ Qual* 39(6): 1909-1924. <https://doi.org/10.2134/jeq2009.0462>
13. Widal BC, Deivaraj TC, Yang J, Too HP, Chow GM, et al. 2005. Stability and hybridization-driven aggregation of silver nanoparticle-oligonucleotide conjugates. *New J Chem* 29(6): 812-816. <https://doi.org/10.1039/b417683a>
14. Stebounova LV, Guio E, Grassian VH. 2011. Silver nanoparticles in simulated biological media: a study of aggregation, sedimentation, and dissolution. *J Nanopart Res* 13: 233-244. <https://doi.org/10.1007/s11051-010-0022-3>
15. Tolaymat TM, El Badaway AM, Genaidy A, Schekel KG, Luxton TP. 2010. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: a systematic review and critical appraisal of peer-reviewed scientific papers. *Sci Total Environ* 408(5): 999-1006. <https://doi.org/10.1007/s11051-010-0022-3>
16. Vigneshwaran N, Ashtaputre N, Varadarajan P, Nachane R, Paralikar KM, et al. 2007. Biological synthesis of silver nanoparticles using fungus *Aspergillus flavus*. *Mater Lett* 61: 1413-1418. <https://doi.org/10.1016/j.matlet.2006.07.042>
17. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, et al. 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloid Surf B Biointerfaces* 28(4): 313-318. [https://doi.org/10.1016/S0927-7765\(02\)00174-1](https://doi.org/10.1016/S0927-7765(02)00174-1)
18. Nain R, Chauhan RP. 2009. Colloidal synthesis of silver nanoparticles. *Asian J Chem* 21: 113-116.
19. Jaiswal S, Duffy B, Jaiswal AK, Stobie N, McHale P. 2010. Enhancement of the antibacterial properties of silver nanoparticles using β -cyclodextrin as a capping agent. *Int J Antimicrob Agent* 36(3): 280-283. <https://doi.org/10.1016/j.ijantimicag.2010.05.006>
20. Mohanty S, Mishra S, Jena P, Jacob B, Sarkar B, et al. 2012. An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles. *Nanomedicine* 8(6): 916-924. <https://doi.org/10.1016/j.nano.2011.11.007>
21. Chen Z, Zhang X, Cao H, Huang Y. 2013. Chitosan-capped silver nanoparticles as a highly selective colorimetric probe for visual detection of aromatic ortho-trihydroxy phenols. *Analyst* 138(8): 2343-2349. <https://doi.org/10.1039/c3an36905f>
22. Solomon S, Bahadory M, Jeyarajasingam AV, Rutkowsky S, Boritz C. 2007. Synthesis and study of silver nanoparticles. *J Chem Educ* 84(2): 322-325. <https://doi.org/10.1021/ed084p322>
23. Jaiswal S, Bhattacharya K, McHale P, Duffy B. 2015. Dual effects of β -cyclodextrin-stabilised silver nanoparticles: enhanced biofilm inhibition and reduced cytotoxicity. *J Mater Sci Mater Med* 26(1): 5367. <https://doi.org/10.1007/s10856-014-5367-1>
24. Jana NR, Gearheart L, Murphy CJ. 2001. Wet chemical synthesis of silver nanorods and nanowires of controllable aspect ratio electronic supplementary information (ESI) available: UV-VIS spectra of silver nanorods. *Chem Commun* 7: 617-618. <https://doi.org/10.1039/B100521I>
25. Mendis P, de Silva RM, de Silva KN, Wijenayaka LA, Jayawardana K, et al. 2016. Nanosilver rainbow: a rapid and facile method to tune different colours of nanosilver through the controlled synthesis of stable spherical silver nanoparticles. *RSC Adv* 6(54): 48792-48799. <https://doi.org/10.1039/C6RA08336F>
26. Diegoli S, Manciuola AL, Begum S, Jones IP, Lead JR, et al. 2008. Interaction between manufactured gold nanoparticles and naturally occurring organic macromolecules. *Sci Total Environ* 402(1): 51-61. <https://doi.org/10.1016/j.scitotenv.2008.04.023>
27. Abdelgawad AM, Hudson SM, Rojas OJ. 2014. Antimicrobial wound dressing nanofiber mats from multicomponent (chitosan/silver-NPs/polyvinyl alcohol) systems. *Carbohydrate Polymers* 100: 166-178. <https://doi.org/10.1016/j.carbpol.2012.12.043>
28. Oluwalana AE, Ajibade PA. 2019. Effect of temperature and capping agents on structural and optical properties of tin sulphide nanocrystals. *J Nanotechnol* 2019: 8235816. <https://doi.org/10.1155/2019/8235816>
29. Sondi I, Salopeck-Sondi B. 2004. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. *J Colloid Interface* 275(1): 177-182. <https://doi.org/10.1016/j.jcis.2004.02.012>
30. Liao S, Zhang Y, Pan X, Zhu F, Jiang C, et al. 2019. Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant *Pseudomonas aeruginosa*. *Int J Nanomedicine* 14: 1469-1487. <https://doi.org/10.2147/IJN.S191340>
31. Panáček A, Kvitek L, Prucek R, Kolář M, Večeřová R, et al. 2006. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. *J Phys Chem B* 110(33): 16248-16253. <https://doi.org/10.1021/jp063826h>