

ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLE-
ENCAPSULATED B-CYCLODEXTRIN AGAINST ASPERGILLUS
SPECIES

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ABSTRACT

The silver nano particles were synthesized by the reduction reaction executed on silver acetate in presence of dodecylamine. The encapsulation of silver nano particles in β -cyclodextrin facilitates us to solubilise the metal nano particles in water and in polar solvents. Thus, the synthesis of silver nanoparticles and silver-nano particle encapsulated β -cyclodextrins offers the development of a new class of

water soluble antimicrobial agents which could be largely used for antibacterial and antifungal applications. The present paper describes the synthesis of silver nano particles by a cost effective reduction technique, stabilization of silver nano particles by encapsulating in well-defined cavities of β -cyclodextrin and the anti microbial activity of the system against selected *Aspergillus* fungal strains using the agar well diffusion method. The results are described in detail.

KEYWORDS: Silver nano particles, Antifungal, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus penicillioides*, β -cyclodextrin, Surface plasmon resonance.

INTRODUCTION

Studies on the biological consequences of new nano materials apt for biomedical applications are of importance, particularly those related to the immune system. Highly reactive metal oxide nano particles exhibit excellent biocidal action against bacteria, various fungal strains,

protozoa etc ^[1]. Silver nanoparticles(Ag-NPs) are known to have fabulous antimicrobial effects. With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free to resistance and cost effective. Due to the emergence of infectious diseases caused by different pathogenic microbes the pharmaceutical companies and the researchers are searching for new antimicrobial agents. Some of antimicrobial agents are extremely irritant and toxic to humans. Such problems and needs have led to the use of Ag-based antiseptics that may be linked to broad-spectrum activity and far lower tendency to induce microbial resistance than antibiotics ^[2]. Reducing the particle size of materials is an efficient and dependable tool for improving their biocompatibility ^[3,4].

Cyclodextrins, which are cyclic oligosaccharides with a large number of peripheral hydroxyl groups, are ideal stabilizing agents for nanoparticles ^[5]. Cyclodextrins are a group of structurally related natural products formed during bacterial digestion of cellulose ^[6,7]. The stability of the silver nano particles is greatly increased on encapsulating them into cyclodextrins ^[8].

Aspergillus species are among the most successful groups of fungi with important roles in natural ecosystems and the human economy. *Aspergillus* species are highly aerobic and are found in almost all oxygen-rich environments. Some *Aspergillus* species cause serious disease in humans and animals. *Aspergillosis* is the name given to all animal diseases caused by growth of any member of the genus on a living host. Immuno suppression is generally a prerequisite for systemic *Aspergillus* infections in humans. The size and arrangement of the conidial heads as well as the colour of the spores they bear are important identifying characteristics. The most common pathogenic species can contaminate foods such as nuts. Some other common species causing allergic diseases, while others are important as agricultural pathogens. Members of the genus are also sources of natural products that can be used in the development of medications to treat human disease ^[9,10].

Aspergillus niger is also commonly used for the production of native and foreign enzymes, including glucose oxidase and hen egg white lysozyme ^[11]. *A.niger* causes black mold of onions. Infection of onion seedlings by *A.niger* can become systemic, manifesting only when conditions are conducive. *A. niger* causes a common postharvest disease of onions, in which the black conidia can be observed between the scales of the bulb. The fungus also causes disease in peanuts and in grapes. *A. niger* is one of the most common causes of otomycosis

(fungal ear infections), which can cause pain, temporary hearing loss, and, in severe cases, damage to the ear canal and tympanic membrane ^[12].

Aspergillus flavus is the second most important *Aspergillus* species causing human infections. The importance of this fungus increases in regions with a dry and hot climate. Many *A. flavus* isolates produce aflatoxin B1, the most toxic and potent hepatocarcinogenic natural compound ever characterized. *A. flavus* seems to be more virulent and more resistant to antifungal drugs than most of the other *Aspergillus* species ^[13].

Aspergillus nidulans is one of many species of filamentous fungi in the phylum Ascomycota. It is one of the several species of *Aspergillus* with low pathogenicity. The significant infections of *A. nidulans* in human have rarely been reported, exclusively in patients with chronic granulomatous disease (CGD) ^[14,15].

Aspergillus penicillioides is a true halophile, having highest fungal index ^[16] present in diverse niches from the hypersaline athalassohaline Dead Sea and the thalassohaline solar salterns, to the polyhaline estuaries and mangroves of Goa-India. They had an obligate need for a low water activity and were unable to grow on a regular defined medium or on various nutrient rich agar medium; however, growth was obtained on all these media when amended with 10 % solar salt ^[17]. Here we report the growth and inhibition induced by silver nano particles–encapsulated β -cyclodextrins against the *Aspergillus* species.

EXPERIMENTAL

MATERIALS

The *Aspergillus* species: Four fungal strains, namely *A. flavus*, *A. nidulans*, *A. niger* and *A. penicillioides* were used for the 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.

(a) Synthesis of silver nano particles

Silver nano particles were synthesized by the reduction of silver acetate with dodecylamine following the procedure reported by Hiroki and Osterloh ^[18]. Silver acetate (50mg) was dissolved in 2ml dodecylamine and the resulting solution was quickly injected into 50ml of refluxing toluene with continuous nitrogen flushing. The reaction mixture was refluxed at 110°C for 12 hr. It was concentrated to 10ml in a vacuum rotatory evaporator. Methanol

(200ml) was added to precipitate the product. The particles were isolated by centrifugation and were dissolved in hexane and precipitated again with methanol (40ml). The process was repeated twice and the black solid was dried in vacuum.

(b) Encapsulation of silver nano particles

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

(c) Antifungal susceptibility test

Antifungal susceptibility test was done by the agar well diffusion method ^[19,20]. In this technique, 0.1 ml of the fungal spore suspension was thoroughly mixed with 20 ml of melted 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 30–35⁰C for 7 days. All culture plates were examined after 24–96 hrs.

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 test sample was compared with the control ^[21,22].

RESULTS AND DISCUSSIONS

The silver nano particles were synthesized by the reduction reaction performed on silver acetate and dodecylamine in presence of toluene (scheme1). It is a high temperature reaction and the formation of nanoparticles is confirmed by the colour change of the reaction mixture^[23] from yellow to reddish yellow, then finally to dark brown. The colour changes of the reaction after 8 hrs, the UV-Visible spectrum shows an absorption peak at 405 nm indicating the presence of silver nano particles with its typical surface plasmon resonance ^[24](figure 1).



Scheme1. Synthesis of silver nano particles

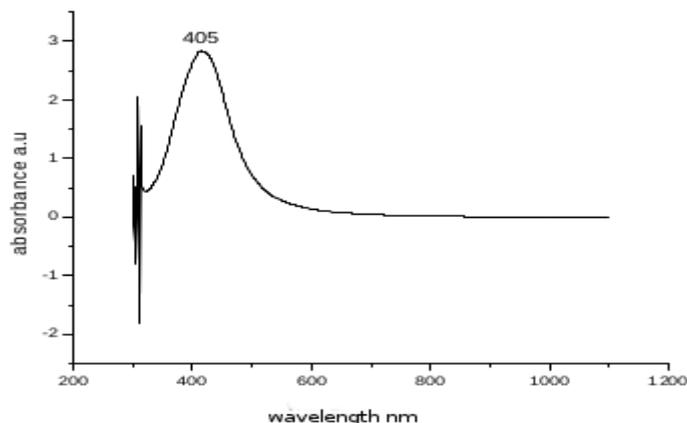


Figure.1 UV -Visible absorption spectrum of silver nano particles

Silver nano particles are very difficult to stabilise and they easily agglomerate when exposed to environment. Thus, it is necessary to stabilise silver nanoparticle in suitable matrices. Cyclodextrins are cyclic oligo- saccharides composed of hydrophobic cavities that can form complexes with various organic molecules and hydrophilic edges of hydroxyl groups. The encapsulation of nano particles in β -cyclodextrin facilitates us to solubilise the metal nano particles in water and in polar solvents. Thus, the silver-nano particle encapsulated cyclodextrins offers the development of a new class of water soluble antimicrobial agents which could be largely used in antibacterial and antifungal applications.

The SEM analysis was also conducted to study the surface morphology of the nanoparticle and nanoparticle – β -CD encapsulation composite. No evident 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 preserved on encapsulation (figure 2).

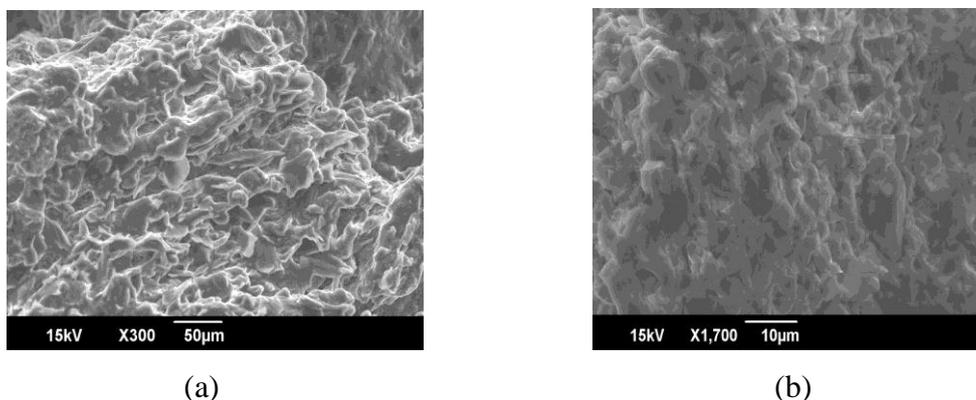


Figure 2. SEM images of (a) silver nanoparticles (b) silver nanoparticle-encapsulated β -CD.

Antifungal activity of Silver nanoparticle dispersed β -CD against various fungal stains

The antifungal activity of the β -CD silver nanoparticle insertion complex was tested by well diffusion method. Table 1 and Figure 3 shows 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 enclosure complexes exhibited marked inhibition against all the tested strains. The antifungal activity was evaluated by measuring the zone of inhibition in millimetres. Experiments were performed thrice and the average diameter was calculated. The zone of inhibition produced by the testing sample was compared with the control.

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

Serial No	<i>A. niger</i>	<i>A. flavus</i>	<i>A. nidulans</i>	<i>A. penicilloides</i>
1	22	18	18	17
2	20	18	11	13
3	21	21	12	17
4	21	20	13	15
5	22	17	16	12
6	18	19	15	13
Solvent/control	0	0	0	0

Among the fungal strains maximum susceptibility was shown by *Aspergillusniger*, and the least activity was shown by *Aspergilluspenicilloide*. The diameter of inhibition zones for *Aspergillusniger*, *Aspergillusflavus*, *Aspergillusnidulans*, and *Aspergilluspenicillioides* were 22mm, 21mm, 18mm and 17mm respectively. These striking results demonstrate the ability of the β -CD silver nanoparticle system as a strong antifungal agent (Figure 2). The distinct inhibition exhibited by silver nanoparticle encapsulated β -CD could be tested on more fungal strain under various conditions and it can provide a more generalised picture of the antifungal activity of these innovative systems.



(a)



(b)

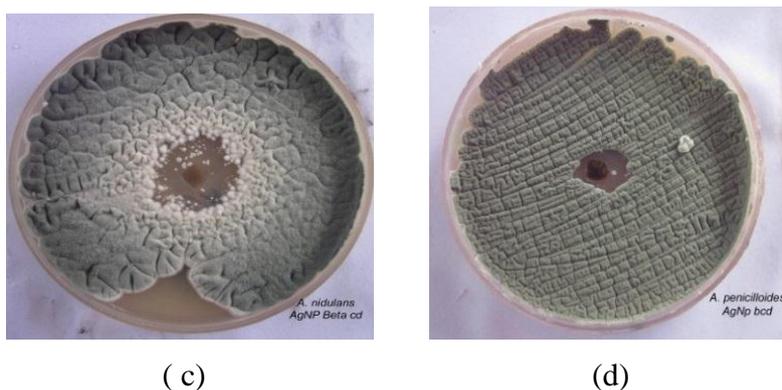


Figure 2. Antifungal activity exhibited by silver nanoparticles-encapsulated- β - CD against *A. niger* (b) *A. flavus* (c) *A. nidulans* (d) *A. penicilloide*

From this analysis it is clear that all the fungi give characteristic inhibitory zone. This means that both silver nano particles and nanoparticle encapsulated β -CDs also possess very good antifungal activity. Thus we can use the stable nanoparticle encapsulated oligosaccharides for anti microbial applications.

CONCLUSION

The usefulness of silver as an antimicrobial agent has been known since ancient times. With recent advance in metal nanoparticles, the use of nano sized silver particles as antimicrobial agents has received special attention. 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 period of time. The antifungal activity of the encapsulated silver nanoparticles was investigated against the four *Aspergillus species*, namely *A. flavus*, *A. nidulans*, *A.niger* and *A.penicilloides*. Among the fungal strains maximum susceptibility was shown by *Aspergillusniger* and the least activity was shown by *Aspergilluspenicilloides*. All the four fungal strains exhibit a very good anti fungal activity with an appreciable inhibitory zones. Advances in *Aspergillus* genomics are giving us new tools for understanding this extremely diverse genus. The proficient fungal activity of silver nanoparticle embedded in cyclodextrin against these *Aspergillus species* suggest the use of the system in future biological and biomedical applications.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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