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PAPER

Toward quantitatively fluorescent carbon-based “quantum” dots

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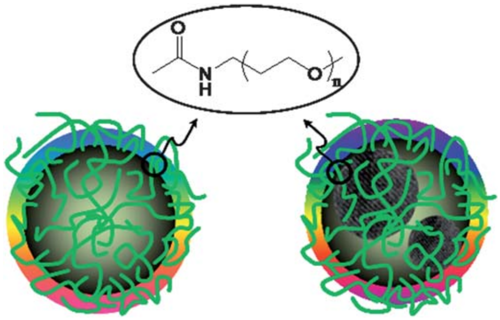
Carbon-based “quantum” dots (or “carbon dots”) are generally defined as surface-passivated small carbon nanoparticles that are brightly fluorescent. Apparently, the carbon particle surface passivation in carbon dots is critical to their fluorescence performance. An effective way to improve the surface passivation is to dope the surface of the precursor carbon nanoparticles with an inorganic salt, followed by the typical functionalization with organic molecules. In this work we passivated small carbon nanoparticles by a combination of the surface-doping with nanoscale semiconductors and the organic functionalization, coupled with gel column fractionation to harvest the most fluorescent carbon dots, which exhibited fluorescence emission quantum yields of up to 78%. Experimental and mechanistic issues relevant to potentially further improve the performance of carbon dots toward their being quantitatively fluorescent are discussed.

Introduction

Among optical materials, those that are strongly fluorescent have attracted much attention for their variety of applications from display technologies to biology and medicine. In the development of fluorescent nanomaterials, a major milestone was the discovery of semiconductor quantum dots (QDs), which are defined in general as nanoscale semiconductor particles with physical dimensions smaller than the exciton Bohr radius for the quantum-confinement effect.[1–4] It seems that the rationale for the use of QDs over organic dyes is now generally accepted in the literature.[5,6] However, despite the many widely acknowledged advantages of QDs, such as bright fluorescence, high photostability, and resistance to metabolic degradation in bio-applications,[7–10] most of the high-performance QDs require the use of heavy metal elements such as cadmium, whose known toxicity may prove to be a major disadvantage.[11–13] In fact, a number of studies have suggested that QDs containing heavy metals are toxic to vertebrate systems at relatively low concentrations,[14,15] and that there may also be risks with the bioaccumulation of these toxic materials in organs and tissues.[13,16] Extensive efforts have been made on encapsulating QDs in benign delivery vehicles to minimize their harmful biological and environmental effects, as well as on the development of non-toxic or much less toxic fluorescent nanomaterials as alternatives to the semiconductor QDs. In this regard, the recent finding[17] and subsequent development of carbon-based “quantum” dots (dubbed “carbon dots”) are particularly encouraging, with their known optical properties and already available performance demonstrations suggesting great application potentials.[18–24]

Carbon dots are surface-passivated small carbon nanoparticles (Scheme 1),[17] from which the fluorescence is likely due to surface energy trapping sites and their associated radiative recombinations.[20,25] Therefore, despite the absence of the traditional quantum confinement effect in carbon dots, the requirement for particles to be “quantum-sized” in semiconductor QDs is also applicable to the core carbon particles in order to ensure a very large surface-to-volume ratio for bright fluorescence emissions.[17] Carbon dots are highly stable photochemically, exhibiting no optical blinking.[17] According to available results from biological evaluations *in vitro* and *in vivo*, carbon dots are non-toxic to cells and mice,[23,26,27] amenable to serving as fluorescence probes in optical bioimaging.[18,28]

The carbon particle surface passivation in carbon dots is apparently critical to their fluorescence performance. For carbon dots with the surface passivation by oligomeric poly(ethylene



Scheme 1 Cartoon illustrations of non-doped (left) and doped (right) carbon dots.

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glycol) diamine (PEG_{1500N}) molecules in well-controlled amidation reactions, strong green fluorescence emissions were observed, with fluorescence quantum yields close to 20% in as-prepared samples.[25,26] Further processing of the samples to harvest the dots of better surface passivation resulted in PEGylated carbon dots of fluorescence quantum yields more than 50%.[25] Separately, improved carbon particle surface passivation was achieved by doping the surface of the precursor carbon nanoparticles with inorganic salts such as ZnS or ZnO, followed by the same functionalization with PEG_{1500N} molecules to obtain surface-doped carbon dots (denoted as C_{ZnS}-dots or C_{ZnO}-dots, respectively, Scheme 1).[29] These dots with their core carbon nanoparticles surface-passivated by a combination of inorganic and organic moieties exhibited significantly enhanced fluorescence performance. A logical question was then how much the surface passivation could be further improved toward quantitatively fluorescent carbon dots. In the work reported here, we passivated small carbon nanoparticles by a combination of doping (surface coating but not to the level of forming a shell) with nanoscale semiconductors and organic functionalization, coupled with gel column fractionation to harvest the most fluorescent carbon dots, which exhibited fluorescence emission quantum yields of up to 78%. Experimental and mechanistic issues relevant to potentially further improving the performance of carbon dots are discussed.

Results and discussion

Neat carbon nanoparticles were refluxed in aqueous nitric acid to oxidize some of the surface carbons into carboxylic acids. The resulting particles in aqueous suspension were fractionated gravimetrically for the harvesting of those mostly smaller in size (5 nm or less). These small carbon nanoparticles could be suspended in aqueous solutions in a relatively stable fashion, thus serving as nucleation centers in the titration for the formation of ZnS or TiO_2 to yield ZnS- or TiO_2-doped carbon nanoparticles, respectively. The surface-doping was probably not uniform among different nanoparticles, more likely with varying doping levels from particle to particle. A surfactant-assisted dispersion procedure in favor of the carbon particles with more ZnS or TiO_2 doping was used to exclude those with no or a negligible level of doping. Subsequently, those carbon nanoparticles with the surface completely covered by ZnS or TiO_2 (thus no accessible carboxylic acid moieties on the particle surface) were discriminated in the functionalization chemistry with organic molecules.

The ZnS- or TiO_2-doped carbon nanoparticles (with the surface not completely covered by the inorganic salts, thus still some exposed carboxylic acid moieties) were functionalized by PEG_{1500N} molecules to yield ZnS- or TiO_2-doped carbon dots (denoted as C_{ZnS}-dots or C_{TiO_2}-dots, respectively).[29] According to thermogravimetric analysis (TGA) results on the as-prepared C_{ZnS}-dots and C_{TiO_2}-dots samples (10 °C min^{-1}, first to 600 °C in nitrogen flow to remove the surface functional groups and then to 800 °C in air flow to oxidize the core carbon nanoparticles into carbon dioxide and at the same time ZnS into ZnO), the estimated core compositions in terms of C : ZnS and C : TiO_2 molar ratios in the C_{ZnS}-dots and C_{TiO_2}-dots samples were on the order of 20 : 1 and 40 : 1, respectively.

UV/vis absorption spectra of the as-prepared C_{ZnS}-dots and C_{TiO_2}-dots samples in aqueous solutions are shown in Fig. 1. The spectra are characterized by a similar shoulder in the blue (420–450 nm), which has been observed in other surface-doped carbon dots and also in some non-doped carbon dots (Fig. 1).[25,29] The excitation into the absorption shoulder resulted in strong fluorescence emissions around 510 nm for both C_{ZnS}-dots and C_{TiO_2}-dots solutions, again similar to those observed previously for other carbon dots (Fig. 1).[25,29] There were some variations in fluorescence quantum yields for the as-prepared C_{ZnS}-dots and C_{TiO_2}-dots samples from batch to batch, due likely to the inhomogeneity in each sample (containing various fractions of dots with different surface morphologies in terms of the surface-doping and organic functionalization chemistry), but all consistently higher than 30%.

The as-prepared C_{ZnS}-dots and C_{TiO_2}-dots samples in aqueous solutions were fractionated on a gel column (Sephadex™ G-100) to harvest the most fluorescent fraction from each of the samples. As shown in Fig. 1, the blue shoulder in the absorption spectra of these fractions is more pronounced than that in the as-prepared C_{ZnS}-dots and C_{TiO_2}-dots samples, though the corresponding fluorescence spectra remain similar. However, the observed emission quantum yields for the most fluorescent C_{ZnS}-dots and C_{TiO_2}-dots were much higher than those for the as-prepared samples, up to 78% for C_{ZnS}-dots and 70% for C_{TiO_2}-dots. In fact, the bright fluorescence from these solutions could be appreciated visually under natural light. Shown in Fig. 2 are comparisons of the C_{ZnS}-dots and C_{TiO_2}-dots solutions with the nearly quantitatively fluorescent fluorescein solution under sunlight and with the excitation of monochromated light from a xenon arc source.

Fluorescence decays of C_{ZnS}-dots and C_{TiO_2}-dots were measured by using the time-correlated single photon counting (TCSPC) technique. For the most fluorescent C_{TiO_2}-dots, as an example, the observed decay curve was similar to that of the

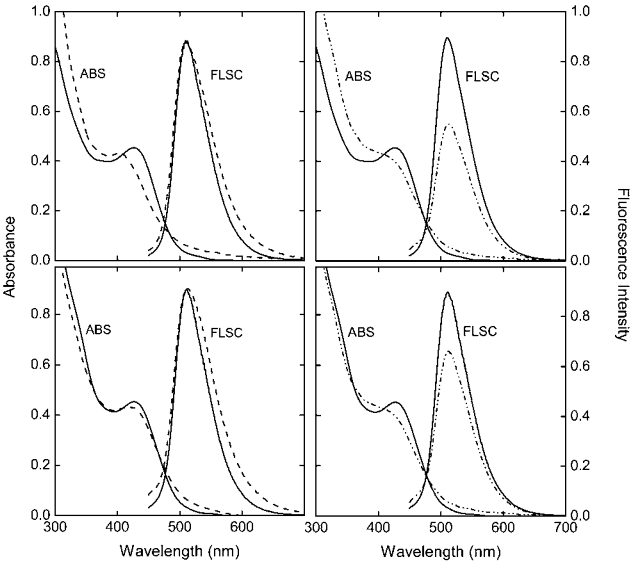

Fig. 1 Absorption (ABS) and fluorescence (FLSC, 440 nm excitation) spectra of the most fluorescent C_{ZnS}-dots (upper, —) and C_{TiO_2}-dots (lower, —) are compared with those of their corresponding as-prepared samples (left, ---) and also with those of the most fluorescent non-coated carbon dots (right, -..-..-, from ref. 25).

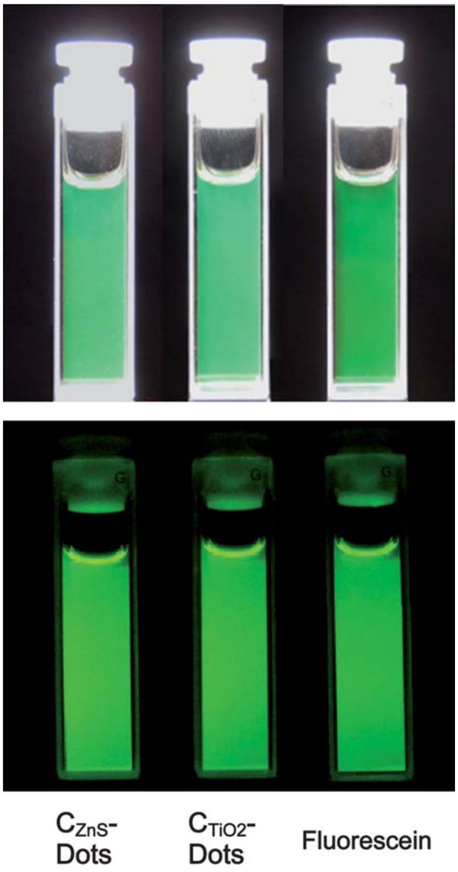

Fig. 2 Photographs of aqueous C_{ZnS}-dots and C_{TiO_2}-dots solutions are compared with a fluorescein solution in ethanol (fluorescence quantum yield ~80%) under sunlight (upper) and with monochromated light from a xenon arc source (lower, with matching optical density at the 440 nm excitation wavelength).

highly fluorescent non-doped carbon dots reported previously (Fig. 3).[25] The decay could only be fitted by a multi-exponential function with an average lifetime of about 6 ns. On average, therefore, the fluorescence lifetimes are not long, despite the observed high fluorescence quantum yields. The corresponding average fluorescence radiative rate constant k_F is more than 1×10^8 s^{-1}, which is considerably larger than those typically found in organic fluorophores, suggesting extraordinarily high electronic transition probabilities in these carbon-based fluorescent dots.[30]

The highly fluorescent samples of C_{ZnS}-dots and C_{TiO_2}-dots were characterized by microscopy techniques for an understanding of their structural parameters. As shown in Fig. 4 for results from the transmission electron microscopy (TEM) analysis of the C_{ZnS}-dots sample, the individual dots were well-dispersed, with an average particle size of about 5 nm. The ZnS doping in the dots was visible in the high-resolution TEM images (Fig. 4), so was the TiO_2 doping in C_{TiO_2}-dots (Fig. 5). The sizes of C_{TiO_2}-dots were also around 5 nm according to atomic force microscopy (AFM) imaging results (Fig. 5).

Mechanistically, the fluorescence emissions in carbon dots are attributed to radiative recombinations of the carbon particle surface-trapped electrons and holes, where the large surface (relative to the particle volume) and diverse surface energy trapping sites in the small carbon nanoparticles are stabilized by the surface passivation agents. Within such a mechanistic framework, the enhanced fluorescence performance in nanoscale semiconductor-doped carbon dots (more specifically C_{ZnS}-dots and C_{TiO_2}-dots in this study) may be rationalized in terms of improved surface passivation by a combination of inorganic surface-doping and organic functionalization, as well as probably more diverse surface sites as a result of the doping (thus facilitating more effective trapping of electrons and holes on the particle surface). There seem to be several issues (or opportunities at the same time) for further investigations to improve the presently highly fluorescent carbon dots toward being quantitatively fluorescent (quantum yields close to one hundred percent). One is on the selection of the dopant, for which nanoscale semiconductors are advantageous but apparently not necessary.[31] Structural and/or material parameters such as the compatibility of the selected inorganic salt with the carbon nanoparticle surface may play major roles in determining the fluorescence performance of the resulting carbon dots. Another issue is on the relative quantities of the inorganic and organic surface passivation moieties, or perhaps even their mutual

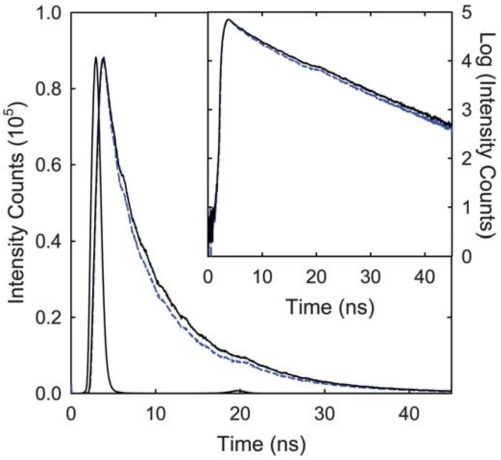

Fig. 3 Fluorescence decays (407 nm laser excitation, and monitored through 470 nm bandpass filter) of the most fluorescent C_{TiO_2}-dots (—) and the highly fluorescent non-coated carbon dots from ref. 25 (---).

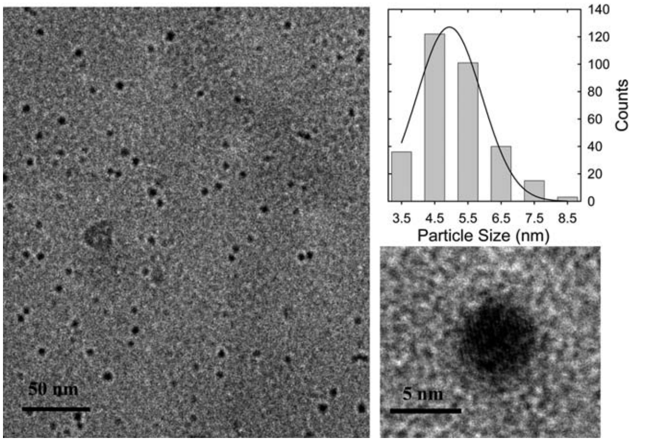

Fig. 4 A representative TEM image of the most fluorescent C_{ZnS}-dots (left) with the result from statistical size analyses of multiple images (upper right) and a high-resolution image of an individual dot (lower right).

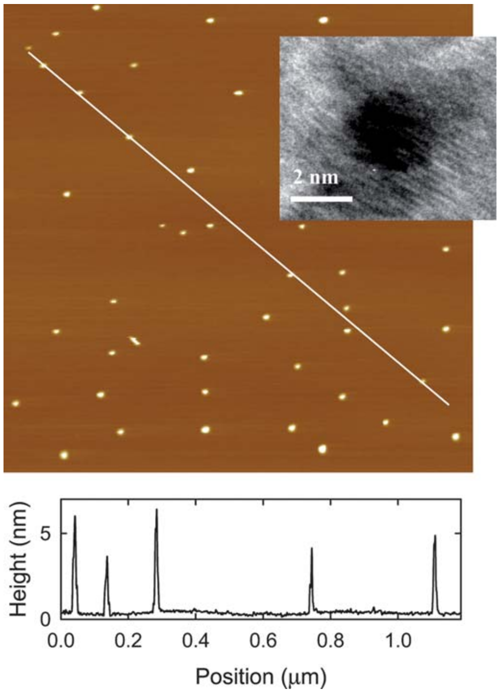

Fig. 5 A representative AFM topography image of C_{TiO_2}-dots on mica substrate, with the height profile analysis along the line in the image. Inset: a high-resolution TEM image of an individual dot.

compatibility on the carbon nanoparticle surface. These are obviously not easy issues, but nonetheless potentially explored for carbon dots of further enhanced fluorescence properties.

Again within the same mechanistic framework, the increase in fluorescence quantum yields as a result of surface-doping the carbon nanoparticles with ZnS or TiO_2 in carbon dots may be rationalized in terms of two possible effects. One is more effective energy trapping and radiative recombinations (essentially a more effective formation of emissive states), and the other is the suppression of processes competing with the fluorescence emissions (namely less "quenching"). According to the comparison between the most fluorescent carbon dots with and without the inorganic doping, the fluorescence decays in the doped carbon dots did not slow in parallel to the increases in fluorescence quantum yields (Fig. 3), suggesting that the doping effect primarily enhances the formation of emissive states. Interesting further studies may include a probing of such formation processes by using picosecond or femtosecond optical spectroscopy techniques.

Experimental section

Materials

O,*O*′-Bis(3-aminopropyl) polyethylene glycol ($M_w \approx 1500$, PEG_{1500N}), thionyl chloride (>99%), and $Ti(OC_2H_5)_4$ (97%) were purchased from Aldrich; $Zn(OOCCH_3)_2 \cdot 2H_2O$ (>98%) and $Na_2S \cdot 9H_2O$ (>98%) from Alfa; and nitric acid (60–70%), ethanol (>99%), *N*,*N*-dimethylformamide (DMF, 99%), sodium dodecyl sulfate (SDS, 99%) from VWR; and Sephadex G-100™ gel from GE Healthcare. Carbon-coated copper grids were supplied by Electron Microscopy Sciences; Millipore Durapore membrane filters (0.22 μm pore size) by Fisher Scientific; and the dialysis membrane tubing (molecular weight cutoff ≈ 1000) by Spectrum Laboratories. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

Measurements

Baxter Megafuge (model 2630), Beckman Coulter ultracentrifuge (Optima L-90K), and VWR bath sonicater (model 250D) were used. Thermogravimetric analysis (TGA) measurements were performed on a TA Instruments Q500 TGA analyzer. Transmission electron microscopy (TEM) images were obtained on Hitachi 9500 TEM and Hitachi HD-2000 S-TEM systems. Atomic force microscopy (AFM) analysis was carried out in the acoustic AC mode on a Molecular Imaging PicoPlus AFM system equipped with a multipurpose scanner and a NanoWorld Pointprobe NCH sensor. The height profile analysis was assisted by using the SPIP software distributed by Image Metrology.

UV/vis absorption spectra were recorded on a Shimadzu UV2101-PC spectrophotometer. Fluorescence spectra were obtained on a Spex Fluorolog-2 emission spectrometer equipped with a 450 W xenon arc source and a detector consisting of a Hamamatsu R928P photomultiplier tube (PMT) operated at 950 V. Fluorescence quantum yields were determined by the relative method[30,32] with the use of quinine sulfate and 9,10-bis(phenylethnyl)-anthracene as well-characterized fluorescence standards. Fluorescence decays were measured on a time-correlated single photon counting (TCSPC) setup with a Hamamatsu stabilized picosecond light pulser (PLP-02) for 407 nm excitation (<100 ps pulses at 1 MHz repetition rate), coupled with a Phillips XP2254/B PMT in a thermoelectrically cooled housing as a detector for an overall instrument time resolution better than 500 ps.

Doped carbon nanoparticles

A carbon nanopowder sample (2 g) obtained from laser ablation was refluxed in an aqueous nitric acid solution (2.6 M, 200 mL) for 24 h. After being cooled to room temperature, the sample was dialyzed against fresh water, followed by centrifuging at 1000*g* to retain the supernatant, from which surface-oxidized carbon nanoparticles were recovered (about 1 g).

The surface-oxidized carbon nanoparticles (600 mg) were dispersed in DMF (200 mL) *via* sonication for 30 min. To the suspension was added $Zn(OOCCH_3)_2 \cdot 2H_2O$ (680 mg, 3.1 mmol) under vigorous stirring, followed by slow dropwise addition of an aqueous Na_2S solution (0.62 M, 5 mL) at room temperature. The mixture was centrifuged at 3000*g*, and the precipitate was retained and repeatedly washed with distilled water to obtain the ZnS-doped carbon nanoparticles (881 mg).

In a typical experiment for the TiO_2 doping, a sol–gel solution was prepared by mixing $Ti(OC_2H_5)_4$, ethanol, water, and nitric acid in the ratio of 1/70/1.9/0.2, followed by refluxing at 80 °C with constant stirring for 1 h. To the solution (32 mL) was added the surface-oxidized carbon nanoparticles (200 mg), and the mixture was sonicated for 1 h, stirred for 12 h, and then filtrated. The filter cake was grounded and annealed at 250 °C for 1 h to obtain the TiO_2-doped carbon nanoparticles (230 mg).

C_{ZnS}-dots and C_{TiO_2}-dots

ZnS-doped carbon nanoparticles (200 mg) were dispersed in an aqueous SDS solution (1 wt%, 120 mL) *via* sonication for 30 min. Upon filtration, the filter cake was washed repeatedly with water and then dried. The solid sample was mixed thoroughly with PEG_{1500N} (1.9 g), and the mixture was heated to 110 °C and stirred for 72 h under nitrogen protection. Upon being cooled to room temperature, the reaction mixture was dispersed in water, and then centrifuged at 25 000*g* to retain the supernatant as an aqueous solution of C_{ZnS}-dots.

The same procedure and reaction conditions were used for the functionalization of TiO_2-doped carbon nanoparticles with PEG_{1500N} to obtain C_{TiO_2}-dots.

The as-prepared C_{ZnS}-dots and C_{TiO_2}-dots samples were further processed in terms of gel column fractionation to harvest the most fluorescent dots. For the gel column, Sephadex G-100™ gel (15 g) was soaked in water for 3 days. The supernatant (including the suspended ultrafine gel) was discarded, and the remaining gel was washed until no gel was suspended in the supernatant. Air bubbles were removed with vacuum. Separately, a glass column was filled with water to remove air bubbles, and then closed. The gel suspension was poured into the column, and as the gel precipitation reaching about an inch in height, the column was opened for the continuous addition of the gel suspension. The gel-filled column was washed until no changes in height, followed by the testing and calibration of the column. In the fractionation experiment, an aqueous solution of the as-prepared sample was added to the gel column and eluted with water. Fractions (typically 5–10) were collected and evaluated for their absorption and fluorescence properties.

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