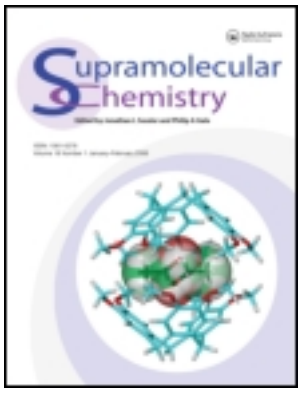

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Antifungal activity of silver nanoparticle-encapsulated β-cyclodextrin against human opportunistic pathogens

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Antifungal activity of silver nanoparticle-encapsulated β-cyclodextrin against human opportunistic pathogens

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Silver nanoparticles were synthesised by reducing silver acetate with a long-chain aliphatic amine. β-Cyclodextrin (CD)-stabilised silver nanoparticles were successfully synthesised and characterised by the UV–vis spectroscopy and scanning electron microscopy analysis. This system was examined for their antifungal activity against opportunistic human pathogens such as *Aspergillus fumigatus*, *Mucor ramosissimus* and *Chrysosporium species*. This study clearly demonstrates that the present system is a powerful antifungal agent against human opportunistic pathogenic fungi.

Keywords: silver nanoparticle; β-cyclodextrin; encapsulation; antifungal activity

Introduction

Micro-organisms produce a wide range of diseases. Latest investigations revealed that most of the microbes are developing an important resistance to current antibiotics (*1*). Thus, there is an urgent need for the development of a highly effective, low-cost and resistant-free antimicrobial agent. Although considering different microbial infections, fungal infections are of particular importance as they are found to be increasing rapidly (*2*). Recent researches revealed that most of the saprophytic fungi of the early days have now changed to opportunistic human pathogens especially among patients with immunological disorders (*3*). The present antifungal drugs are inadequate for the treatment of these pathogens and thus there is an urgent need for the development of more efficient antifungal agents with fewer limitations, less side effects and a broad spectrum of antifungal activity.

Silver and silver-based compounds have high antimicrobial activity. Silver-based antimicrobial agents receive much attention due to the low toxicity of silver ions to human cells and enhanced activity against a wide spectrum of microbes. As the size of the silver particles decreases down to the nanoscale regime, their antimicrobial efficacy increases because of their larger total surface area per unit volume. On the basis of this enhanced effectiveness, nanoparticles of silver have been aptly employed to fight human pathogens (*4*). Although an efficient antibacterial agent, their complex multistep preparation methods, high production cost, low stability and easy oxidation often fail to reach commercial needs.

Silver particles in the nanometre scale could be stabilised by incorporating in matrices with polar terminal groups (*5*). Micelles (*6*), linear polymers (*7*), small organic molecules (*8*), mesoporous materials (*9*), dendritic polymers (*10*, *11*), etc. are widely used as stabilisers for nanoparticles. In this work, we adopted a simple low-cost procedure for the synthesis of silver nanoparticles. These silver nanoparticles were stabilised by entrapping into β-cyclodextrin (CD) aggregates involving many CD molecules. CDs are a class of non-toxic oligosaccharides with a hydrophobic exterior and a hydrophilic interior. They have been extensively investigated in host–guest chemistry for the construction of versatile supramolecular aggregations (*12*). The antibacterial property of the silver nanoparticle stabilised in β-CD aggregates has previously been reported (*13*). In this investigation, we report the antifungal property of silver nanoparticle-encapsulated β-CD against human opportunistic pathogens such as *Aspergillus fumigatus*, *Mucor ramosissimus* and *Chrysosporium species*.

Experimental

Materials

Three fungal strains, namely *A. fumigatus*, *M. ramosissimus* and *C. species* were subjected to this analysis. Analytical grade reagents from Merck India Ltd (Worli, Mumbai, India), Himedia (LBS Marg, Mumbai, India) and Loba (Colaba, Mumbai, India) were used for the synthesis and encapsulation of silver nanoparticles.

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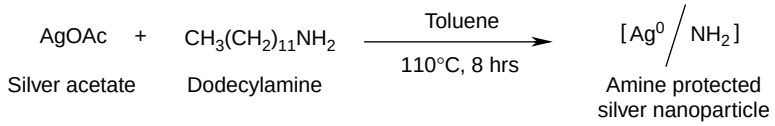
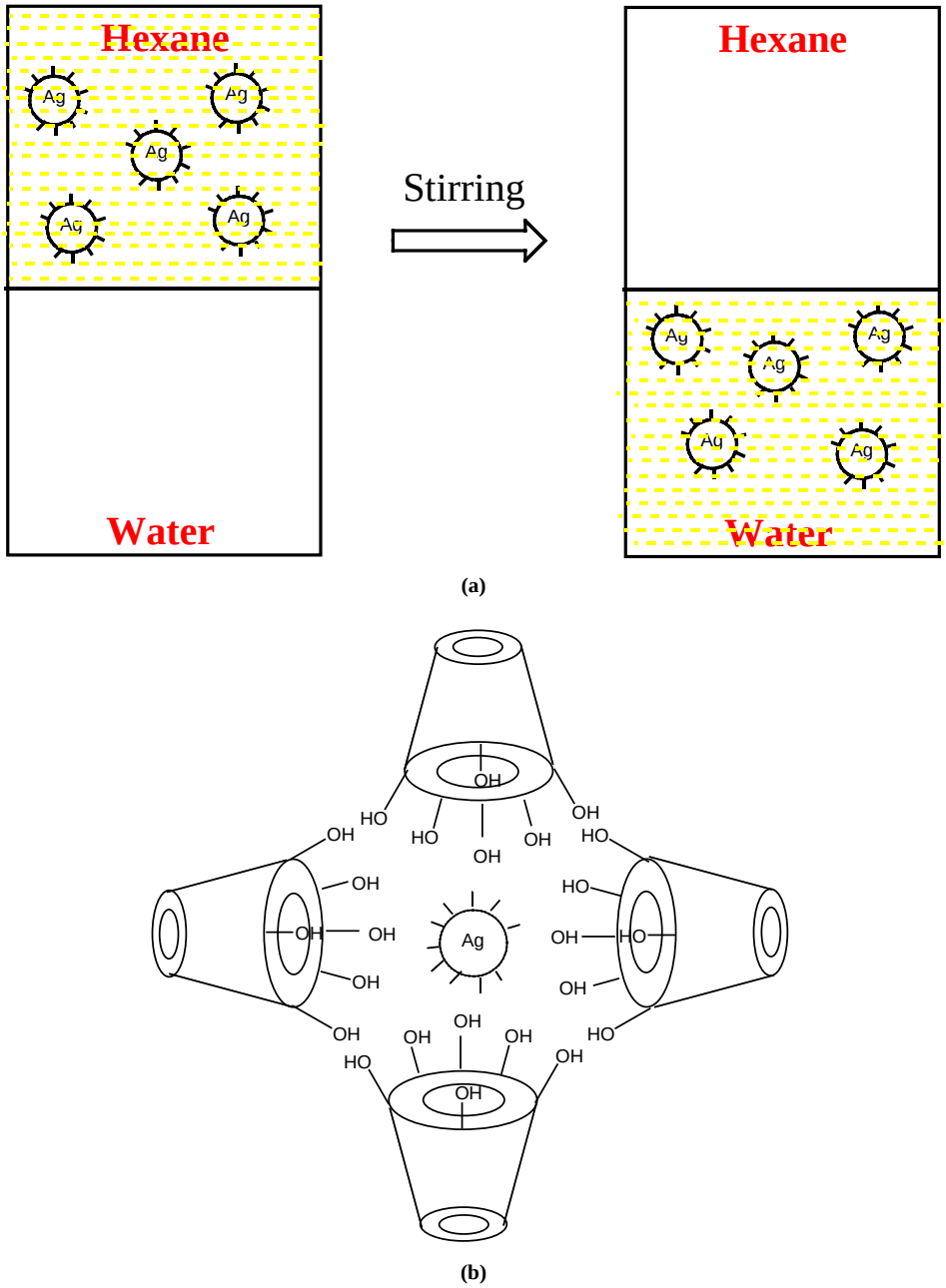


Scheme 1. Synthesis of silver nanoparticles.

Synthesis of silver nanoparticles

Silver nanoparticles were prepared according to the procedure described in the literature (*14*). Briefly, silver nanoparticles were obtained by dissolving 0.30 mmol of silver acetate in 7.5 mmol of dodecylamine and by injecting the solution quickly into 50 ml refluxing toluene. The colour of the solution slightly changed from light yellow to reddish brown and then to dark brown. The



Scheme 2. Schematic illustration of (a) phase transfer (b) β-CD–silver nanoparticle inclusion complex.

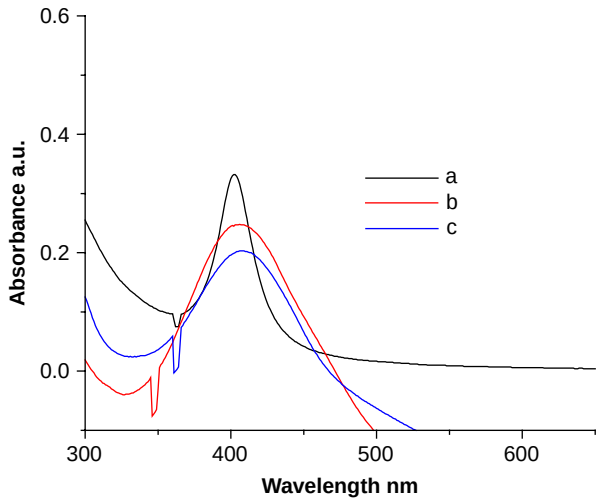


Figure 1. UV–vis spectrum of (a) silver nanoparticles (b) silver nanoparticle-encapsulated β-CD and (c) silver nanoparticle-encapsulated β-CD after 2 months.

reaction was continued for 8 h and the nanoparticles were isolated by precipitating in methanol.

Encapsulation of silver nanoparticles in β-CD

Silver nanoparticles (50 mg) were dissolved in hexane (20 ml), and added to an aqueous solution of β-CD (10 mM). The mixture was stirred at room temperature (for 4 h), which caused the transfer of silver nanoparticles from organic phase to aqueous phase with subsequent formation of nanoparticle-encapsulated β-CD.

Antifungal susceptibility test

Antifungal susceptibility test was performed by the agar well diffusion method (*15*, *16*). In this technique, 0.1 ml of the fungal spore suspension was thoroughly mixed with 20 ml of melted potato dextrose agar (PDA) and poured into sterilised Petri plates. When the agar was set, a bore was made on each of the seeded plates. These holes are filled with the testing sample. The Petri plates were incubated at 30–35°C for 7 days. All culture plates were examined after 24–96 h. The antifungal activity was evaluated by measuring the zone of inhibition in millimetres. Experiments were performed three times and the average diameter was calculated. The zone of inhibition produced by the testing sample was compared with the control.

Results and discussion

Stable silver nanoparticles were successfully synthesised by chemical reduction of silver acetate using dodecylamine. The reaction scheme is shown in Scheme 1.

Silver particles in the nanometre range are very difficult to stabilise and they easily agglomerate when exposed to environment. Thus, it is necessary to stabilise silver nanoparticle in suitable matrices. CDs are cyclic oligosaccharides composed of hydrophobic cavities that can form complexes with various organic molecules and hydrophilic rims of hydroxyl groups. Thus, CDs and their derivatives have been used in the modification of metal nanoparticles (*17–20*). Here, the nanoparticles were stabilised by incorporating in β-CD aggregates (*13*). For this, the hexane solution of nanoparticle was vigorously stirred with the aqueous solution of β-CD at room temperature. A schematic illustration of the transfer of silver nanoparticles from organic phase to aqueous phase using β-CD and the resultant inclusion complex are given in Scheme 2(a) and (b), respectively.

The nanoparticles and β-CD-capped nanoparticles were characterised by the UV–vis spectroscopy and scanning electron microscopy (SEM) analysis. The UV–vis spectrum of silver nanoparticles was recorded in hexane and the spectra exhibited a well-defined absorption peak at 402 nm (Figure 1(a)). This peak is characteristic of the surface plasmon resonance of silver nanoparticles. When incorporated into β-CD, this absorption peak was shifted to 407 nm (Figure 1(b)). The surface plasmon resonance of silver nanoparticles depends on the size and shape of the particles. For non-spherical particles, the absorption spectra consist of more than one SPR bands.

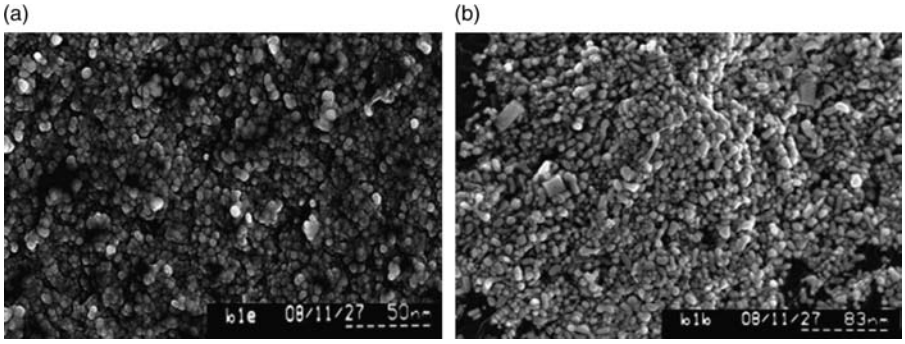


Figure 2. SEM images of (a) silver nanoparticles and (b) silver nanoparticle-encapsulated β-CD.

Table 1. Diameter of inhibition zone developed by nanoparticle dispersed β-CD against various fungal stains.

Sl. no.	Fungal strain tested	Diameter of zone inhibition				
		Test I	Test II	Test III	Test IV	Mean value
1	*Aspergillus fumigatus*	46	50	47	45	47
2	*Mucor ramosissimus*	44	42	42	40	42
3	*Chrysosporium species*	No growth	No growth	No growth	No growth	No growth

Here, we got only a single SPR band in the visible region. This observation suggests that the particles are spherical in shape.

The aqueous suspension of nanoparticles was found to be very stable. No obvious change was found after 2 months under atmospheric conditions. The UV–vis spectra of the aqueous suspension of nanoparticle after keeping under atmospheric conditions for a period of 2 months are given in Figure 1(c).

The SEM analysis was conducted to study the surface morphology of the nanoparticle and nanoparticle – β CD inclusion complex. No obvious change in size or shape was found in the samples before and after encapsulation. The results show that the physical properties of nanoparticles are completely conserved on encapsulation.

Moreover, the stability of the silver nanoparticles is greatly increased on encapsulating them into the β-CDs. The SEM analysis also confirmed the spherical shape of the nanoparticles. The SEM images are given in Figure 2.

Aspergillus is the most common genus of fungi in our environment with more than 160 different species of mould. It is a saprophytic mould living in different habitats such as water, soil, organic matter and so on. Sixteen of these species have been documented as causing human disease. The important diseases caused by them are aspergillosis, and different forms of allergies. *A. fumigatus* produces a carcinogenic and a histotoxic secondary metabolite called 'Aflatoxin'. When it infects nuts and other food grains contamination through its toxin and generate causes troubles in humans and animals.

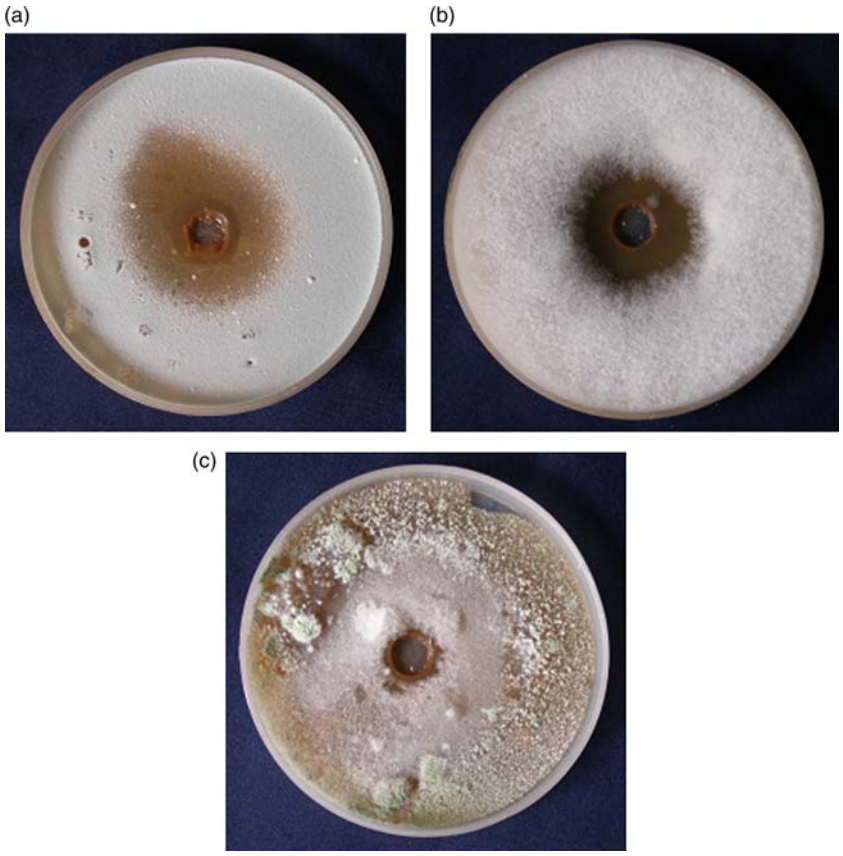


Figure 3. Antifungal activity exhibited by silver nanoparticles-encapsulated CD against (a) *A. fumigatus* (b) *M. ramosissimus* and (c) *C. species*.

A. fumigatus also causes lung aspergillosis in patients having lung diseases. It may also cause blood clot in lungs, allergies and rhinitis. *Mucor sp*. is generally saprophytes found in soil, plant body and on humans. Among different species, *M. ramosissimus* is reported to be pathogenic to humans and animals. In birds, it causes feather loss and dermatitis. In humans, its spore activates a complementary system. *C. species* is a keratin-dependent saprophytic fungus found in birds, soil, plant materials and dung. For their nutrition, they digest the keratin of feathers and hairs that fall on the ground. Occasionally, they develop as a human pathogen affecting skin. All these indicate the medical significance of the fungi under investigation.

The antifungal activity of the β-CD silver nanoparticle inclusion complex was tested by diffusion plate method. Table 1 and Figure 3 show the antifungal activities of silver nanoparticle-encapsulated β-CD. Control experiments were also conducted and they did not show any inhibitory zone. However, the nanoparticle CD inclusion complexes exhibited pronounced inhibition against all the tested strains.

Maximum susceptibility was shown by *C. species* in which almost 100% inhibition was observed. Among the susceptible strains, least activity was shown by *M. ramosissimus* with an average inhibition zone of 42 mm. The growth of *A. fumigatus* was also powerfully inhibited by nanoparticle-encapsulated CD which showed an inhibition zone of 47 mm. These striking results demonstrate the ability of the β-CD silver nanoparticle system as a strong antifungal agent (Figure 3). The pronounced inhibition exhibited by silver nanoparticle-encapsulated β-CD could be tested on more fungal strains under various conditions and it can provide a more generalised picture of the antifungal activity of these novel systems.

Conclusion

Silver is an effective germ fighter and silver nanoparticles are widely recognised as being especially effective because of their increased surface area. Although being relatively non-toxic to human cells, silver possesses antibacterial properties for a broad spectrum of bacterial and fungal strains. The main problem in the synthesis and utilisation of this system is its easy oxidation and aggregation in solution. Here, silver nanoparticles were synthesised by a reduction approach and stabilised by incorporating in β-CD aggregates. The antifungal activity of the newly developed system was analysed on different saprophytic fungi that cause opportunistic infections in humans. The different fungal species used in this study were *A. fumigatus*, *M. ramosissimus* and *C. species*. The study revealed that this inclusion complex is a potential antifungal agent against these opportunistic pathogens. Previously, we reported the antibacterial efficacy of β-CD silver nanoparticle complex against bacterial stains such as *Pseudomonas aeruginosa*, *Staphylococcus aureus, Serratia marcescens, Escherichia coli* and *Klebsiella pneumoniae*. This study showed that these systems could be used for the development of a new generation of antifungal agents.

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