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Facile synthesis of carbon dot and residual carbon nanobeads: Implications for ion sensing, medicinal and biological applications

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ABSTRACT

Synthesis of carbon dots (Cdots) *via* chemical route involves disintegration of carbon materials into nano-domains, wherein, after extraction of Cdots, the remaining carbon material is discarded. The present work focuses on studying even the leftover carbon residue namely, carbon nanobeads (CNBs) as an equally important material for applications on par with that of carbon dot. It employs oxidative treatment of carbonised gum olibanum resin (GOR) to produce the carbons namely Cdots and CNBs (as the residue). The Cdots (~5–10 nm) exhibit blue-green fluorescence with an optical absorption at ~300 nm unlike the CNBs (40–50 nm) which fail to exhibit fluorescence. The fluorescence behaviour exhibited by Cdots were utilized for heavy metal ion sensing of Pb^{2+}, Hg^{2+} and Cd^{2+} ions in aqueous media. Interestingly, both Cdots and CNBs are biocompatible to normal cell lines but cytotoxic to cancer cell lines, observed during several *in vitro* experiments (cell viability assay, cell cycle assay, apoptosis assay, ROS determination assay, caspase-9 activity assay). Additionally, Cdots exhibit bright green fluorescence in B16F10 cells. The Cdots and CNB's demonstrate multifunctional activities (sensor, cellular imaging and cancer therapy) in biomedical applications.

1. Introduction

Carbon, due its exceptional thermal, mechanical and electrical properties, serves as interesting and promising material for material scientists [1–3]. The never-ending search for better materials with superior properties has catered the development of several new carbon allotropes unknown before. The present research mainly focuses on obtaining materials that can add additional functionalities to carbon nanomaterials like fluorescence, water solubility or high conductivity depending on the end application [4]. As a result, application specific synthesis or modification to carbon nanomaterial is employed so as to compensate for the deficient property in the original material. Amongst many carbon nanomaterials developed, Cdots are a relatively new addition, which have secured a significant place in the carbon family [5]. They are superior to semi-conductor quantum dots due to having low toxicity, better biocompatibility and chemical inertness [6–11]. They are also easy to synthesize, cost effective, resistant to photo-bleaching and exhibit tunable size-dependent luminescence. Many methods have been proposed for the synthesis of Cdots including laser ablation of graphene, electrochemical treatment of graphite, thermal oxidation of molecular precursors, proton beam irradiation of nanodiamond, acid treatment on candle soot and oxidative treatments on camphor soot [2,3,8–17]. They exhibit potential applications in bio-imaging, solar cells and medical diagnostics. Further, several reports are available wherein Cdots are utilized for drug delivery or as theranostics agents for different biomedical applications [8,10,11, 13,14,18,19].

The synthesis of Cdots *via* chemical routes involves disintegration of carbon materials into particles of smaller domains. Once Cdots are obtained the remaining carbon material is discarded. It is innovative of the present study to consider the left over carbon as a useful resource and utilise it for biological and medicinal applications. In this regard the present work analyses the CNBs which are obtained after the filtration of carbon dots. The CNBs are black in colour and quasi spherical. Their size ranges from ~40–50 nm. They do not exhibit fluorescence. The π-π interactions between the mutual carbon nanoparticles make them stack like a group of beads attached together. Sharon et al. has reported CNBs formation from camphor *via* pyrolysis, suitable for energy applications [20]. In another report, synthesis of carbon nanoparticles from camphor has been reported by Gaddam et al. [1]. These nanobeads exhibit wide range of applications in electronics, coatings, textile, solar

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cells, batteries, drug delivery, bio-imaging *etc.* [12] The CNBs and Cdots are generally reported to be synthesized individually under different conditions. However, the present work reports simultaneous synthesis of CNBs and Cdots from carbonised gum olibanum resin (GOR) as the materials were obtained from the same precursor by a simple filtration step to separate them and no loss of compound elsewhere were observed. The properties of the obtained carbon materials are dependent on the geometry and surface functional groups. Here, the starting material is bio-sourced polymeric gum extruded from the incense tree *Burseraceae* [21]. In the classical Indian medicine it is used as an *anti*-inflammatory remedy and several recent studies have shown a positive impact of the gum in rheumatism and anti-cancer activity [22]. From the standpoint of sustainability, carbon materials derived from *gum olibanum* eradicates the reliance on fossil fuel precursors for material fabrication. It would be worthwhile to mention that this is the first report on the simultaneous preparation of carbon dot and carbon nanobeads from GOR.

In the current report GOR is initially prepared from GO and then carbonised. The obtained product is treated with piranha solution. The ash of the gum is treated with oxidising agents and filtered out to obtain Cdots and CNBs. The formations of these materials are confirmed from microscopic and spectroscopic analysis. The fluorescent properties were studied and the effect of Cdots in sensing heavy metal ions was investigated. Further, we checked the *in vitro* cytotoxicity of both CNBs and Cdots in different normal and cancer cells. Both CNBs and Cdots show excellent biocompatibility in normal cells. However, they exhibit cytotoxicity in cancer cells. Furthermore, Cdots exhibit bright green fluorescence inside live cells, which can be utilized for live cell imaging. The results altogether suggest that Cdots demonstrate multifunctional activities (sensor, cellular imaging, and cancer therapy) in biomedical applications.

2. Experimental procedures

2.1. Materials

Gum olibanum (GO) was obtained from the local market. Sulphuric acid (98%), 30% hydrogen peroxide solution (AR), Lead acetate, Mercuric chloride (AR grade, 99.5% pure) and Cadmium Chloride (AR grade, 99.9% pure) were purchased from SD Fine chemicals, Mumbai. All the chemicals were used as received without any purification.

2.2. Extraction of resin from gum-olibanum (GO) powder

12 g of GO was powdered and solubilised in hexane. The solubilised material was placed in a round bottom flask and stirred under inert atmosphere for 48 h. Subsequently, the mixture was filtered out and the hexane from the mixture evaporated under reduced pressure. A highly viscous honey coloured material was initially obtained which was immediately transferred onto a Teflon dish and allowed to cool. It was then dried for 28 h in a vacuum oven. Finally, 10 g of bright yellow solid coloured resin (GOR) was obtained.

2.3. Synthesis of carbon nanobeads and carbon dots

The bright yellow coloured solid GOR obtained in the previous step was then crushed and taken into a 500 mL round-bottom flask and heated to around 350–400 °C to ensure complete degradation of the material. The charred GOR was then refluxed with 45 mL of piranha solution (an oxidative mixture of sulphuric acid and hydrogen peroxide in 7:3 ratio) for 5 h. Subsequently, the mixture was diluted with deionised water and filtered using a Whatman filter paper, Grade 93 with a pore size of 10 μm. The CNBs remained as black coloured residue on the filter paper while the Cdots were obtained as the bright yellow filtrate. The CNBs were washed with water repeatedly, then with acetone and finally dried in vacuum oven for 48 h. The Cdot solution was neutralised with sodium hydroxide which lead to the formation of salt. The salt was removed by evaporation of the dispersion and then the re-dispersion of the Cdots in N,N dimethyl formamide. Then the DMF was filtered out and the filtrate was evaporated to get pure Cdots. The Cdots were then redispersed in water for further analysis.

2.4. Preparation of salt solutions

AR samples of mercuric chloride, cadmium chloride and lead acetate were used for studies on fluorescence quenching of carbon dots upon addition of these metal ions. A 0.1 M stock solution of each of these substrates was prepared. 2 mL solution of Cdots was taken in the cuvette for fluorescence measurement. To this, increasing amounts of Hg^{2+}, Pb^{2+} and Cd^{2+} (0.5, 1, 1.5 mL and so on) were added as the case may be. With each addition, the solution was stirred and the fluorescence measured.

2.5. Characterization techniques

Fourier Transform Infrared (FTIR) spectroscopy was recorded using Thermo Nicolet Nexus 670 spectrometer. Fluorescence spectroscopy was performed with a Horiba Fluoromax 4 spectrophotometer at different excitation wavelengths. UV–vis absorption spectra were obtained using a Shimadzu 220 V (E) UV–vis spectrophotometer. Transmission electron micrographs (TEM) were recorded on a JEOL JEM-100CX electron microscope. The fluorescence intensity of biological samples was measured using spectrofluorometry (Biotek Synergy).

2.6. Cell culture experiments

All cell lines [NIH-3T3: mouse fibroblast cell lines, CHO: Chinese hamster ovarian cell lines, B16F10: murine melanoma cancer cell lines and MDA-MB-231: human breast epithelial cancer cell lines] were purchased from ATCC. All normal and cancer cell lines were cultured in DMEM (Dulbecco's Modified Eagle Medium) media supplemented with 10% fetal bovine serum (FBS), penicillin-streptomycin antibiotics (50 mg each/Lt) and 5% L-glutamine, in a humidified 5% CO_2 incubator at 37 °C.

2.7. Cell viability assay using MTT reagent

All samples were kept under UV irradiation for 15–20 min before doing *in vitro* experiments. Cells (1×10^4) were seeded in 96 well plates and kept for 24 h in the CO_2 incubator to grow. Cell viability assay was carried out by incubating NIH-3T3, CHO, B16F10 and MDA-MB-231 cells using Cdots and CNBs in a dose dependent manner (Cdots: 130–1300 $\mu g \cdot mL^{-1}$; CNBs: 100–1000 $\mu g \cdot mL^{-1}$) for 24 h using MTT reagents according to already published protocol [21,23]. Results were calculated and expressed as percent of cell viability = $([A570_{(\text{treated cells}) - \text{background}}] / [A570_{(\text{untreated cells}) - \text{background}}]) \times 100$.

2.8. Cell imaging study using fluorescence microscopy

B16F10 cells (2×10^4) were seeded in 24 well plates and kept for 24 h to grow in the CO_2 incubator. These cells were incubated with Cdots (1 $mg \cdot mL^{-1}$) for 12 h. All the treated cells and control untreated cells were extensively washed by phosphate buffer saline (PBS; pH = 7.4) for 4 times and kept in Hank's Balanced Salt Solution (HBSS buffer; pH = 7.4). Finally, the fluorescence images were monitored and captured in a fluorescence microscope (Nikon Eclipse TE2000-E). The green fluorescence emission (λ_{Em} = 525 nm) was collected with a 10× and 20× microscope objective after excitation at λ_{Ex} = 420–495 nm [24].