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Photoinduced Antifungal Studies Of Carbon Nanoparticle And Functionally Modified Hyperbranched Polyglycerol-Carbon Nanoparticle Aggregates

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Abstract

This paper describes the synthesis of carbon nanoparticle (CNP) from natural sources such as kitchen soot, synthesis of a chromophoric system {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid and its incorporation into hyperbranched polyglycerol (HPG) 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 HPG system, characterization of the products by UV-visible, FT-IR, NMR, fluorescence spectroscopic methods, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD) methods, and study of their antifungal action. The antifungal activity of CNP and functionally modified HPG-CNP aggregates were tested against various pathogenic fungal strains such as Aspergillus niger, Aspergillus fumigates, Aspergillus flavus, Penicillium janthinellum, and Mucor ramosissimus by well diffusion method. The results proved that the CNPs and functionally modified product have excellent antifungal activity against selected pathogenic fungal strains.

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

Introduction

Nano materials have potential applications in biomedical nanotechnology. Nanoparticles can be used as an anti microbial

because of its physical and chemical properties. Nano particles can be integrated into a variety of materials and assembled into many different shapes. Carbon nanoparticles exhibit significantly distinct physical, chemical and biological properties from their bulk counter parts because of small particle dimension, high surface area, quantum confinement and other effects. Carbon nanoparticles have different surface interactions compared to micron sized particles. CNPs have an extremely high tendency of adhesion and aggregation [12]. Carbon nanoparticles encapsulated in hyperbranched polymers has the ability to increase water solubility and improve light stability.

Hyperbranched polymers constitute a special class of branched macromolecules characterized by their randomly branched topology. Hyperbranched polymers are attractive because they resemble dendrimers, but they can be prepared on a large scale and at a reasonable cost in a single step synthesis. Hyperbranched polyglycerol represents the first hyperbranched polymer that can be prepared in a controlled manner. Hyperbranched polymers have attracted much attention in nanotechnology, because these polymers exhibit special characteristic features, higher solubility, and higher amount of terminal groups.

Dendritic macromolecules, such as hyperbranched polyglycerol are increasingly being studied in surface modification. Hence, in the past few years, hyperbranched polyglycerol have been employed as carriers for various guest molecules such as dyes, pharmaceuticals and nanoparticles. Hyperbranched polyglycerol which possess scaffolds or channels are best suited for encapsulating nanoparticles [10, 11]. It prevents degredation and protects the nanoparticles from aggregation and can help preserve different properties of the material [18, 19].

Functional modification of HPG with the photoactive system 2-(5-(4-dimethylamino-benzylidin)-4-oxo-2-thioxo-thiazolidin-3-yl) acetic acid nature friendly, photo responsive system. The HPG core is prone to functional transformation due to the presence of free hydroxyl functions in its backbone. CNPs encapsulated with functionally modified HPG are of great interest because of its noticeable antimicrobial properties. It can certainly be marked down as a hot spot in nanobiotechnology. In the present work, we adopted a simple low-cost method for the synthesis of CNP from natural sources. We studied the antifungal activity of CNP and functionally modifided HPG-CNP complex against selected fungal strains such as Aspergillus niger, Aspergillus fumigates, Aspergillus flavus, Penicillium janthinellum, and Mucor ramosissimus. These pathogenic fungal strains cause serious health problems.

Materials and methods

(i) Synthesis of carbon nanoparticles by chemical methods

Carbon nanoparticles were prepared by refluxing a sample of kitchen soot (2g) in nitric acid (200ml of 5M) for 6 hr. 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 [9].

(ii) 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 80^0C 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.

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

HPG 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 80^0C 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.

(iv) Encapsulation of CNP in the cavities HPG functionalised with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

CNP in water (20 ml) was added to HPG functionalised with chromophoric system in chloroform (20 ml). The reaction mixture was stirred at room temperature for 5 hours. The CNP was transferred to the chloroform layer by phase transfer on encapsulation which was separated from the aqueous layer using a separating funnel. The solvents were removed in a vacuum rotory evaporator, dried and was kept under vacuum.

(vi) Antifungal study

(a) Agar well diffusion method

The 'Agar Diffusion Technique' was used to test the antifungal activity. The 'well method' was employed for the studies. In this method 20 ml of sterilized SDA was poured into sterile petriplate, after solidification, 100µl of pathogenic fungal strain were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher and 200µl sample solution were added to the wells. The plates were incubated at 30-35^{o}C and all culture plates were examined after 24-96 hours. After incubation the diameter of inhibitory zones formed around each well were measured in millimeter and recorded [16,17]. For in vitro screening, fungi, such as Aspergillus niger, Aspergillus fumigates, Aspergillus flavus, Penicillium janthinellum, and Mucor ramosissimus were selected.

(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 loaded in the disc and applied in the launed plate. All the plates were incubated at 28 ^{o}C for 24hrs. The lowest concentrations which will inhibit the growth of cultured plates were considered as MIC [18].

Results and discussion

(i) Synthesis and characterisation of carbon nanoparticles

CNPs were synthesized from kitchen soot by thermal refluxion 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 (λmax) of CNP was obtained at 412 nm. This is due to the $\pi - \pi^{*}$ transitions. The fluorescence emission maximum for CNP was observed at 443nm. SEM was used for surface analysis of the CNP in order to investigate the morphology of the particles. The SEM image shows almost uniform particle size 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 atoms within a crystal and also the size of the carbon nanoparticle. The results are shown in figure 1.

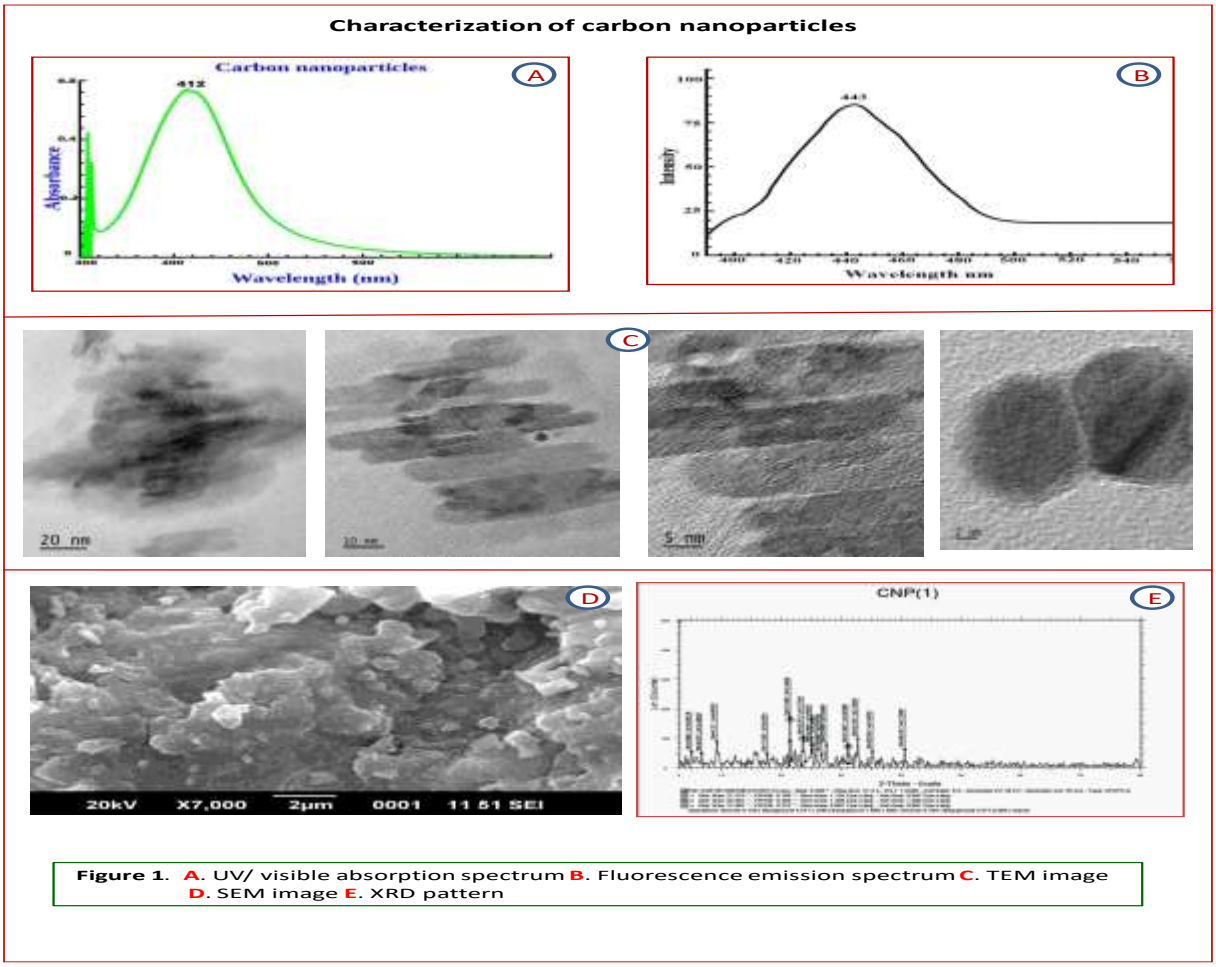
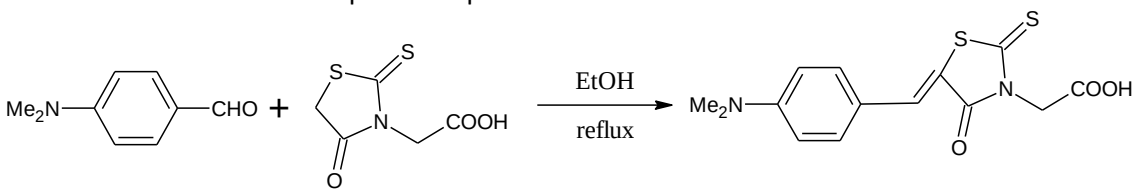


Figure 1. **A**. UV/ visible absorption spectrum **B**. Fluorescence emission spectrum **C**. TEM image **D**. SEM image **E**. XRD pattern

3.2 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, 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 (λmax) of the dye was obtained at 464.5 nm. This is due to the n-π^* transition of the conjugated carbonyl group present in

the chromophoric system. The fluorescence emission maximum for the chromophoric system was observed at 507nm (figure 2). 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 4000–400cm^{-1} (figure 2). FT-IR (KBr) : 3300-3500 cm^{-1}(broad): ν_{O-H} (str), 2922 cm^{-1}: ν_{C-H} of CH_2, 1714 cm^{-1}: $\nu_{C=O}$(str) , 1610 cm^{-1}: $\nu_{C=C}$(str) ,1562 cm^{-1}: $\nu_{N=N}$ (str), 1360 cm^{-1}: ν_{C-N} (str), 1315 cm^{-1}: $\nu_{C=S}$ (str), 1186 cm^{-1}: ν_{C-O} (str). ^{1}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 2). The molecule has 6 sets of chemically different protons which give six NMR signals. ^{1}H-NMR: 10.372 ppm (-COOH, H, s), 6.747 ppm (Ha, 2H, d), 7.780 ppm (Hb, 2H,d), 7.317 ppm(Hc, 1H,s) and 4.817 ppm (Hd, 2H, s) and 3.117 ppm (NMe_2, 6H, s).

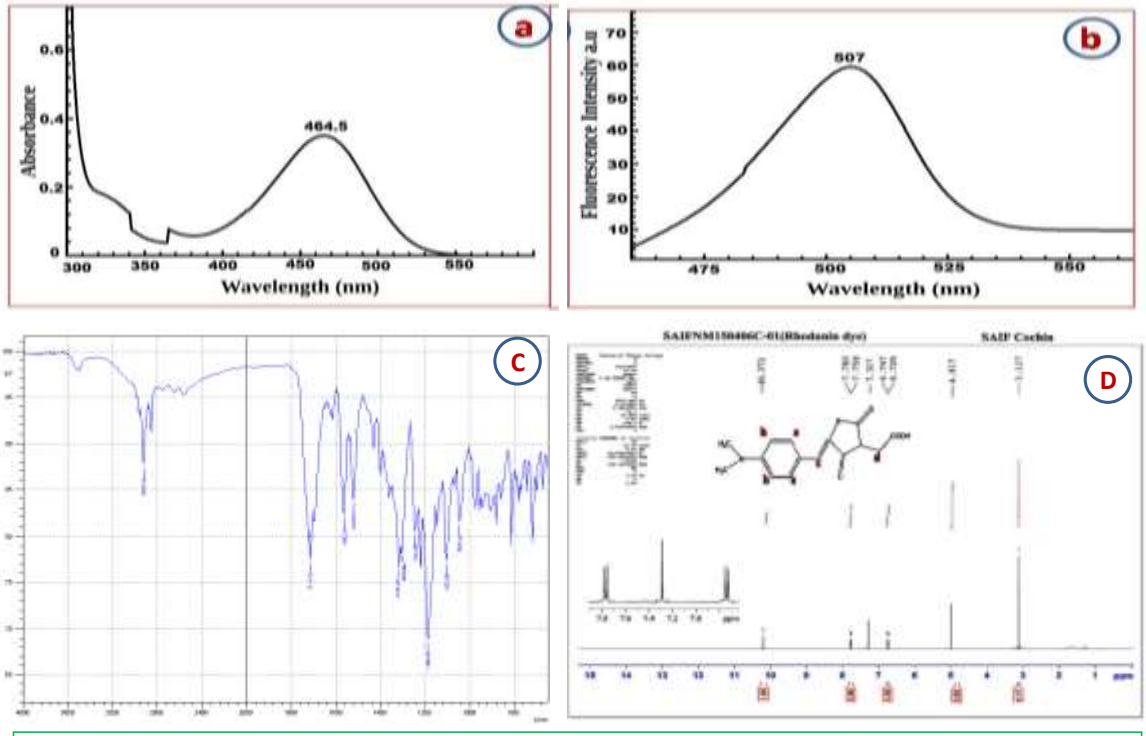
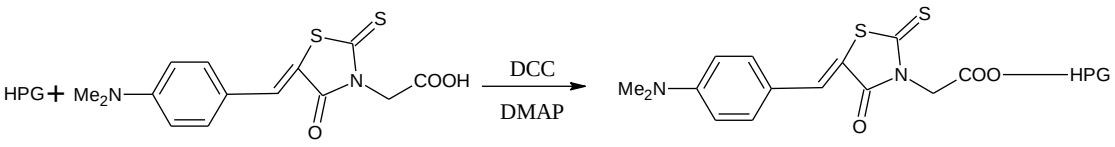


Figure 2. (a) UV-visible absorption (b) fluorescence emission © FT-IR and (D) ^{1}H-NMR spectra of {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

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

The hydroxyl groups of hyperbranched polyglycerol 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 hyperbranched polyglycerol with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

The UV-visible absorption and fluorescence emission spectra of HPG functionalised with 2-(5-(4-dimethylaminobenzylidin)-4-oxo-2-thioxo-thiazolidin-3-yl) acetic acid were recorded in chloroform (figure 5.5). The absorption maximum (λmax) of the original dye was obtained at 464.5 nm and the signal was shifted to 494 nm on attaching to CD. The peak at 494 nm is due to n-π^* transition of the HPG supported chromophoric system. The fluorescence emission maximum for the functionalized CD system was observed at 524nm. The fluorescence emission efficiency and intensity were greatly enhanced on attaching to the cyclodextrin core system. On attaching to β-cyclodextrin core material, notable increase in wavelength and emission intensities were observed. On attaching the choromophore the energy gap of the molecular system reduced and this led to the formation of an excimer, and this is the reason for red shift. FT-IR spectrum of HPG functionalised with 2-(5-(4-dimethylaminobenzylidin)-4-oxo-2-thioxo-thiazolidin-3-yl)acetic acid was recorded in the solid state as KBr discs in the operating frequency range 4000–400 cm^{-1}(figure 5.6). FT-IR (KBr): 3325 cm^{-1}(broad): ν_{O-H}(str), 2927 cm^{-1}: ν_{C-H} of CH_2, 1708 cm^{-1}: $\nu_{C=O}$(str), 1568 cm^{-1}: $\nu_{C=C}$(str), 1344 cm^{-1}: ν_{C-N}(str), 1359 cm^{-1}: $\nu_{C=S}$(str), 891 cm^{-1}: ν_{C-S}(str). ^{1}H-NMR spectrum of the product was recorded in a 400 MHz instrument using $CDCl_3$ as solvent (figure 5.25). ^{1}H-NMR: 6.528 ppm(Ha, 2H, d), 7.764 ppm (Hb, 2H, d), 7.284 ppm(Hc, 1H, s), 5.013 ppm (Hd, 2H, s), and 3.107 ppm (NMe_2, 6H, s), 3.218-2.915ppm (OHgroup of HPG) 1.287-1.943ppm(aliphatic protons of HPG core). The COOH proton gave a signal at 9-11 ppm in the dye and this was absent in the coupled product due to complete esterification of the carboxylic group.

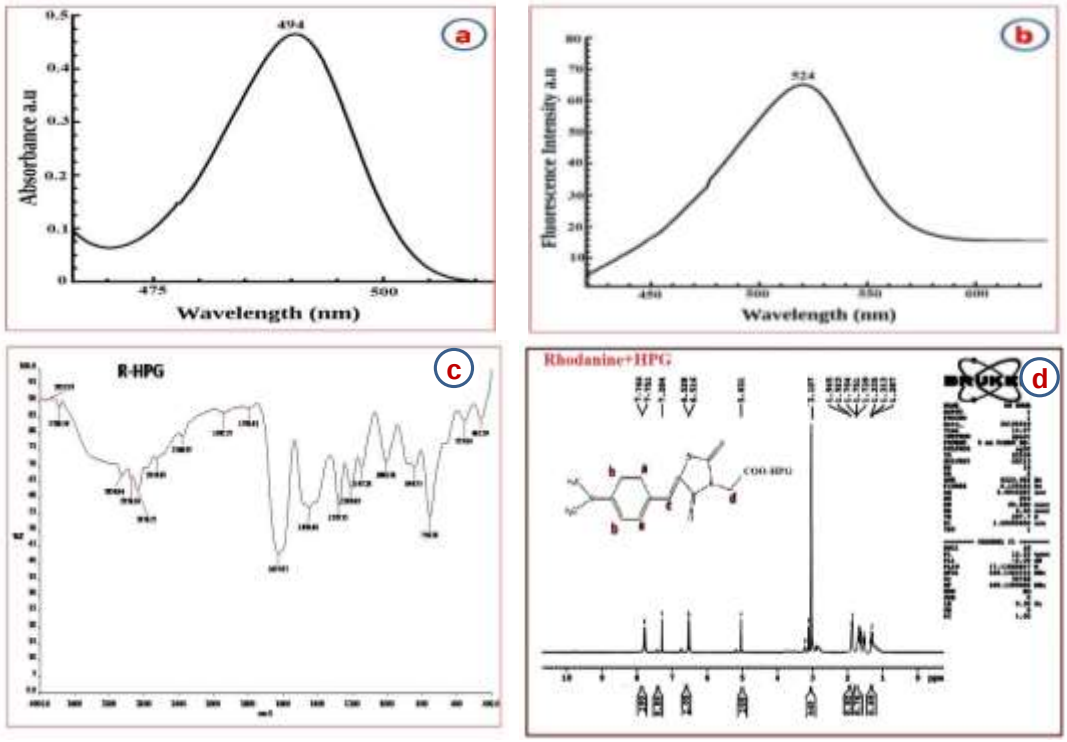
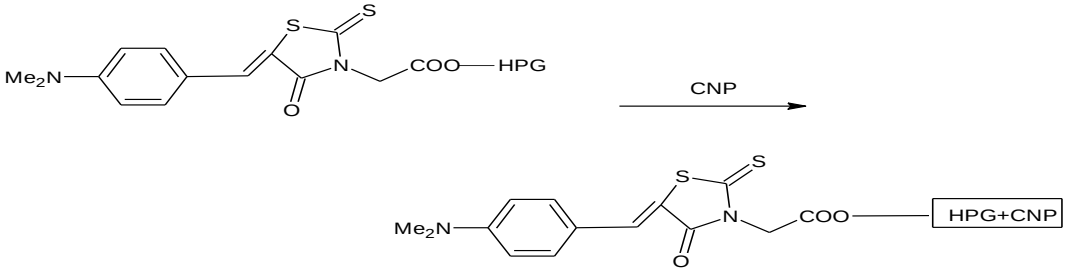


Figure 3. (a) UV-visible absorption (b) fluorescence emission (c) FT-IR and (d) ^{1}H-NMR spectra of HPG functionalised with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

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

CNPs in water and functionally modified HPG in chloroform were stirred at room temperature and HPG-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 hyperbranched polyglycerol functionalized with {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid

The UV-visible absorption and fluorescence emission spectra of CNP encapsulated in the cavities of HPG functionalised

with 2-(5-(4-dimethylaminobenzylidin)-4-oxo-2-thioxo-thiazolidin-3-yl) acetic acid were recorded in chloroform figure 5.9). The λmax of the system was found to be shifted from 494 nm to 510 nm on encapsulation of carbon nanoparticles. The red shift observed 16nm is due to the effect of incorporation of carbon nanoparticles. The fluorescence emission maximum for the functionalised HPG-CNPs complex was observed at 578nm. The enchanced fluorescence efficiency and intensity is due to the incorporation of CNPs in the functionalized HPG system. IR spectrum was recorded in the solid state as KBr discs in the operating frequency range 4000–400 cm^{-1}(Figure 5.10). IR(KBr): 3427 cm^{-1}(broad): ν_{O-H}(str), 2926 cm^{-1}: ν_{C-H} of CH_2, 1682 cm^{-1}: $\nu_{C=O}$(str), 1568 cm^{-1}: $\nu_{C=C}$(str), 1344 cm^{-1}: ν_{C-N}(str), 1373 cm^{-1}: $\nu_{C=S}$(str), 891 cm^{-1}: ν_{C-S}(str). ^{1}H-NMR spectrum of the product was recorded in a 400 MHz instrument using $CDCl_3$ as solvent (figure 10). ^{1}H-NMR: 6.743 ppm(Ha, 2H, d), 7.719 ppm (Hb, 2H, d), 7.492ppm(Hc, 1H, s), 4.897 ppm (Hd, 2H, s), and 3.112 ppm (NMe_2, 6H, s), 3.218-2.915ppm (OHgroup of HPG) 1.287-1.943ppm(aliphatic protons of HPG core). The COOH proton gave a signal at 9-11 ppm in the dye and this was absent in the coupled product due to complete esterification of the carboxylic group.

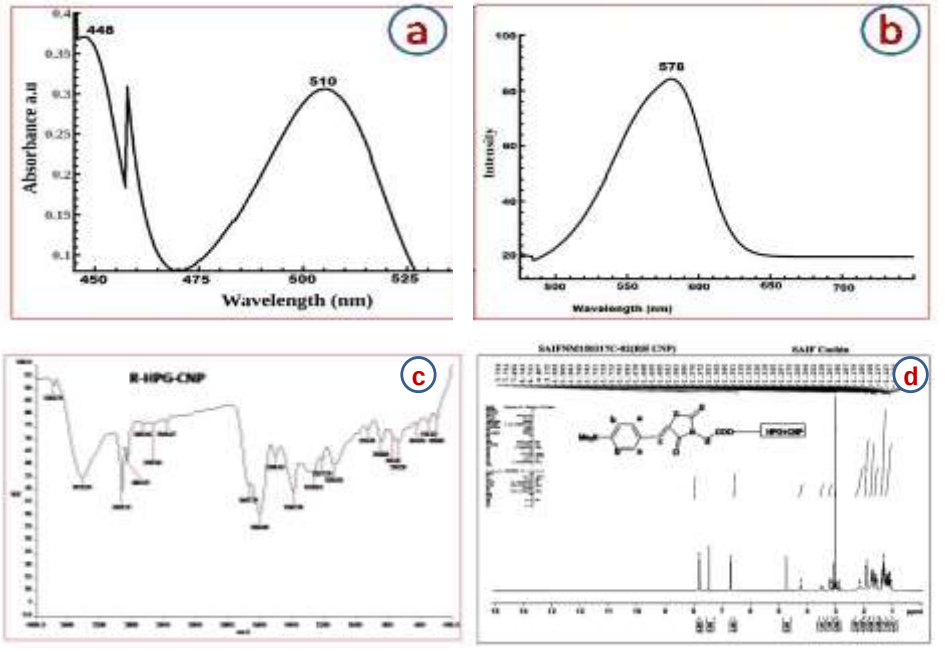


Figure 4. (a) UV-visible absorption (b) fluorescence emission (c) FT-IR and (d) ^{1}H-NMR spectra of functionally modified HPG–CNP system

(V) Photoinduced Antifungal activity of CNP and functionally modified HPG- CNP aggregates

The antifungal activity of CNP and functionally modified HPG-CNP systems were investigated against various pathogenic fungal strains such as Aspergillus niger, Aspergillus fumigates,

Aspergillus flavus, Penicillium janthinellum, and Mucor ramosissimus using well method. Antifungal activity of functionally modified HPG-CNP systems irradiated with light (1hr) also examined. Triplicates and control tests were also conducted. After incubation times the zone of inhibition was measured. 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 launed plate. The lowest concentrations which will inhibit the growth of fungal strain are considered as MIC. The minimum inhibitory concentrations (MIC) of CNP and R-HPG-CNP samples against Aspergillus niger, A. fumigatus and Aspergillus flavus was 300µg/ml and 200µg/ml respectively. For Penicillium janthinellum, and Mucor ramosissimus was 400µg/ml and 300µg/ml respectively. The minimum inhibitory concentrations (MIC) were examined by a micro dilution method. MIC results are shown in table 2.

Table 2. MIC values of CNP and functionally modified HPG-CNP systems

Fungal strain	MIC index of CNP					MIC index of Dye+HPG+CNP				
	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
A.niger	+	+	–	–	–	+	–	–	–	–
A.fumigatus	+	+	–	–	–	+	–	–	–	–
A.flavus	+	+	–	–	–	+	–	–	–	–
Penicilliu m jan…..	+	+	+	–	–	+	+	–	–	–
Mucor.ram	+	+	+	–	–	+	+	–	–	–

-: no growth; + : growth

The strains susceptible to functionally modified HPG-CNP system exhibited larger zone of inhibition in Aspergillus niger, and Penicillium janthinellum. Collision between nanoparticles and a fungal cell is unlikely to introduce direct physical damage. CNPs and functionally modified HPG-CNP 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. 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 HPG enhance the biological applications such as its antifungal activity. The results are presented in figure 5.

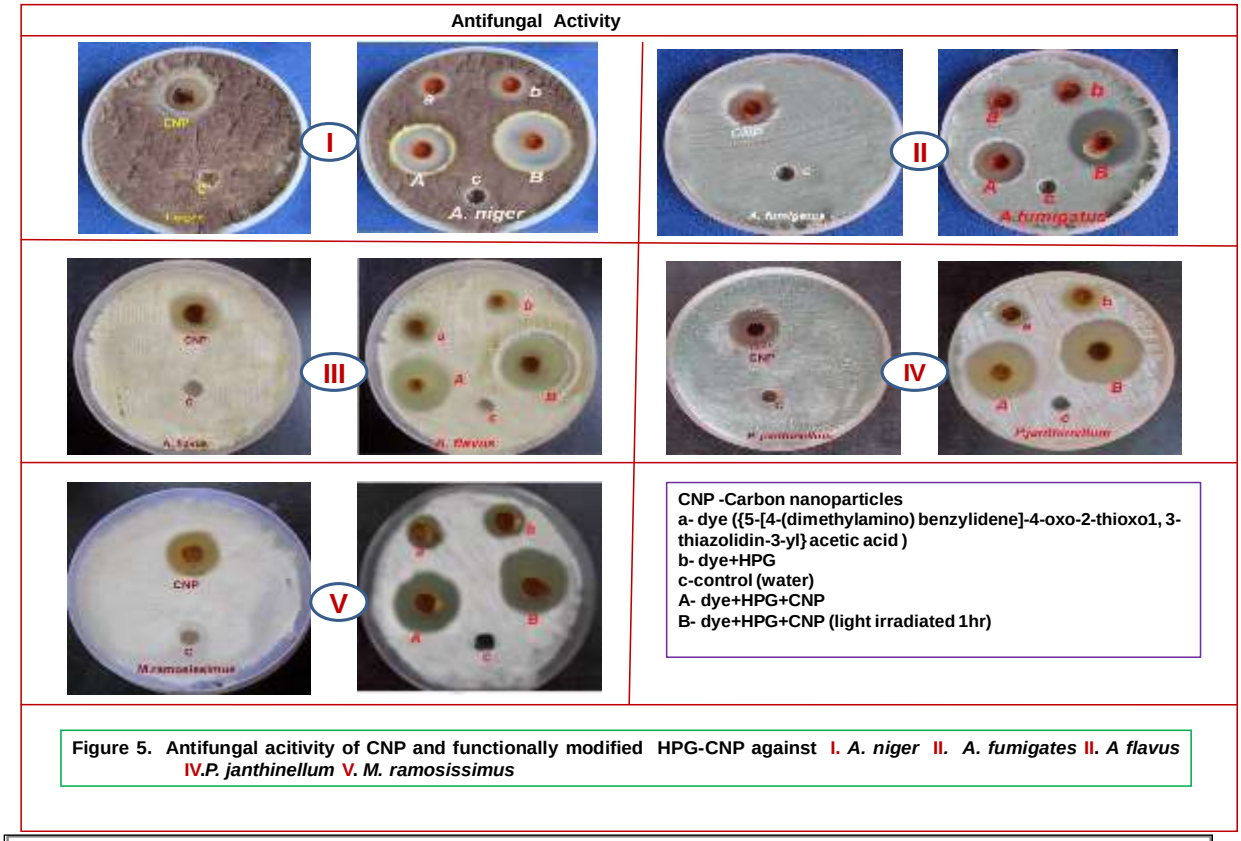


Figure 5. Antifungal acitivity of CNP and functionally modified HPG-CNP against I. *A. niger* II. *A. fumigates* II. *A flavus* IV.*P. janthinellum* V. *M. ramosissimus*

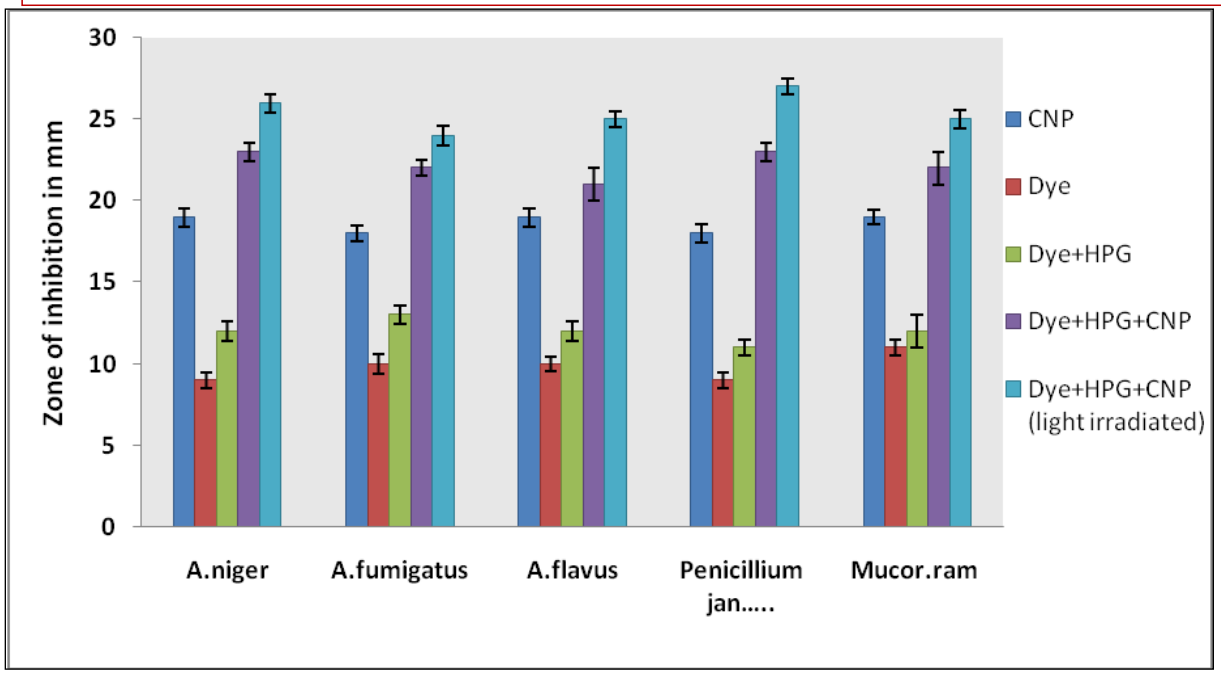


Figure 6. Antifungal activity {Results are the means of three repetitions ± standard deviation}

CONCLUSION

In this work we present a novel, effective and inexpensive nanosystem derived from natural sources by green methods, which is a highly efficient antifungal agent and can show biomedical applications. The present work gave thrust on synthesis of CNPs from natural sources such as kitchen soot and their encapsulation in the well defined cavities of functionally modified HPG aggregates. The photochromic group, {5-[4-(dimethylamino) benzylidene]-4-oxo-2-thioxo1, 3-thiazolidin-3-yl} acetic acid was introduced with a view to make the system able to absorb light and to combine the properties of nanoselectivity and photosensitivity. The newly developed systems were soluble in polar solvents, nature friendly, and green in their properties. The CNPs and functionally modified β-CD-CNP complex were characterized by SEM, XRD, TEM, UV/visible, fluorescence, FT-IR, NMR spectroscopic methods. Spectral studies showed that chromophoric system was successfully introduced in to the scaffolds of HPG aggregates, and CNP effectively encapsulated in to the functionally modified systems. The antifungal effects of CNP and functionally modified HPG-CNP system were tested against selected pathogenic fungal strains such as Aspergillus niger, Aspergillus fumigates, Aspergillus flavus, Penicillium janthinellum, and Mucor ramosissimus. Functionally modified HPG-CNP system demonstrated good antifungal activity. The antifungal activity of functionally modified HPG-CNP complex was greatly enchanced. This can provide dramatic effects in photodynamic therapy and in photodynamic antimicrobial applications. The work presented in this paper can be extended further to develop a series of antifungal agents of natural origin. This work explores the immense possibilities of our natural resources as biomedical and photodynamic agents if these are formulated into nanosized and nanostructured materials.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGEMENT

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REFERENCE

1. Georgakilas, V., Perman, J. A., Tucek, J., & Zboril, R. (2015). Broad Family of Carbon Nanoallotropes: Classification, Chemistry, and Applications of Fullerenes, Carbon Dots, Nanotubes, Graphene, Nanodiamonds, and Combined Superstructures. Chemical reviews.
2. Ray, S. C., Saha, A., Jana, N. R., & Sarkar, R. (2009). Fluorescent carbon nanoparticles: synthesis, characterization, and bioimaging application. The Journal of Physical Chemistry C, 113(43), 18546-18551.
3. Li, H., He, X., Liu, Y., Huang, H., Lian, S., Lee, S. T., & Kang, Z. (2011). One-step ultrasonic synthesis of water-soluble carbon nanoparticles with excellent photoluminescent properties. Carbon, 49(2), 605-609.
4. Liu, H., Ye, T., & Mao, C. (2007). Fluorescent carbon nanoparticles derived from candle soot. Angewandte Chemie International Edition, 46(34), 6473-6475.
5. Yang, Y., Yu, Z., Nosaka, T., Doudrick, K., Hristovski, K., Herckes, P., & Westerhoff, P. (2015). Interaction of carbonaceous nanomaterials with wastewater biomass. Frontiers of Environmental Science & Engineering, 1-9.
6. Veerapandian, M., & Yun, K. (2011). Functionalization of biomolecules on nanoparticles: specialized for antibacterial applications. Applied microbiology and biotechnology, 90(5), 1655-1667.
7. Yan, D., Gao, C., & Frey, H. (Eds.). (2011). Hyperbranched polymers: synthesis, properties, and applications (Vol. 8). John Wiley & Sons.
8. Irfan, M., & Seiler, M. (2010). Encapsulation using hyperbranched polymers: from research and technologies to emerging applications. Industrial & Engineering Chemistry Research, 49(3), 1169-1196.
9. Sun, F., Luo, X., Kang, L., Peng, X., & Lu, C. (2015). Synthesis of hyperbranched polymers and their applications in analytical chemistry. Polymer Chemistry, 6(8), 1214-1225.
10. Liu, X., Li, H., Xu, Z., Peng, J., Zhu, S., & Zhang, H. (2013). Development of hyperbranched polymers with non-covalent interactions for extraction and determination of aflatoxins in cereal samples. Analytica chimica acta, 797, 40-49.
11. Chandran, A., Kuriakose, S., & Mathew, T. (2012). Thermal and Photoresponsive Studies of HPG Modified with 2-(5-(4-Dimethylamino-benzylidine)-4-oxo-2-thioxo-thiazolidin-3-yl) acetic Acid. International Journal of Carbohydrate Chemistry, 2012.
12. Cao, L., Wang, X., Meziani, M. J., Lu, F., Wang, H., Luo, P. G., & Sun, Y. P. (2007). Carbon dots for multiphoton bioimaging. Journal of the American Chemical Society, 129(37), 11318-11319.

13. Varghese, S., & Kuriakose, S. (2014) Synthesis, Characterization and studies on Antifungal Activity of Carbon Nanoparticles from Natural Sources. European Journal of Biomedical and Pharmaceutical Sciences, Vol. 1, 204-214.
14. Nieddu, M., Rassu, G., Boatto, G., Bosi, P., Trevisi, P., Giunchedi, P., & Gavini, E. (2014). Improvement of thymol properties by complexation with cyclodextrins: In vitro and in vivo studies. Carbohydrate polymers, 102, 393-399.
15. Chen, Z. H., Zheng, C. J., Sun, L. P., & Piao, H. R. (2010). Synthesis of new chalcone derivatives containing a rhodanine-3-acetic acid moiety with potential anti-bacterial activity. European journal of medicinal chemistry, 45(12), 5739-5743.
16. Alizadeh, A., Rostamnia, S., Zohreh, N., & Hosseinpour, R. (2009). A simple and effective approach to the synthesis of rhodanine derivatives via three-component reactions in water. Tetrahedron Letters, 50(14), 1533-1535.
17. Yüce, A. O., Solmaz, R., & Kardaş, G. (2012). Investigation of inhibition effect of rhodanine-N-acetic acid on mild steel corrosion in HCl solution. Materials Chemistry and Physics, 131(3), 615-620.
18. Foley, S., Crowley, C., Smaihi, M., Bonfils, C., Erlanger, B. F., Seta, P., & Larroque, C. (2002). Cellular localisation of a water-soluble fullerene derivative.Biochemical and biophysical research communications, 294(1), 116-119.
19. Benincasa, M., Pacor, S., Wu, W., Prato, M., Bianco, A., & Gennaro, R. (2010). Antifungal activity of amphotericin B conjugated to carbon nanotubes.ACS nano, 5(1), 199-208.
20. Sunder, A., Hanselmann, R., Frey, H., & Mülhaupt, R. (1999). Controlled synthesis of hyperbranched polyglycerols by ring-opening multibranching polymerization. Macromolecules, 32(13), 4240-4246.