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Electrochemically generated green-fluorescent N-doped carbon quantum dots for facile monitoring alkaline phosphatase activity based on the Fe^{3+}-mediating ON-OFF-ON-OFF fluorescence principle

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A B S T R A C T

Herein, green-fluorescent N-doped carbon quantum dots (N-CQDs) with quantum yield of 30.6% were prepared via a simple bottom-up electrochemical (EC) method using the mixtures of pyrocatechol and ethylenediamine as both precursors and electrolytes. The ON-OFF fluorescence (FL) principle that the FL intensity of the N-CQDs was quenched in the presence of Fe^{3+} was further extended to the ON-OFF-ON and ON-OFF-ON-OFF ones for pyrophosphate anions (PPi) and alkaline phosphatase (ALP) detection, respectively, based on the specific competitive interaction of N-CQDs with Fe^{3+} and PPi and the hydrolysis of PPi in the presence ALP. Under the optimized conditions, limit of detection (LOD) of 1.2 μM and 0.5 μM were achieved for assays of Fe^{3+} and PPi, respectively. Meanwhile, measurable evaluation of ALP activity was reached in a wide range from 5 to 360 U/L with a LOD of 1.1 U/L. The as-proposed sensors showed many advantages including label free, rapid, simple, low-cost, high sensitive, which has great promising application potential *in vivo* and *in vitro* clinical diagnosis and will promote the application of EC derived carbon nanomaterials.

1. Introduction

Carbon quantum dots (CQDs), a new class of carbon nanomaterials, have been attracting intense interest due to their excellent fluorescence (FL) properties, high chemical stability, low toxicity, anti-photobleaching, environmental friendliness, low cost and good biocompatibility [1–3]. All these unique and novel properties made them as new-generation fluorescent nanomaterials in optoelectronics, photocatalysis, bioimaging, chemical sensing, drug delivery, etc. [4–7] To date, various methods have been developed for the synthesis of CQDs such as laser ablation, microwave treatment, hydrothermal carbonization, electrochemical (EC) methods. Among them, the EC ones have satisfactory features for CQDs synthesis including simple operation, mild reaction conditions, low cost, mass production, and so on [8,9]. In addition, the bottom-up EC methods, which are employed for CQDs generation through electro-oxidation, electro-polymerization, carbonization and passivation from small molecular precursors including ethanol [10,11] and nitriles [12], amino acid [13], possess obvious superiorities of accurately regulating the morphology, size distribution, surface functionalization or heteroatom (e.g. N or/and S elements) doping of the CQDs [8,13]. It is a common phenomenon that the FL intensities of the EC-produced CQDs are significantly quenched in the presence of Fe^{3+}. Based on this FL principle, the sensing applications of these CQDs can be used for but mostly limited in the specific Fe^{3+} assays till now [12,13]. Thus, it will greatly widen the applicability of the CQDs if Fe^{3+}-mediating principle can be efficiently designed for sensing of more analytes such as anions or biological molecules [14].

Pyrophosphate anions (PPi), a kind of hydrolysis product of adenosine triphosphate, play important roles in various biological processes [15–17] such as cellular metabolism [17], enzymatic reactions [15], cancer research [18], etc. Accordingly, assay of PPi is of great significance in aqueous physiological system. Alkaline phosphatase (ALP), is one popularly used hydrolase enzymes, whose participation is vital in cell growth, apoptosis and signal transduction [19–22] as well as in diagnostics of cancers [23], liver

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dysfunction, diabetes [24] and bone diseases [24,25]. Thus, it is in great need to seek convenient and sensitive ways for monitoring ALP activity/level. Meanwhile, that PPi can be hydrolyzed by ALP shows great importance in vascular calcification and skeletal mineralization, and also make PPi as its natural substrate in the analysis of ALP [26]. Various strategies including colorimetry, surface enhanced Raman scattering, chromatographic analysis, fluorometric methods have been developed for detection of PPi or/and ALP activity [27–31]. Among them, fluorometric analysis is considered to be the more satisfying one because of its high sensitivity, convenience and simple instrument requirement, in which organic dyes [32,33], heavy-metal semiconductive quantum dots [34], and noble metal nanoparticles [26,35] are used as the fluorometric indicators. Despite of the advantages, these fluorophores usually suffer from some drawbacks including with poor photostability, complex synthesis procedure, high toxicity, or high cost [36], which greatly impedes their practical applications. Thus, it is still in urgent need to explore efficient fluorescent probes with good photostability, excellent biocompatibility, low toxicity, simple preparation process and cost-effectiveness for fluorometric assays of PPi and ALP. Subsequently, CQDs synthesized via EC methods were suggested to be promising fluorescent alternatives, but have not been reported till now.

In this study, green-fluorescent N-doped CQDs (N-CQDs) were prepared via a simple bottom-up EC method using the mixtures of pyrocatechol and ethylenediamine as both precursors and electrolytes. The as-prepared N-CQDs were characterized by transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), etc. The ON-OFF principle that the FL intensity of the N-CQDs was quenched in the presence of Fe^{3+} was further extended to the ON-OFF-ON and ON-OFF-ON-OFF modes for PPi and ALP detection, respectively, based on the turn-on and turn-off signal caused by disaggregation and reaggregation of N-CQDs. PPi thereinto acted as the substrate for ALP activity assessment simultaneously. Under the optimized conditions, sensitive and specific quantifications of Fe^{3+} and PPi and monitoring the ALP activity were achieved accordingly.

2. Experimental section

2.1. Materials

All the reagents were of analytical grade and used as received. Alkaline phosphatase (ALP) and pyrophosphate anions (PPi) were purchased from Sigma-Aldrich (Steinheim, Germany). Ethylenediamine, pyrocatechol, sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), mono potassium phosphate (KH_2PO_4) were purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Dehydrogenase (GDH), galactosidase (Gal), glucose oxidase (GOx), thrombin, L-cysteine (Cys), L-aspartic acid (Asp), L-serine (Ser), L-methionine (Met), L-histidine (His), L-arginine (Arg) and the MD34 dialysis bag with cut-off molecular weight of 3500 Da were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Ultrapure water (18.2 MΩ cm^{-1}) was obtained with a Milli-Q ultrapure system (Qingdao, China).

2.2. Apparatus

RXN-305D DC power supply was supplied by Shenzhen Zhaoxin Yuan Electronics Co., Ltd (Shenzhen, China). The FL and ultraviolet–visible (UV–Vis) absorption spectra were measured on an Edinburgh instruments spectrofluorometer FS5 (Edinburgh, U. K.) and a Mapada UV-1800PC spectrophotometer (Shanghai, China), respectively. The TEM morphology of N-CQDs was observed on a JEOL Ltd JEM-2010 transmission electron microscope (JEOL Ltd., Japan). AFM images were obtained by using a SPI3800N microscope operating in the tapping mode (Seiko Instruments Inc., Japan). AFM samples were prepared by depositing the N-CQDs dispersions on freshly cleaved mica surfaces through a drop-casting method, respectively, and then were left to air-dry. FTIR were recorded on a Nicolet 5700 FTIR spectrometer (Thermo Electron Scientific Instruments Corp., USA). XPS data were obtained on an ESCALab220i-XL electron spectrometer (VG Scientific, West Sussex, U. K.) using 300 W Al Kα radiation.

2.3. Synthesis of the N-CQDs

A facile EC system has been developed for the synthesis of N-CQDs (Fig. 1). Two Pt sheets (1.0 cm^2) were employed as the working and auxiliary electrodes, respectively. The electrolytes were prepared by adding 0.11 g pyrocatechol in 10 mL ultrapure water containing 5 mM ethanediamine. A constant potential of 10 V was applied to advance the EC reaction using a DC power. The color of the electrolyte mixture changed from colorless to yellow and then to dark brown in 30 min. In order to purify the N-CQDs, the dark brown aqueous solution was subjected to dialysis against ultrapure water through a membrane (MWCO of 3500) for 24 h. The purified aqueous suspension of N-CQDs was diluted for 20 times with a phosphate buffer solution (PBS) (0.1 M, pH = 6.0) used for the following characterizations.

2.4. Quantum yield (QY) measurements

The QY of the N-CQDs were determined on the basis of previously established procedure. Typically, quinine sulfate (literature quantum yield 0.54) in 0.1 M H_2SO_4 aqueous solution was used as the reference [37,38]. In order to minimize the re-absorption effects, the aqueous suspension of N-CQDs and the aqueous solution of reference sample were diluted to keep the absorption intensity below 0.1 and 0.05, respectively, at the excitation wavelength of 375 nm. The QY of the N-CQDs was calculated using the equation below [39]:

$$\Phi_X = \Phi_{ST}\left(\frac{\mathrm{Grad}_X}{\mathrm{Grad}_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

where the subscripts ST and X refer to quinine sulfate and N-CQDs, respectively, Φ represents the QY. Grad represents the gradient from the plot of integrated FL intensity *vs.* absorbance, and η is the refractive index of the solvent.

2.5. Selectivity assessment N-CQDs or N-CQDs-Fe^{3+} based sensing for Fe^{3+} or PPi detection

Different metal ions (Ag^+, Al^{3+}, Cd^{2+}, Co^{2+}, Hg^+, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+}) were chosen to assess the selectivity of the quenched FL of N-CQDs towards Fe^{3+}. Each metal ion with final concentration of 400 μM was added and incubated for 3 min at room temperature before the spectral measurements. For the selectivity test to PPi, eight kinds of different anions including F^-, Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, NO_3^- and Ac^- were chosen to evaluate the influence of anions on the FL of N-CQDs-Fe^{3+} system. Each anion at 1500 μM was introduced into the above N-CQDs-Fe system and incubated for 3 min at room temperature. Tris-HCl buffer (10 mM, pH 7.4, 100 mM NaCl) was selected as the butter for selectivity assessment experiments. The FL spectra were recorded under excitation at 410 nm.