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Nitrogen-rich functional groups carbon nanoparticles based fluorescent pH sensor with broad-range responding for environmental and live cells applications

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ABSTRACT

A nitrogen-rich functional groups carbon nanoparticles (N-CNs) based fluorescent pH sensor with a broad-range responding was prepared by one-pot hydrothermal treatment of melamine and triethanolamine. The as-prepared N-CNs exhibited excellent photoluminescence properties with an absolute quantum yield (QY) of 11.0%. Furthermore, the N-CNs possessed a broad-range pH response. The linear pH response range was 3.0 to 12.0, which is much wider than that of previously reported fluorescent pH sensors. The possible mechanism for the pH-sensitive response of the N-CNs was ascribed to photo-induced electron transfer (PET). Cell toxicity experiment showed that the as-prepared N-CNs exhibited low cytotoxicity and excellent biocompatibility with the cell viabilities of more than 87%. The proposed N-CNs-based pH sensor was used for pH monitoring of environmental water samples, and pH fluorescence imaging of live T24 cells. The N-CNs is promising as a convenient and general fluorescent pH sensor for environmental monitoring and bioimaging applications.

1. Introduction

Minute pH changes negatively impact the lives of plants and animals. Therefore, there is considerable concern regarding pH changes in all life forms (Wu et al., 2014). For example, large amounts of sewage discharged into the environment may cause changes in the environmental pH, which not only pollute drinking water, but also have a range of negative health effects (Yang et al., 2013). In the life science, intracellular pH is of great concern to cell, enzyme and tissue activities, which plays a critical role in modulating many biological reactions (Han and Burgess, 2010). Abnormal intracellular pH in living cells can cause improper cell function and growth, resulting in various diseases and neurological disorders (Dennis et al., 2012). Thus, the facile and accurate pH monitoring in the environment and on the cellular level is of increasing importance.

In recent years, fluorescence-based pH measurement techniques, such as organic dyes (Yang et al., 2013), carbon dots (Dennis et al., 2012; Jin et al., 2015), silicon nanodots (Feng et al., 2014), Cu nanoclusters (Liao et al., 2015), and quantum dots (Jia et al., 2012; Shen et al., 2013; Wan et al., 2014), have received increasing attention owing to their high rapidity, sensitivity, and wide applicability to cells. Among them, organic dyes-based pH sensors are efficient and accessible. Unfortunately, successful application of organic dye-based pH sensors is usually limited because of their complex synthesis/lable processes (Dennis et al., 2012; Wan et al., 2014). More recently, considerable attention has been given to fluorescent carbon nanomaterials owing to their low toxicity, good biocompatibility, chemical inertness, stable photoluminescence and tunable luminescence emission (Li et al., 2011; Liu et al., 2011; Peng et al., 2012), which are advantageous over the organic dye-based fluorescence probes used for pH detection (Xu et al., 2004). For instance, Sun and co-workers have reported a one-pot hydrothermal method to prepare carbon dots using threonine as carbon source, and their fluorescence was sensitive to pH in the 2.13–9.34 range, a decrease in fluorescence intensity occurred as pH increased (Jin et al., 2015). Shi et al. have proposed a one-pot

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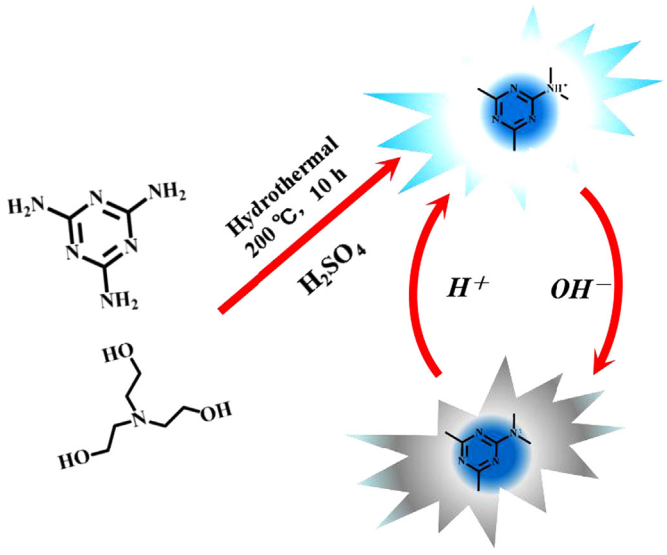
solid-phase synthesis method to synthesize a high quantum yield of oxygen-rich N-doped graphene quantum dots (N-GQDs), and used as pH sensor to obtain good linearity in the 3.0–8.0 pH range (Shi et al., 2015). Huang and co-workers have developed a facile hydrothermal route to synthesize N-GQDs with pH-sensitive response from 1.81 to 8.96 (Wu et al., 2014). Although these developed techniques are excellent for pH sensing, there are somewhat problems existed, such as the range of response and linear relation for quantification (Wu et al., 2014). Therefore, developing simple, broad range of response and sensitive pH sensor is still challenging.

To date, many strategies for synthesizing carbon dots have been reported (Hu et al., 2009; Wang et al., 2011; Han et al., 2012; Ma et al., 2012; Yang et al., 2012; Zhang et al., 2012). Among them, the hydrothermal route is attractive due to the eco-friendly, the good stability and biocompatibility of as-prepared carbon dots resulting from the O-rich groups on their surface (Zhang et al., 2013). Moreover, protonation and deprotonation of these O-and N-rich groups in aqueous solution is attributed to pH-dependent fluorescence property of carbon dots (Dong et al., 2012). Herein, we reported a facile hydrothermal method to synthesize nitrogen-rich functional groups carbon nanoparticles (N-CNs) using melamine and triethanolamine as carbon source and nitrogen source, respectively. The synthesis process of N-CNs is shown in Scheme 1. The as-prepared N-CNs exhibited blue photoluminescence with an absolute quantum yield (QY) of 11.0%. Impressively, the N-CNs possessed distinct pH-sensitive photoluminescence properties in the pH range from 3.0 to 12.0, which is much wider than that of previously reported fluorosensors. The mechanism responsible for the pH-sensitive properties involved photoinduced electron transfer (PET) of aniline to the N-CNs. Moreover, the proposed fluorosensors were successfully applied to detect pH level in real water samples and living cells.

2. Experimental

2.1. Reagents and materials

Melamine ($C_3N_6H_6$, 99.0%) and Triethanolamine ($C_6H_{15}NO_3$, 98.0%) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Other chemicals used in the tests were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All of the reagents were used as received without further purification. Water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA) and used throughout the work.



Scheme 1. Schematic illustrations for the synthesis process of N-CNs and pH-responsive.

2.2. Synthesis of the N-CNs

In a typical preparation process, melamine (0.3 g), triethanolamine (3.0 mL) and 10.0 mL H_2SO_4 (1.0 M) were mixed in a 50 mL Teon-lined stainless steel autoclave and heated at 200 °C for 10 h under vigorous stirring. After the reaction was completed, the reaction solution was cooled down naturally. Next, the aqueous solution was centrifuged at 12,000 rpm for 15 min to dislodge the non-fluorescent deposit and obtain the upper aqueous solution containing the N-CNs. The obtained mixture was dialyzed in a dialysis membrane (1000 MWCO, Shanghai Green Bird Science & Technology Development Co., China) for 48 h to remove residual melamine, triethanolamine and fluorescent precursors. The as-prepared N-CNs were dried under vacuum at room temperature for 48 h and stored at 4 °C for further use.

2.3. Characterization

Absorption measurements were performed with a Cary 60 UV–vis spectrometer (Agilent Technologies, USA). Fluorescence spectra were recorded on a Perkin-Elmer LS55 luminescence spectrometer (Perkin-Elmer, USA). Fourier transform infrared spectroscopy (FTIR) analyses were conducted using KBr pellets on a Perkin-Elmer FT-IR spectrophotometer (Perkin-Elmer, USA). Transmission electron microscopy (TEM) images were taken on a Tecnai G2 F20 transmission electron microscope (TEM) (FEI, USA) operating at 200 kV. X-Ray photoelectron spectra (XPS) were obtained on a Thermo ESCALAB 250Xi Multitechnique Surface Analysis (Thermo, USA). Fluorescence lifetime experiments were recorded on a time-resolved fluorescence spectrometer FL3-P-TCSPC (HORIBA JOBIN JVON, France). Cell imaging was performed by confocal laser scanning microscopy (CLSM, Olympus FluoView FV1000). Cytotoxicity evaluation was performed using an enzyme linked immunosorbent assay (ELISA) reader (Multiskan Mk3, Thermofisher).

2.4. pH measurements

A sample containing 12 mg of N-CNs was diluted to become an aqueous solution of 12 mg N-CNs/mL. Then, 20 μL of the diluted N-CNs aqueous solution were added into Britton-Robinson (BR) buffer of different pH (3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0) to give a final volume of 200 μL. Afterwards, the fluorescence intensities were recorded in the wavelength range of 390～590 nm at an excitation wavelength of 365 nm. The fluorescence intensities of N-CNs were used to evaluate the pH value in BR buffer of a different pH. All of the measurements were performed in triplicate, and the standard deviation was plotted as the error bar.

2.5. pH detection for environmental water samples

Water samples from rainwater, tap water, spring water and river water were filtered through a 0.22-μm membrane and centrifuged at 12,000 rpm for 20 min. The obtained water samples were then analyzed using the N-CNs based pH sensor.

2.6. Cytotoxicity investigation

The cytotoxicity of N-CNs was evaluated by a MTT assay. T24 cells were seeded in a 96-well plate, and 1×10^4 cells per well were mixed in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, invitrogen). The cells were cultured first for