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Research Article

Augmentation of Antimicrobial Behaviour of Silver Nanoparticles using α-Cyclodextrin as Encapping Agent

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Abstract

This work reports a simple, novel, cost effective and eco-friendly synthesis of silver nanoparticles (AgNPs) using α-cyclodextrin as the reducing agent. Here α-cyclodextrin act as reducing agent as well as capping agent. This involves synthesis of silver nanoparticles by capping with different concentrations of α-cyclodextrin. The synthesized nanoparticles were characterized using Ultraviolet Visible spectroscopy (UV-VIS), X-ray Diffraction studies (XRD), Fourier Transform Infra Red (FT-IR) Spectroscopy and Transmission Electron Microscopy (TEM). UV-VIS Spectroscopy and X-ray diffraction studies confirmed the formation of nanoparticles. FT-IR Spectroscopy confirmed the binding of α-cyclodextrin to the AgNPs. Transmission electron microscopic studies also reveal the existence of spherical shaped particles of nano dimension. Antibacterial and antifungal activities of uncapped and capped AgNPs were investigated. It was found that capped AgNPs exhibited more antibacterial activity and antifungal activity when compared to the uncapped one. The capping with α-cyclodextrin showed much pronounced enhancement in antibacterial and antifungal behaviour in silver nanoparticles. α-cyclodextrin encapsulated AgNPs ensures its potential ability as an antimicrobial agent for therapeutic purposes as well as for antimicrobial coating materials.

Keywords: Silver nanoparticles; α-Cyclodextrin; Capping agent; Antibacterial activity; Antifungal activity.

Introduction

Nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance [1,2]. In particular, silver nanoparticles (AgNPs) have attracted much attention in the scientific field [3-5]. The biological application of AgNPs is a rapidly developing area of nanotechnology that raises new possibilities in the diagnosis and treatment of various diseases. Silver has a broad spectrum of biocide activity against 650 bacteria, fungi and viruses[6-8], that this excellent property makes them applicable in various fields of marketing as well as for medicinal applications [9,10].

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) causing cell death [11,12]. 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 release silver ion is key to their antibacterial activity [13]. In spite of AgNPs with different shapes and sizes successfully obtained in various aqueous solution systems, most of the reported synthetic methods depend heavily on the use of organic solvents and toxic reducing agents. All these chemicals are highly reactive and can induce potential environmental and biological pollution [14,15]. So a more efficient, simple and reproducible method could be used instead of the conventional methods. And we think about stabilized metal nanoparticles for antimicrobial applications by manipulating the host-guest protocols.

Metal nanoparticle encapsulated macromolecular systems need special attention in this regard. So encapsulation of AgNPs in

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suitable macromolecular systems like starch, cyclodextrins etc provide high stability and excellent antimicrobial coating effect. They can effectively stabilize AgNPs because of the presence of large number of easily accessible functional groups present in these systems and the unique scaffolds or pockets provided by them [16]. Cyclodextrins (CDs) are a family of soluble, nontoxic 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 and 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 [17] CDs too induce nanoparticle assembly via host-guest interactions because of their relatively weak capping ability for metal nanoparticles [18].

Although the hydroxylic groups are poor electron donor ligands to silver, in relatively high concentrations, α-cyclodextrin is able to stabilize AgNPs. Herein, we report the controlled synthesis of AgNPs by directly reducing silver nitrate with α-CD in an alkaline aqueous solution. The reduction behavior of α-CD in the synthesis of AgNPs was investigated employing UV/visible Spectrophotometry, FT-IR Spectroscopy, X-ray diffraction (XRD), and Transmission Electron Microscope (TEM). The augmentation of antimicrobial behavior after and before capping with α-CD was studied.

Materials and methods

Silver nitrate (Merck) and α-cyclodextrin (Sigma Aldrich) were the chemicals purchased of high purity grade. All solvents were of analytical grade. Double distilled water was used for the preparation of stock solutions.

Sample Characterization

The UV-visible spectra were measured on a Systronics Smart 2203 UV-visible double beam spectrophotometer operating in the range of 200-1100 nm. FT-IR spectra were measured using a SCHIMADZU IR Affinity I FT-IR Spectrophotomer, operating range 400-4000 cm^{-1} by KBr pelleting X-ray diffraction measurements were carried out using an XPERT-PRO X-ray diffractometer (PANalytical BV) with CuKα radiation in θ-2θ configuration. All measurements were performed at room temperature. TEM measurements were done by Jeol/JEM 2100 LaB6 operating at 200KV.

Synthesis of α-CD capped Ag nanoparticles

Synthesis of α-CD capped AgNPs was simply achieved by the reduction of silver nitrate with α-CD in alkaline aqueous solution. Working solutions of silver nitrate with concentration 10^{-3}M was prepared from stock solution of 10^{-1}M in doubly distilled water. Aqueous α-CD solutions (5, 10 and 15mM) were stirred into equal volumes of 2mM $AgNO_3$ for 15 minutes. Then ice cold NaOH solution was added and magnetically stirred continuously until silver ions were reduced to silver metal of nano dimensional range. During reduction process the temperature was kept at 30-35ºC and α-CD free control was similarly prepared.

Antimicrobial Analysis:

For the evaluation of antibacterial activity four bactrial strains were selected namely *Klebsiella pneumoniae*, *Pseudomonas aeruginos*a, *Staphylococcus aureus* and *E. coli*. For the evaluation of antifungal activity two fungal strains viz-*candida albicans* and *Aspergillus niger* were selected.

Antibacterial Activity

Antibacterial activity was carried out by agar disc diffusion method.0.1 ml of 24 hrs old bacterial culture was spread in Muller -Hinton agar plate by spread plate technique. The sterile disc containing respective solvent extract was placed on the surface of the medium and the antibiotic disc was used as positive control. The plates were incubated at 37°C for 24 hrs. The plates were observed after 24 hrs for zone of inhibition [19].

Antifungal Activity

Antifungal activity was determined using a modified Kirby Bauer disc diffusion method. Briefly, 100 µl of the test fungi was spread onto the potato-dextrose agar plate. The different test solvent extracts were loaded to the sterilized sterile 6 mm discs, allowed to dry and then the impregnated discs placed on the surface of medium. Plates were incubated at 37ºC at room temperature for 3-4 days. The diameters of the

inhibition zones were measured in mm. Standard antibiotic flucanazole served as positive control [19].

Result and discussion

UV-visible spectroscopic studies

UV-visible spectroscopy is the most widely used techniques for structural characterization of AgNPs. From Fig. 1 the absorption spectrum of the pale yellow-brown silver colloid prepared by α-CD reduction showed a Surface Plasmon absorption band with a maximum of 420nm indicating the presence of spherical or roughly spherical AgNPs. In metal nanoparticle such as silver, the conduction band and valence band lie very close to each other. The reaction mixture showed colour change from yellowish brown to reddish brown which indicated the formation of AgNPs. The absorption peak obtained in the visible range 420 nm wavelength is a clear evidence of formation of AgNPs from the silver nitrate solution. The frequency and width of the Surface Plasmon absorption depends on the size and shape of the metal nanoparticles as well as the dielectric constant of metal itself and the surrounding medium [20-22].

This is the reason that when incorporated into α-CD, the absorption peak was blue shifted to 408nm. The absorption intensity increased with increase in α-CD concentration as shown in Fig. 1.

X-ray Diffraction Analysis

Fig. 2 (a), (b) and (c) shows the XRD pattern of the prepared AgNPs capped with α-cyclodextrin at three different concentrations. The XRD pattern of the AgNPs synthesized using various concentrations of α-CD exhibited sharp peaks indicating that the nanoparticles are crystalline in nature. The peaks at 2θ=20.37º, 39.93º, 44.58º, 64.69º, reveal that it is a face centre cubic (FCC) structure. The discernible peaks can be indexed to (100), (111), (200), (220) planes of a cubic unit cell, which corresponds to cubic structure of silver (JCPDS card No.89-3722).

Crystallite size calculation was done using Debye-Scherrer formula $D = \frac{K\lambda}{\beta Cos\theta}$ where 'λ' is wave length of X-ray (0.1541nm), 'β' is FWHM (full width at half maximum), 'θ' is the diffraction angel and 'D' is particle diameter size. Results shows that 15 mm of α-CD capped AgNPs has crystalline structure and crystallite size was less when 15 mm of β-CD was used as capping agent.

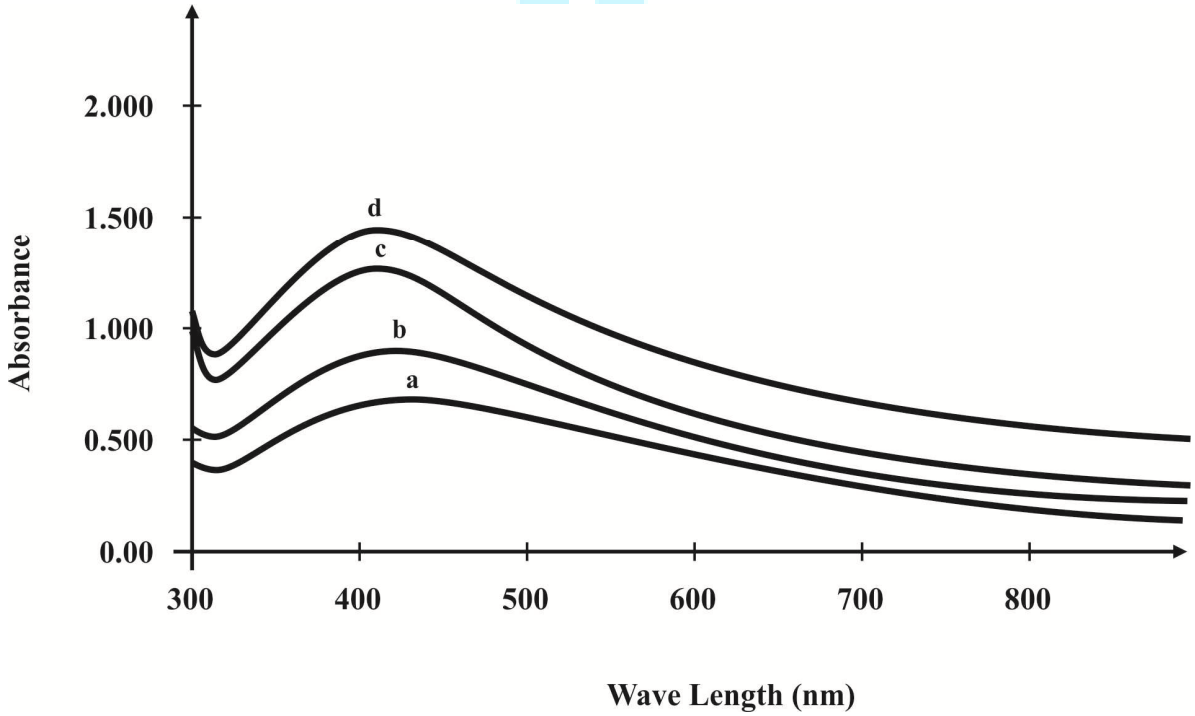

Fig. 1. UV-Visible spectrum of AgNPs (a) 420 nm for uncapped Ag (b) 416 nm for α-CD (5 mM) capped Ag (c) 410 nm for α-CD (10mM) capped Ag and (d) 408 nm for α-CD (15 mM) capped Ag

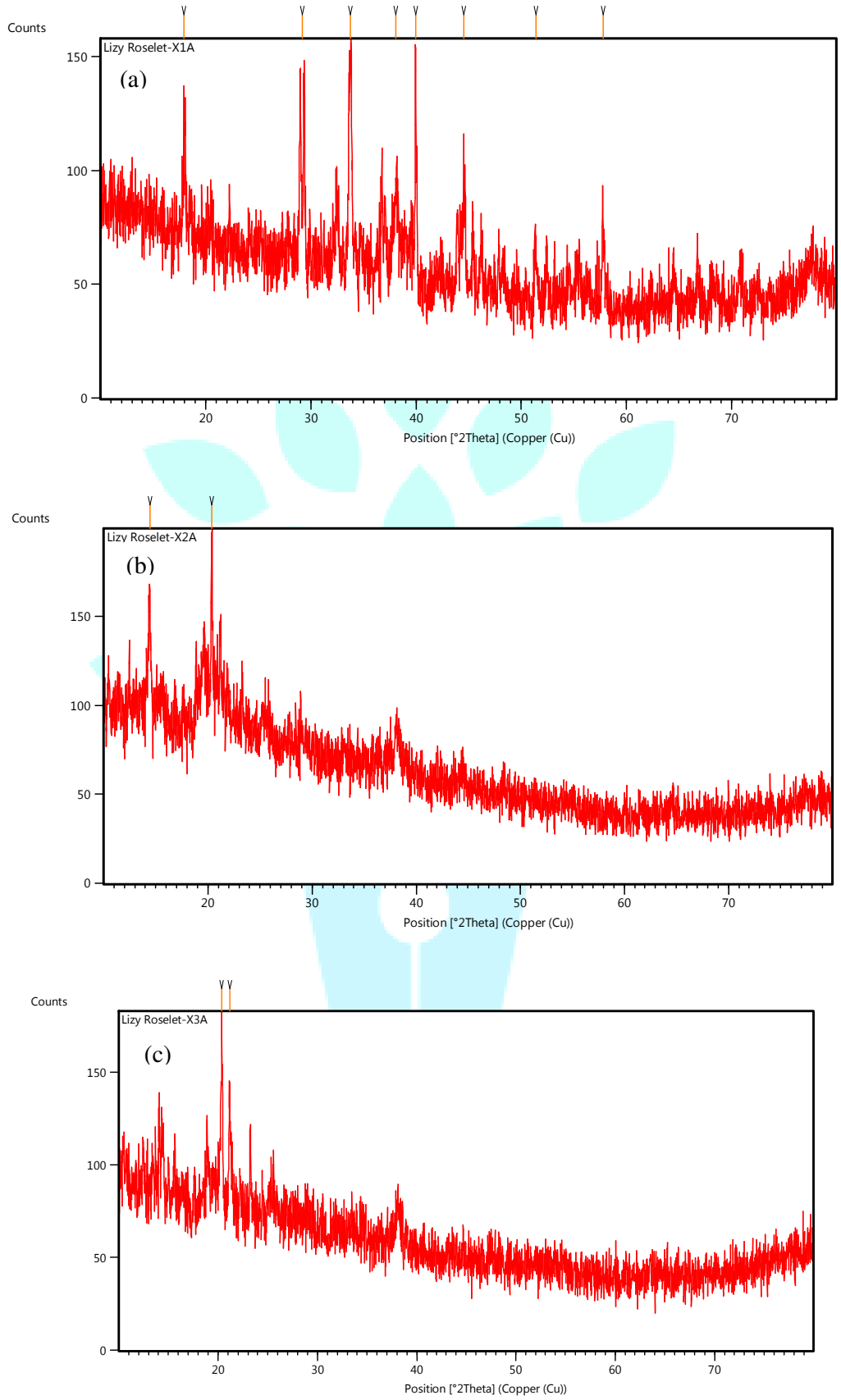

Fig. 2. XRD spectrum of AgNPs (a) uncapped Ag, (b) α-CD (5 mM) capped Ag and (c) α-CD (10 mM) capped Ag

Fourier Transform Infra Red Spectroscopic Studies

FT-IR confirmed α-CD binding to the AgNPs surface. It can be clearly observed from Fig. 3(a) that the two absorptions around 1411 cm^{-1} & 1639 cm^{-1} characteristic of carboxylate group (Ag-C00$^-$) appeared for 15 mM α-CD capped AgNPs.

The free α-CD spectrum showed bands at 997 cm^{-1} (skeletal vibration involving α 1-4 linkage) and 759.95 cm^{-1} (ring breathing vibration) whilst bands at 709.8 cm^{-1} and 570.93 cm^{-1} were assigned to pyrannose ring vibration as depicted in Fig. 3(b). The spectra of 15 mM α-CD capped AgNPs showed a relative decrease in the intensities of transmittance band at 997 cm^{-1}, 759.95cm^{-1}, 709.9cm^{-1} and 570.93cm^{-1} and a suppression of the –OH vibrational band at 3392.79cm^{-1}, indicating the deprotonation of the hydroxyl groups at the rim of the α-CD structure. This suggests that there are chemisorptions between the α-CD molecules and AgNPs via the rim hydroxyl groups.

Sorption of α-CD might be due to the binding of the AgNPs to hydroxyl positions at C_2 or C_3. Here the hydroxyl groups of α-CD should act as the reducing species for the reduction of Ag^+ into metallic silver and they were self oxidised to carboxylic acid. The oxidation product of α-CD molecules bearing a negative charge would provide a more efficient capping on the surface of AgNPs due to Ag-C00$^-$ interaction.

Transmission Electron Microscopic Analysis of silver nanoparticles

TEM Micrographs confirmed the presence of spherical or roughly spherical AgNPs. Fig. 4(a) implies that in the presence of α-CD, AgNPs are nearly spherical and their average size ranges from 10 nm – 30 nm. Fig. 4(b) suggests that the particles are poly disperse and are mostly spherical in shape. The torroidal hydrophobic cavity of α-CD is able to have inclusion effects on the silver nitrate in aqueous solution.

The interactions such as Vander Waals force and hydrophobic interaction between guest and host are generally accepted as the driving force for the bonding of guest molecules or ions to CD cavity. Nanoparticles with high surface area remain separated from each other. Thus the interactions between silver nitrate and α-CD are important factors responsible for the production of weakly agglomerated and uniformly dispersed AgNPs. The aggregation of the capped nanoparticles is probably driven by both hydrophobic effect and the hydrogen bonding on the capping agent used for the nanoparticle preparation.

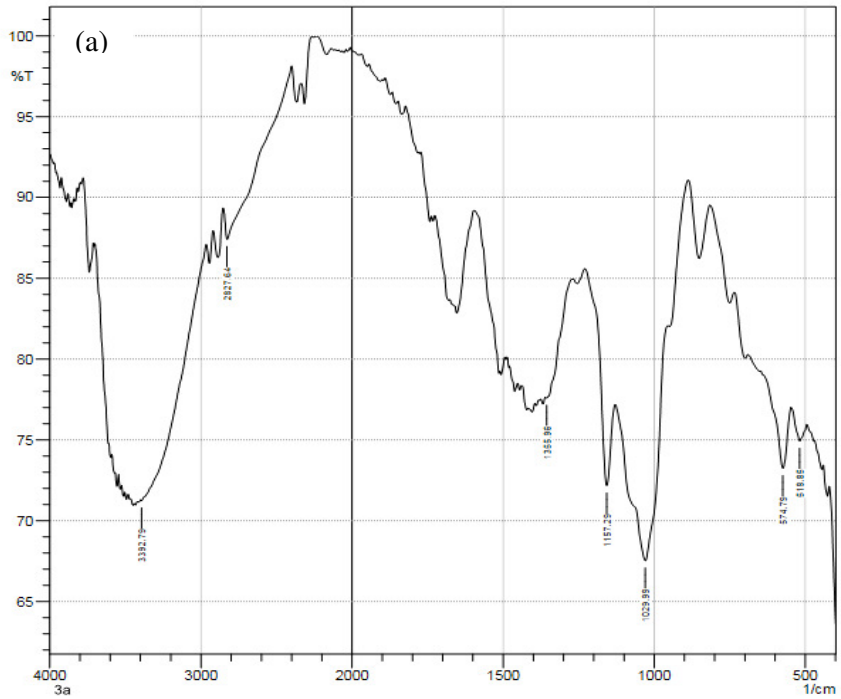

Fig. 3(a). FT-IR spectrum of α-CD

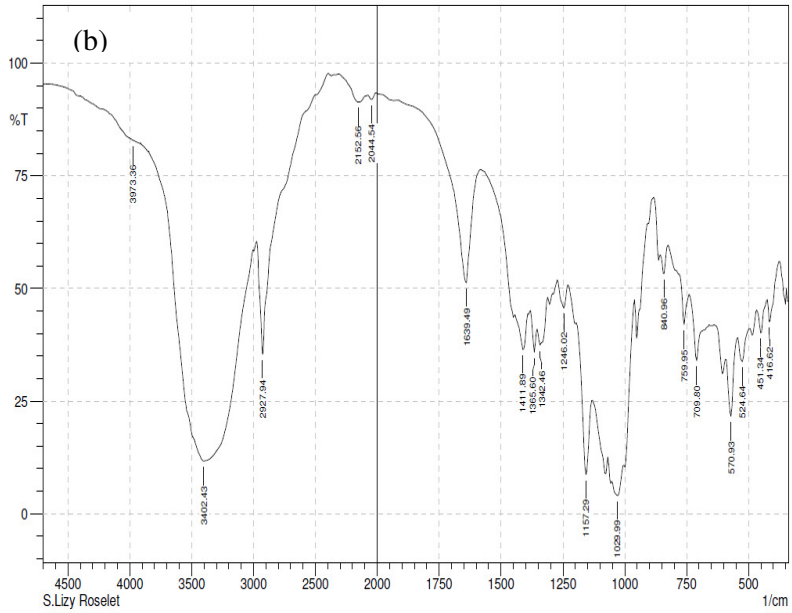

Fig. 3(b). FT-IR spectrum of α-CD (15 mM) capped Ag

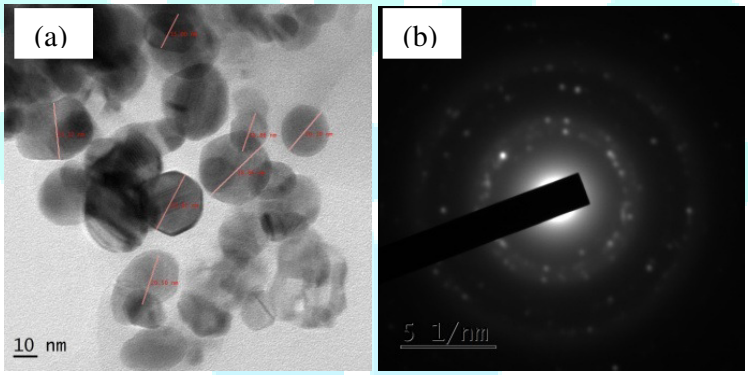

Fig. 4. TEM pictures of Spherical silver nanoparticles (a) 5 mM α-CD capped, AgNPs (b) Selected area diffraction images of randomly selected AgNPs

Antibacterial Activity

In the present investigation the antibacterial effect of synthesized AgNPs is studied on different types of bacteria such as *K. pneumoniae, P. aeruginosa* (Gram negative), *S. aureus and E. coli* (Gram Positive). The antibacterial activities of three different concentration of α-CD capped AgNPs with four micro organisms were studied. From Fig. 5(a), (b), (c), (d) and Table 1 it was found that the synthesized AgNPs had the highest antibacterial activity against *P. aeruginosa* > *E. coli* > *K. pneumoniae*. Lesser antibacterial activity or no zone of inhibition is formed by AgNPs with *S. aureus*. While the concentration of α-CD was increased antibacterial activity of AgNPs with 15mM concentration was found to be pronounced against *K. pneumoniae* and 10 mM concentration α-CD against *P. aeruginosa*. The degree of antibacterial activity was not much altered with higher concentration of α-CD capped AgNPs for *E. coli.*

Antifungal activity

Antifungal activity of AgNPs was studied with two fungal strains *C. albicans* and *A. niger*. Among the tested strains *Candida albicans* showed maximum zone of inhibition. From the Table 2 and Fig. 6 (a) and (b) it was clearly revealed as the concentration of α-CD increased the antifungal activity also increased. For *A. niger*, the zone of inhibition was observed only at 10 mM concentration of α-CD capped AgNPs. The inhibitory zone indicates the disruption of the fungal cell wall membrane by the action of AgNPs. This result shows the efficiency of AgNPs encapsulated α-CD to kill the pathogenic fungal strains.

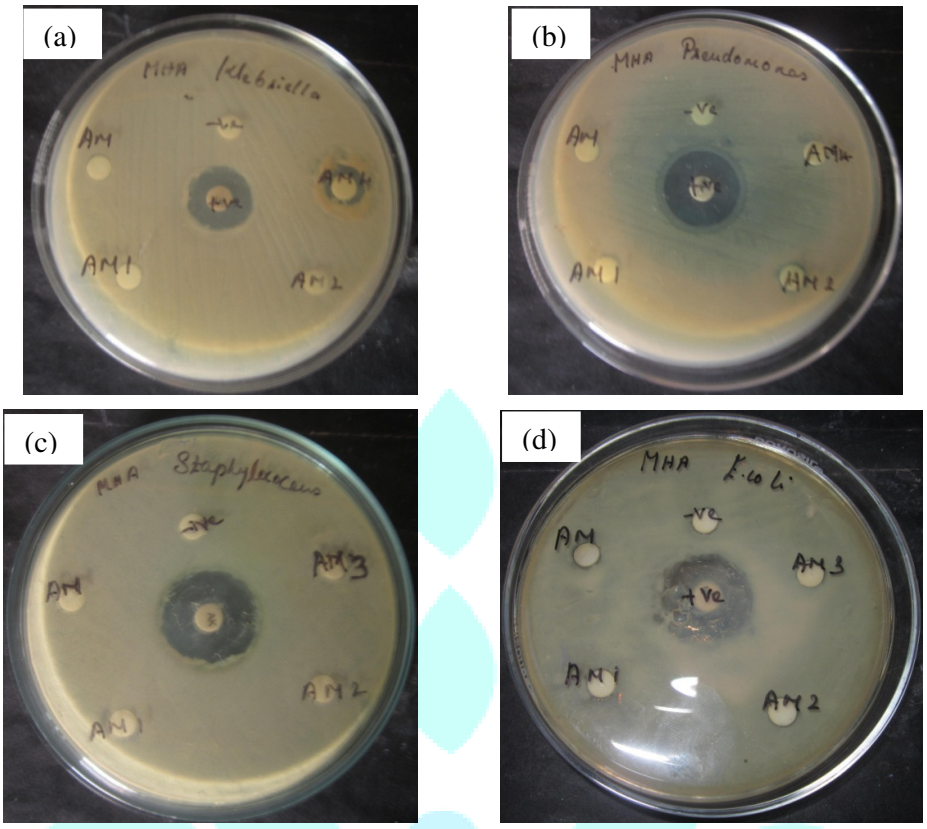

Fig. 5. Antibacterial activity of α-Cyclodextrin capped silver nanoparticles against (a) *K. pneumonia* (b) *P. aeruginosa* (c) *S. aureus* (d) *E. coli*

Table 1. Antibacterial activity of α-Cyclodextrin capped silver nanoparticles

Bacteria	Sample's Antimicrobial Activity (mm)				Reference (mm)
	AM	AM1	AM2	AM3	Positive Control (Amikacin)
K. pneumonia	8	8	NZ	13	18
P. aeruginosa	8	8	9	8	22
S. aureus	NZ	NZ	NZ	NZ	22
E. coli	8	8	8	8	23

AM - Ag Nanoparticles; AM1 - 5 mM α-CD capped Ag Nanoparticles
AM2 - 10 mM α-CD capped Ag Nanoparticles; AM3 - 15 mM α-CD capped Ag Nanoparticles

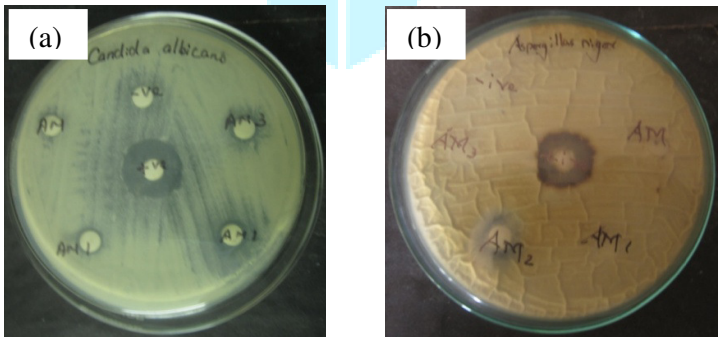

Fig. 6. Antifungal activity of α-Cyclodextrin capped silver nanoparticles against (a) *C. albicans* (b) *A. niger*

Table 2. Antifungal activity of α-Cyclodextrin capped silver nanoparticles

Fungi	Sample's Antimicrobial Activity (mm)				Reference (mm)
	AM	AM1	AM2	AM3	Positive Control (Flucanazole)
C. albicans	10	10	13	13	18
A. niger	NZ	NZ	NZ	NZ	22

AM - Ag Nanoparticles; AM1 - 5 mM α-CD capped Ag Nanoparticles
AM2 - 10 mM α-CD capped Ag Nanoparticles; AM3 - 15 mM α-CD capped Ag Nanoparticles

The binding of the particles to the fungal cell wall depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more fungicidal effect than the larger particles. The antimicrobial activity of AgNPs has been previously reported [8,23,24]. 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 α-CD promotes the release of silver ion [25]. Such interactions have been described as Trojan-Horse mechanisms and have been reported in the literature where fetal bovine serum was used to improve AgNPs efficiency [26]. These results are in accordance with a previous report in which AgNPs synthesised by dissaccharides had higher antibacterial activities than those synthesised by mono saccharides [27].

Conclusion

This method is eco-friendly, of low cost and capable of producing AgNPs at room temperature. Here α-cyclodextrin acts as both capping agent and reducing agent. The AgNPs were characterized by UV-VIS, FTIR, XRD and TEM Analysis. The UV-VIS spectral studies confirmed the Surface Plasmon Resonance of synthesised AgNPs. FTIR spectra confirmed the binding of α-CD to AgNPs. XRD pattern showed fcc crystal structure. The average particle size was calculated to be 10nm. TEM Analysis confirmed the presence of spherical AgNPs ranging from 10nm – 30nm. The synthesized AgNPs were found to have antibacterial activity and much pronounced antifungal activity against *Candida albicans* and *Aspergillus niger*. There was an increase in the antibacterial activity and antifungal activity of α-CD capped AgNPs compared with their uncapped equivalents. This activity increased as the concentration of α-CD increased. Thus α-CD capping can be used to achieve higher anti microbial efficacies at lower metal ion concentrations. The obtained uniform AgNPs functionalized by α-CD molecules would find a wide range of biomedical application by virtue of biologically compatible characteristic as well as the special inclusion ability of the α-CD molecules. α-cyclodextrins are proved to be the good alternatives for polymers which are the commonly used capping agents in nanoparticle synthesis. Thus it opens the possibility of formulation of a new generation of antimicrobial materials.

Conflict of Interest

Authors declare there are no conflicts of interest.

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