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Synthesis, characterisation and antibacterial applications of water-soluble, silver nanoparticle-encapsulated β-cyclodextrin

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This paper describes a simple method for the synthesis of water-soluble, silver nanoparticle-encapsulated β-cyclodextrin by phase transfer of silver nanoparticles from organic to aqueous phase using β-cyclodextrin as a capping agent. β-Cyclodextrin–silver nanoparticle inclusion complex was purified, characterised by spectroscopic and microscopic analyses and tested *in vitro* against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli* and *Klebsiella pneumoniae*. The silver nanoparticle-encapsulated β-cyclodextrin inclusion complex displayed considerable antimicrobial activity and stability.

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

Introduction

The emergence and increase of micro-organisms resistant to antibiotics is a serious problem to public health. Some antimicrobial agents are extremely irritant and toxic to humans. Thus, the formulation of new effective, resistance-free and low-cost antimicrobial agents is of great interest (*1*, *2*). It has been previously reported that nanosized antimicrobial formulations could be used as effective bactericidal materials (*3*, *4*). Highly reactive metal oxide nanoparticles exhibit excellent biocidal action against both gram-positive and gram-negative bacteria (*5*).

Silver has been known as a very effective disinfecting agent for a long time. Silver exhibits strong toxicity to a wide range of micro-organisms and due to this reason silver-containing compounds and materials have been extensively used to prevent the attack of micro-organisms. The strong biocidal effects of silver nanoparticles along with their low toxicity to mammalian cells make them suitable antimicrobial candidates (*6*, *7*). Yet the stability of silver nanoparticles is a serious problem. They can be stabilised by incorporating into suitable matrices. Different matrices such as micelles (*8*), linear polymers (*9*), small organic molecules (*10*), mesoporous materials (*11*) and dendritic polymers (*12*, *13*) can be used for this purpose. It is found that matrices with polar groups such as amine, hydroxyl and amide are more suitable for stabilising nanoparticles (*14*). Cyclodextrins, which are cyclic oligosaccharides with a large number of peripheral hydroxyl groups, are ideal stabilising agents for nanoparticles (*15*).

To ensure the specific biological applications of nanoparticles, it is required that these silver nanoparticles be present in aqueous media. But most of the methods developed to date for the preparation of nanoparticles render nanoparticles in the organic solution. In recent years, a large number of studies have been reported on the phase transfer of nanoparticles from organic to aqueous phase. Several works by Rotello and co-workers (*16*), Gittins and Caruso (*17*, *18*), Sastry and co-workers (*19*) as well as Rao and co-workers (*20*) have reported various methods for the phase transfer of metal nanoparticles from organic to aqueous phase. Diao and co-workers have reported the phase transfer of oleic acid-stabilised silver nanoparticles from organic to aqueous phase using *p*-sulphonated calix[4]arene (*21*). Yang and co-workers have reported the phase transfer of silver nanoparticles to aqueous phase using α-cyclodextrin as capping agent (*22*). The poor solubility of these systems in aqueous phase restricts their biological applications. The encapsulation of nanoparticles in β-cyclodextrin enables us to solubilise the metal nanoparticles in water and in polar solvents. Thus, the synthesis of silver nanoparticle-encapsulated β-cyclodextrins offers the development of a new class of water-soluble antimicrobial agents which could be largely used in antibacterial and antifungal applications. Here, we also report the growth inhibition induced by silver nanoparticle-encapsulated β-cyclodextrin against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli* and *Klebsiella pneumoniae*.

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Experimental

Materials and methods

β-Cyclodextrin was purchased from Himedia (LBS Marg, Mumbai, India). Silver acetate and dodecylamine were obtained from Loba, Colaba, Mumbai, India. All the solvents were purchased from Merck, Worli, Mumbai, India and used without further treatment. UV–vis analysis was carried out on a Shimadzu-160 UV–vis spectrophotometer operating in the range 190–1100 nm. The surface analyses of the samples were carried out with a HITACHI S-4200 scanning electron microscope operated at 10 kV.

Synthesis of silver nanoparticles

Silver nanoparticles were synthesised by the reduction of silver acetate with dodecylamine following the procedure reported by Hiroki and Osterloh (*23*). Silver acetate (50 mg) was dissolved in 2 ml dodecylamine and the resulting solution was quickly injected into 50 ml of refluxing toluene with continuous nitrogen flushing. The reaction mixture was refluxed at 110°C for 12 h. It was concentrated to 10 ml in a vacuum rotary evaporator. Two hundred millilitres of methanol were added to precipitate the product. The particles were isolated by centrifugation and were dissolved in hexane and precipitated again with 40 ml methanol. The process was repeated twice and the black solid was dried in vacuum.

Phase transfer of silver nanoparticles from organic to aqueous phase

The transfer of silver nanoparticles from organic to aqueous phase was achieved by vigorously stirring equal volumes of hexane solution of the silver nanoparticles and aqueous solution of β-cyclodextrin at room temperature. After stirring for 4 h, the hexane layer became colourless and the aqueous layer became yellow. The aqueous layer was collected and the transparent yellow solution of nanoparticles was recovered.

Analysis of the antibacterial activity

For *in vitro* screening, bacterial strains such as *P. aeruginosa*, *S. aureus*, *S. marcescens*, *E. coli* and *K. pneumoniae* were used. The strains were subcultured and stocked on the semi-solid nutrient agar slants and kept at 4°C in the refrigerator. The micro-organisms were transferred to the nutrient broth prior to inoculation.

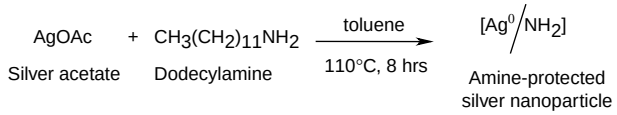
Preparation of medium and antibacterial assay by disc diffusion method

Nutrient agar powder (3.7 g) was dissolved in 100 ml of water by gently boiling in a conical flask. The agar solution was sterilised by autoclaving at 121°C for 15 min. Then, it was cooled to 40–45°C in a laminar hood, which was disinfected following the same procedure as described earlier. Two millilitres of the above culture broth medium were added to both the gram-positive and gram-negative bacteria, separately after taking all precautions to avoid any contamination, and mixed thoroughly by hand shaking. The content was then poured into Petri dishes with almost equal agar thickness (2.5 mm). The dishes were cooled for sufficient time (25–35 min) to solidify the agar medium. Filter papers dipped in silver nanoparticle-encapsulated cyclodextrin were introduced into the Petri dishes. The covered dishes were wrapped well by a paraffin film to seal them. The entire handling was done inside the laminar hood in front of a flame of a spirit lamp. Then, the dishes were kept in an incubator oven at 37°C for 24 h to test the antimicrobial activity of the polymer solution. After the test, inhibition zone diameter was measured from the clear zone of the agar dish.

Results and discussions

The silver nanoparticles were synthesised by a reduction reaction performed on silver acetate. A long chain aliphatic amine such as dodecylamine was used for the reduction and the reaction was performed in toluene. The long hydrocarbon chains of the aliphatic amine prevent the nanoparticle from aggregation and are responsible for the hydrophobicity of the nanoparticles. It is a high-temperature reaction. As the reaction proceeded, the colour of the reaction mixture slightly changed from pale yellow to reddish brown, then to dark brown. The nanoparticles formed are spherical, uniform in size and are soluble in hexane. The spherical shape of the silver nanoparticle is also evident from the UV–vis spectrum and scanning electron microscopic (SEM) studies. The schematic representation of the reaction is shown below (Scheme 1).

The formation of nanoparticles is confirmed by the change in the colour of the solution from yellow to brown. At the beginning of the reaction, the reaction mixture was colourless. After 10 min, the reaction mixture became pale yellow indicating that the reduction has begun. As the reaction proceeded, the solution became more intensely coloured and after 30 min the reaction mixture became reddish brown. The intensity of colour increased gradually with elapse of time and after 8 h the reaction mixture appeared to be dark brown. The UV–vis spectra of the



Scheme 1. Synthesis of silver nanoparticles.

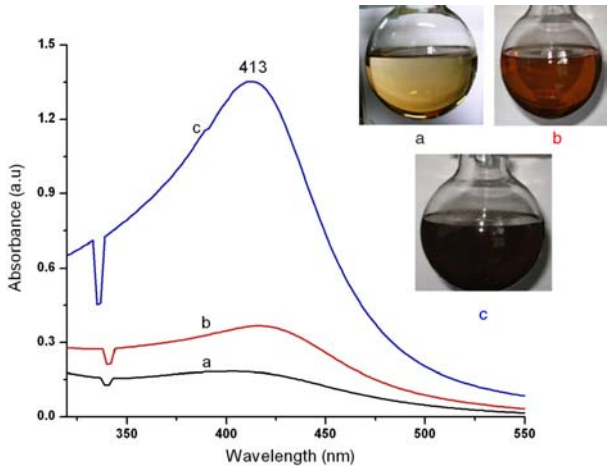


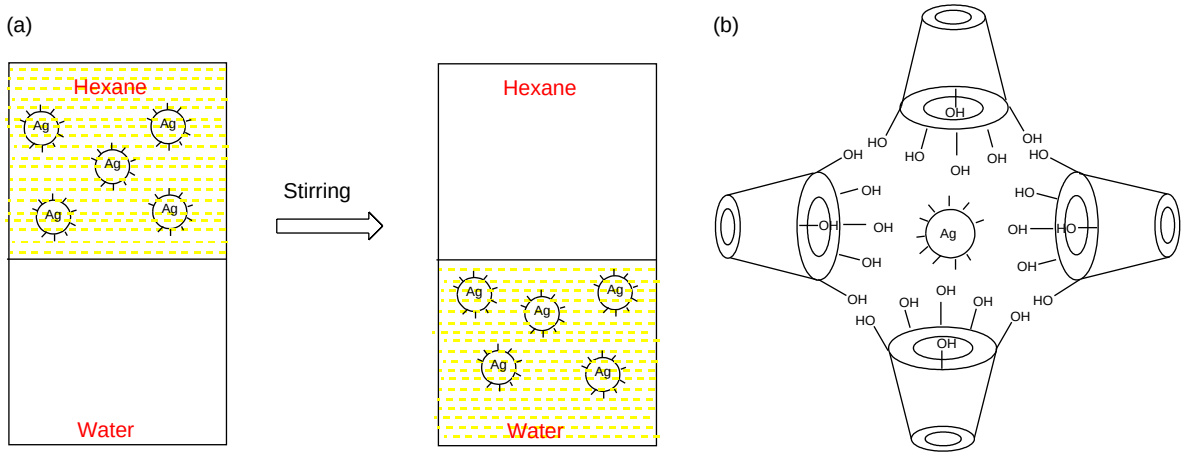
Figure 1. UV–vis spectra of the reaction mixture during different stages of nanoparticle formation and their corresponding colour changes: (a) after 10 min, (b) 30 min and (c) 8 h.

reaction mixture were recorded on a time-dependant manner. The spectrum recorded after 10 min shows a shallow absorption band (Figure 1(a)). As the reaction proceeded, this band became well defined and more and more intense. The spectra recorded after 30 min and 8 h are shown in Figure 1(b) and (c), respectively. The colour changes of the reaction mixture during the course of the reaction and the corresponding UV–vis spectra are shown in Figure 1. The spectrum recorded after 8 h shows a strong and well-defined peak at 413 nm.

The phase transfer of the nanoparticles was achieved by vigorously stirring the hexane solution of the nanoparticle and the aqueous solution β-cyclodextrin at room temperature. The transport of nanoparticles from organic phase to aqueous phase was followed by the change of the colours of the mixture. The yellowish colour of the top hexane layer gradually disappeared and at the same time the aqueous layer became yellowish. The cavity size and dimension of one β-cyclodextrin molecule is not large enough to entrap the silver nanoparticles. The cyclodextrin aggregates, however, can encapsulate the nanoparticle and the resultant host–guest complex is stabilised by the polar interaction of the peripheral hydroxyl groups of β-cyclodextrin and amine-protected silver nanoparticles. A schematic illustration of the transfer of silver nanoparticles from organic to aqueous phase using β-cyclodextrin and the resultant inclusion complex is given in Scheme 2(a) and (b), respectively.

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 just after phase transfer and after keeping under atmospheric conditions for a period of 2 months are given in Figure 2. The UV–vis spectra show the plasmon resonance signal at 407 nm. The well-defined signal corresponding to the plasmon resonance obtained at 407 nm further confirms the spherical shape of silver nanoparticles.

The silver nanoparticles before and after phase transfer were studied by SEM analysis. Figure 3(a) and (b) shows the SEM images of the nanoparticles prepared from the hexane suspension and those from aqueous suspension after phase transfer, respectively. No obvious size or shape change was found between the samples before and after phase transfer. 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-cyclodextrins. This observation suggests that the phase transfer is possible through the formation of an



Scheme 2. (a) Schematic illustration of transfer of silver nanoparticles from organic to aqueous phase (b) β-cyclodextrin–silver nanoparticle inclusion complex.

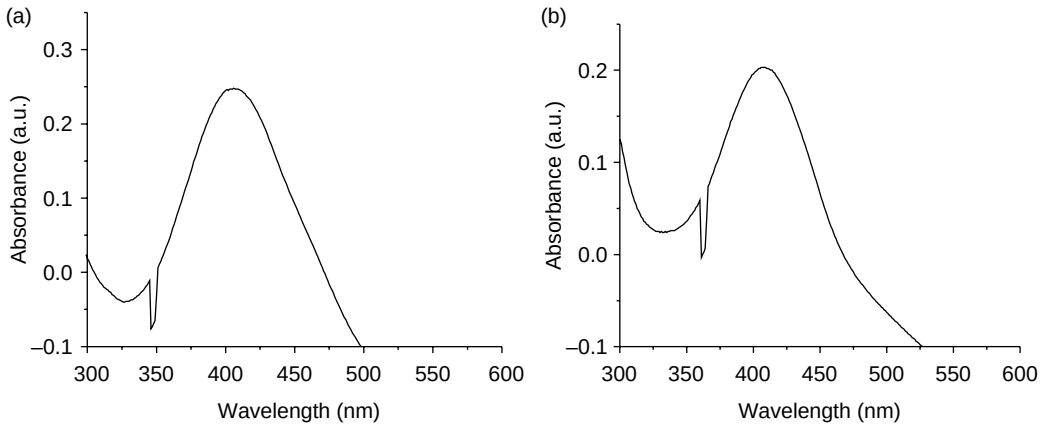


Figure 2. UV–vis spectrum of silver nanoparticle-encapsulated β-cyclodextrin (a) immediately after phase transfer and (b) after 2 months.

inclusion complex between β-cyclodextrin and the nanoparticles.

The antibacterial activity of silver nanoparticle-encapsulated β-cyclodextrin was investigated on different bacterial strains. The disc diffusion method was used. In this test, β-cyclodextrin–silver nanoparticle inclusion complexes were examined for antimicrobial activity by placing the sample onto a filter paper disc and applying the disc to a test organism distributed over an agar growth medium. The size of bacterial colonies grown on plates was significantly reduced by the action of silver nanoparticle-encapsulated cyclodextrin (Figure 4). β-Cyclodextrin–silver nanoparticle inclusion complexes displayed approximately the same activity against *P. aeruginosa* and *S. aureus* and the minimum activity was obtained against *S. marcescens*. The studies show that nanoparticle-encapsulated cyclodextrin exhibits efficient antibacterial activity against pathogenic bacteria. These systems show appreciable solubility in aqueous medium and in polar solvents and this property can be exploited in the development of many antimicrobial systems from water-soluble silver nanoparticles. Moreover, the stability of the silver nanoparticle can be greatly enhanced by encapsulating them in the well-defined cavities of β-cyclodextrins and this new host–guest system offers wide utility in antimicrobial applications.

Conclusion

Non-covalent modifications of macromolecules are very relevant for the development of new materials with biomedical applications as well as in coating formulations. Most of these modifications make use of electrostatic interactions or host–guest interactions. In the present work, we synthesised silver nanoparticles and successfully encapsulated them into the β-cyclodextrin matrix taking advantage of host–guest interactions. The cyclodextrin-modified silver nanoparticles prepared in this work are remarkably stable in aqueous media. No obvious change was observed on keeping for a long

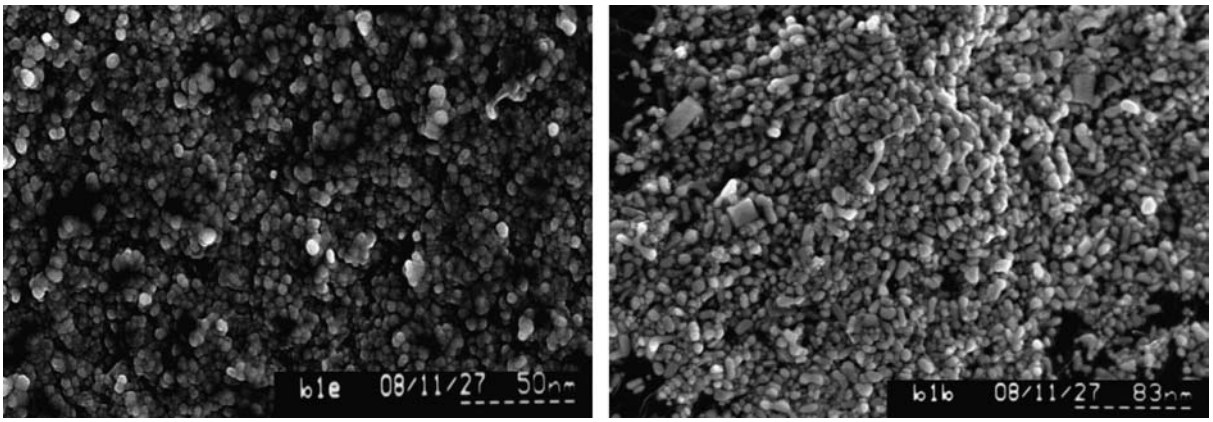


Figure 3. SEM images of silver nanoparticles (a) before and (b) after phase transfer.

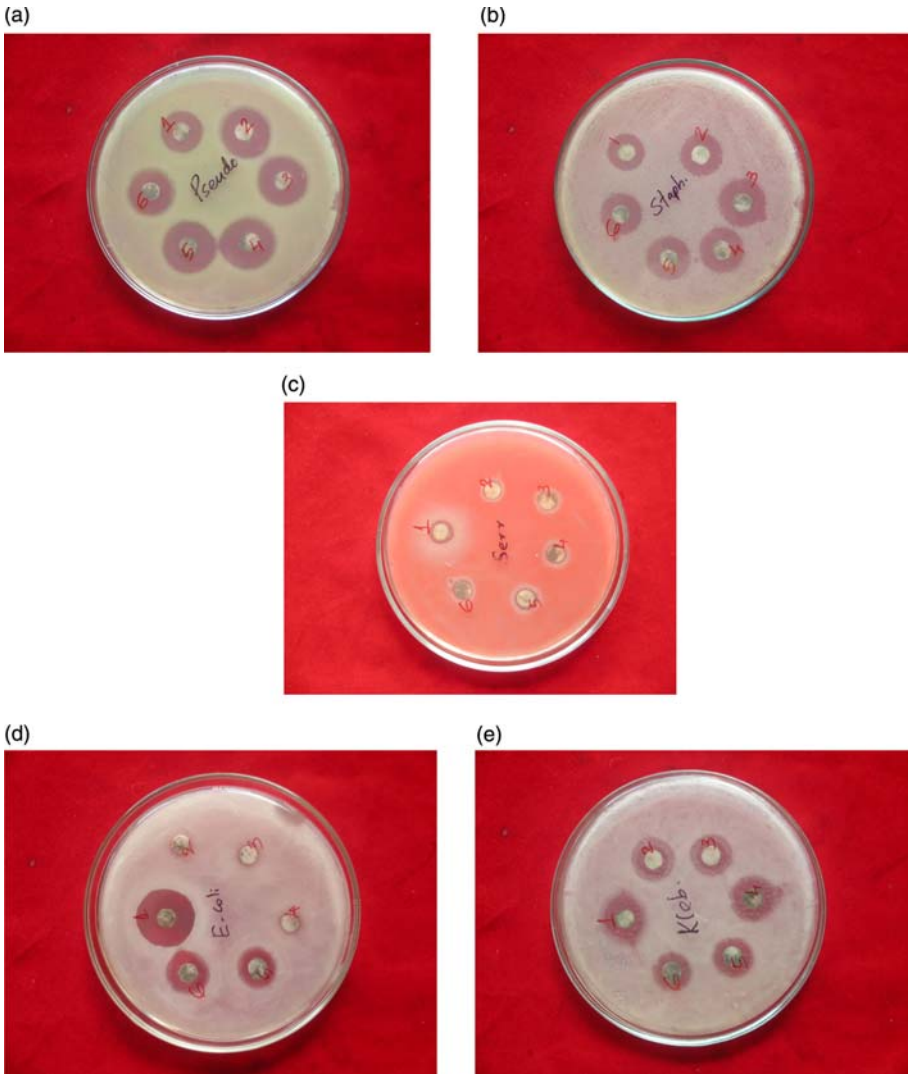


Figure 4. Antibacterial activity exhibited by silver nanoparticle-encapsulated cyclodextrin against (a) *P. aeruginosa*, (b) *S. aureus*, (c) *S. marcescens*, (d) *E. coli* and (e) *K. pneumoniae*.

period of time. The antibacterial activity of the encapsulated silver nanoparticles was investigated against *P. aeruginosa*, *S. aureus*, *S. marcescens*, *E. coli* and *K. pneumoniae*. The results obtained here clearly demonstrate the efficient antibacterial activity of silver nanoparticle-encapsulated cyclodextrin. The proficient bactericidal activity of silver nanoparticle-embedded cyclodextrin against both gram-positive and gram-negative bacteria suggests the use of the system in future biological and biomedical applications.

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