Carbon "Quantum" Dots

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Bandgap-Like Strong Fluorescence in Functionalized Carbon Nanoparticles**

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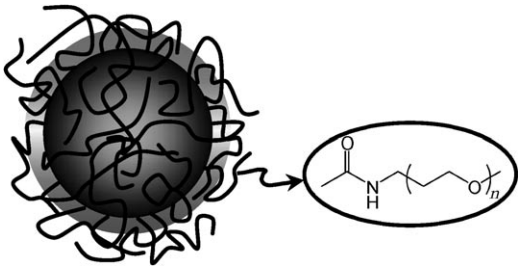
Semiconductor quantum dots (QDs), especially the highly fluorescent CdSe-based core-shell nanostructures, have generated much excitement for their variety of potential applications in optical bioimaging and beyond.[1,2] These QDs are widely considered as being more advantageous than conventional organic dyes and genetically engineered fluorescent proteins in terms of optical brightness and photostability.[1,3–5] However, a serious disadvantage with these popular QDs is that they contain heavy metals, such as cadmium, whose significant toxicity and environmental hazard are well-documented.[6–9] Therefore, alternative benign (nontoxic) QD-like fluorescent nanomaterials have been pursued, including the recent finding of fluorescent carbon nanoparticles (dubbed "carbon dots").[10,11]

Carbon dots are surface-passivated small carbon nanoparticles and the surface passivation is most effective following functionalization with organic or biomolecules[10–16] (though other passivation schemes are also possible for weaker emissions[17–19]). In addition to sharing some of the major advantageous characteristics of semiconductor QDs, including high photostability,[1,10,13] large two-photon excitation cross-sections,[11,20] and their applicability as optical imaging agents in vivo,[20,21] carbon dots are also nonblinking,[10,13] readily water soluble,[10,11,13–16] and nontoxic according to currently available cytotoxicity and in vivo toxicity evaluation results.[18,22] The as-produced carbon dots have so far exhibited fluorescence quantum yields of up to 20% in the green region of the spectrum,[22] which are somewhat lower than those of the best-performing commercially available CdSe/ZnS QDs for the comparable spectral region.

Herein, we report that the as-prepared carbon dots sample could be fractionated simply on an aqueous gel column and the most fluorescent fractions achieved emission yields close to 60%, comparable to those of the best commercial CdSe/ZnS QDs in solution and brighter at the individual dot level (owing to the carbon dots being significantly higher in absorptivities). Interestingly, both the absorption and fluorescence results of the carbon dots resembled those of band-gap transitions, typically found in nanoscale semiconductors. The prospect of carbon particles on the nanoscale acquiring essentially semiconductor-like properties that are enhanced by surface functionalization is discussed.

The synthesis of carbon dots with an oligomeric PEG diamine (PEG_{1500N}) as the surface passivation agent (Scheme 1) was based largely on the previously reported procedure,[10,22] except for a more rigorous control of the functionalization reaction conditions (critical to the enhanced fluorescence performance in the resulting carbon dots). The precursor carbon nanoparticles were treated with thionyl chloride to generate acyl chlorides on the particle surface and then reacted in the melt of PEG_{1500N} at 110 °C, for which the reaction temperature was found to significantly influence the fluorescence yield of carbon dots. The sample of carbon dots was processed in aqueous solution, and the resulting colored aqueous solutions at various concentrations remained stable indefinitely. The blue optical absorption shoulder (around 450 nm, Figure 1) was characteristic of these sample solutions, whilst the excitation resulted in equally characteristic green fluorescence emissions (centered around 510 nm, Figure 1) with quantum yields Φ_F of 16–20% (representing variations from batch to batch).



Scheme 1. Representation of a carbon dot containing an oligomeric PEG diamino surface passive agent.

The as-prepared sample of carbon dots was loaded onto an aqueous gel column packed with Sephadex G-100 (supplied by GE Healthcare)[23] for fractionation. With water as eluent, the fractions were collected and their optical absorption spectra were measured. As in the pre-fractionation

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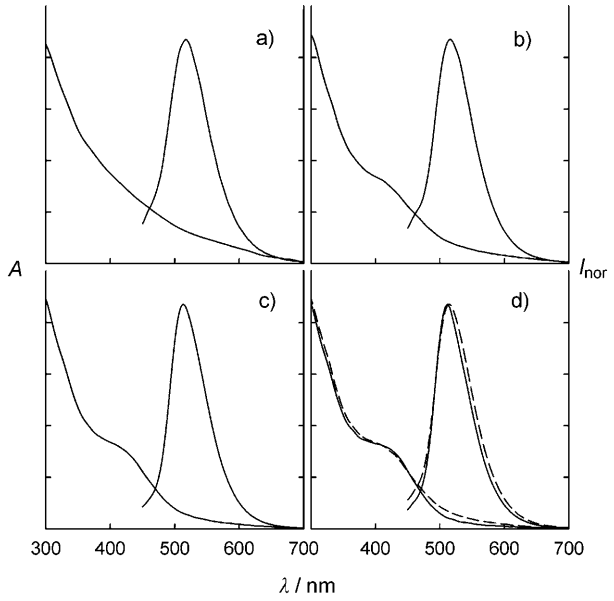

Figure 1. Absorption (*A*) and normalized fluorescence intenstiy (I_{nor}; 440 nm excitation) spectra of fraction 1 (a), 3 (b), 5 (c), and the most fluorescent fraction 7 (d). Dashed lines in (d) represent the spectra of the "as-prepared" sample for comparison.

sample, later fractions featured an increasingly well-defined absorption shoulder in the blue region (in the first fraction, the shoulder, which had a relatively lower intensity, was masked by other broad absorptions; Figure 1), and the excitation resulted in strong green fluorescence emissions. Whilst the observed fluorescence spectra were all rather similar (Figure 1), their quantum yields were significantly different, becoming progressively higher in the later fractions, and reaching Φ_F of 55–60% in the most fluorescent last fraction (Figure 2).

For comparative analyses on the nanoscale, the pre-fractionation sample and the most fluorescent fraction were deposited onto substrates for imaging using transmission electron microscopy (TEM) and atomic force microscopy (AFM). The TEM images suggested no major differences between the two samples under comparison, except for the latter sample containing on average slightly smaller particles, and a narrower distribution according to statistical analyses (Figure 3). These conclusions were generally supported by the AFM imaging results and the associated height analyses (see the Supporting Information).

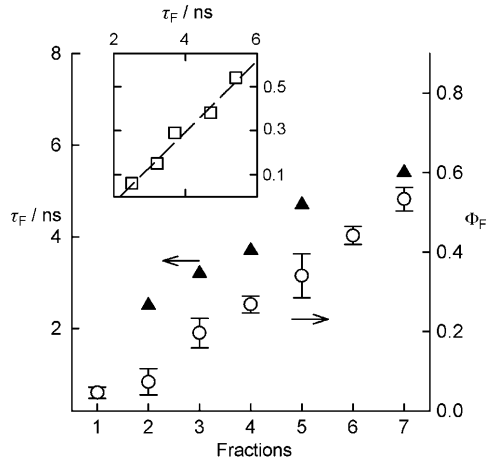

Figure 2. Fluorescence quantum yields (○) and lifetimes (▲) of the different fractions, and the linear relationship between the observed yields and lifetimes (inset).

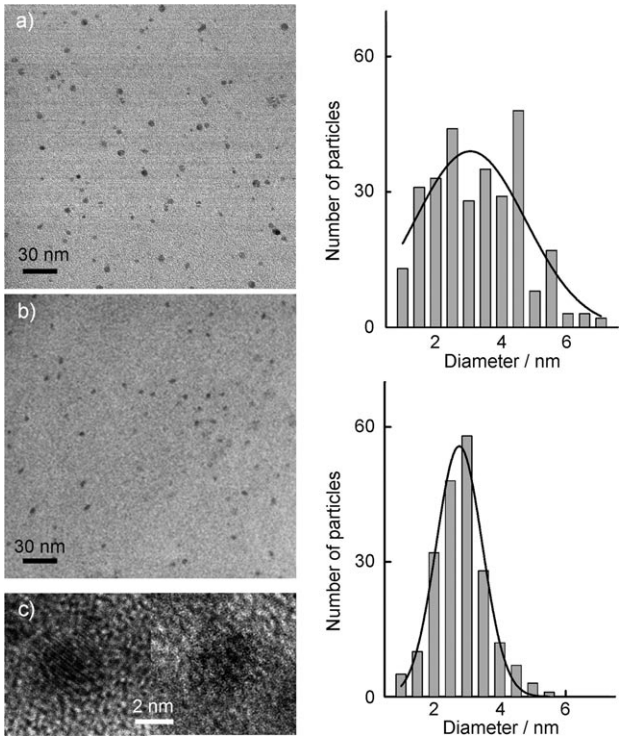

Figure 3. Representative TEM images of carbon dots in the as-produced sample (a) and in the most fluorescent fraction (b) with their corresponding statistical size analysis results based on multiple images. c) High-resolution image of two dots.

The fluorescence decay in the fractions could only be deconvoluted with a multiexponential function,[24] to give an average fluorescence lifetime for each of the fractions. The variation in the lifetime values was consistent with that in the observed fluorescence quantum yields from different fractions (Figure 2), thus suggesting a relatively uniform fluorescence radiative process throughout the fractions (namely, that the observed fluorescence quantum yield variations were due predominantly to changes in the competing nonradiative processes from fraction to fraction). The fluorescence radiative rate constants ($k_F = \Phi_F/\tau_F$) were very large throughout the fractions, on average $1 \times 10^8\ s^{-1}$, which suggests very strong electronic transitions.[25,26] For reference, anthracene as a strongly fluorescent organic dye has a radiative rate constant k_F of less than $5 \times 10^7\ s^{-1}$, to which the corresponding molar absorptivity of the 0–0 transition is more than $8000\ M^{-1}cm^{-1}$.[26] Also, for comparison, the commercially supplied best-performing CdSe/ZnS QDs ("QD525PEG" from Invitrogen) were found to have a k_F value of approximately $0.3 \times 10^8\ s^{-1}$ for the similar spectral region ($\Phi_F \approx 0.6$

and $\tau_F \approx 18.5$ ns; determined experimentally under the same conditions).

According to well-established photophysical principles,[24–26] the radiative rate constant is proportional to the integrated molar absorptivities in a particular absorption band, and in the first approximation proportional to the molar absorptivity at the band maximum.[26] Therefore, ratio of the absorbance at the band maximum (A_{max}) to k_F is approximately proportional to the numbers of dots in the solution; i.e., in a comparison between solutions of carbon dots and QDs, the same A_{max}/k_F value essentially represents the same number of dots in both solutions. Such a comparison, shown in Figure 4, suggests that at the individual dot level the carbon dots in the most fluorescent fraction could fluoresce more

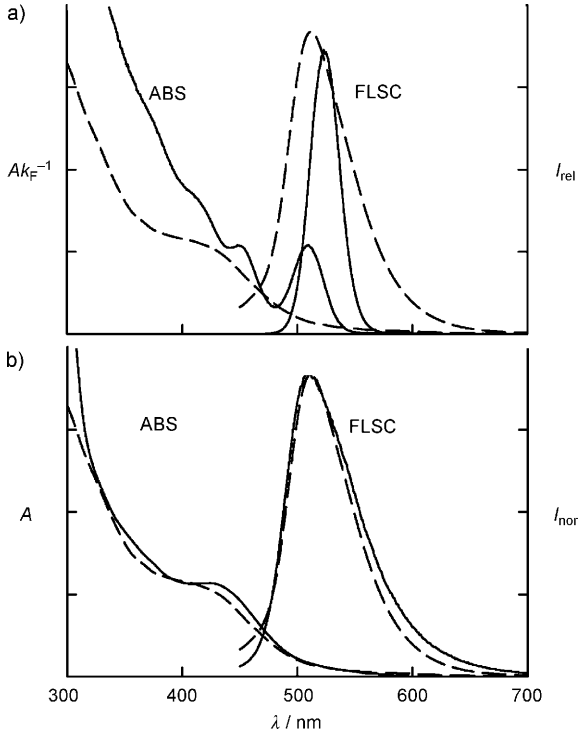

Figure 4. Absorption (ABS) and fluorescence (FLSC) spectra (I_{nor} = normalized intensity, I_{rel} = relative intensity) of carbon dots in the most fluorescent fraction (-----) are compared with those of Invitrogen QD525PEG QDs (——) in aqueous solutions. a) FLSC intensities corresponding to excitations at matching first band maximum Ak_F^{-1} values, and b) with those of ZnS-doped carbon dots.[34]

than twice as brightly as the reference CdSe/ZnS QDs in the same spectral region. This supposition was supported by results from the single-dot fluorescence imaging experiments described below.

The carbon dots were dispersed on cover glass used as a substrate in infinite dilution to allow confocal microscopy imaging of individual dots. The deposition conditions for the preparation of the specimens were essentially the same as those for TEM and AFM imaging, and the results confirmed the dispersion of individual dots in the specimens. For the pre-fractionation sample, fluorescence images of carbon dots that had a wider range of brightness were observed (Figure 5),

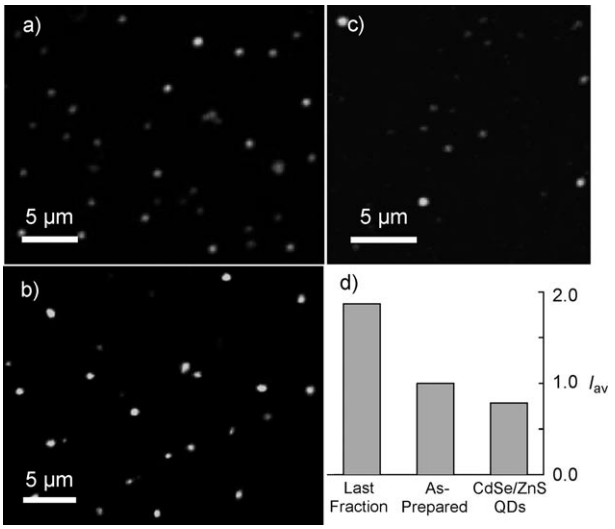

Figure 5. Fluorescence microscopy images (458 nm excitation) of carbon dots in the "as-prepared" sample (a) and in the most fluorescent fraction (b), and images of Invitrogen QD525PEG QDs (c). The bar-chart comparison was based on averaging the 300 most fluorescent dots in each of the three samples. I_{av} = average intensity.

which was consistent with the fact that the sample contained fractions of different fluorescence quantum yields. As expected, the carbon dots in the specimen from the most fluorescent fraction were more uniform in terms of fluorescence brightness (Figure 5). Also as expected from the conclusion in the comparison between bulk solutions of the same A_{max}/k_F ratio (discussed above), the individual carbon dots in this fraction had a noticeably brighter fluorescence (mostly by 2–2.5 fold; Figure 5) than the CdSe/ZnS QDs.

The carbon dots are comparable in size with, or somewhat smaller than, the commercially available aqueous-compatible CdSe/ZnS QDs (especially when the surface-capping agents are included in the dot sizes). Therefore, the brighter fluorescence emissions in individual carbon dots make these dots particularly valuable for optical bioimaging in vitro and in vivo, especially with regard to the emerging needs for molecular probes in high-resolution cellular imaging.[27,28]

Mechanistically, the fluorescence in carbon dots was thought to be associated with passivated surface defects of the core carbon particles.[10,11] In previous reports on the trapping of excited-state energy by surface defects in the nanoparticles, the emissive states were generally different from the initially excited state.[29,30] For nanoscale semiconductors such as CdS, as a classical example, the excitation into the band-gap absorption band resulted in exciton fluorescence and, in most cases, surface-defect emissions.[29–32] These surface-defect emissions may even be overwhelming in the observed fluorescence spectra of many CdS nanoparticles.[30,33] In carbon dots, on the other hand, there are no classical band-gap absorptions, so the surface-defect states must be accessed directly from the ground state. Therefore, the trapping of excited-state energy probably occurs between the defects responsible for absorptions and those for emissions (instead of between the excitonic state and the emissive

defect states found in CdS and other semiconductor nanoparticles). One may thus expect a broad distribution of excitations, corresponding to mostly featureless absorption spectra, as are typically observed for carbon dots.[10,13,14] Interestingly and importantly, however, the spectroscopic results reported here suggest that the electronic transitions in carbon dots are not necessarily broadly distributed.

The absorption shoulder in the blue-light region (Figure 1) is in fact surprisingly well-defined and specific in all of the more-fluorescent later fractions and in the pre-fractionation sample as well, in which the more rigorously controlled reaction conditions in the synthesis of carbon dots apparently enhanced the absorption shoulder at the expense of broad absorptions at other colors. The same absorption feature was also observed previously in the "doped" carbon dots (Figure 4), in which the carbon core was doped with an insoluble inorganic salt, such as ZnO or ZnS.[34] Of particular interest is that the ZnO or ZnS doping also resulted in substantially more-fluorescent carbon dots,[34] rather similar to the fractionated carbon dots obtained previously in terms of both optical absorption and fluorescence properties (Figure 4). It seems that the absorption shoulder around 450 nm and the corresponding fluorescence band around 510 nm represent "sweet spots" in the electronic transitions, because they are apparently shared by the carbon dots of different surface functionalities. These preferred transitions in the carbon dots are almost as specific as the band-gap transitions that are characteristic of quantum-confined nanoscale semiconductors. Phenomenologically at least, nanoscale carbon particles that have the appropriate surface functionalization (as in the later fractions reported here) or other forms of surface passivation, such as a combination of doping with inorganic salt and organic functionalization,[34] could become semiconductor-like to exhibit band-gaplike electronic transitions. In terms of optical properties at least, the surface-passivated small carbon nanoparticles seem no different from quantum-confined semiconductors.

An interesting question with potentially far-reaching implications is whether such specific electronic transitions in the carbon dots in this work could be found or even tuned to other colors. At present, we have insufficient experimental data available to provide an affirmative answer to this question, although the broad absorption and fluorescence spectra (covering the entire visible spectral region and extending into the near-IR region) observed in the preparations of other carbon dots do suggest that carbon dots are, at least in principle, capable of direct electronic transitions at many other wavelengths.

The changes in fluorescence quantum yield and lifetime among the different fractions might be explained by varying the degree of surface passivation by PEG_{1500N} molecules, both covalently through amide linkages and noncovalently through strong surface adsorption, and an influence from the differences in particle size. Because the free PEG_{1500N} molecules eluted slowest from the gel column, we expect that the later fractions probably consisted of carbon dots that were somewhat smaller in size and well passivated with PEG_{1500N} molecules (thus making the dots behave more similarly to free PEG_{1500N} molecules). However, we have not yet obtained the quantitative results required to confirm or disprove this theory, as structural elucidation of the carbon dots using NMR and FTIR analysis has been rather difficult. For example, ^{13}C NMR spectra were generally simple but not informative, exhibiting only the expected weak carbonyl signals (other particle surface carbons were not detected owing to their being too diverse). Further investigations are necessary and will be pursued.

Even without a clear structural understanding of the carbon dots in the most fluorescent fraction, the existence of these dots itself is very important fundamentally and mechanistically, and the successful isolation of these brightly fluorescent carbon dots reported here may be highly valuable technologically. The fact that these carbon dots are individually much brighter than their comparable semiconductor QDs, coupled with their nontoxicity (at least on the basis of presently available results),[18,22] should lead to significant applications in bioimaging and beyond.

Experimental Section

The preparation of precursor carbon nanoparticles and the synthesis of carbon dots were based on the previously reported procedures,[10,22] with slight modifications and more rigorous controls of the experimental conditions for improved fluorescence properties. The carbon soot was refluxed in aqueous nitric acid solution (2.6 M) for 12 h, dialyzed against fresh water, and then centrifuged at 1,000 *g* to retain the supernatant. The recovered sample was refluxed in neat thionyl chloride for 6 h, followed by the removal of excess thionyl chloride under reduced pressure. The treated carbon particle sample (100 mg) was mixed well with carefully dried PEG_{1500N} (1 g) in a flask, heated to 110 °C, and vigorously stirred under nitrogen for 3 d. The reaction mixture was cooled to room temperature, dispersed in water, and then centrifuged at 25000 *g* to retain the supernatant.

The gel column for the fractionation of carbon dots was prepared with the commercially supplied Sephadex G-100 gel.[23] The gel (15 g) was soaked in water for 3 d, and the supernatant (including the suspended ultrafine gel) was discarded. The remaining gel was washed until no gel was suspended in the supernatant. Air bubbles were removed under vacuum. Separately, a glass column (25 mm inner diameter) was filled with water to remove air bubbles, and then closed. The gel suspension described above was poured into the column until it reached about 2 cm in height, the column was then opened for the continuous addition of the gel suspension. The gel-filled column was washed until no change in height (36 cm) was observed, followed by the testing and calibration of the column.[23] In the fractionation, an aqueous solution of the as-prepared carbon dots was added to the gel column and eluted with water. Colored fractions were collected for characterization and further investigation.

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