STUDIES ON THE BIOMEDICAL APPLICATIONS OF CARBON NANOPARTICLES-DISPERSED IN MACROMOLECULAR SCAFFOLDS

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INTRODUCTION

Nanoparticles are at the leading edge of the rapidly developing field of nanotechnology. Their unique size-dependent properties make these materials superior and indispensable in many areas of human activity. Once particle becomes small enough they start to exhibit quantum mechanical behavior. Materials reduced to the nanoscale can show different properties compared to what they exhibit on a macroscale, enabling unique applications. The increase in the surface area to volume ratio, which happens as the particle get smaller, leads to an increase dominance of the behavior of atoms on the surface of a particle over that of those in the interior of the particle. The large surface area of nanoparticle also results in a lot of interactions between the inter mixed materials in nanocomposites, leading to special properties such as increased strength, increased chemical properties and increased heat resistance.

Biomedical monitoring applications have taken considerable advantage of using nanoparticles for sensitive optical imaging in fixed cells and tissues, living cells and animal models. Electronic applications of nanoparticles are envisaged in future high-speed electronic and photonic devices. Nanoparticles provide a promising way forward for a new generation of lasers infrared photo detectors photovoltaic devices and optical data storage media. Nanotechnology will fundamentally restructure the technologies currently used for manufacturing, medicine, communication, computation, transportation and many other application areas.

Carbon is responsible for creating the most diverse variety of compounds. It has more allotropes than any other elements. The most recent addition to the list is fullerenes and carbon nanotubes. The sp^2 hybridised state of carbon makes two-dimensional structures and the most studied of is its allotrope, graphite. The other well- known allotrope, diamond has sp^2 hybridized atoms. The two-dimensional sheets made of sp^2 hybridized carbon can curl, just like a piece of paper, and makes cylinders. By using

hexagons alone, carbon cannot yield closed three-dimensional structures. The inclusion of pentagons results in a closed-cage structure; at least six pentagons are needed on each sides of the cylinder, thereby making a closed pipe. This is called a carbon nanotube as the diameter of such a tube is typically in the nanometer range.

The present work is aimed to give thrust on the isolation, characterization and antimicrobial studies of carbon nanoparticles of natural origin. The emphasis is given to explore the chemistry and biology behind the medicinal application of carbon soot which was widely and popularly used as an antiseptic from ancient days. To explore the science behind this, we designed method to collect carbon soot from natural origin, isolation of carbon nanoparticles from the collected soot, synthesis of carbon nanoparticles by simple chemical methods, characterization of CNP using UV/visible spectroscopy, XRD, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), stabilization of CNP in aggregates made up of macromolecular scaffolds and the studies on the antimicrobial action of CNP against various bacterial and fungal strains.

OBJECTIVES

The main objectives of the work are:

1. *Synthesis of carbon nanoparticles from natural sources such as kitchen soot.*
2. *Characterization of carbon nano systems by UV-visible and fluorescence spectroscopic methods, scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction studies (XRD) and thermogravimetric techniques such as TGA-DSC.*
3. *Encapsulation of CNPs into the scaffolds of dendritic polymers or macromolecular systems such as HPG and starch and in the well defined cavities of supramolecular systems such as β-CD.*
4. *Characterization of the CNP-encapsulated HPG, β-CD, and starch by UV-visible, fluorescence, FTIR and ^{1}HNMR spectroscopic studies and SEM and thermogravimetric techniques such as TGA-DSC.*

5. *Studies on the antimicrobial properties of CNPs, HPG-CNP, β-CD-CNP and starch-CNP aggregates.*

MTHODOLOGY

Carbon nanoparticles were isolated from the soot that deposit on the glass plate by the burning of wood. Glass plates of square shape was cut and kept above hearth with a string and smoked continuously. Natural wood were used for the burning purpose. The soots at different intervals were collected by scratching the glass plate and were stored for further use by wrapping in aluminum foil. Its solubility was tested with different solvents, and was found to be soluble in methanol. By centrifuging the solution, the undissolved part was removed. The solvent was removed by vacuum rotatory evaporator

The carbon nanoparticles obtained were characterized by scanning electron microscopy (SEM), XRD and UV visible spectroscopy. Surface analysis of the carbon nanoparticles was carried out by scanning electron microscopy in order to investigate the morphology of the particle. UV/visible analysis were carried out on a Shimadzu-160 UV/Vis spectrophotometer operating in the range 190-1100 nm. The UV visible spectrum was recorded by suspending the nanoparticles in methanol. The carbon nanoparticle was dissolved in methanol and subjected to UV visible spectral analysis. The spectrum showed peaks at 240 nm, and 439 nm. The peak at 240 is due to π-π^* transition. The maximum absorbing wavelength was in the visible region around λ_{max} 439 nm and this is due to the surface plasma resonance of carbon.

The UV absorption of carbon nanoparticles is caused by electronic transitions between the bonding and antibonding orbitals. The (σ-σ^*) transitions are expected to produce a band in the FUV, peaking between 60 and 100 nm, whereas the (π-π^*) transitions provide an absorption maximum located in the range between 180 and 280 nm. The incorporation of hydrogen into the internal structure of carbon black leads to an increase of the fraction of sp^3 hybridized carbon. The position of the (π-π^*) transition is extremely sensitive to very small changes of the preparation conditions, which correspond to small variations in the internal electronic structure of the carbon nanoparticles. There have been only a limited number of systematic experimental

investigations on the relation between the internal structure and their UV absorption behavior. The width of the peak depends also on the state of agglomeration.

It was also aimed to explore the synthesis of CNP-macromolecular and CNP-supramolecular aggregates and to study the stability of these aggregates. This study focused on three core systems such as a dendritic polymer like hyperbranched polyglycerol (HPG) a macromolecular system such as starch and a supramolecular host system like β-cyclodextrin (β-CD). The incorporation of nanoparticles into the scaffolds or cavities of dendritic systems and other macromolecular or supramolecular systems offer improved stability, better physicochemical properties and wide application in biomedical fields. CNPs encapsulated in the channels of macromolecules can prevent CNP from aggregation. These core systems have scaffolds or cavities which are suitable for nanoparticles.

CNPs were encapsulated into HPG, β-CD and starch by stirring at room temperature. Characterizations of the products were done by UV-visible, NMR, IR and fluorescence spectroscopic methods, SEM, XRD and TGA-DSC analysis. Antibacterial activity of different samples of CNPs were tested against *S.haemolyticus, S. aureus, Bacillus cereus, V. parahaemolitics V. cholera, Proteus refrigere, K. pneumonia, S. marcescens, E.Coli (MTCC1687)* and *Pseudomonas aeruginosa* by Agar disc diffusion method. Antibiotics were used as positive control in antibacterial studies. The 'Agar well method' was used to test the antifungal activity. For antifungal study, fungal strains, such as *Aspergillus niger, Aspergillus fumigates, Aspergillus flavus, Penicillium janthinellum, Mucor ramosissimus, Neosartriya fischeri, Paecilomyces variotii, Microsporum cookie, Gliocladium species and Epidermophyton floccosum* were selected.

IMPORTANT RESULTS

CNPs-HPG, CNPs-β-CD and CNPs-starch inclusion complexes were prepared by phase transfer mechanism. The λmax and fluorescence emission maximum of CNP was found to be at 412 nm. The λmax was recorded at 432 nm, 424 nm and 429 nm on encapsulating CNP in HPG, β-CD and starch respectively. The size of CNP was found to be in the range of 2nm-20nm. DSC-TGA studies indicated that the products are thermally stable for very long period.

CNP-HPG, CNP-CD and CNP-starch aggregates showed enhanced absorption and emission characterists. The λ_{max} of 412 nm obtained in the UV-visible absorption spectrum of CNPs was shifted to 432 nm, 424 nm and 429 nm on encapsulating in the scaffolds or cavities of HPG, β-CD and starch respectively. The corresponding red shifts of 20 nm, 12 nm and 17 nm are due to the incorporation of CNPs in HPG, β-CD and starch. The peaks observed in the UV-visible spectrum is assigned as various π-π* transition of core-CNP aggregates. The fluorescence emission observed at 433 nm for CNPs was shifted to 454 nm, 443 nm and 448 nm on HPG-CNP, β-CD-CNP and starch-CNP systems respectively. The corresponding enchancement of fluorescence emission maxima were 21 nm, 10 nm and 15 nm are due to the encapsulation of CNPs in HPG, β-CD and starch aggregates respectively. SEM, TEM and XRD analysis HPG-CNP, β-CD-CNP and starch-CNP aggregates showed no apparent change in size or shape of the nanoparticles on encapsulation. The TEM photograph confirms nanoscale structure of carbon nanoparticles. The results show that the physical properties of nanoparticles are completely conserved on encapsulation in the core systems indicating enhanced stabilization of the CNPs. TGA-DSC analysis further indicated that the complete thermal decomposition of CNP-HPG, CNP-CD and CNP-starch aggregates were observed at 480°C, 535°C and 510°C respectively. The thermal stability of these CNP-core aggregates was much better than simple CNP (460°C).

One important objective of the present work was to investigate the antibacterial and antifungal activities of the newly developed seven different carbon nanosystems viz, HPG-CNP, β-CD-CNP and starch-CNP and simple CNP. The unique chemical, physical and biological characteristics, electronic structure, size and surface area of nanoparticles are responsible for their antimicrobial action. We could improve the stability of chromophoric systems by incorporating these moieties onto macromolecular backbones and it enhances the stability and light absorption properties. The carbon nanoparticles encapsulated in HPG, β-CD and starch seem to be promising and effective photoactive antimicrobial agents against the multidrug resistant strains of bacteria and fungi.

The antibacterial activity and minimum inhibitory concentrations of CNPs were investgated on ten different bacterial strains such as *Streptococcus haemolyticus* (+ve), *Staphylococcus aureus* (+ve), *Bacillus cereus* (+ve), *Vibrio parahaemolitics* (-ve), *Vibrio*

cholera (-ve), *Proteus refrigere* (-ve), *Klebsiella pneumonia* (-ve), *Serratia marcescens* (-ve), *E.Coli MTCC1687* (-ve) and *Pseudomonas aeruginosa* (-ve). The antibacterial activities of the samples were examined by disc diffusion method. 200µlts of the sample solutions (5mg/ml) were poured into the disc and it was applied in the lawned plate. After incubation times, the zones were measured. The diameter of inhibition zone reflects magnitude of susceptibility of microbes. The strains susceptible to CNP exhibited larger zone of inhibition, while resistant strains exhibit smaller zone of inhibition. CNPs exhibited larger zone of inhibition in *Staphylococcus aureus* (19.33±0.471 mm), *Streptcoccus haemoliticus* (18.66±0.577 mm), *Klebsiella pneumonia* (18.66±0.577 mm) and *E.ColiMTCC1687* (19.00±1.00 mm) while least sensitivity towards *Vibrio parahaemolitics* and *Vibrio cholera* (13.00±1.00 mm) among the tested microbes. The inhibition zones were measured and it was found that the CNPs from natural sources are active against these Gram negative and Gram positive bacterial strains.

MIC of the CNP sample was examined by a micro dilution method. 200µl solutions of fraction of samples (100µg/ml, 200µg/ml, 500µg/ml, 750µg/ml and 1000µg/ml) were added in the disc in the lawned plate. The lowest concentrations that would inhibit the growth of bacterial strain were taken as MIC. MIC of CNP samples against *Streptcoccus haemoliticus* (+ve), *Staphylococcus aureus* (+ve), *Klebsiella pneumonia* (-ve) and *E. coli* (-ve) was 500µg/ml. For *Proteus refrigrie, Serratia marcescens and Pseudomonas aeruginosa* was 1000 µg/ml and above 1000 µg/ml for *Bacillus cereus* (+ve), *Vibrio parahaemolitics* and *Vibrio cholera* (-ve). According to zone of inhibition and MIC value of bacterial strains, Gram positive *Staphylococcus aureus* and *Streptcoccus haemoliticus* and Gram negative *Klebsiella pneumonia* and *E.ColiMTCC1687* were selected for antibacterial studies of newly developed carbon nanosystems.

The antibacterial effects and MIC of HPG-CNP, CD-CNP and starch-CNP aggregates were investigated against four selected different pathogenic bacterial strains such as *E. coliMTCC1687 (-ve), Klebsiella pneumonia (-ve), Streptcoccus haemoliticus* (+ve) and *Staphylococcus aureus* (+ve) using disc diffusion method and microdilution methods respectively. Results showed that HPG-CNP system showed excellent activity and exhibited larger zone of inhibition in *Streptococcus haemolyticus* (21.66±0.577) and *Staphylococcus aureus* (21.00±1.00) whereas β-CD-CNP system showed highest activity

towards *Klebsiella pneumonia (*21.66±0.577) and starch-CNP aggregates showed higher activity towards *E.ColiMTCC1687 (*21.33±0.471). The antibacterial effects of all the three CNP-core aggregates were enhanced significantly in inhibitory zone (≤3mm) on encapsulation of CNP in the cavities or scaffolds of the core systems. The MIC values of 500µg/ml obtained in CNPs against all the four bacterial strains were changed to 400µg/ml for CNP-core aggregates. The results showed that all the three systems are effective to control bacterial infections. Carbon nanoparticles embedded in the polymer matrix exhibited noticeable antibacterial properties and it can certainly be marked down as a vital development in biomedical field.

CONTRIBUTION TO THE SOCIETY

The results obtained from this minor research project may find potential applications in medical and biomedical fields since the systems developed in this project are active against a series of pathogenic bacteria. Bacterial infection and increased resistance of bacteria towards antibiotics are major problems in recent years and developing new antimicrobial systems are challenges in the present scenario.

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