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Facile and green approach to prepare fluorescent carbon dots: Emergent nanomaterial for cell imaging and detection of vitamin B_2

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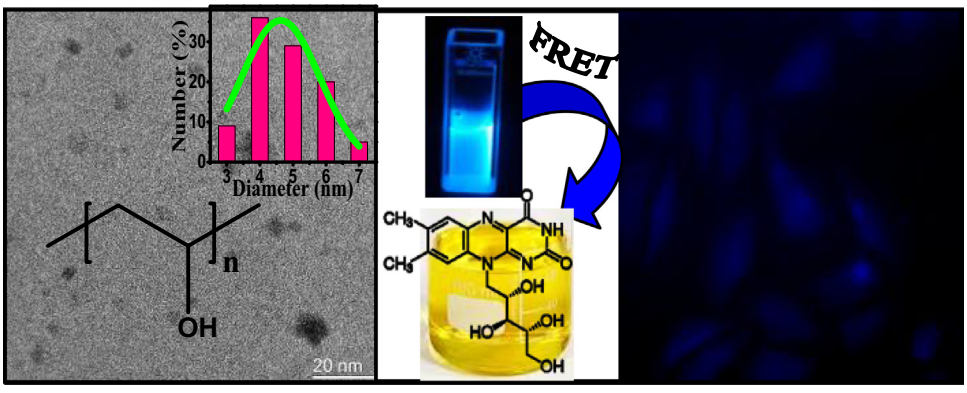
HIGHLIGHTS

- Facile and green approach to prepare fluorescent CDs.
- CDs are biocompatible and used in cell imaging.
- Detection of Vitamin-B_2 via FRET using CDs.
- The limit of detection is 1.2 nM, lowest ever reported in the literature.
- The detection is possible even in presence of different biomolecules.

GRAPHICAL ABSTRACT



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ABSTRACT

Carbon dots (CDs) are a new representative in carbonaceous family and have initiated remarkable research interests over the past one decade in a large variety of fields. Herein, we have utilized a facile, one-step carbonization method to prepare fluorescent carbon dots using poly(vinyl alcohol) (PVA) both as a carbon source and as a surface passivating agent. The as prepared CDs emit bright blue fluorescence under ultraviolet illumination. The structure and optical properties of the CDs are thoroughly investigated by several methods such as high-resolution transmission electron microscopy; dynamic light scattering; UV–vis, fluorescence and Fourier transform infrared spectroscopy. The CDs exhibit excellent water solubility and demonstrate average hydrodynamic diameter of 11.3 nm, holding great promise for biological applications. The biocompatibility evaluation and *in vitro* imaging study reveals that the synthesized CDs can be used as effective fluorescent probes in bio-imaging without noticeable cytotoxicity. In addition, a unique sensor for the detection of vitamin B_2 in aqueous solution is proposed on the basis of spontaneous fluorescence resonance energy transfer from CD to vitamin B_2. These findings therefore suggest that the CDs can find potential applications in cellular imaging along with sensing of vitamin B_2.

1. Introduction

Nanomaterials have remarkable impact on the growth of a wide range of fields including electronics, photonics, energy, catalysis, medicine and so on. The discovery of semiconductor quantum dots (QDs) is considered to be a major breakthrough for the development of fluorescent nanomaterials. Semiconductor QDs usually possess strong and tunable fluorescence emission properties, high photostability and resistance to metabolic degradation which enable their applications in biosensing, bioimaging and biodiagnostics [1,2]. This prompted scientists of diverse fields to extensively investigate on semiconductor QDs for many years,

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although, these QDs contain heavy metals (Cd, Pb, Hg), whose well known toxicity and potential environmental hazards limit their further applications [3–5]. Hence, extensive efforts have been paid on the development of nontoxic and benign fluorescent nanomaterials as alternatives to the semiconductor QDs. In this regard, carbon nanomaterials such as nanodiamonds, carbon nanotubes, fullerene and graphene QDs [6–9] have appeared as promising candidates for numerous exciting applications, for example in bioimaging, biosensing, catalysis, and photovoltaic devices [4,10,11]. Fluorescent carbon nanoparticles (CNPs) or carbon dots (CDs), a novel class of heavy metal-free nanomaterials have emerged recently and have harvested much interest as potential competitors to conventional semiconductor QDs due to low toxicity, environmental friendliness, low cost and simple synthetic routes. Again, CDs render unique optical and physicochemical properties which encourage the development of novel imaging probes as well as biosensing platform for identifying challenging issues raised by biological research [12–14]. The aforementioned unique properties of CNPs/CDs have recently attracted considerable research interest to achieve wide range of applications. In the past few years, several techniques like laser-ablation methods [15], electrochemical synthesis [16], and microwave/ultrasonic synthesis [17,18] have been adopted to synthesize CDs. But, most of the above methods involve the use of expensive starting materials, strong acid, sophisticated instruments and complicated processes. Numerous carbon based materials like candle soot [19], bulk graphite [20], polymers [21–23], carbohydrates [24], and sugars [25] are used to produce CNPs/CDs. Again, in most cases the synthesized CDs generally require further surface passivation to improve their water solubility and fluorescence properties, which may limit their applications. Recently, CDs are enormously used for both cellular imaging and biosensing purposes [26–32]. For example, CDs produced from garlic is used for cell imaging of A549 cells by incubating with CDs [26] and the CDs produced from amino-terminated poly(ethylene glycol) is used for fluorescence imaging of stem cells [27]. N-doped CDs are utilized for the detection of catechol, dopamine, aflatoxin B_1, etc. [28,29,32] whereas amino functionalized green fluorescent CD is being used as a biosensing platform to detect hyaluronidase [30].

It has been known that starch, cellulose, polylactide, poly(vinyl alcohol) (PVA), etc. can be degraded using alkaline solution in presence of oxygen [33–36]. However, to the best of our knowledge, there is no report yet exist to produce CDs from the above polymers using alkaline medium. Here we chose PVA degradation in alkaline medium to study whether CDs can be produced in environment friendly condition. In this article, a facile and one-step hydrothermal route is designed to synthesize CDs from water soluble, biocompatible [37] polymer PVA without further surface modification. Here, PVA acts both as a carbon source and surface passivating agent due to the presence of hydroxyl groups. The resultant CDs display good dispersion in aqueous solution, strong blue fluorescence and low toxicity. These advantages indicate that the as synthesized CDs can be used as an excellent fluorescent probe for cellular imaging. In a recent article we have reported the sensitive and selective detection of Riboflavin (RF, commonly known as vitamin B_2) using sulfonated graphene (SG), based on fluorescence resonance energy transfer (FRET) from SG to RF at pH 4 [38]. But, the major disadvantage of this method is that we were unable to detect RF at biological pH due to low quantum yield of SG at this pH. To overcome this problem, in the present article, we have tactfully utilized our synthesized CDs as effective fluorescent sensing platform for RF owing to the spontaneous FRET process from CDs to RF at neutral pH. In addition, this method has high selectivity for detecting RF at pH = 7, even in presence of other biologically relevant components such as nucleic acids (DNA, RNA), and protein (human serum albumin, HSA), amino acid (lysine). Conventional methods such as high performance liquid chromatography [39,40], capillary electrophoresis [41,42] and immunoassay [43] are used for the determination of RF. But the present approach for the detection of RF is proved to be superior to the above mentioned methods since FRET-based approach has many advantages including faster response, greater simplicity, lower cost and higher sensitivity and selectivity.

2. Experimental section

2.1. Materials

PVA ($M_w \sim 9000$), Riboflavin, Calf thymus DNA (Type 1; sodium salt), RNA (diethyl amino ethanol salt, type IX from Torula Yeast), human serum albumin (HSA), lysine were purchased from Sigma Aldrich (USA). Sodium hydroxide (NaOH) was purchased from Merck (Mumbai). All the reagents were used as received.

2.2. Synthesis of CDs

For the synthesis of CDs, 500 mg PVA was added to 20 mL concentrated sodium hydroxide (1.25 M) solution and stirred to get a homogenous solution. Then the solution was refluxed in a preheated oil bath at 120 °C for 5 h in air. After the completion of reflux process, the transparent viscous liquid turned into low viscosity liquid with pale yellow color. Then the resulting solution was cooled to room temperature and neutralized. The neutralized solution was dialyzed (MWCO = 12 kDa) against double distilled water and golden yellow colored solution was obtained which implies the formation of pure CDs. Finally, this solution was lyophilized to collect dry CDs for further use.

2.3. Preparation of sample

The synthesized CD was taken in double distilled water and it was sonicated for 15 min, using an ultrasonic bath (60 W, frequency 28 kHz, Model AVIOC, Eyela) to make a homogenous dispersion (1 mg/mL). DNA, RNA, HSA, lysine solutions (2 mg/mL) were prepared by dissolving the required amount of them in water. For determination of RF, the concentration of CD was kept constant while the concentration of RF was varied from 0.13 μM to 2.4 μM.

2.4. Quantum yield measurements

The relative fluorescence quantum yields (QY) of CD was measured using quinine sulfate in 0.1 M H_2SO_4 (quantum yield 58% at 340 nm) as a standard. The value of QY was calculated according to the following equation [44,45]:

$$QY_{sample} = QY_{std.}[(I/A)_{sample} \times (A/I)_{std.}]\left(\eta^2_{sample}/\eta^2_{std.}\right)$$

where A is the absorbance at the excitation wavelength, η is the refractive index of solvent and I is the integrated emission intensity calculated from the area under the emission peak on the same wavelength scale. The absorbance values of both sample and standard solutions were kept below 0.1 to minimise the re-absorption effects at the excitation wavelength (340 nm).

2.5. In vitro cellular imaging

Human cervical cancer cells (HeLa cell) were seeded into 4-well chamber slides (cell seeding density: 2×10^4 per well) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2 for overnight. For fluorescent imaging, CD solution was added to reach final concentration of