Facile preparation of low cytotoxicity fluorescent carbon nanocrystals by electrooxidation of graphite†

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Received (in Cambridge, UK) 21st July 2008, Accepted 26th August 2008
First published as an Advance Article on the web 24th September 2008
DOI: 10.1039/b812420e

A simple and facile method was developed to prepare fluorescent carbon nanocrystals (CNCs) with low cytotoxicity and no photobleaching, by electrooxidation of graphite in aqueous solution.

Fluorescent semiconductor nanocrystals have been widely used in biology and medicine, for example, in immunolabeling,[1] as cell markers,[2] in cell motility assays[3] and in biological assembly,[4] due to their photostability and other excellent optical properties. Semiconductor quantum dots such as CdSe and the related core–shell nanocrystals are usually used. However, the release of a Cd^{2+} ion arouses cytotoxicity and is potential environmental hazard, which limits the application of quantum dots.[5] Therefore, it is urgent to search environment-friendly fluorescent nanoparticles.

Recently, fluorescent carbon-based nanomaterials have been developed including nanotubes,[6] fullerenes[7] and nanoparticles.[8–15] Among these fluorescent carbon-based nanomaterials, carbon nanoparticles (CNPs) are highly promising due to their relatively high quantum yield and small size. CNPs can be prepared by laser ablation of graphite,[8,9] carboxylation of carbon nanotubes[10–12] or candle soot,[13] and proton-beam irradiation of nanodiamonds.[14,15] Although nanodiamonds are of low cytotoxicity and do not photobleach, their large size of about 100 nm diameter and high cost would limit their use. For other CNPs, tedious preparation methods such as refluxing in 3–5 M nitric acid over night[8,9,12,13] or expensive raw materials[10–12] make them difficult to obtain. Sun's group and Zhou *et al.* prepared CNPs with high quantum yield, but their emission spectra were dependent on excitation wavelengths.[8–10] Therefore, it is difficult to use these CNPs for multicolor imaging. Mao's group obtained multicolor fluorescent CNPs from candle soot by PAGE gel separation, but it is still difficult to get pure CNPs with different colors (with a full width at a half maximum (FWHM) of larger than 120 nm) as mentioned.[13] An electrochemical method was used to prepare CNPs from multiwalled carbon nanotubes (MWCNTs) in acetonitrile solution, which is unsuitable for both aqueous solution and bulk carbon materials.[10] Herein, we report a simple method to facilely prepare fluorescent carbon nanocrystals (CNCs) with low cytotoxicity and no photobleaching by electrooxidation of graphite in aqueous solution, followed by a convenient separation.

In this work, a graphite column electrode (GE) was electrooxidized at 3 V against a saturated calomel electrode (SCE) with a Pt wire counter electrode in 0.1 M NaH_2PO_4 aqueous solution as the supporting electrolyte to prepare fluorescent CNCs. With increasing oxidation time, the electrolyte solution changed from colorless to yellow and finally to dark brown. The dark brown solution was centrifuged (28 000*g*) for 30 min to remove the non-fluorescent deposit. The supernatant was then ultrafiltered through centrifugal filter devices respectively with three different molecular weight cutoff membranes (Amicon Ultra-4, Millipore) to obtain products equivalent to <5, 5–10, 10–30 and >30 kDa. The 10–30 and >30 kDa fractions exhibited no fluorescence. The <5 and 5–10 kDa fractions emitted blue and yellow fluorescence, respectively, when irradiated with a UV lamp (Fig. 1 inset). The emission spectra showed a peak at 445 nm and 510 nm for the <5 and 5–10 kDa fractions, respectively (Fig. 1). The FWHM for the blue fluorescent fractions was only 68 nm smaller than those previously reported.[8–13] The maximum excitation wavelengths for the blue and yellow fractions were at 330 nm and 370 nm, respectively (Fig. 1).

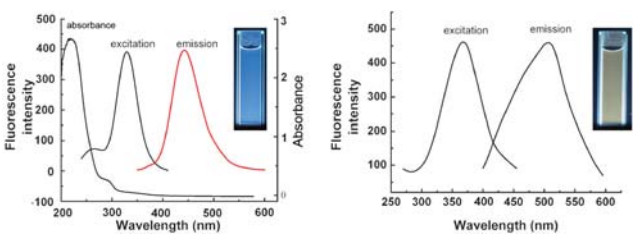

Fig. 1 (Left) UV-vis absorption and fluorescence spectrum of <5 kDa fraction in aqueous solution. The emission spectrum was obtained under excitation at 330 nm, and the excitation spectrum was obtained at the maximum emission wavelength of 445 nm. Inset: digital photo for the product, illuminated with a UV lamp. (Right) Fluorescence spectrum of 5–10 kDa fraction in aqueous solution, excitation wavelength: 370 nm; the excitation spectrum collected at 510 nm. Inset: digital photo for the product, illuminated with a UV lamp.

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† Electronic supplementary information (ESI) available: XPS result, determination of quantum yield, additional fluorescence spectra, photostability of CNCs, effect of ionic strength on fluorescence intensity, effect of pH on the fluorescence spectra and intensity, Fourier-transform infrared spectra, and the protocol of cytotoxicity tests. See DOI: 10.1039/b812420e

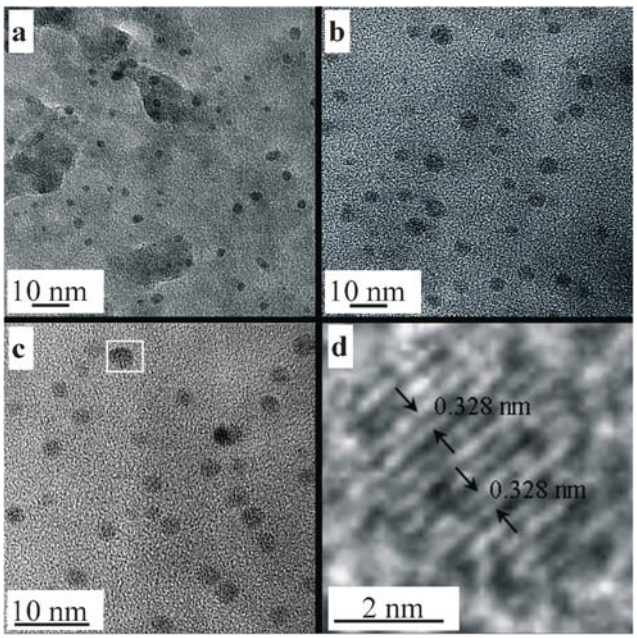

Fig. 2 HRTEM images of CNCs. (a): Blue fluorescent CNCs (<5 kDa), (b) and (c): yellow fluorescent CNCs (5–10 kDa), (d) enlargement of the CNC highlighted with a rectangle in (c).

The X-ray photoelectron spectroscopy (XPS) result showed that the fluorescent fractions contained mainly carbon and oxygen (Fig. S1 in the ESI†). High-resolution transmission electron microscopy (HRTEM) images of the fluorescent fractions (Fig. 2) revealed clearly that they were monodisperse nanocrystals with a lattice spacing of 3.28 Å (Fig. 2, inset), which is very close to that for the 002 facet of graphite.[16] Therefore, the fluorescent fractions obtained in this work were graphite-structured CNCs. The nanocrystals had a uniform spherical shape and a narrow size distribution of 1.9 ± 0.3 nm and 3.2 ± 0.5 nm in diameter, respectively, for the blue and yellow CNCs. The fluorescent emission peak of CNCs red shifted from 445 nm to 510 nm with increase in the diameter, indicating that the emission spectra of CNCs were size-dependent, similar to those of quantum dots.

The quantum yield of the blue CNCs was determined to be 0.012, when excited at 330 nm (Fig. S2 in ESI†). The value could be comparable to those of CNCs obtained by other groups,[12,13] and with varying excitation wavelength, the fluorescence intensity of the CNCs changed, while the emission peak did not shift (Fig. S3 in the ESI†). The emission spectra of the CNCs were excitation-independent compared to those obtained by other groups.[8–10] The CNCs showed excellent photostability, as the fluorescence intensity did not change even after continuous excitation of 6 h with a Xe lamp (8.3 W) (Fig. S4 in ESI†). The fluorescence intensity of the CNCs did not change even in a solution at a high ionic strength of 2 M KCl (pH 7) (Fig. S5 in ESI†), indicating that the CNCs aqueous solution was stable.

The fluorescence intensity of the CNCs was pH dependent (Fig. 3), and decreased when the solution pH was higher or lower than 4.5, and recovered totally when the pH value was adjusted back to around 4.5 (Fig. S6 in ESI†). At the same time, the fluorescence emission peak did not shift with varying pH. The fluorescence intensity of CNCs was linear with the solution pH over the range of 7–14. These pH-sensitive CNCs could be used to monitor reactions that may lead to a minor change in pH.

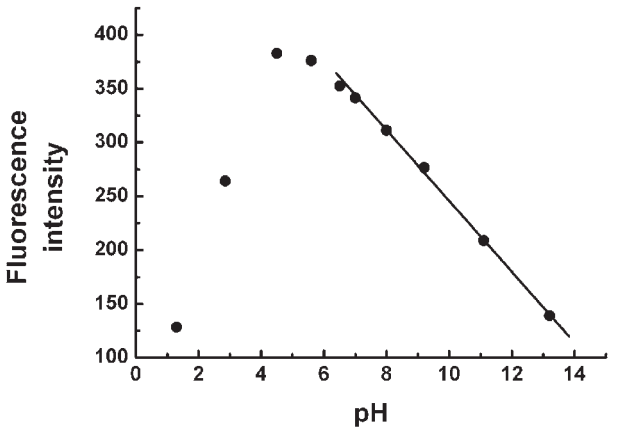

Fig. 3 Effect of the solution pH on the fluorescence intensity of the CNCs.

Besides the high photostability and monodispersity, low cytotoxicity was another advantage of CNCs. As revealed by the MTT assay (see ESI for details†), adding CNCs, up to 400 μg, to 100 μL of the culture medium (containing 8×103 293T human kidney cells) did not obviously diminish the cell viability (Fig. 4). The low cytotoxicity of CNCs was related to the properties of the raw material, chemically inert and non-cytotoxic graphite, which does not release any toxic species even in harsh environment. So, the low cytotoxicity would make CNCs suitable for *in vivo* labeling and imaging.

The luminescence mechanism of the CNCs is still unclear. In this work, it has been found that the emission spectrum of the CNCs is size-dependent. With increasing particle size from 1.9 ± 0.3 nm to 3.2 ± 0.5 nm, the νC═O shifted from 1630 to 1620 cm^{-1} (Fig. S7 in ESI†), indicative of an increase in the conjugation system. The increase in conjugation system usually results in a red shift in the emission wavelength. In this work, the emission spectra of CNCs red shifted with an increasing

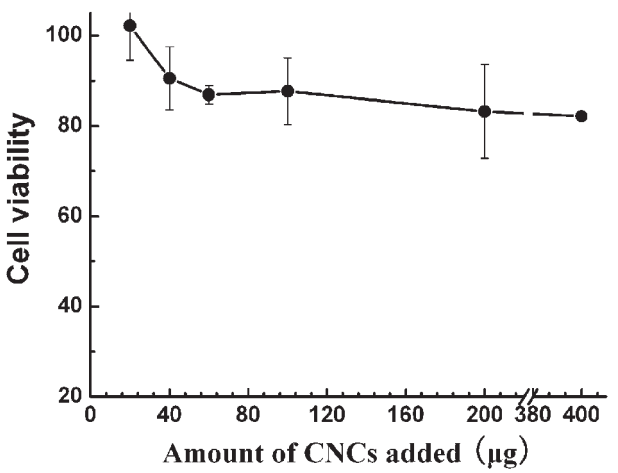

Fig. 4 Effect of CNCs on 293T human kidney cell viability.

conjugation system. Therefore, the luminescence of graphite-structured CNCs may be related to the conjugation system.

In conclusion, a facile method has been developed to prepare fluorescent CNCs by electro-oxidation of graphite in an aqueous solution. The resultant CNCs are monodisperse, photostable and lowly toxic, which are promising in biological labeling, imaging and disease diagnosis.

This work was supported by the National Key Scientific Program (973)—Nanoscience and Nanotechnology (No. 2006CB933100), the Science Fund for Creative Research Groups of NSFC (No. 20621502), the National Natural Science Foundation of China (Grant No. 30570490), and the Ministry of Education (Nos. 306011 and IRT0543). The authors wish to thank Min Liu for his help in cytotoxicity test and Prof. Dong-Shan Zhao for HRTEM.

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