

Photoinduced Antifungal Studies of Functionally Modified Beta Cyclodextrin- Carbon Nanoparticle System

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KEYWORDS

Carbon nanoparticle, {5-[4-(dimethylamino)benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid, DCC coupling, β -cyclodextrin, antifungal, *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Penicillium janthinellum* and *Mucor ramosissimus*.

ABSTRACT

paper describes the synthesis of carbon nanoparticle (CNP) from natural sources such as kitchen soot, synthesise of a chromophoric system {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid and its incorporation into β - cyclodextrin (β -CD) through the esterification of the hydroxyl group with the free carboxyl function of the chromophoric system by DCC coupling. Encapsulation of CNP in to functionally modified β -CD system was achieved. Characterization of the products was undertaken by UV-visible, FT-IR, NMR, fluorescence spectroscopic methods, SEM, TEM, and X-ray diffraction methods. It is also aimed to study the thermal stability, light fastening ability and photoinduced antifungal action of the products. The antifungal activity of functionally modified CD-CNP systems was tested against pathogenic fungal strain such as *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Penicillium janthinellum* and *Mucor ramosissimus* by well method. The results showed that and functionally modified product have excellent photoinduced antifungal activity against selected fungal strains.

INTRODUCTION

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 [1, 2]. Nanomaterials are difficult to be maintained as individual particles due to their tendency to agglomerate [3-8]. Surface modification of nanoparticles is one of the accepted methods to maintain the stability of nanoparticles. Macromolecules possess scaffolds or channels which are best suited for surface modification of nanoparticles [9-10]. Cyclodextrins are cyclic oligosaccharides which made up of sugar molecules have recently been considered as pharmaceutical excipients [11]. Cyclodextrins like other supramolecular systems act as molecular chelating agents owing to cage-like supramolecular structure. Chemical reactions of these supramolecular systems are ‘host–guest’ types involving intermolecular interactions [12, 13]. The presence of large number of hydroxyl groups makes cyclodextrins a functionally modified system by DCC coupling. Thus they can form water soluble and bioavailable compounds. This is of high interest for pharmaceutical applications in which hydrophobic compounds shall be delivered [14, 15]. Appending a chromogenic group to a CD is

making a photosensitive cyclodextrin system, which could be further developed as biomedical agents for photodynamic therapy.

A significant property of CNPs is its antimicrobial activity. CNPs are effective to control the microorganism infection [2, 3]. The combination of nanoparticles and macromolecules can provide the ideal coating system with excellent antimicrobial properties. The truncated cone or torus molecular structures of cyclodextrins are able to form water-soluble complexes with CNPs that entrap in the cavity of cyclodextrin [9, 11]. Thus they can enhance the solubility and bioavailability of such compounds. Functional modification of cyclodextrin with the chromophoric system {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid develop aqueous soluble photoactive system [16-18]. The incorporation of nanoparticles into β -CD loaded with chromophoric system offers new photodynamic materials with wide applications in photochemistry, and biomedical fields [16].

In the present work, we adopted a simple low-cost method for the synthesis of CNP from natural sources. It is also aims on the synthesis of chromophoric system, functional modification of β -CD with photochromic system and improving the stability of CNPs by encapsulating in functionally modified β -CD [19]. We studied the antifungal activity of CNP and functionally modified β -CD-CNP complex against selected Gram positive fungal strains such as *Aspergillus flavus* and *Mucor ramosissimus*. *Aspergillus flavus* and *Mucor ramosissimus*, cause skin and wound infections [20, 21].

MATERIALS AND METHODS

(i) Synthesis of carbon nanoparticles by chemical methods

The samples were collected from ordinary kitchen soot. Carbon nanoparticles were prepared by refluxing a sample of kitchen soot (2g) in nitric acid (200ml of 5M) for 6 hr [3]. After thermal refluxing in acid, the carbon nanoparticles became water soluble. It was then 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.

(iii) Synthesis of {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

P-Dimethylaminobenzaldehyde (1g) and rhodanin-N-acetic acid (1.25g) were dissolved in ethanol (50 ml). The mixture was stirred thoroughly for a few minutes. The temperature was raised to 80°C and the mixture was refluxed for 4 hours. The product was filtered. It was purified by recrystallisation from absolute ethanol. The yield was noted as 80%. It was further purified by column chromatography using 10:3 hexane-ethyl acetate solvent systems and dried in vacuum [19].

(iv) Synthesis of β -Cyclodextrin functionalised with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

Beta CD and the dye in molar ratio (1g), DMAP (200 mg), and DCC (1 g) were separately dissolved in DMF and introduced into an R.B flask fitted with a reflux condenser and a magnetic stirrer cum heater. The mixture was stirred at room temperature for 2 hours and at 80°C for 6 hours. The by-product dicyclohexyl urea (DCU) was removed by warming-cooling-filtration processes and the solvent was

removed in a vacuum rotory evaporator and dried. It was purified by column chromatography using chloroform-methanol and dried in vacuum.

(v) Encapsulation of CNP in the cavities of β -CD functionalised with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo-1, 3-thiazolidin-3-yl} acetic acid

CNP in water (20 ml) was added to β -cyclodextrin functionalised with chromophoric system in chloroform (20 ml). The reaction mixture was stirred at room temperature for 5 hours. The encapsulated CNP was in the chloroform layer and was separated from the aqueous layer using a separating funnel. The solvents were removed in a vacuum rotory evaporator, dried and kept under vacuum. The proposed scheme of the encapsulation of CNP in the cavity of functionally modified CD aggregate is shown in figure 1.

(vii) Photoinduced antifungal study

The Agar well-diffusion method was used to test the antifungal activity. For *in vitro* screening, fungi, such as *Aspergillus flavus* and *Mucor ramosissimus* were selected. The photoinduced antifungal effects of functionally modified CD-CNP systems were tested pathogenic fungal strains. In this study the sample was irradiated with visible light for 0.5 hr, 1 hr, 2 hrs and 4 hrs durations. Samples of the irradiated system were immediately withdrawn and the activity studies were conducted. Using sterile micro pipette 200 μ lts of the sample solutions (irradiated and nonirradiated) were poured into each well. Three replications and control tests were conducted. Controls used for the measurement were water, dye, and -dye. After incubation times (96 hrs) the zones of inhibition (in mm) were measured [20, 21]. MIC of the samples was examined by a micro dilution method. 200 μ l solutions of fraction of samples (100 μ g/ml, 200 μ g/ml, 300 μ g/ml and 400 μ g/ml) were added in the well in the lawned plate. All the plates were incubated at 30-35°C for 24-96 hours. The lowest concentrations that would inhibit the growth of fungal strain were taken as MIC [20].

RESULTS AND DISCUSSION

(i) Synthesis and characterisation of carbon nanoparticles

CNPs were synthesized from kitchen soot by refluxing with nitric acid. The carbon nanoparticles were characterized by UV/visible spectroscopy, fluorescence spectroscopy, SEM, TEM and XRD. The UV/visible absorption and fluorescence emission spectra of CNP were recorded in chloroform. The absorption maximum (λ_{max}) of CNP was obtained at 412 nm which was assigned as $\pi - \pi^*$ transitions. The fluorescence emission maximum for CNP was observed at 433nm. SEM was used for surface analysis of the CNP in order to investigate the morphology of the particles. The SEM image shows almost uniform sized particles and distribution of particles. The TEM image shows particle size in the range 2-20nm. XRD was used as a method of determining the arrangement of CNPs in arrays and also the size of the carbon nanoparticle. The results are shown in figure 2.

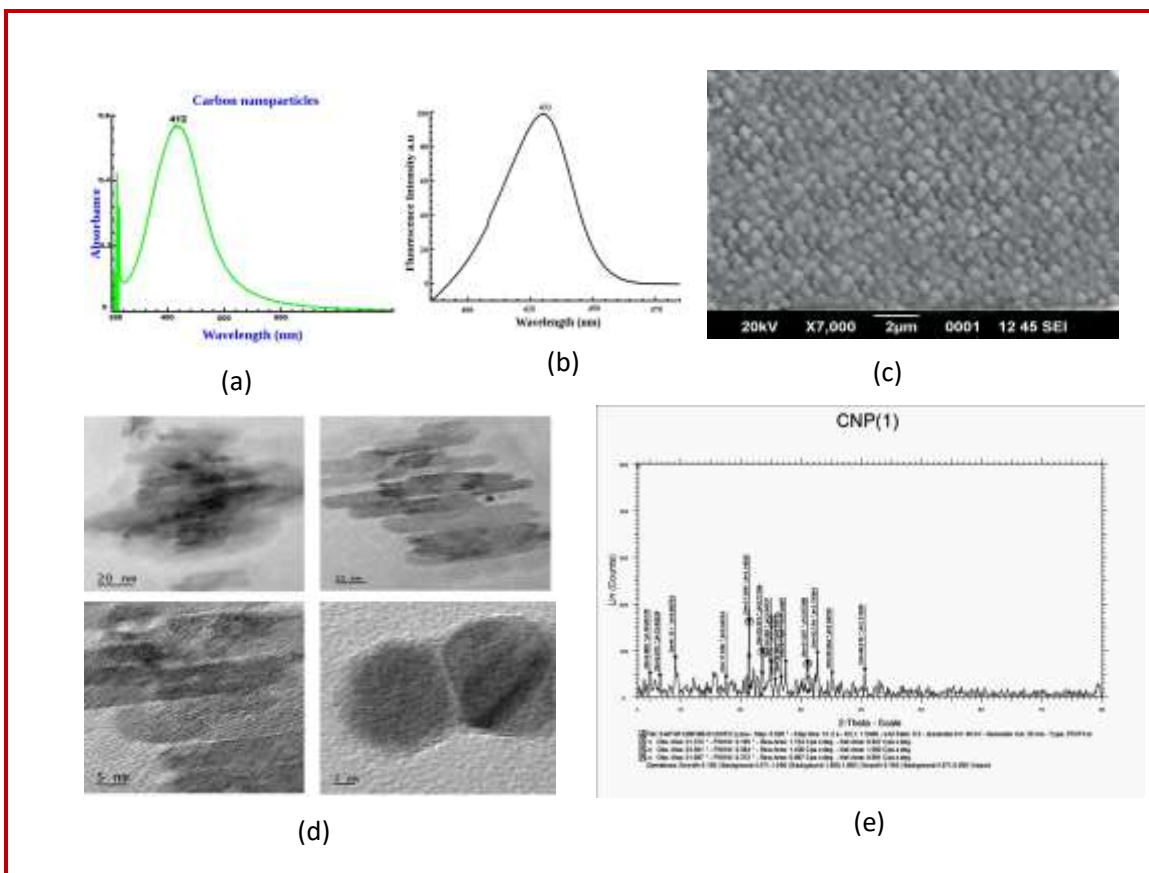
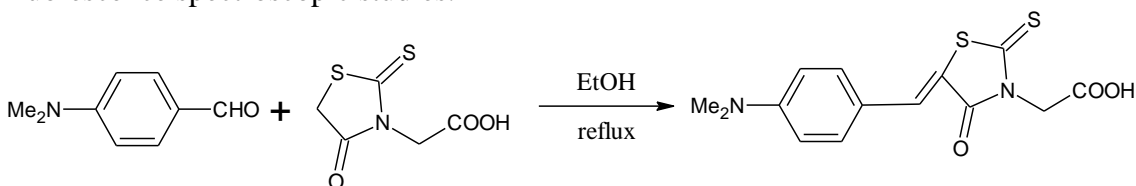


Figure 2 (a) UV-visible absorption spectrum (b) fluorescence emission spectrum (c) SEM image (d) TEM image and (e) XRD pattern of carbon nanoparticles

(ii) Synthesis and characterisation of {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

The photoactive {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid was prepared from p-dimethylaminobenzaldehyde and rhodanin-N-acetic acid (scheme 1). The products were characterized by UV-visible, FT-IR, ¹H-NMR and fluorescence spectroscopic studies.



Scheme 1. Synthesis of {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

The UV-visible absorption and fluorescence emission spectra of {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid were recorded in chloroform. The absorption maximum (λ_{max}) of the dye was obtained at 464.5 nm. This is due to the $n-\pi^*$ transition of the conjugated carbonyl group present in the chromophoric system. The fluorescence emission maximum for the chromophoric system was observed at 507nm (figure 3). FT-IR spectrum of {5-[4-(dimethylamino)

benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid was recorded in the solid state as KBr discs in the operating frequency range $4000\text{--}400\text{cm}^{-1}$ (figure 4). IR(KBr) : $3300\text{--}3500\text{ cm}^{-1}$ (broad): $\nu_{\text{O-H}}$ (str), 2922 cm^{-1} : $\nu_{\text{C-H}}$ of CH_2 , 1714 cm^{-1} : $\nu_{\text{C=O}}$ (str) , 1610 cm^{-1} : $\nu_{\text{C=C}}$ (str) , 1562 cm^{-1} : $\nu_{\text{N=N}}$ (str), 1360 cm^{-1} : $\nu_{\text{C-N}}$ (str), 1315 cm^{-1} : $\nu_{\text{C=S}}$ (str), 1186 cm^{-1} : $\nu_{\text{C-O}}$ (str). $^1\text{H-NMR}$ spectrum of {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid was recorded in a 400 MHz instrument using CDCl_3 as solvent (figure 5). The molecule has 6 sets of chemically different protons which give six NMR signals. $^1\text{H-NMR}$: 10.329 ppm (-COOH, H, s), 6.7 ppm (Ha, 2H, d), 7.4 ppm (Hb, 2H,d), 7.8 ppm (Hc, 1H,s) and 4.817 ppm (Hd, 2H, s) and 3.09 ppm (NMe_2 , 6H, s).

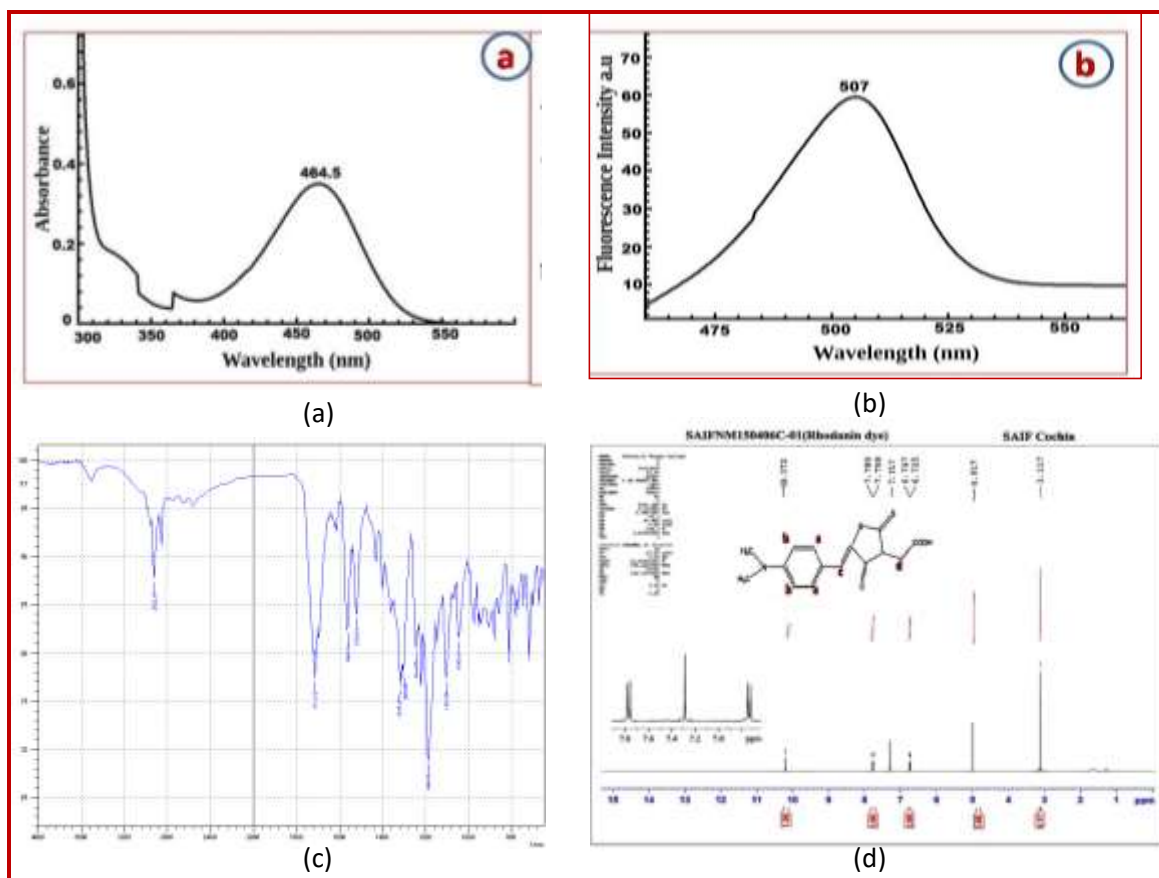
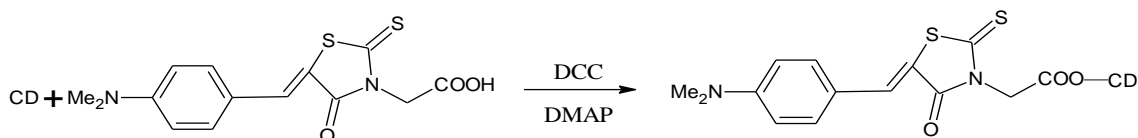


Figure 3. (a) UV-visible absorption spectrum (b) fluorescence emission spectrum (c) FT-IR spectrum (d) $^1\text{H-NMR}$ spectrum of {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

(iii) Synthesis and characterisation of β -CD functionalised with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

The hydroxyl groups of cyclodextrin were esterified with the free carboxyl group of {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid through DCC coupling using DMAP as the catalyst (scheme 2). The products were characterized by UV-visible, FT-IR, NMR and fluorescence spectroscopic studies.



Scheme 2. Functional modification of cyclodextrin with {5-[4-(dimethylamino)benzylidene]-4-oxo-2-thioxo-1,3-thiazolidin-3-yl} acetic acid

The UV-visible absorption and fluorescence emission spectra of CD functionalised with {5-[4-(dimethylamino)benzylidene]-4-oxo-2-thioxo-1,3-thiazolidin-3-yl} acetic acid were recorded in chloroform (figure 6). The λ_{max} of the original dye was obtained at 464.5 nm and the signal was shifted to 483 nm on attaching to β -CD. The peak at 483 nm is due to $n\text{-}\pi^*$ transition of the β -CD supported chromophoric system. The fluorescence emission maximum for the functionalized β -CD system was observed at 515 nm. The fluorescence emission efficiency and intensity were enhanced on attaching the dye onto cyclodextrin core system.

FT-IR spectra of β -CD and β -CD functionalised with {5-[4-(dimethylamino)benzylidene]-4-oxo-2-thioxo-1,3-thiazolidin-3-yl} acetic acid were recorded in the solid state as KBr discs in the operating frequency range 4000–400 cm^{-1} (figure 7). FT-IR (KBr) of β -CD: 3300–3653 cm^{-1} (broad- inter and intra molecular hydrogen bonding): $\nu_{\text{O-H}}$ (str); 2912–2897 cm^{-1} : $\nu_{\text{C-H}}$ of CH_2 and CH_3 ; 1155 cm^{-1} : $\nu_{\text{C-O-C}}$ (str); 1028 cm^{-1} : $\nu_{\text{C-O-H}}$ (str). FT-IR (KBr) of β -CD-dye: 3325 cm^{-1} (broad- inter and intra molecular hydrogen bonding): $\nu_{\text{O-H}}$ (str), 2927 cm^{-1} : $\nu_{\text{C-H}}$ of CH_2 , 1708 cm^{-1} : $\nu_{\text{C=O}}$ (str), 1568 cm^{-1} : $\nu_{\text{C=C}}$ (str), 1344 cm^{-1} : $\nu_{\text{C-N}}$ (str), 1359 cm^{-1} : $\nu_{\text{C=S}}$ (str), 891 cm^{-1} : $\nu_{\text{C-S}}$ (str). Spectral data clearly indicated the incorporation of photochromic system in to β -cyclodextrin (β -CD) core through the esterification of the hydroxyl group of β -CD with the free carboxyl function of the chromophoric system by DCC coupling.

$^1\text{H-NMR}$ spectrum of the product was recorded in a 400 MHz instrument using CDCl_3 as solvent (figure 8). $^1\text{H-NMR}$: 6.673 ppm (Ha, 2H, d), 7.756 ppm (Hb, 2H, d), 7.284 ppm (Hc, 1H, s), 5.031 ppm (Hd, 2H, s), and 3.107 ppm (NMe_2 , 6H, s), 3.756–3.352 (OH group of β -CD), 1.735–1.242 ppm (aliphatic protons of β -CD). The absence of a peak at 9–11 ppm shows that the carboxylic proton is absent in the product and is evident of the coupling of free carboxylic group of the dye with hydroxyl function of the β -CD.

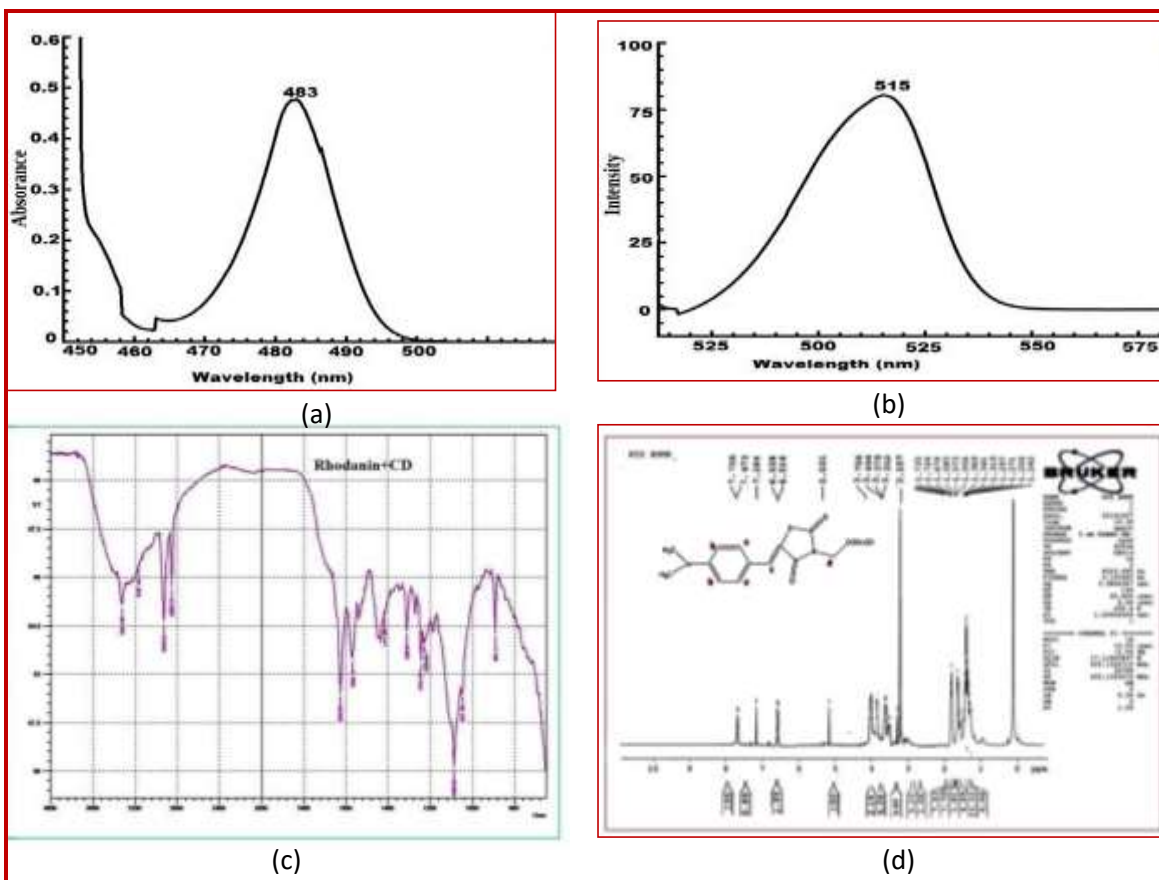
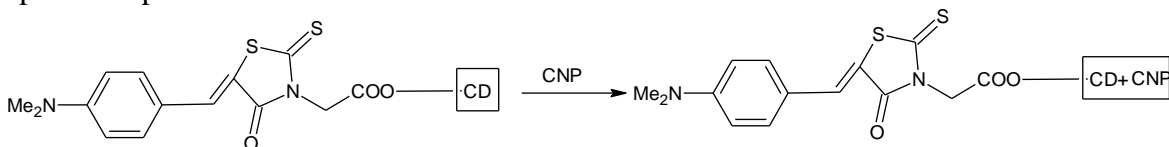


Figure 6. (a) UV-visible absorption spectrum (b) fluorescence emission spectrum (c) FT-IR spectrum (d) ^1H -NMR spectrum of β -CD functionalised with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

(iv) Synthesis and characterisation of CNP encapsulated in the cavities of β -CD functionalised with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

CNPs in water and functionally modified β -CD in chloroform were stirred at room temperature and β -CD-CNP complex was collected from the chloroform layer (scheme 3). The encapsulated systems were characterised by UV-visible, NMR, and FT-IR spectroscopic methods.



Scheme 3. Encapsulation of CNP in β -CD functionalised with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

The UV-visible absorption and fluorescence emission spectra of functionally modified β -CD-CNP products were recorded in chloroform (figure 9). The λ_{max} of the system was found to be shifted from 483 nm to 512 nm on encapsulation of carbon nanoparticles. The red shift observed (29nm) is because of incorporation of CNP. The

fluorescence emission maximum for the functionalised β -cyclodextrin-CNPs complex was observed at 572nm. The enhanced fluorescence efficiency and intensity is due to the incorporation of CNPs in the functionalized β - cyclodextrin system. On attaching CNP to intensely functionalized β -CD core, the energy gap of the molecular system reduced and led to the formation of an excimer, and this is the reason for red shift.

FT-IR spectrum was recorded in the solid state as KBr discs in the operating frequency range 4000–400 cm^{-1} (figure 10). FT-IR (KBr): 3323 cm^{-1} (broad): $\nu_{\text{O-H}}$ (str), 2926 cm^{-1} : $\nu_{\text{C-H}}$ of CH_2 , 1682 cm^{-1} : $\nu_{\text{C=O}}$ (str), 1568 cm^{-1} : $\nu_{\text{C=C}}$ (str), 1344 cm^{-1} : $\nu_{\text{C-N}}$ (str), 1373 cm^{-1} : $\nu_{\text{C-S}}$ (str), 891 cm^{-1} : $\nu_{\text{C-S}}$ (str). $^1\text{H-NMR}$ spectrum of the product was recorded in a 400 MHz instrument using CDCl_3 as solvent (figure 11).

$^1\text{H-NMR}$: 6.673 ppm (Ha, 2H, d), 7.756 ppm (Hb, 2H, d), 7.284 ppm (Hc, 1H, s), 4.756 ppm (Hd, 2H, s), and 3.190 ppm (NMe_2 , 6H, s), 3.756-3.352 (OHgroup of β -CD)1.735-1.242ppm (aliphatic protons of β -CD). The absence of a peak at 9-11ppm shows that carboxylic proton is absent in the product and this shows the coupling of free carboxylic group of the dye with hydroxyl function of the β -CD. The UV-visible absorption and fluorescence emission spectra of functionally modified β -CD-CNP products showed red shift of 29nm in absorption spectra and 63nm in fluorescence emission spectra due to the effect of incorporation of CNP.

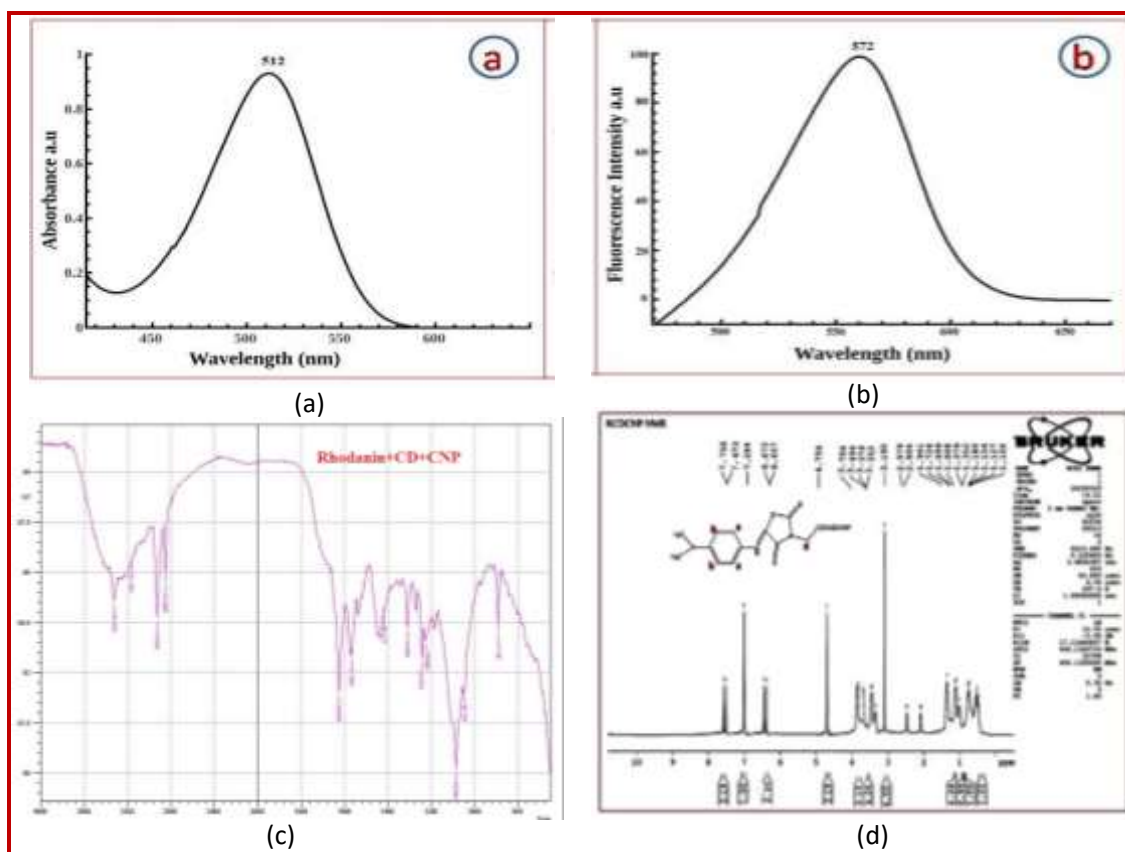


Figure 6. (a) UV-visible absorption spectrum (b) fluorescence emission spectrum (c) FT-IR spectrum (d) $^1\text{H-NMR}$ spectrum of β -CD-CNP functionalised with {5-[4-

(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

(v) Photoinduced Antifungal Studies of Functionalised CD-CNP System

The photoinduced antifungal activity and minimum inhibitory concentrations (MIC) of the functionally modified products were examined. Pathogenic fungal strains such as *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Penicillium janthinellum* and *Mucor ramosissimus* were selected for studies.

(a) Minimum inhibitory concentrations (MIC) of functionalised CD-CNP systems

The minimum inhibitory concentrations (MIC) were examined by a micro dilution method. 200µl solutions of fraction of samples (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500µg/ml) were added in the well in the lawned plate. All the plates were incubated at 28°C for 96 hrs. The lowest concentrations that would inhibit the growth of fungal strains were taken as MIC. MIC values of functionalised CD-CNP (non irradiated sample) against all the pathogenic fungal strains were found to be 300µg/ml and irradiated sample (2 hrs) showed MIC 200 µg/ml. MIC results of the functionalised products against selected fungal strains are shown in table 1.

Table 1. MIC values of functionalised -CNP, CD-CNP and -CNP systems (nonirradiated sample and sample irradiated with visible light)

Systems	Fungal Strains	MIC values (nonirradiated)				MIC values (light irradiated 2hrs)			
		100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml
Functionally modified CD-CNP	<i>A.niger</i>	+	+	+	–	+	+	–	–
	<i>A.fumigatus</i>	+	+	+	–	+	+	–	–
	<i>A.flavus</i>	+	+	+	–	+	+	–	–
	<i>Penicillium</i>	+	+	+	–	+	+	–	–
	<i>Mucor</i>	+	+	+	–	+	+	–	–

+: growth, -: no growth

(b) Photoinduced antifungal activity of functionalised CD-CNP and system

The photoinduced antifungal effects of functionally modified CD-CNP system was tested with *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Penicillium janthinellum* and *Mucor ramosissimus* using well diffusion method. In this study the sample was irradiated with visible light by 0.5 hr, 1 hr, 2 hrs and 4 hrs. Using sterile micro pipette 200µlts of the sample solutions (irradiated and nonirradiated) were poured into each well. Three replications and control tests were conducted. Controls were used as water, dye, CD and CD-dye. After incubation time (96 hrs) the zones of inhibition (in mm) were measured.

Results revealed that the functionally modified, CD-CNP, system irradiated with visible light showed greater antifungal activity than the nonirradiated system, in fact there was no appreciable change of inhibitory effect with different time intervals up to 2 hrs

exposures.. The strains susceptible to functionally modified CD-CNP aggregates exhibited higher activity in *Aspergillus niger* (26.66±0.577mm) and *Penicillium janthinellum* (28.66±0.577mm) The results of antifungal studies are summerised in table 6.11, figure 6.14, figure 6.15 and figure 6.16).

Table 6.11 Antifungal activities of functionalised CD-CNP system (nonirradiated sample and sample irradiated with visible light)

Fungal strains	Functionally modified CD-CNP Zone of inhibition in mm at different time intervals				
	Zero hr	0.5 hr*	1 hr*	2 hrs*	4 hrs*
<i>A.niger</i>	25.00±1.00	25.66±0.577	26.66±0.577	26.66±0.577	26.66±0.577
<i>A.fumigatus</i>	23.00±1.00	24.00±1.00	25.00±1.00	25.00±1.00	25.00±1.00
<i>A.flavus</i>	23.33±0.471	25.00±1.00	26.00±1.00	26.00±1.00	26.00±1.00
<i>Penicillium</i>	26.00±1.00	26.66±0.577	28.66±0.577	28.66±0.577	28.66±0.577
<i>Mucor ramosissimus</i>	23.00±1.00	24.66±0.577	25.66±0.577	25.66±0.577	25.66±0.577

Results are the means of three replications ± standard deviation

Zero hr- non irradiated sample

0.5 hr*- sample irradiated with visible light by 30 minutues

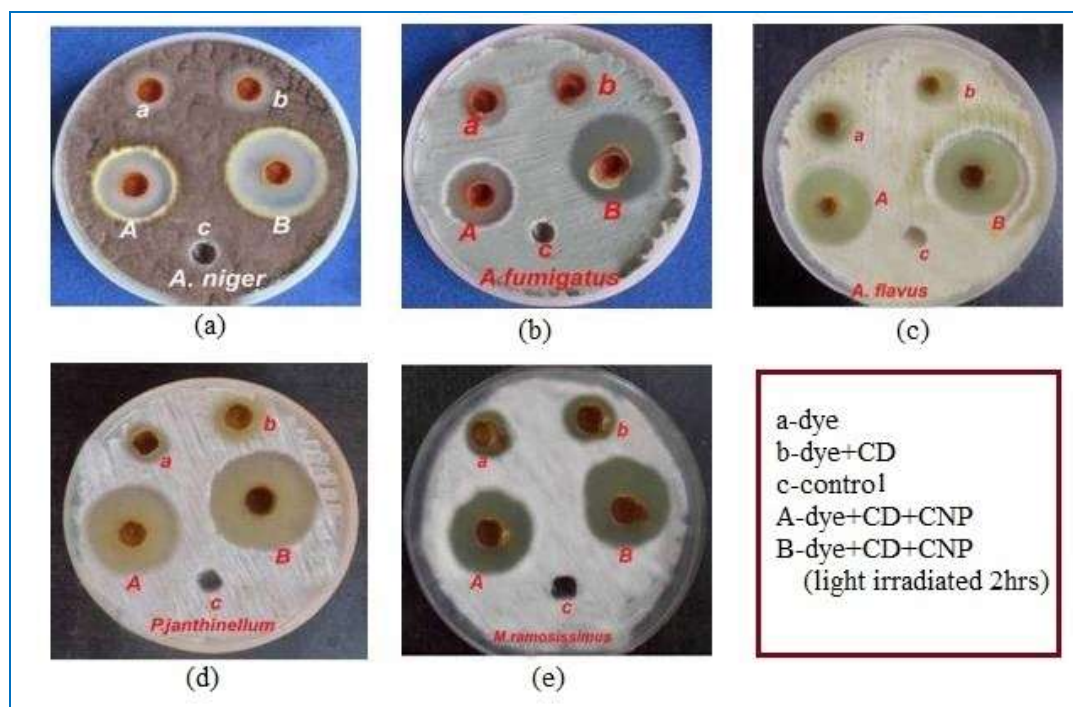


Figure 7. Antifungal activity of functionally modified CD-CNP against (a) *A. niger* (b) *A. fumigates* (c) *A. flavus* (d) *P. janthinellum* (e) *M. ramosissimus*.

The antifungal activity of the various CNP system in light induced conditions are found to be greater by ≥2mm (inhibition zone) than those in dark condition. All

pathogenic fungi gave similar results in dark condition. When irradiated with visible light the surface electrons are excited and become more active. On irradiation, collision between nanoparticles and a fungal cell is increased unlikely to cause direct physical damage. These system act as antifungal agents that restrain 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. As the size of CNPs decreases down to nanoscale range, their antifungal activity increases because of their larger surface area per unit volume. The surface coating of carbon nanoparticles with functionally modified system enhances the biological applications such as its antifungal activity. A growing interest has been developed in water-soluble polymers for their various applications potential in physical and biological system. The process of cell membrane destruction depends mainly on the effectiveness of the samples. The functionally modified system prevent fungal growth and cell division and finally kill the organism. Functionally modified products induced with light exhibited good antifungal effects because of lower MIC values ($\geq 300\mu\text{g/ml}$), notable inhibitory zone ($< 24\text{mm}$). The newly developed biocompatible, nature friendly, photo responsive system showed very promising antifungal activity towards all fungal strains under investigation. Comparative study of antifungal activity of different CNP system gave the following results (table 6.12).

Table 2. Comparative study of antifungal activity of different CNP system

System	Zone of inhibitions (mm)				
	<i>A.niger</i>	<i>A.fumigatus</i>	<i>A.flavus</i>	<i>Penicillium</i>	<i>Mucor</i>
CNP	18.33 \pm 0.471	20.66 \pm 0.577	18.33 \pm 0.471	20.33 \pm 0.471	18.66 \pm 0.577
CNP-CD	22.33\pm0.471	21.00 \pm 1.00	19.33 \pm 0.471	22.66\pm0.577	20.33 \pm 0.577
CD-dye-CNP	24.33\pm0.471	23.00 \pm 1.00	23.33 \pm 0.471	26.00\pm1.00	23.00 \pm 1.00
[CD-dye-CNP]*	26.66\pm0.577	25.00 \pm 1.00	26.00 \pm 1.00	28.66\pm0.577	25.66 \pm 0.577

Results are the means of three replications \pm standard deviation

[CD-dye-CNP]* -sample irradiated with visible light by 2 hrs

Results showed that CNP-CD, CD-dye-CNP and [CD-dye-CNP]* showed maximum antifungal effect towards *A. niger* and *P. janthinellum*.

Conclusion

We present novel inexpensive nanosystem derived from natural sources, which are highly efficient antimicrobial agents and can be used for biomedical applications. Since pathogenic microbes fungi have always been considered as major cause of diseases in humans, there is a pressing need to develop new and effective antifungal agents. CNP from soot presents as a bioactive compound that can be of interest in therapeutic applications because of their low toxicity. There is a long tradition of carbon in its nano form in remedial treatment of microbial infectious diseases. The functionally modified, supramolecular CD-CNP aggregates which are very effective against pathogenic microbes without affecting their properties. CNPs have been known for a long time to possess antimicrobial properties, and also to be non toxic and environment friendly. The study showed that CNPs encapsulated in functionally modified

system exhibits enhanced antimicrobial activity on light exposure. A goal of this work was the development of new antimicrobial agents which offer dramatic effects in photodynamic therapy and photodynamic antimicrobial applications.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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