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Pentaethylenehexamine and D-penicillamine co-functionalized graphene quantum dots for fluorescent detection of mercury(II) and glutathione and bioimaging

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ABSTRACT

Pentaethylenehexamine and D-penicillamine co-functionalized graphene quantum dots (PEHA-GQD-DPA) was made via one two-step thermal pyrolysis. The resulting PEHA-GQD-DPA is composed of the graphene sheets with an average size of 3.16 nm and the rich of functional groups. It gives an ultra strong fluorescence emission with the fluorescent quantum yield of 90.91% and sensitive and selective optical response towards Hg^{2+}. The fluorescence intensity linearly decreases with the increase of Hg^{2+} in the range of 1.0×10^{-10}–2×10^{-4} M with the detection limit of 4.6×10^{-11} M (S/N = 3). No species tested interfere with detection of Hg^{2+}. The fluorescence quenched by Hg^{2+} can be well recovered by glutathione. The fluorescence intensity linearly increases with the increase of glutathione in the range of 5×10^{-8}–2.5×10^{-6} M with the detection limit of 1.7×10^{-8} M (S/N = 3). The PEHA-GQD-DPA as a fluorescence probe has been successfully applied in determination of Hg^{2+} in natural water and glutathione in human serum and SW480 cell imaging.

1. Introduction

Mercury(II) ion (Hg^{2+}) is one of the most ubiquitous heavy metal pollutant that is serious threat to human health, because of its highly reactive nature that binds thiol groups in some proteins and enzymes, and a lesser degree to hydroxyl, carboxyl and phosphoryl groups [1]. Hg^{2+}contamination may come from natural sources and human activities, including oceanic and volcanic emissions, solid waste incineration and combustion of fossil fuels [2]. The annual release amount of Hg^{2+} was about 4400–7500 metric tons estimated by the United Nations Environment Programme [3]. Hg^{2+} easily passes through skin, respiratory and gastrointestinal tissues and leads to DNA damage, mitosis impairment and permanent damage to the central nervous system [4]. Even at a low concentration, Hg^{2+} can enter food chain and constitute a serious threat to human health and natural environment [5]. Developing effective method for detection of Hg^{2+} is highly desirable.

Mostly used methods for the detection of Hg^{2+} are inductively coupled plasma-mass spectrometry [6], atomic absorption/emission spectroscopy [7] and selective cold vapor atomic fluorescence spectrometry [8]. These methods often require the use of expensive and sophisticated instrumentation or complicated sample preparation processes [9]. In the recent years, many modern technologies have also been developed for the detection of Hg^{2+}, such as gold nanorods enhanced resonance Rayleigh scattering [10], surface-enhanced Raman scattering [11], colorimetric sensor [12], quartz crystal microbalance [13], near-infrared chemosensor [14], electrochemical sensors [15] and fluorescent methods [16]. The fluorescence analysis with high sensitivity, fast detection and non-sample destructing features has been proved to be an alternative method for detection of Hg^{2+} [17]. Metal nanoparticles [18], semiconductor quantum dots [19], carbon nanoparticles [20] and organic small molecules [21], g-C_3N_4 nanoparticles [22] and Schiff base [23] have been explored and synthesized for optical detection of Hg^{2+}. However, these fluorescent materials suffer from involving toxic reagents, poor selectivity or tedious synthesis. Consequently, it is urgent to develop the eco-friendly and low-cost Hg^{2+} fluorescent probes with a lower detection limit and higher selectivity.

Graphene quantum dot (GQD) is one zero-dimensional graphitic nanocrystals with the size of only several nanometers, strong quantum confinement and edge effects [24,25]. Due to outstanding fluorescent properties with tunable excitations and emissions, low toxicity and high chemical stability, GQDs have been widely applied in optical detection [26,27], biological imaging [28], electrochemical sensor [29], nanomaterial synthesis [30] and lithium ion batteries [31]. To date, considerable efforts have been devoted to synthesis of GQDs. One method is to make GQDs by cutting large graphene sheets via the acidic oxidation

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[32]. The method is time-consuming and the resulted GQDs lack of the rich of functional groups and special structures. Another method is to make GQDs by thermal pyrolysis of organic acids. The method is simple and effective and it can achieve to the formation of graphene sheets and their functionalization in one-step reaction. More importantly, the method is easy to introduce functional groups and structures [33]. The studies have demonstrated that the introduction of functional groups may alter the electrical characteristics, leading to unusual properties and related applications of GQDs [34].

In the study, we report a facile synthesis of pentaethylenehexamine (PEHA) and penicillamine (DPA) co-functionalized graphene quantum dots, which is termed as PEHA-GQD-DPA. The results show that the use of two-step synthesis strategy effectively circumvents producing impurities caused by the reactions between citric acid, PEHA and DPA. The introduction of PEHA and DPA greatly improves the fluorescence emission of GQD. The unique structure of PEHA-GQD-DPA achieves to a sensitive and selective fluorescence response towards Hg^{2+}. It has been successfully applied in optical determination of Hg^{2+} and glutathione and cell bioimaging.

2. Experimental

2.1. Materials

Citric acid, pentaethylenehexamine, D-penicillamine, glutathione and mercury chloride ($HgCl_2$) were purchased from Sigma-Aldrich (Mainland, China). Other reagents were purchased from Shanghai Chemical Company (Shanghai, China). All chemicals used were of analytical grade. Phosphate-buffered saline (PBS, pH 5.5, Na_2HPO_4-KH_2PO_4-NaCl-KCl, 0.01 M) was prepared in the laboratory. Ultra pure water (18.2 MΩ cm) purified from Milli-Q purification system was used throughout experiment.

2.2. Apparatus

Transmission electron microscope (TEM) images were conducted on a JEOL 2010 FEG microscope at 200 keV. The samples were prepared by dispensing a small amount of dry powder in the water. Then, one drop of the suspension was dropped on 300 mesh copper TEM grids covered with thin amorphous carbon films. Infrared spectra (IR) were recorded on a Nicolet FT-IR 6700 spectrometer. Atomic force microscopic (AFM) image was obtained on a Multimode 8 force microscope (Bruker, Germany). X-ray photoelectron spectroscopy (XPS) measurement was carried out on PHI 5700 ESCA spectrometer using Al HR radiation ($h\nu$ = 1486.6 eV). Fluorescence spectrum and intensity were recorded on Cary Eclipse fluorescence spectrophotometer (Agilent, Japan). pH was measured on PHS-3D pH meter (Shanghai Precision Scientific Instruments Co., Ltd., China). UV–Visible absorption spectrum was measured on Shimadzu UV-2550 UV/visible spectrophotometer.

2.3. PEHA-GQD-DPA Preparation

The PEHA-GQD-DPA was synthesized by one two-step continuous thermal pyrolysis. In a typical procedure, citric acid (4.2 g) was dissolved in ultra pure water (200 mL). Slowly dropped pentaethylenehexamine (1.86 g) into the solution under stirring. The mixed solution was transferred into the autoclave pressure vessel of 250 mL. Followed by heating at 200 °C for 1.5 h and cooling to room temperature. After added D-penicillamine (1.2 g) into the above solution under stirring, it was heated at 200 °C for 2 h and cooled to room temperature. The obtained PEHA-GQD-DPA sample was dissolved in ultra pure water to form a transparent PEHA-GQD-DPA solution (40 mg·mL^{-1}). The solution was dialyzed in a dialysis bag with a 3 kDa cut-off molecular weight with change of water every 6 h before the precipitate occurred in the bag. To obtain a solid PEHA-GQD-DPA sample, the solution in the bag was dried by free-drying. In addition, three control samples, GQD, PEHA-GQD and DPA-GQD, were also made by thermal pyrolysis of citric acid, the mixture of citric acid and pentaethylenehexamine, and the mixture of citric acid and D-penicillamine, respectively.

2.4. Quantum Yield Measurement

Quantum yield measurement was carried out by dissolving quinine sulphate in 0.1 M H_2SO_4 (used as a reference). The PEHA-GQD-DPA dispersion was used as such. The absorbance of respective sample was measured in the spectrophotometer. Quantum yield was calculated from following Eq. (1) [35]:

$$Q = Q_R \frac{m_s}{m_r}\frac{n_s^2}{n_r^2} \quad (1)$$

where, Q is the quantum yield of PEHA-GQD-DPA, Q_R is the quantum yield of quinine sulphate, m_s is slope of the plot integrated fluorescence intensity vs. absorbance of the PEHA-GQD-DPA, m_r is the slope of the plot of integrated fluorescence intensity vs. absorbance of reference quinine sulphate, n_s and n_r are the refractive indices of the sample and reference, respectively, in the distilled water, which are assumed to be equal to that of water (1.33). The emission spectra for the sample were recorded at the excitation wavelength of 356 nm, keeping slit width at 2 nm.

2.5. Fluorescent Measurement for Hg^{2+}

The PEHA-GQD-DPA dispersion (600 μL, 20 μg·mL^{-1}) was mixed with the PBS (3 mL, pH 5.5). Then, added the standard solution of Hg^{2+} with a known concentration. The mixed solution was equilibrated for 5 min and subsequently subjected to fluorescence measurement on fluorescence spectrophotometer with excitation wavelength of 360 nm. For the each of Hg^{2+}detections, the fluorescence measurement was repeated thrice, and average fluorescence signal was obtained.

2.6. Fluorescent Measurement for Glutathione

The Hg-PEHA-GQD-DPA dispersion was prepared by using the same procedure described in Section 2.5. The formed complex dispersion (1 mL) was mixed with the glutathione standard solution. After equilibrated for 5 min, the fluorescence spectrum was measured on fluorescence spectrophotometer with excitation wavelength of 360 nm. For the each of glutathione detections, the fluorescence measurement was repeated thrice, and average fluorescence signal was obtained.

2.7. Live Cell Imaging

SW480 cells were cultured in the DMEM containing 10% FBS and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air. Before the imaging experiment, the cells were seeded in 24-well plates for 24 h. The cells were incubated with the PEHA-GQD-DPA (20 μg·mL^{-1}) at 37 °C under 5% CO_2 for 6 h, washed 3 times to remove the remaining probe and bathed in the DMEM containing no FBS prior to imaging. Then, the standard Hg^{2+} solution was added in the growth medium for 15 min at 37 °C, washed 3 times with the PBS. The cells were imaged under an inverted fluorescence microscopy. After that, the cells were washed 3 times using the PBS of pH 7. The fluorescence images were characterized with an excitation wavelength of 450 nm.