Reversible fluorescence modulation of spiropyran-functionalized carbon nanoparticles†

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Fluorescent carbon nanoparticles (CNPs) with diameters of about 3 nm which can emit blue-green light were synthesized through the hydrothermal carbonization of ethylenediaminetetraacetic acid disodium salt (EDTA-2Na). Then, the CNPs were functionalized with spiropyrans to obtain the spiropyran-functionalized CNPs. The emission of the spiropyran-functionalized CNPs centered at 510 nm could be switched off, while being turned on at 650 nm via energy transfer after UV light irradiation. The process could be reversed by using visible light irradiation. The optical switching of the fluorescence was repeated 10 times without apparent “fatigue”, showing excellent photoreversibility and high stability. Spiropyran-functionalized CNPs may find potential applications in biological imaging and labeling, reversible data storage/erasing, as well as individual light-dependent nanoscale devices.

Introduction

Fluorescent carbon nanoparticles (CNPs) are a new kind of fluorescent material, whose photoluminescence behavior is similar to that of semiconductor quantum dots. However, compared with semiconductor quantum dots, fluorescent CNPs have advantages such as stable photoluminescence, low cytotoxicity, and excellent environmental and biological compatibility.1–8 Therefore, fluorescent CNPs have attracted much attention in recent years because they can potentially be applied in biological labeling and imaging, sensors and other different optoelectronic device fields.9–13 Currently, fluorescent CNPs are prepared by a variety of methods including high-energy ion beam irradiation of diamond particles,1,9 laser ablation,1,2,12 electrooxidation of graphite,14,15 thermal decomposition of organic compounds,7 oxidation of candle soot with nitric acid,16,17 microwave synthesis18 and aqueous solution routes.19 To date, despite remarkable advances made in the preparation of CNPs and their applications, there have been few reports on the functionalization of CNPs with other functional groups.20 If CNPs could be functionalized, it would change their luminescence properties and even widen the scope of their applications. For example, the fluorescence properties of CNPs were improved due to functionalization by fluorescein, rhodamine B and α-naphthylamine.21 Usually, there are some carboxyl and hydroxyl groups on the surface of the fluorescent CNPs prepared with most of the reported methods. Therefore, it is very easy to functionalize the CNPs with other functional groups. Spiropyran is a kind of photoisomeric dye, which can generally be used to functionalize some low dimensional materials such as semiconductor quantum dots,22 noble metal nanoclusters23,24 and carbon nanotubes25 to gain multifunctional materials to widen the field of their applications. However, CNP–spiropyran systems have not been reported to the best of our knowledge. Herein, we report such spiropyran-functionalized CNPs, which were prepared by covalently linking CNPs and carboxyl spiropyrans. The spiropyran-functionalized CNPs featured reversible fluorescence modulation properties, which can be potentially used for reversible data storage/erasing, individual light-dependent nanoscale devices, etc.

Experimental section

Materials

Ethylenediaminetetraacetic-acid–disodium salt (EDTA·2Na) and ethylenediamine were obtained from Aldrich. N-Hydroxysuccinimide (NHS) was received from ACROS (New Jersey, USA). N-Ethyl-N-[[(dimethylamino)propyl] carbodiimide (EDC) were from Avocado Research Chemicals Ltd (Lancashire, UK), while the carboxyl-containing spiropyran 1-(β-carboxyethyl)-3',3'-dimethyl-6-nitrospi[indoline-2',2-[2H-1]-benzopyran]
(SPCOOH) was synthesized according to a procedure reported in ref. 25.

The water used was double-distilled water, and other chemicals used as received were of analytical reagent grade.

Synthesis of the fluorescent CNPs

The fluorescent CNPs were synthesized by a hydrothermal method as follows: a certain amount of EDTA-2Na was added to 20 mL of water in a hydrothermal reactor and sealed, then heated at 220 °C for 24 h. After the reaction, the reactants were cooled down naturally to room temperature. The brown mixture was obtained and dried by vacuum rotary evaporation at 70 °C. The resulting brownish black solid was dissolved in 20 mL of ethanol. Then the obtained solution was centrifuged at a high speed (16,000 r min⁻¹) for 10 min to remove the deposits. The upper yellow solution exhibited strong blue-green luminescence with irradiation by light at 365 nm, and was a solution containing CNPs. The CNP solution was dried to obtain powder for further use.

Preparation of ethylenediamine-functionalized CNPs

Functionalization of the CNPs with ethylenediamine was carried out according to the following method: 100 mg of the CNP powder was first dispersed in 100 mL of water \( (C_{\text{CNPs}} = 1 \text{ mg mL}^{-1}) \) and activated by NHS (15 mmol L⁻¹) and EDC (50 mmol L⁻¹) at a pH of 5.0 for 30 min. An excess amount of ethylenediamine \( (C = 0.05 \text{ mg mL}^{-1}) \) was added to the CNP solution to functionalize the CNPs for 1 hour. The solution of the functionalized CNPs was then dialyzed against water for 48 h (molecular weight cut off was 500) and dried by vacuum rotary evaporation at 70 °C for use.

Preparation of spiropyran-functionalized CNPs

20 mg of ethylenediamine-functionalized CNPs was dispersed in 20 mL of ethanol \( (C_{\text{CNPs}} = 1 \text{ mg mL}^{-1}) \), and then mixed with an equal volume of carboxyl-containing spiropyran ethanol solution \( (C_{\text{spiropyran}} = 0.3 \text{ mg mL}^{-1}) \), activated by 15 mmol L⁻¹ NHS and 50 mmol L⁻¹ EDC ethanol solution in the dark, and maintained for 1 hour at a pH of 5.0. The CNP/spiropyran mixed solution was dialyzed against ethanol for 48 h (molecular weight cut off was 500), and stored in the dark at 2–8 °C.

Characterization methods

Transmission electron microscopy (TEM) was performed on a Hitachi-7650 electron microscope operating at 100 kV, using a micro grid as a support membrane. Fluorescence spectra were recorded with a Hitachi F-4500 fluorescence spectrophotometer at room temperature. UV-visible spectra were recorded with a Perkin-Elmer Lambda 25 instrument. FTIR spectra were obtained by using a Nicolet Avatar 360 FTIR spectrophotometer. X-ray Photoelectron Spectra (XPS) were recorded with an Escalab 250 XI X-ray photoelectron spectrometer. Time-resolved fluorescence was measured with an FLS920 combined fluorescence lifetime and steady state spectrometer, using 405 nm light for excitation.

Results and discussions

Fig. 1 shows the TEM images of the CNPs, ethylenediamine-functionalized CNPs and spiropyran-functionalized CNPs. The CNPs are almost monodispersed and have diameters of about 3 nm. The ethylenediamine-functionalized CNPs can be well dispersed, and are of the same size as the CNPs. The spiropyran-functionalized CNPs show little aggregation, but most of them are dispersed. This may be due to the surface modification of the hydrophobic spiropyran which changed the nature of the surface of the CNPs, and resulted in the aggregation.

Fig. 2 shows the FTIR results for the CNPs, ethylenediamine-functionalized CNPs and spiropyran-functionalized CNPs. For the fluorescent CNPs, the peaks at 3423, 2926 and 1383 cm⁻¹ clearly demonstrate the presence of \( –\text{OH} \), \( –\text{CH}_2 \), and \( –\text{CH}_3 \), respectively, while the absorption bands at 1601 cm⁻¹ and 1410 cm⁻¹ are from the antisymmetric and symmetrical stretching vibrations of \( –\text{COO}^– \), respectively. Obviously, the FTIR results of the CNPs are different from those of the pure EDTA-2Na (Fig. S1†), which indicates that the EDTA-2Na was completely carbonized and turned into CNPs during the hydrothermal process. For the ethylenediamine-functionalized CNPs, the band at 1632 cm⁻¹ of the amine group \( (–\text{NH}_2) \) can be seen, while the peaks at 1600 and 1410 cm⁻¹ of \( –\text{COO}^– \) have disappeared, indicating that the CNPs were functionalized with ethylenediamine.

Meanwhile, for the spiropyran-functionalized CNPs, the typical stretching band of the aryl nitro group \( (–\text{NO}_2) \) at 1340 cm⁻¹, and the peaks of the cyclic ether C–O–C group at 1264, 1160 and 1090 cm⁻¹ can be observed, indicating the presence of spiropyran.26,27 Furthermore, two additional peaks at 1651 and 1563 cm⁻¹ can be observed, showing that the formation of such amide bonds was due to the bonding between the \( –\text{COOH}/–\text{COO}^– \) groups of the CNPs or the spiropyran with the \( –\text{NH}_2 \) group of ethylenediamine. We also used XPS to characterize the as-prepared functionalized CNPs as shown in Fig. S2.† The amount of amide groups in the spiropyran-functionalized CNPs...
is far higher than that in the ethylenediamine-functionalized CNPs. These results confirm that the fluorescent CNPs were covalently linked with spiropyran.

The UV-vis absorption and fluorescence emission spectra of the CNPs dispersed in ethanol are shown in Fig. 3. One weak absorption peak centered at about 270 nm can be seen. The fluorescence emission spectra of the CNPs are dependent on the excitation wavelength in the range 280–480 nm, similar to that of CNPs reported by Yang et al.28 It can be seen that the optimal emission wavelength is 480 nm, while the optimal excitation wavelength is 380 nm. The luminescence behavior of the CNPs dispersed in ethanol was the same as that of those dispersed in water (Fig. S3†). The quantum yield of the CNPs was estimated to be 15% when excited at 360 nm, (based on quinine sulfate as a reference at room temperature). The ethylenediamine-functionalized CNPs feature similar UV-vis absorption and fluorescence properties to the as-prepared CNPs (Fig. S4†) when dispersed in ethanol. However, compared with the as-prepared CNPs, the absorption was blue shifted and sharpened, and the emission spectra red shifted. This indicates that the functionalization with ethylenediamine changed the optical properties of the CNPs.

Fig. 4 displays the absorption spectra of the spiropyran-functionalized CNPs dispersed in ethanol. There is one characteristic absorption peak of spiropyrans centered at 340 nm which can be seen before UV irradiation, which originates from the closed-ring spiro (SP) forms of the spiropyran molecules. However, after UV irradiation for 4 min (365 nm, 15 W), the absorption of the spiropyran red-shifts to about 355 nm, and at the same time, a new absorption peak at 550 nm appears due to the formation of the open-ring merocyanine (MC) form because of UV irradiation.21,22 The results further indicate that the surfaces of the CNPs were successfully modified with spiropyran.

Usually, it is impossible for the fluorescence emission intensity of CNPs to be modulated only by UV/vis light irradiation. Nevertheless, the spiropyran-functionalized CNPs could exhibit an on–off function as shown in Fig. 5. Upon UV light irradiation, the fluorescence intensity at 510 nm could be gradually decreased with an increase of the irradiation time. Meanwhile, a new fluorescence emission at 650 nm appeared and its intensity gradually increased (Fig. 5a). The emission at 650 nm arises from the MC form of the spiropyran molecules,21 the emission at 510 nm from CNPs. However, the progress can be reversed when the system is irradiated with visible light (525 nm, 5W LED). The fluorescence intensity at 650 nm could be decreased, and simultaneously, the fluorescence emission at 510 nm could be gradually recovered (Fig. 5b). Moreover, the position of the maximum emission of the spiropyran-functionalized CNPs shown in Fig. 5 seems to blue shift upon UV irradiation and red shift upon visible light irradiation, which may be caused by the appearance/disappearance of the fluorescence emission band of the MC-state of the spiropyran moiety which partly overlapped with the emission of the CNPs, together with the increase/decrease of energy transfer, resulting in the phenomena of blue and red shifting.

In this study, the fluorescence emission band (400–650 nm, excited at 420 nm) of the CNPs can overlap significantly with the absorption band (500–650 nm) of the MC form of spiropyran in the hybrid system as shown in Fig. 6. According to the principles of fluorescence resonance energy transfer (FRET), the energy of the donor and acceptor can overlap, and the distance between...
them should be less than 10 nm), here, energy transfer from the CNPs (donor) to the open-ring state MC (acceptor) is possible. As Scheme 1 illustrates, upon UV irradiation, the spiropyran can turn into the open-ring MC form, and the energy transfer from the CNPs to the MC-form of the spiropyran in the hybrid system is efficient. Therefore, the fluorescence emission of the CNPs can be quenched by the MC form, and the color of the spiropyran-functionalized CNPs observed under a 365 nm UV lamp can change to red from the original blue-green (Fig. S4†). However, when the system was irradiated with visible light, the MC-form could be converted to the closed-ring SP form, and the energy transfer from the CNPs to the spiropyran was inhibited because the energies of the CNPs and the SP form in the hybrid system could not overlap. Thus, the fluorescence emission at 510 nm of the CNPs could recover, and the spiropyran-functionalized CNPs under 365 nm UV lamp irradiation could exhibit blue-green fluorescence again (Fig. S5†). Furthermore, the lifetime of the emission at 510 nm of the spiropyran-functionalized CNPs when spiropyran molecules were in the SP form is longer than that when in the MC form (the results and discussion are shown in Fig. S6†). As a result, the reversible fluorescence modulation of the spiropyran-functionalized CNPs can be ascribed to FRET from the CNPs to the open-ring state MC and the structural transformation between the SP and MC forms of the spiropyran molecules.

The typical photoresponse of the spiropyran-functionalized CNPs is given in Fig. 7. The fluorescence intensity at 510 nm from the spiropyran-functionalized CNPs could be gradually
decreased by about 80% within 4 min upon UV irradiation, indicating that the fluorescence quenching of CNPs occurs due to the transformation of spiropyran moieties from the SP form to the MC form in 4 min, while its fluorescence intensity gradually recovers upon irradiation with 525 nm visible light within 130 seconds, which means that the photoinduced conversion from the MC form to the SP form of spiropyran moieties can be completed in 130 seconds. Compared with some fluorescent polymer nanoparticles containing spiropyrans previously reported,29 the switching speed rate of the spiropyran-functionalized CNPs is much faster. However, the inherent rate constants for photoinitiated SP–MC interconversion usually result in half-lives typically on the subpicosecond time scale.31 Under the present experimental conditions, 4 min of UV illumination was required to quench the fluorescence of the CNPs and 130 seconds of visible light irradiation was required to reverse the process, indicating a slow interconversion rate constant. It is the polar solvent ethanol and residual water in the system that probably contribute to such a slow rate of fluorescence switching because a polar solvent such as water can tend to stabilize the zwitterionic MC form.30

The reversible nature of fluorescence modulation for the spiropyran-functionalized CNPs upon exposure to alternating cycles of UV/vis light irradiation was also investigated. As shown in Fig. 8, the optical switching of the fluorescence was repeated 10 times without the apparent “fatigue” effect caused by the photodegradation of dyes due to UV irradiation. Moreover, in our case, the photoreversible fluorescence modulation of the spiropyran-functionalized CNPs could be repeated for many cycles even after they were stored at room temperature in the dark for more than one month, which indicates that the obtained spiropyran-functionalized CNPs were stable, and the reversible change of the photoluminescence could be repeated for many cycles even a

![Graph](image)

**Fig. 8** Fluorescence intensity of the spiropyran-functionalized CNPs at 510 nm upon alternate UV and visible light irradiation cycles.

**Conclusions**

In summary, spiropyran-functionalized CNPs exhibiting reversible fluorescence modulation can be obtained by linking CNPs prepared by the hydrothermal carbonization of EDTA-2Na with carboxyl spiropyran. The reversible modulation of blue-green and red fluorescence emission of the spiropyran-functionalized CNPs is due to the FRET from the CNPs to the open-ring state MC and the transformation of spiropyran moieties structures upon UV and visible light irradiation. The spiropyran-functionalized CNPs not only show good photo-reversibility but also possess high stability and relatively fast photoresponsivity. This suggests that the photoresponsive spiropyran-functionalized CNPs can be potentially applied in biological imaging and labeling, reversible data storage/erasing, as well as individual light-dependent nanoscale devices.

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**Notes and references**