Talanta 150 (2016) 253–264

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

One pot synthesis of intriguing fluorescent carbon dots for sensing and live cell imaging

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ARTICLE INFO

Article history:
Received 17 October 2015
Received in revised form
16 December 2015
Accepted 17 December 2015
Available online 19 December 2015

Keywords:
Carbon dots
Fluorescence
Acetone
Sensing
Cytocompatible
Cell imaging

ABSTRACT

We report a simple one-pot synthesis of highly fluorescent carbon dots (CDs) via modified hydrothermal (MHT) treatment of alkaline solution of dopamine and cysteine. These CDs (λ_{ex}=320 nm, λ_{em}=390 nm, and quantum yield ~5.1%) are of ~2–3 nm in diameter. Further attempt of synthesizing CDs in some common water-miscible solvents ends up the fact that the MHT product from acetone medium is non-fluorescent. However, CDs, produced in aqueous medium, are so stable that they can be dried as a deliverable solid (WCD) without any alteration of fluorescing property if reversibly dispersed in water. Fluorescence of WCD is quenched selectively in acetone. Quenching occurs presumably due to the disruption of radiative recombination along with the hindrance in quantum confinement of the emissive energy traps to the particle surface. Successive quenching of fluorescence of WCD in different acetone concentration admixed in water paves the way to selective acetone sensing (LOD=8.75×10^{-7} M). The synthesized CDs (in aqueous medium) are cytocompatible and are efficient fluorescent probe for cell imaging. Only living cells are recognized exclusively from fluorescence imaging leaving aside dead cells, while cells are treated with CDs.

1. Introduction

Carbon dots (CDs) have created immense interest to scientists since their discovery out of electrophoretic analysis and subsequent purification of fluorescent carbon nanotube fragments [1]. Generally, CDs attain dimension of about 10 nm. They are often compared with known quantum dots (QDs). CDs are more biocompatible and less cytotoxic [2] than the well known QDs. CDs posses different intrinsic structures e.g. carbon quantum dots (CQDs) [3], carbon nanodots (CNDs) [4] and polymer dots (PDs) [5] etc. CQDs and some CNDs possess distinct lattices, whereas other CNDs and PDs generally have amorphous nature. Several techniques have been adopted for the synthesis of CDs [6–10]. Most of the techniques include hydrothermal treatment [11], solvothermal treatment [12], microwave pyrolysis [13], plasma treatment [14], laser ablation/passivation [15], electrochemical method [16] etc. The prepared CDs need further surface passivation to show better emissive property with higher quantum yield. The photoluminescence/fluorescence property of CDs is quite complicated due to quantum size effect [17], triplet carbenes at the zigzag edges [18], radiative recombination of the excitons [19] and the molecular state (as for organic dyes). Also the factors those affect the energy states (HOMO and LUMO) and the band gap between the energy states generally and directly affect the emissive nature of CDs. The optical properties of CDs are the consequences of either combination or competition amongst these factors. The emission mechanism of CDs may change depending upon the nature of precursor compounds and also on the CD preparation procedures [20]. Now CDs are widely employed in the field of drug delivery [21], biomedicine [22], bioimaging [23,24], optical imaging [25] sensing [26] and certain optical devices [27]. Bioimaging or cell imaging is an important application of CDs. Among the fluorescent agents for optical cell imaging/ bioimaging [23,24,28]. CDs prove to be one of the best suited candidates due to their ready aqueous solubility, physicochemical and photochemical stabilities, eminent optical performance including non-blinking behavior, and significantly superb biocompatibility.

Acetone is a common organic solvent, miscible with water in all proportions. Commonly it is used in nail polish remover, varnish removers, adhesives, plastics and paints, and used to make other

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http://dx.doi.org/10.1016/j.talanta.2015.12.047
0039-9140/

chemicals such as acetylene. Low level of acetone is present naturally in the human body, plants, trees and volcanic gases also [29]. Higher amount of acetone, because of its efficient solubilizing property; can be harmful in human body. Presence of higher concentrations of acetone in human body is reported to be produced during fasting or crush dieting. When carbohydrate intake becomes low, ketosis occurs which in turn produces high amount of acetone in our body. Acetone exposure may cause skin dryness, dermatitis, throat and eye irritation etc. Acetone, if present in humans, can be measured from the breath, blood, and urine etc. Also a noninvasive breath acetone detection makes the way of the detection of type-I diabetes and helps to arrange the dose of insulin. Hence, acetone detection in blood sample is a good alternative measure for pathological analysis [30]. There are a good numbers of acetone vapor sensors like screen printed TiO_2 nanoparticles [31], Si doped WO_3 nanoparticles [32], thin-walled WO_3 hemitubes [33], etc. Detection of acetone in solution is equally significant not only for health information purpose but also for analytical laboratories [34]. Acetone is considered as a hazardous waste. Decomposition of acetone produces toxic carbon monoxide [35]. Also its high flammability makes it hazardous. Presence of any kind of interference in terms of external particle or other water miscible solvents in aqueous phase reaction causes hazards and sometimes experimental failure. Hence it is important to use distilled and pure water for laboratory experiments. It is very difficult to detect any water miscible solvent in water while present in trace level. Porter et al. [36] reported the detection of the triplet state of acetone in solution by fast conventional flash techniques. Again there is other report of solvatochromogenic fluorimetry method for detection of water in acetone by Liu et al. [37]. Our strategy of acetone sensing is simple without any sophisticated instrumentation. It is a fluorometric technique exhibiting intriguing sensitivity as well as selectivity in solution phase. It is the first report, according to the best of our knowledge, of acetone sensing involving the quenching of fluorescence of carbon dots in solution.

Water soluble CDs with high emissive property have been prepared from a reaction between dopamine and cysteine precursors. They can effectively detect trace level of acetone in aqueous solution in terms of "turn off" fluorescence phenomenon. Moreover, the as prepared CDs have been found to be capable of working as an agent for selective live cell imaging (Scheme 1).

2. Experimental

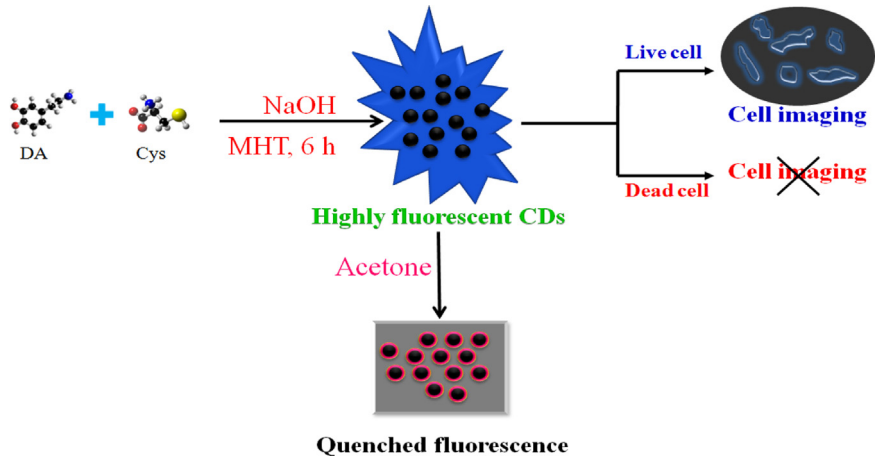
2.1. Chemicals and characterization

All the reagents used throughout the experiment were of AR grade. Triple distilled water was employed during the experiment. Dopamine s (DA), L-DOPA (L-3,4- dihydroxyphenyl alanine), L-phenylalanine, L-tyrosine, cysteine (cys), N-acetyl cysteine and all solvents were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH) was obtained from HiMedia Laboratories Pvt. Ltd. All the solid reagents were used in the experiment without further purification. All solvents were used after distillation. All glass wares were cleaned with freshly prepared aqua regia, rinsed with sufficient amount of distilled water, and dried well before use.

The fluorescence measurement was performed at room temperature using a LS55 fluorescence spectrometer (Perkin-Elmer, Waltham, MA). The samples were taken in a quartz cuvette of 1 cm path length for fluorescence measurement. Absorbance was measured using Evolution 201 UV spectrometer, Thermo Scientific, United States. The samples were taken in a quartz cuvette of 1 cm path length for absorbance measurement. XPS analysis was carried out with a VG Scientific ESCALAB MK II spectrometer (U.K.) equipped with a Mg Kα excitation source (1253.6 eV) and a five-channeltron detection system. The samples were fridge dried before measurement. FTIR studies were carried out with a Thermo-Nicolet continuum FTIR microscope. The sample in solution phase was taken for measurement. TEM analysis was performed with a H-9000 NAR instrument, Hitachi, using an accelerating voltage of 300 kV. The samples in solution phase were drop-cast onto a carbon-coated copper grid, and the grid was air dried at room temperature (25 °C) before loading into the microscope. Fluorescence lifetimes were measured with Easy life^R (Optical Building Blocks Corporation) equipped with a 295 nm LED excitation source. The samples were taken in a quartz cuvette of 1 cm path length for fluorescence measurement.

2.2. Synthesis of carbon dots (CDs)

Carbon dots (CDs) were prepared from DA and cys. In the typical synthesis 25 μL DA (5×10^{-3} M) and 100 μL cys (5×10^{-3} M) were mixed together and the mixture was diluted to 3 mL using triple distilled water taken in a screw cap test tube (final concentration of DA in 10^{-5} M order). The solution was made alkaline by adding NaOH (10^{-1} M). Then a screw capped test tube was kept in front of 200 W bulb (Philips India) at a distance of 3 cm for 6 h. The whole system was kept in a 1 ftx1 ftx1 ft closed wooden box. This method of heating a reaction mixture is reported as modified



Scheme 1. Intriguing fluorescence of carbon dot solution is utilized in live cell imaging. This fluorescence is quenched exclusively in the presence of acetone.