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ORIGINAL ARTICLE

Biogenic Synthesis of Fluorescent Carbon Dots at Ambient Temperature Using Azadirachta indica (Neem) gum

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Abstract Synthesis of fluorescent Carbon Dots (CDs) from various carbonaceous materials apparently has acquired lots of interest amongst researchers as the corollary of the properties of CDs; which are subsequently getting unveiled. In this study we report the use of *Azadirachta indica* (Neem) Gum as a novel natural pre-cursor for synthesis of CDs at room temperature. Water soluble CDs of around 5–8 nm were obtained after treatment of the gum with ethanol and NaOH. These CDs exhibited green fluorescence in UV-light (λ = 365 nm). These CDs were found to be stable, having many bio-linkers attached on their surface, making it suitable for drug attachment and hence can serve as potential candidates for applications like drug delivery vehicles as well as for biosensors.

Keywords *Azadirachta indica* · Carbon-dots · Carbon materials · FTIR · Nanoparticles

Introduction

The word quantum dots (QD) usually implies to quantum dots of metals like CdSe, CdS etc. Usually they are synthesized by chemical methods [1, 2], which accounts for presence of heavy metals in QDs making them unfavourable to be used for Bioimaging. Moreover, such QDs are found to have high cytotoxicity [3, 4].

After the discovery of Carbon Dots (CDs) in 2004 [5], it has fetched lot of attention because of its properties like small size, photoluminescence, biocompatibility, water solubility and excellent stability. Till date these properties of CDs have been explored in various fields like drug delivery [6], gene delivery [7], bio-imaging [8], biosensors [9] etc. Biogenically synthesized CDs have been proved to be worthy over chemically synthesized CDs [10], as it overcomes several drawbacks of Quantum Dots (QDs). There are many reports that focus on the low toxicity of CDs and excellent Photoluminescence properties of biogenic CDs, which are idyllic for imaging application [10–12].

Till date, many studies have reported synthesis of CDs by Physical, Biological as well as Chemical approach using various methods e.g. Combustion/Microwave assisted synthesis [13], Laser ablation/Passivation [14], supported synthesis [15] and electrochemical oxidation [12]. However, these methodologies require use of expensive instruments, chemicals and rigorous experimental conditions as well as these produce a low yield of CDs which makes them tedious and unfavourable. Additionally, many of the chemicals used are environmentally detrimental and hence additional precautions are needed to be taken. Ample of studies reported CDs synthesis at higher temperature using above mentioned methods [13–15] however, so far only our lab has reported CDs synthesis at room temperature [16].

Of late, natural pre-cursors like Sugar cane juice [16], Orange juice [17], Watermelon peel [18], *Trapa bispinosa* peel [19], Bread [20], Rice husk [21] have been used for production of eco-friendly CDs because of their high carbon content. These CDs get coated with natural linkers onto whom

Chinmay Phadke and Ashmi Mewada have equal contribution.

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molecules can be directly loaded for the drug delivery purpose without the need of surface functionalization. CDs prepared from natural materials are found to be thermodynamically stable, biocompatible and cost effective for bulk production and naturally functionalized with peptides [22]. Being stable, they can be used in drug delivery applications, where release of the drug is ought to be sustained [23].

In this study we report a simple, eco-friendly and cost effective method of synthesizing fluorescent CDs using novel natural pre-cursor "Azadirachta indica gum" at ambient temperature. CDs obtained were purified by conventional dialysis method against nanopure water in order to use them for further studies.

Materials and Methods

Materials

Neem gum (*A. indica*) was obtained from a tree growing in the institute campus. Nano-pure water was obtained from Merck Specialities Pvt. Ltd. Ethanol was purchased from Jiangsu Huaxi International Trade Co. Ltd. Sodium hydroxide was purchased from Fisher Scientific India Pvt. Ltd. To avoid contaminations, all the glasswares were prewashed with chromic acid and rinsed thrice with deionised water.

Synthesis of CDs

1 g of Neem gum was dissolved in 20 ml of nano-pure water and to it 40 ml of 1 M sodium hydroxide and absolute ethanol in ratio 1:1 was added. This reaction mixture was incubated at RT for 3 h. After incubation the brown solution was observed under UV-light (λ = 365 nm).

Purification of CDs

Purification of CDs was achieved by conventional dialysis method. Pre-activated dialysis bag having pore size ~20 nm was used and crude CDs solution was dialysed against nanopure water with constant mild stirring for 3 h. The dialyzed solution was dried in vacuum oven at 60 °C till dry powder of CDs was obtained.

Characterization

Sample was analyzed using dual beam UV- Vis spectrophotometer (Perkin Elmer Lambda 25, USA). UV- Vis spectra was recorded using Standard quartz cuvette having path length 1 cm. Morphology and size of the CDs obtained were then assessed using Transmission Electron Microscope (TEM) [Carl Ziess, GmbH, Germany] and Selected Area Electron Diffraction (SAED). Moreover, Fourier Transform Infrared Spectroscopy (FTIR) [MAGNA-550, Nicolet instruments, USA], analysis was done to know whether use of plant material (as source of CD) has caused attachment of any specific functional groups or peptides on to the CDs. Dynamic Light Scattering (DLS) was performed to determine the size distribution using Malvern : Zen 1600.

Results and Discussions

Addition of ethanol and sodium hydroxide to Neem gum with at R.T caused change in reaction mixture color from light brown to dark brown. This solution exhibited green fluorescence when observed under UV light (λ = 365 nm). The change in colour as well as fluorescence indicated the formation of CDs, which was further confirmed by UV–Vis spectra. It is well known that the ethanol helps in oxidation whereas the alkaline nature of NaOH helps in surface passivation of CDs which is one of the major reasons for its florescence.

UV-Vis Spectrophotometric Analysis

The UV–visible spectra of purified CDs showed peaks at 216 nm, 248 nm and 275 nm which is a characteristic of CDs (Fig. 1) these peaks mainly represent π → π* electron transition of C = C bonds. Inset a of Fig. 1 represent photoluminescence spectra of CDs showing peaks at 350 nm, 362 nm, 357 nm and 488 nm after excitation at 225 nm, 245 nm, 290 nm and 420 nm respectively. The red shift was observed (350 nm to 488 nm) in PL spectra with almost same intensity of peaks. Only decrease in intensity of peak was observed after CDs were excited at 420 nm which may be due to insignificant absorption of CDs in visible range. This excitation dependent emission spectra is a signature

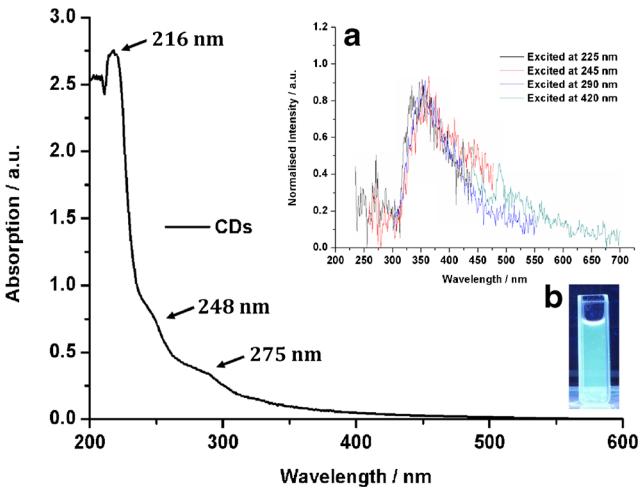

Fig. 1 UV–Vis spectra of Neem gum CDs with hump at 262 nm, inset (a) showing photoluminescence spectra of CDs at 300 nm excitation wavelength and inset (b) showing the fluorescence of CDs

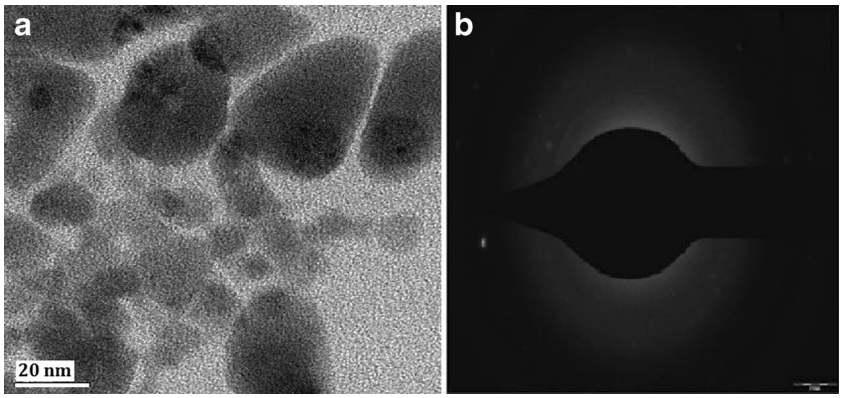

Fig. 2 **a** TEM image of CDs obtained from Neem gum **b** SAED image of Neem gum CDs

characteristic of CDs [24]. The fluorescence property of CDs is considered to be the result of surface energy traps and quantum confinement [10].Upon exposure to UV light (λ = 388 nm) CDs solution showed green turbid fluorescence (Inset b of Fig. 1).

TEM and SAED Analysis of CDs

TEM analysis (Fig. 2a) showed that the CDs obtained were spherical and oval in shape. The size of the CDs was in the range of 5–10 nm. SAED pattern demonstrated the amorphous nature of the CDs obtained from the Neem gum (Fig. 2b).

FTIR Analysis

Neem gum

FTIR spectra of Neem gum displayed in Fig. 3a, showed a broad peak at 709.03 cm^{-1}, which corresponds to -CH_2 bend of alkanes. A minor peak at 1041.17 cm^{-1} depicts C-O bonding of alcohol. An intense and narrow peak at 1638 cm^{-1} represents –NH bend and C = O stretching of amide linkages; this is expected to be due to the proteinaceous content of the Neem gum i.e. amino acids present in gum like alanine, aspargine, glycine etc. [25] and sugars present viz. Fucose, galactose etc. [25] respectively. A broad and weak peak at 2068 cm^{-1} corresponds to the C to C bond stretch of alkynes. A broad and intense peak at 3450.36 cm^{-1} was obtained due the –OH stretching, that is mainly due the water molecules present in the aqueous extract used for analysis.

CDs from Neem gum

FTIR spectra of CDs synthesized from Neem gum there showed a shift in the peak at 1041.17 cm^{-1} to 1028.90 cm^{-1} along with decrease in the intensity, which may be due to the electrostatic interactions with other functional groups (Fig. 3b). Also appearance of new peak at 1365.11 cm^{-1} was observed, which corresponds to C-C and C-O stretch as well as CH_3 bend. All these explain the functional groups attached to the surface of CDs emerging after the fabrication of CDs using Neem gum. These functional groups can be very useful and work as linkers for drug attachment.

DLS

TEM analysis shows 5–10 nm of C-dots while DLS analysis as depicted in Fig. 4 show hydrodynamic diameter to be

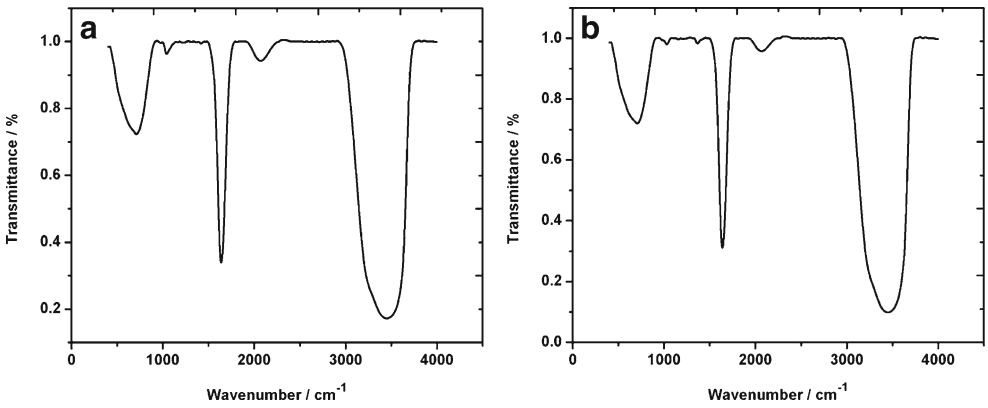

Fig. 3 FTIR spectra of **a** Neem gum and **b** CDs obtained from Neem gum

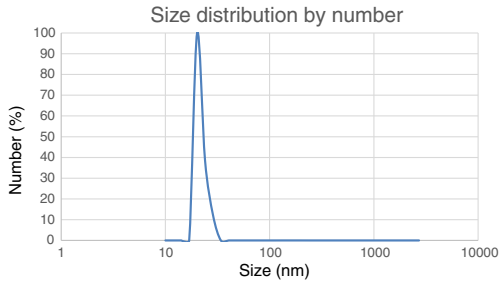

Fig. 4 DLS spectra of C-dots synthesized using Neem gum

24 nm which indicate the diameter along with attached functional groups as shown by FITR spectrum (−C = O, −OH, $-NH_2$, $-CH_3$).

Stability Studies of CDs-

In order to be used as drug carrier or for any other biological applications, it's mandatory for CDs to be stable under different circumstances. Stability of CDs was checked at different temperature, pH and in different solvents. CDs were found to be stable at 4 °C, 37 °C, 60 °C and 100 °C for up to 72 h. Also at different pH conditions CDs were found to be stable. Methanol, Acetone and HCl can be considered as the good solvents for the CDs (Figure. S1, S2 and S3 from supporting information).

Conclusion

Neem gum is a good precursor for biogenic synthesis of CDs. The method used for synthesis has many advantages over other conventional methods e.g. Neem gum produces biocompatible CDs, which can be used for the applications like drug delivery, gene delivery and bio-imaging, also the size of the CDs obtained was 5–10 nm which is ideal for these applications. These CDs can be functionalised with targeting molecule and suitable drug can be loaded. It can also serve as platform for bimodal imaging, gene delivery. Moreover, it is a simple and cost effective method that does not need expensive instruments and chemicals.

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The entire work is original, unpublished and is not submitted to any other journal for reviewing purpose.

Consent to submit the presented work is received collectively from all co-authors as well as the funding authorities.

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