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Antibacterial Activity of Nano-Silver capped by β-Cyclodextrin

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Silver nanoparticles were prepared by chemical reduction method using sodium citrate as reducing agent, followed by capping with various concentrations of β-Cyclodextrin (β-CD) and characterized by various physic-chemical characterization techniques. Antibacterial activity of Pseudomonas aeruginosa (Gram-negative) and Staphylococcus aureus (Gram-positive) was determined by Well-Diffusion method. The nano-silver were spherical under Scanning electron microscopy (SEM) and the XRD result shows average diameters of capped particles are smaller than their equivalent uncapped particles. Capped nano silver particles of four different concentrations were demonstrated as superior for photo stability, when exposed to intense ultraviolet (UV-Vis) radiation for 4 hours, as well as significantly higher antibacterial activity. The influence of β-CD concentration (5 mM, 10 mM and 15 mM) was seems to be delay in bacterial growth, showing that a Trojan horse mechanism may be owing to occur bacterial affinity, thereby improving silver ion absorption.

1. Introduction : The persistence of antibiotic-resistant bacteria has renewed interest in the use of silver and silver-based components, including silver nanoparticles (AgNPs), as alternative antibacterial agents. Such compounds can reduce infections in burns patients and prevent bacterial colonization of prostheses, catheters, dental materials and human skin. The high thermal stability, low toxicity to human cells and effective broad-spectrum antibacterial activity of AgNPs **[1]** has been exploited in a range of commercial products.

There are several hypotheses to explain the antibacterial activity of AgNPs. Their rapid breakdown releases ionic silver that inactivates vital bacterial enzymes by interacting with essential thiol groups. Silver ions can inhibit bacterial DNA replication, damaging bacterial cytoplasmic membranes, depleting levels of intercellular adenosine triphosphate (ATP) and causing cell death **[2]**. The high specific surface-to-volume ratio of AgNPs increases their contact with microorganisms, promoting the dissolution of silver ions, thereby improving biocidal effectiveness. The ability of AgNPs to silver ions is a key to their antibacterial activity **[3]**.

AgNPs can be synthesized in a number of ways, with sodium citrate reduction of silver salts being most common. Stabilization is achieved using capping agents that bind to the nanoparticle surface improve stability and water solubility, which are essential to prevent aggregation; examples include water-soluble polymers, oligosaccharides and polysaccharides, sodium dodecyl sulphate (SDS) and sophorolipid (glycolipid) **[4]**.

Cyclodextrins (CDs) are a family of soluble, non-toxic molecules consisting of 6, 7 or 8 D-glucopyranosyl residues (denoted as α-, β- and γ-CDs, respectively) linked in cyclic structure by α-1, 4 glycosidic bonds. They can form inclusion complexes incorporating various molecular guests within their hollow, truncated cone shaped cavity structure, enabling them to be used as drug carriers enzyme mimics. Host-guest interaction has been attributed to a combination of weak interactions such as van der Waals forces, hydrogen bonding and hydrophobic interactions **[5]** of the three cyclodextrins, β-CD, with an internal cavity diameter of 0.78 nm, is the most widely used but is too small to entrap AgNPs and simply binds via

chemisorption to the nanoparticles through rim hydroxyl groups.

The present work investigated AgNP synthesis using different concentrations of unmodified β-CD with Sodium citrate as the reducing agent. AgNPs were characterized by ultraviolet-visible (UV-Vis) Spectrometry, Scanning Electron Microscopy (SEM), X-ray Diffractrometer (XRD). Antibacterial activities of uncapped and β-CD capped AgNPs against Gram-negative (Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus) bacteria were determined.

2. Materials and methods :

Synthesis of silver nanoparticles : Aqueous β-CD solutions (5, 10 and 15mM) were stirred into equal volumes of 2mM $AgNO_3$ for 15 min and equal volumes of ice cold 20mM sodium citrate were added. Resultant solutions were stabilized by stirring (24h). The final molar rations of β-CD: $AgNO_3$ were 2.5, 5 and 7.5 to 1, respectively. A β-CD free control was similarly prepared. All chemicals were purchased from Sigma-Aldrich (Dublin, Ireland) and were used without further purification.

Sample Characterization : UV-Vis absorption spectra of β-CD-capped AgNPs were acquired in 200-800 nm wavelength range using an UV-Vis-NIR spectrophotometer (Perkin-Elmer) operating at a resolution of 2 nm. FT-IR spectra were measured in the 4000-400 cm^{-1} range with a resolution of $1cm^{-1}$ using a Perkin-Elmer Spectrum GX instrument in transmission mode using potassium bromide (KBr) pellets prepared by mixing KBr powder with AgNP solutions and then drying (100^oC, 24 hr). AgNP morphology was determined using a JEM-100CX II scanning electron microscope (JSEOL) operated at an accelerating voltage of 100 Kv. Samples were drop-cast onto standard Formvar-coated copper grids (200-300 nm) and then air dried. Sample photo stability was determined by exposing solutions to an intense UV source (Q-sun Xenon Test Chamber; Q-Lab) operating at 0.4W/m2 (340nm) for 1, 2, 3 and 4h, respectively.

Bacterial strains and growth condition : Gram-negative bacteria (P.aeruginosa ATCC 27852) and Gram- positive bacteria (S.aureus ATTCC 25923) were grown, sub cultured and maintained on Mueller–Hinton agar (lab M) and stored at 4º C. For the experiment, a single colony of each organism was inoculated into 10mL of Mueller –Hinton broth (MHB) and incubated overnight at 37º C with shaking at 200 rpm. The optical density of the overnight culture was adjusted to that of a 0.5 Mcfarland standard using a Densimatphotometer (bioMerieux, France) and diluted with MHB to give a final working concentration of 1x106 CFU/mL.

Antibacterial activity assay : Antibacterial activities of capped and uncapped AgNPs of different silver concentrations were determined by a Well Diffusion method **[6]**. Samples were doubly diluted in water (100 µL) in microtitre wells and bacteria (100 µL; 106 CFU/mL) were added. Negative (AgNPs100 µL + sterile MHB 100 µL) and positive (sterile MHB 100 µL + bacterial suspension 100µL) controls wells and a sterility control bank (sterile MHB 200 µL) were included in each assay. Plates were incubated (18 h) in microtitre plate reader (Power WaveTMMicroplate spectrophotometer; BioTek, Winooski, VT) at 37º C. Samples were tested in duplicate on each plate and each plate was analyzed in triplicate.

3. Results and Discussion :

Characterization of β-Cyclodextrin-capped silver nanoparticles : All AgNPs showed a surface Plasmon absorption band around 400 nm as expected for colloidal AgNPs, indicating roughly spherical nanoparticles, whilst solution absorbance intensities increased with β-CD concentration. 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 **[7]**.

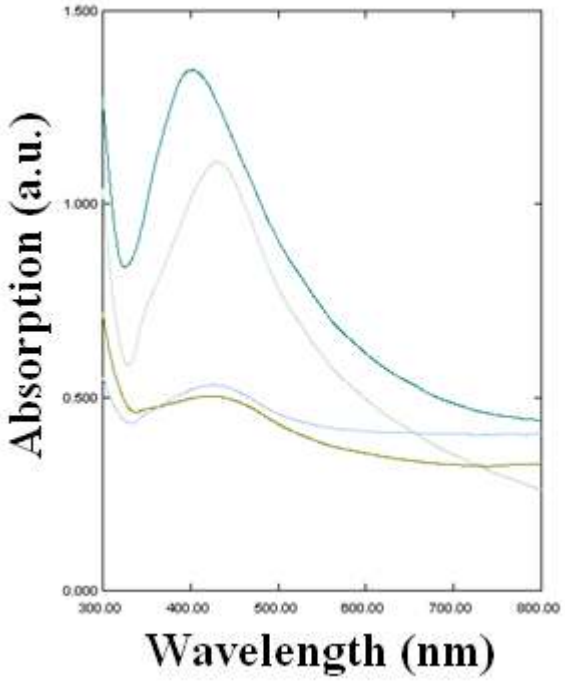


Figure (1) : UV-Visible spectrum of silver nanoparticles a) 425 nm for uncapped Ag, b) 420 nm for CD (5 mM) capped Ag, c) 414 nm for CD (10 mM) capped Ag and d) 402 nm for CD (15 mM) capped Ag.

XRD Analysis : Figure (2) shows that the XRD pattern of the as-prepared silver nanoparticles capped with β-cyclodextrin at three different concentrations. The XRD patterns of the silver nanoparticles synthesized using various concentrations of β-CD exhibit five sharp peaks in the diffractogram which indicates that the silver nanoparticles are crystalline in nature. The peaks at 2º=38.36, 44.55, 64.69, 77.54 and 81.54 reveal that it is a face centered cubic (fcc) structure. The discernible peaks can be indexed to (111), (200), (220), (311) and (222) planes of a cubic unit cell, which corresponds to cubic structure of silver (JCPDS card No. 89-3722).

Crystallite size calculation were done using scherrer equation **[8]** and results shows that 15 mM of β-CD has well crystalline nature and crystallite size was the less when 15 mmol of β-CD was used as capping agent. Therefore, β-CD (15 mmol) was employed as a capping agent for future studies.

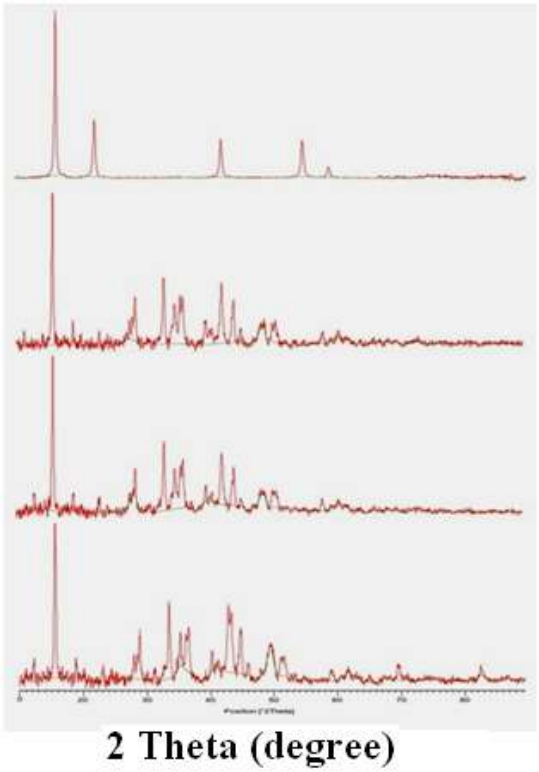


Figure (2) : XRD spectrum of silver nanoparticles (a) uncapped Ag, (b) CD (5 mM) capped Ag, (c) CD (10 mM) capped Ag and (d) CD (15 mM) capped Ag.

SEM Analysis :

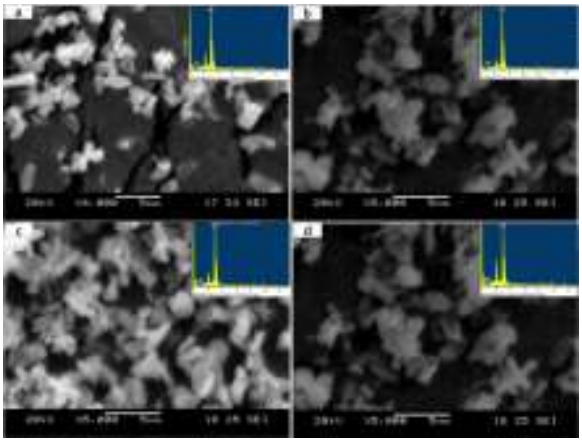


Figure (3) : SEM spectrum of silver nanoparticles (a) uncapped Ag, (b) CD (5 mM) capped Ag, (c) CD (10 mM) capped Ag and (d) CD (15mM) capped Ag

Figure (3) shows the SEM / EDS images of silver nanoparticles prepared using a capping agent β-CD and they show a shape with relatively narrow size distribution. The EDS spectrum shows the elemental peaks of Ag, O and Na. From EDS it was confirmed that there is no other impurities except sodium and oxygen **[9]**.

Antibacterial activity : Most published AgNP antibacterial studies have used plate counting **[10]** and agar diffusion methods. Fewer reports have used the qualitative microliter Well Diffusion method. The effect of β-CD capping on antibacterial activity was determined by comparing the percentage inhibition of bacterial growth by uncapped AgNPs compared with that of capped AgNPs (5, 10 and 15 mM β-CD).

β–CD capped AgNPs displayed significantly ($p<0.05$) greater antibacterial activity against P.aeruginosa than uncapped AgNPs. AgNPs capped with 15mM and 10mM β-CD had the strongest activity of 99.2% and 97.3% inhibition respectively, whereas inhibition for AgNPs capped with 5mM β-CD was 64.5% and for uncapped AgNPs was 13.9%. Both capped and uncapped AgNPs with a [Ag] ≥ 25 ppm significantly inhibited bacterial growth, whilst those with a [Ag] ≤ 6.25 ppm showed no inhibition.

Staphylococcus aureus was 97.1% inhibition by 15mM β-CD-capped AgNPs at a [Ag] of 6.25 ppm compared with 59.5% inhibition by uncapped AgNPs. Antibacterial activity was high (94.0% inhibition) even at a concentration of 10mM β-CD. Similar findings were obtained with a [Ag] of 3.12 ppm.

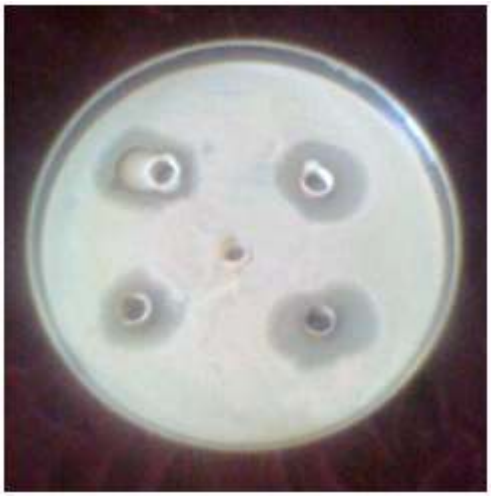
Antibacterial activity increased with corresponding rises in both silver and cyclodextrin concentrations in all cases. Importantly, β-CD alone showed no antibacterial activity.

Bacterial growth curves showed that the AgNPs delayed growth. The 15 mM and 10 mM β-CD capped AgNPs containing a [Ag] of 6.25 ppm completely inhibited P.aeruginosa growth until 18 h compared with uncapped. 10 h for the uncapped AgNPs. AgNPs capped with 15 mM and 10 mM β-CD delayed growth of S.aureus for 17 h. Overall, the duration of the lag growth phase of the test organisms when grown in the presence of AgNPs with different [Ag] increased as the β-CD capping concentration increased.

The increased antibacterial activity of β-CD-capped AgNPs 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 AgNPs, resulting in a higher antibacterial activity.

It is proposed that intimate contact between AgNPs and organisms may enhance the transfer of Ag ions to the bacterial cell, whilst bacterial degradation of the β-CD saccharide promotes the release of silver ions **[11]**. Such interactions have been described as Trojan-horse mechanisms and have been reported in the literature where fetal bovine serum was used to improve AgNP efficacy **[12]**. These results are in accordance with a previous report in which AgNPs synthesized by disaccharides higher antibacterial activities than those synthesized by monosaccharides **[13]**.

Antibacterial activity of β-cyclodextrin (5, 10 and 15 mM)-Capped silver nanoparticles : Silver nanoparticles on Gram-negative bacteria (P.aeruginosa ATCC 27852)



(a)

(b)

(c)

Figure (4) : (a) 15 Mm, (b) 10 mM, (c) 5 mM

Antibacterial activity of β-cyclodextrin (5, 10 and 15 mM)-Capped silver nanoparticles : Silver nanoparticles on Gram- positive bacteria (S.aureus ATTCC 25923).

(a)

(b)

(c)

Figure (5) : (a) 15 Mm, (b) 10 mM, (c) 5 mM

4. Conclusions : In summary, there was an increase in the antibacterial activity of β-CD-capped AgNPs compared with their uncapped equivalents. This activity increased as the concentration of β-CD increased. Thus, β-CD capping can be used to achieve higher antibacterial efficacies at lower metal ion concentrations.

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