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SYNTHESIS, CHARACTERISATION AND ANTIFUNGAL ACTIVITY OF CARBON NANOPARTICLE-BETA-CYCLODEXTRIN AGGREGATES AGAINST *ASPERGILLUS SPECIES*

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ABSTRACT

This paper describes the synthesis of carbon nanoparticle (CNPs) from natural sources such as kitchen soot, encapsulation of carbon nanoparticle in β-cyclodextrin, characterization of CNPs and encapsulated product by UV/visible spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and thermogravimetric analysis (TGA-DSC) and studies on their antifungal action. The antifungal activity of CNPs and CNPs dispersed cyclodextrin were tested against various pathogenic fungal strains such as *Aspergillus niger*, *Aspergillus fumigates* and *Aspergillus flavus* by well method. The results showed that the CNPs and CNPs dispersed β-cyclodextrin have excellent antifungal activity against selected pathogenic fungal strains.

KEYWORDS: Carbon nanoparticle, β-cyclodextrin, Antifungal, *Aspergillus niger*, *Aspergillus fumigates, Aspergillus flavus.*

INTRODUCTION

A wide variety of carbon-based nano materials have been prepared, such as carbon nanotubes, nanofibers, nanodiamond, carbon nanoonions, graphene, and other carbonaceous nanomaterials.[1] The unique chemical and physical properties of carbon nanoparticles are

largely attributed to their high surface area, surface morphology and special electronic, optical and thermal properties. These particles have many potential applications especially in the biomedical field.[2] A significant property of CNPs is its antimicrobial activity. CNPs are effective to control the microorganism infection.[3] The usefulness of nano carbon as an antimicrobial agent has been known since ancient times. Carbon nanoparticles are being explored widely for use in medical treatment.[4, 5]

Controlling the dispersion stability of nanoparticles is an essential issue to control the properties of the final products. Surface modification of nanoparticles is one of the mostly accepted methods to improve the dispersion stability of nanoparticles in those challenging conditions.[6]

For biological applications, the surface coating should be polar to give high aqueous solubility and prevent nanoparticle aggregation. Macromolecules possess scaffolds or channels are best suited for surface modification of nanoparticles.[7] This can generate systems with potential applications in antimicrobial coatings for paints, printing inks and dyes. The combination of nanoparticles and macromolecules can provide the ideal coating system with excellent antimicrobial properties.[8] Surface coating materials are used to protect the surface of materials, commonly nanoparticles. Capping agents like dendrimers, cyclodextrins, hyperbranched polymers and even clay particles protect the nanoparticles.[9] Cyclodextrins are a family of compounds made up of sugar molecules bound together in a ring. They are used in a wide variety of biomedical and biological applications. Cyclodextrins are able to form inclusion complexes with broad range of hydrophobic molecules.[10]

Carbon nanoparticles have different surface structures and surface interactions compared to micron sized particles. CNPs have an extremely high tendency of adhesion and aggregation. Surface modification of CNPs is one of the best suited methods to improve the stability of nanoparticles by preventing aggregation.[11] Cyclodextrins have hydrophobic interior and hydrophilic exterior and they can form complexes with hydrophobic compounds. Thus they can increase the solubility and bioavailability of such compounds. This is of high interest for biomedical as well as antimicrobial applications of carbon nano-β-cyclodextrin complexes.[12]

In the present work, we adopted a simple low-cost method for the synthesis of carbon nanoparticles from natural sources and improve the stability of CNPs by encapsulation in β-

cyclodextrin. We studied the antifungal activity of carbon nano-β-cyclodextrin complexes against *Aspergillus niger, Aspergillus fumigates* and *Aspergillus flavus.*

Aspergillus species are highly aerobic and are found in almost all oxygen-rich environments. Diseases caused by *Aspergillus* are named aspergillosis. *A.niger* causes a disease called black mold on certain fruits and vegetables such as grapes, onions and peanuts and is a common contaminant of food.[13] *A. niger* (commonly known as black *Aspergilli*), was recorded as a most dominating fungal species. *Aspergillus fumigates* causes a wide range of human diseases, such as allergic bronchopulmonary aspergillosis (ABPA), astma, and aspergilloma.[14] *A. flavus* grows and thrives in hot and humid climates. In humans, *A. flavus* aflatoxin production can lead to acute hepatitis and immunosuppression. It is a causative agent of fungal sinusitis and non invasive fungal pneumonia.[15]

MATERIALS AND METHODS

(i) Carbon Nanoparticles by Chemical Synthesis

Carbon nanoparticles were prepared by refluxing the kitchen soot (2g) in nitric acid (200ml of 5M) for 6 hr. After thermal refluxing in acid, the carbon nanoparticles become water soluble. When cooled to room temperature, the brownish yellow supernatant liquid after centrifugation was neutralized by sodium carbonate. The excess solvent was removed on a vacuum rotary flash evaporator at reduced temperature and carbon nanoparticles were separated from the solution by centrifugation. The solid carbon nanoparticles were dried and kept under vacuum.[3, 7]

(ii)Encapsulation of Carbon Nanoparticle in Cyclodextrin

Carbon nanoparticles were encapsulated into β-cyclodextrin by stirring at room temperature. Carbon nanoparticles (2gm) were dissolved in methanol (55ml) and quickly added to β cyclodextrin (1gm) dissolved in water (55ml). The reaction mixture was stirred at room temperature for 4 hours. It was extracted using chloroform, the unreacted particle goes to the aqueous layer and the chloroform layer was collected. The solvents were removed on a vacuum rotary flash evaporator, dried and kept under vacuum.[7]

(iii) Characterisation of CNPs and CNPs Cyclodextrin Complex

The characterization techniques include UV/visible spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and thermogravimetric analysis (TGA-DSC).[3, 5, 16, 17]

(iv) Test for Antifungal Activity

(a) Preparation of culture media for incubation of micro organisms

The 'Agar Well Method' was used to test the antifungal activity. In this technique, 0.1 ml of fungal spore suspension was thoroughly mixed with 20 ml of melted Savoured Dextrose Agar (SDA) and poured into sterilized petri plates. The plates were cooled for sufficient time to solidify the Agar medium. 200µl solution of carbon nano-β-cyclodextrin complexes were applied in the well in the launed plate. The petri plates were incubated at 30-35^{o}C for 4-6 days. All culture plates were examined after 24-96 hours. After the test, inhibition zone diameter was measured from agar dish. Water alone was also used as control. The zone of inhibition produced by the testing sample was compared with the control.[3, 15, 18,19]

(b) Determination of MIC

The minimum inhibitory concentrations (MIC) were examined by a micro dilution method. The test organisms were inoculated into SDA medium. The cultures were incubated at 37 ^{o}C for 12hrs. Then 250µl of culture was added to sterilized petri plates, cooled for sufficient time to solidify the medium. 200µl solutions of fraction of samples (100µg/ml, 200µg/ml, 300µg/ml 400µg/ml and 500µg/ml) were added in the well in the launed plate. All the plates were incubated at 28 ^{o}C for 72hrs. The lowest concentrations which will inhibit the growth of cultured plates were considered as MIC.[20, 21]

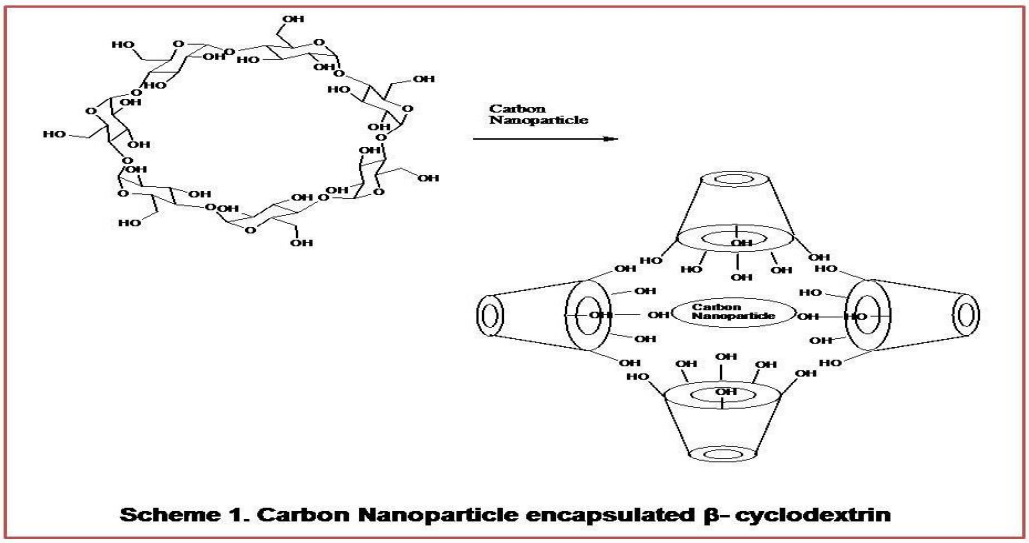
RESULTS AND DISCUSSION

(i) Carbon Nanoparticles by Chemical Synthesis

Carbon nanoparticles were chemically prepared by refluxing carbon soot in nitric acid. After thermal refluxing the water soluble CNPs were collected, cooled, and excess solvent was removed. The solid CNPs were dried and kept under vacuum.[3,8]

(ii) Synthesis of Carbon Nanoparticle –β-Cyclodextrin Complex

Carbon nanoparticles β-cyclodextrin inclusion complex was prepared by stirring the reaction mixture at room temperature for 4 hours **(**Scheme 1).



Scheme 1. Carbon Nanoparticle encapsulated β- cyclodextrin

(iii) Characterization of CNPs and CNPs- β-Cyclodextrin Complex

The CNPs and CNPs-β-cyclodextrin complexes were characterized by UV/visible spectroscopy, SEM, XRD and TGA-DSC. The λ_{max} was obtained at 390 nm for CNPs[3] which was red shifted to 424nm on encapsulation in cyclodextrin. The particle size was calculated using XRD by applying Debye- Scherrer formula and the size was found to be in 20nm-40nm range.[3] SEM images gave detailed information of surface topography and surface characteristics of CNPs[3,5] and CNPs-β-CD complex. It shows the particle size and particle distribution of nanoparticles in β-CD matrix. The results are presented in figure 1.

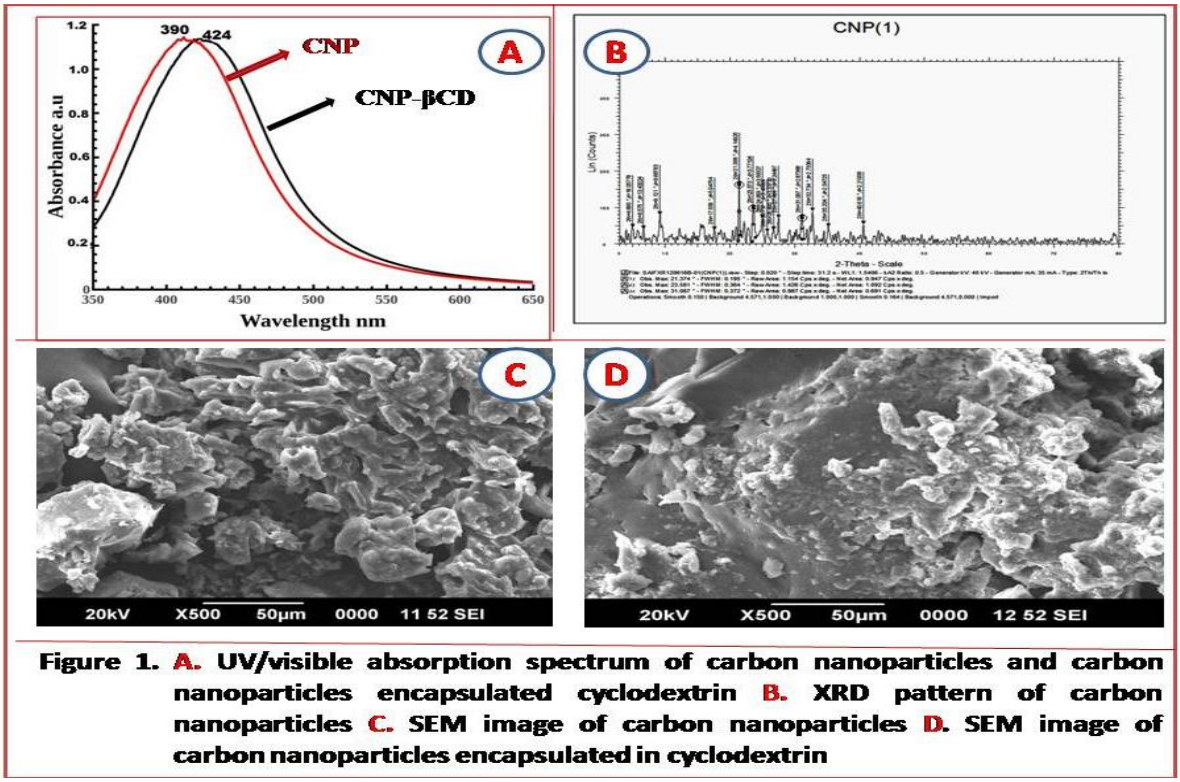


Figure 1. A. UV/visible absorption spectrum of carbon nanoparticles and carbon nanoparticles encapsulated cyclodextrin B. XRD pattern of carbon nanoparticles C. SEM image of carbon nanoparticles D. SEM image of carbon nanoparticles encapsulated in cyclodextrin

Thermal analysis gives information about changes in material properties as a function of temperature. A comprehensive study of a materials thermal behaviour is possible by TGA and DSC.[19] The complete thermal decomposition of CNPs and β-cyclodextrin encapsulated CNPs were achieved at 460°C and 550°C respectively. At a temperature of 220°C the molecular structure of CNPs starts degrading. The degradation of β-cyclodextrin encapsulated CNPs is bit rapid compared to CNPs and the thermal stability of of β-cyclodextrin encapsulated CNPs is much greater than CNPs. The results of the TGA-DSC analysis of CNPs and CNPs-β- cyclodextrine systems are plotted in figure 2.

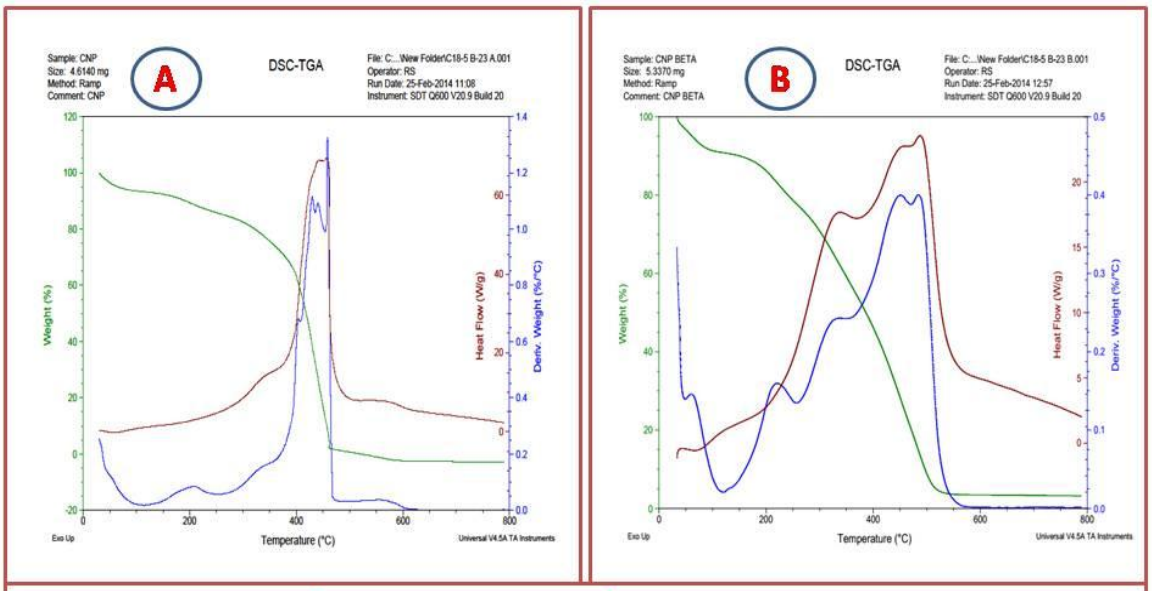


Figure 2 .DSC-TGA of A. carbon nanoparticles B. carbon nanoparticles encapsulated in β-cyclodextrin

(iv) Antifungal Activity of CNPs and CNPs Encapsulated in β-Cyclodextrin

The antifungal activity of CNPs and CNPs encapsulated in β-cyclodextrin was investigated against various pathogenic fungal strains such as *Aspergillus niger*, *Aspergillus fumigates* and *Aspergillus flavus* using Agar Well method. Using sterile micro pipette, 200µlts of the sample solutions (400µg/ml) were poured into the well in the launed plate. After incubation times the different levels of zone were measured. Figure 3 presents the antifungal activity of the CNP samples. The minimum inhibitory concentrations (MIC) of all CNP and CNP-CD samples against *A. niger* was 300µg/ml. For *Aspergillus fumigates* and *Aspergillus flavus*, the MIC of all samples was 400µg/ml.

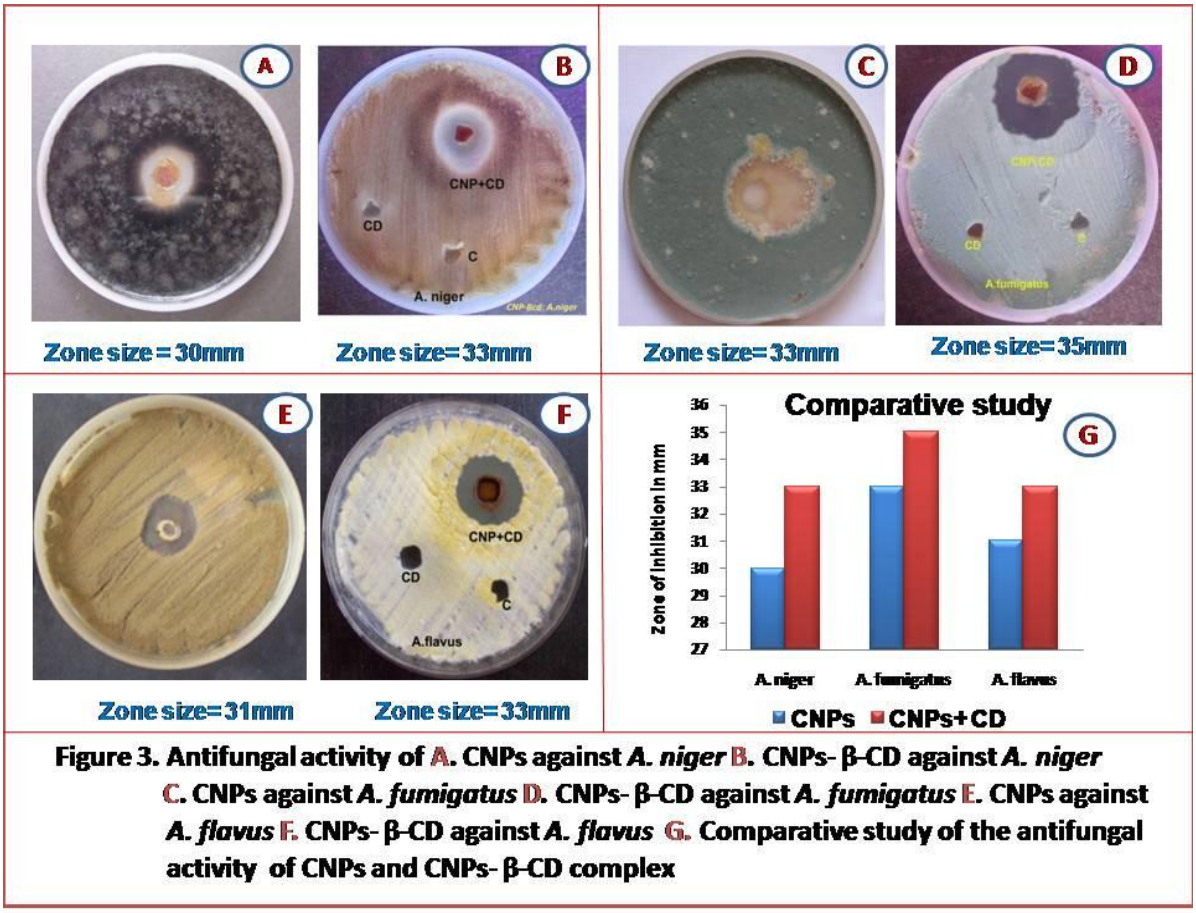


Figure 3. Antifungal activity of A. CNPs against *A. niger* B. CNPs- β-CD against *A. niger* C. CNPs against *A. fumigatus* D. CNPs- β-CD against *A. fumigatus* E. CNPs against *A. flavus* F. CNPs- β-CD against *A. flavus* G. Comparative study of the antifungal activity of CNPs and CNPs- β-CD complex

Results showed that CNPs-β-CD complexes are more active than CNP synthesized by chemical method. As the size of carbon particles decreases down to nanoscale range their antifungal activity increases because of their larger surface area per unit volume. Collision between nanoparticles and a fungal cell is unlikely to introduce direct physical damage. CNPs and CNPs- β-cyclodextrin complex act as antifungal agents that inhibit cell wall formation, cell membrane disruption and inhibition of cell division. Nanoparticles accumulate fungal cell wall which increases its permeability and it results in the death of cell wall. Carbon nanoparticles can be linked to biological molecules such as cyclodextrin which can cause to prevent nanoparticle aggregation. Carbon nanoparticles encapsulated in cyclodextrin has the ability to increase water solubility and improve light stability. The surface coating of carbon nanoparticles with cyclodextrin enhance the biological applications such as its antifungal activity.

CONCLUSION

In this work we present a novel inexpensive nanosystem derived from natural sources by green methods, which is a highly efficient antimicrobial agent and can show biomedical

applications. The present work gave thrust on green synthesis of CNPs from natural sources such as kitchen soot and their encapsulation in the well defined cavities of β- cyclodextrin aggregates. The results of analytical studies confirmed the formation of well dispersed carbon nanoparticles in β-cyclodextrin. The CNPs and CNPs-β-cyclodextrin complex were characterized by UV/visible spectroscopy, SEM, XRD and TGA-DSC. This paper reported the development of a novel, effective and simple CNP and a coating system with excellent antifungal properties. The antifungal activities of CNPs-β-cyclodextrin complex were tested against selected pathogenic *Aspergillus* fungal strains such as *Aspergillus niger*, *Aspergillus fumigates* and *Aspergillus flavus.* The incorporation of CNPs into β-cyclodextrin aggregates offers excellent stability and antifungal applications. The main advantage of this method is that it is simple, convenient and it can be produced at anytime, everywhere without much effort. The thermal stability of nanoparticle was found to be increased upon encapsulation in β-cyclodextrin. From ancient times, the antimicrobial property of ordinary kitchen soot was widely applied in treating infectious. On combining traditional knowledge with modern technology, carbon nanoparticles were found to have noticeable applications in medical and biomedical fields.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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