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ORIGINAL PAPER

Fluorescent Carbon Dots Capped with PEG_{200} and Mercaptosuccinic Acid

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Abstract The synthesis and functionalization of carbon nanoparticles with PEG_{200} and mercaptosuccinic acid, rendering fluorescent carbon dots, is described. Fluorescent carbon dots (maximum excitation and emission at 320 and 430 nm, respectively) with average dimension 267 nm were obtained. The lifetime decay of the functionalized carbon dots is complex and a three component decay time model originated a good fit with the following lifetimes: τ_1= 2.71 ns; τ_2=7.36 ns; τ_3=0.38 ns. The fluorescence intensity of the carbon dots is affected by the solvent, pH (apparent pK_a of 7.4±0.2) and iodide (Stern-Volmer constant of 78± 2 M^{-1}).

Keywords Carbon nanoparticles · Carbon dots · Fluorescence · Functionalization · PEG_{200} and mercaptosuccinic acid

Introduction

Carbon dots are a new class of fluorescent nanoparticles with a carbon based core. These carbon dots possess high stability over time, exceptional resistance to photo and chemical degradation, tunable fluorescence emission and excitation, high quantum yields, large Stokes shifts and since their synthesis is performed in water they are water soluble. Although this new class of quantum dots (QDs) was recently discover they are gaining a lot of attention since they enable fluorescence imaging with both one-and two-photon excitations on the same platform [1, 2]. They are imaging agents with a performance competitive to the traditional CdSe/ZnS quantum dots [2]. Furthermore, these carbon dots have proved to be a valuable tool to overcome the toxicity issues arising from the use of cadmium core based quantum dots. A toxicity assay of these new nanoparticles was performed very recently [2, 3] and it was proved that unlike the traditional cadmium based quantum dots and nanotubes their accumulation level in the liver was very low.

So far, carbon dots have been produced from multi-walled carbon nanotubes with electrochemical methods [4], from candle soot, through thermal oxidation of suitable molecular precursors [5, 6], from commercial lampblack, which is a primary material of Chinese ink [7], and by laser ablation of graphite and subsequent surface oxidation with nitric acid [8]. Despite the different ways of obtaining carbon dots, they have only been functionalized with NH_2-polyethylene-glycol of different molecular weights. This may be due to the fact that it was recently shown that using QDs capped with PEG_{200} in cultured keratinocytes significantly inhibited cytotoxicity and immune responses when compared with QDs without this capping [9], thereby suggesting that PEG coating is an effective approach for the safe use of QDs for in vivo applications [10, 11]. However it is known that quantum dots can be capped with selected molecules according to the intended application, as such, and due to the possibility that carbon dots can overcome the toxicity limitation of the cadmium based quantum dots for biological applications, it is important to develop and analyze the stability of new nanosensors by further functionalization of these PEG coated carbon dots.

Here we report the synthesis and characterization of carbon nanoparticles obtained by direct laser ablation of

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carbon targets immersed in water and the carbon dots that these nanoparticles originate after functionalization with PEG_{200} and mercaptosuccinic acid (MSS).

Experimental section

Functionalization of the carbon dots

All chemicals were purchased from Sigma Aldrich and were used without further purification.

The synthesis of the carbon nanoparticles was performed by laser ablation [UV pulsed laser irradiation (248 nm, KrF)] of carbon targets immersed in deonized water [12]. The carbon nanoparticles obtained by laser ablation are not fluorescent and the following activation/functionalization process is necessary to render them fluorescence [13]: (i) 20 mL of the water solution with the carbon nanoparticles dispersed plus 20 mL of HNO_3 (0.1 M) was refluxed for 12 h in order to activate the carbon nanoparticles surface; (ii) afterwards it was added 20 mL of PEG_{200} and the mixture continue refluxing for 28 h; (iii) after 28 h it was added 2.650 g of mercaptosuccinic acid (MSS) and left refluxing for more 31 h. The color of the solution evolves from colorless to light brown. The obtained carbon dots solution was extracted six times with ethyl acetate in order to eliminate unreacted reagents. 1 mL of this purified solution was diluted to 100 mL water which constituted the sensing solution used throughout the work. For the solvent analyses the carbon dots were dried in vacuum for 2 h resulting in a viscous light brown liquid.

pH and ion titrations

The pH response was obtained through an acid-base titration of the sensing solution with HCl 0.1 M and NaOH 0.1 M. For testing the carbon dots sensitivity towards heavy metals the pH of the sensing solution was adjusted to 6.4± 0.1 using a phosphate buffer solution and the addition of micromolar quantities of all metal ions did not change this value.

Standard aqueous solutions of $Hg(NO_3)_2$, $Pb(NO_3)_2$, $CdCl_2$, $Cu(NO_3)_2$, $NiCl_2$, $CoCl_2$, KI and $Zn(NO_3)_2{\cdot}4H_2O$ from Merck, were prepared in water with concentrations of 5.00×10^{-4} M. Aliquots of these standard solutions were added to 20 mL of a carbon dots solution at pH 6.4–25 mL of the sensing solution and 25 mL of phosphate buffer solution at pH 6.4. For all ion solutions, except iodide, the range of concentrations were between 1.00×10^{-7} and 2.69×10^{-6} M. Iodide concentrations were: 9.70×10^{-4}, 2.90×10^{-3}, 4.83×10^{-3}, 6.75×10^{-3}, 8.66×10^{-3}, 1.06×10^{-2}, 1.15×10^{-2}, 1.34×10^{-2}, 1.53×10^{-2}, 1.72×10^{-2} and 1.90×10^{-2} M.

To perform the dynamic light scattering (DLS) analysis the solutions of carbon dots was diluted in water and passed through two continuous 0.2 μm Fischer Scientific RC filters.

Instrumentation

Fluorescence excitation emission matrices (EEM) [excitation between 300 to 600 nm and emission between 350 to 700 nm] were recorded with a Horiba Jovin Yvon Fluoromax 4 TCSPC using an integration time of 0.1 s and a slit of 5 nm. The emission fluorescence measurements were acquired using the Horiba Jovin Yvon Fluoromax 4 TCSPC using an excitation of 330 nm and an emission range of 300–650 nm, with an integration time of 0.1 s and a slit of 5 nm.

Lifetime measurements were recorded with a Horiba Jovin Yvon Fluoromax 4 TCSPC using the following instrumental settings: 368 nm NanoLED; time range, 200 ns; peak preset 10,000 counts; repetition rate at 1 MHz; synchronous delay of 50 ns. Quartz cells were used.

The size distribution of carbon dots in water was determined by dynamic light scattering analysis using a Malvern Instruments (Malvern, UK) Zeta Sizer Nano ZS, using disposable polystyrene cells from Sigma.

Data analysis

Lifetime deconvolution analysis was done using Decay Analysis Software v6.4.1 (Horiba Jovin Yvon). Fluorescence decays were interpreted in terms of a multiexponential model:

$$I(\mathrm{t}) = \mathrm{A} + \Sigma\,\mathrm{B_i}\,\exp(-t/\tau_i)$$

where B_i are the pre-exponential factors and τ_i the decay times. The fraction contribution (percentage of photons) of each decay time component is represented by P_i.

The variations in the fluorescence intensity of the carbon dots resulting from the ionization reaction can be linearized using a Henderson-Hasselbalch type equation which allows the calculation of the pK_a.

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log[(I_{\max.} - I)/(I - I_{\min.})]$$

where $I_{max.}$ and $I_{min.}$ are respectively the maximum and minimum of the fluorescence intensity of the acid or conjugated base species and I the fluorescence intensity as function of the pH.

In this study quenching of fluorescence by ions [E(II)] was described using the Stern-Volmer equation:

$$I_\mathrm{o}/I = 1 + K_{SV}[\mathrm{E(II)}]$$

where I_o is the fluorescence intensity without ion, I is the fluorescence intensity observed in the presence of an ion

and K_{SV} is the (conditional stability constant) Stern-Volmer constant [14].

Results and discussion

Functionalization and DLS characterization

The effect of functionalization was studied by taking samples overtime. After 1 and 31 h reaction the maximum fluorescence emission remained almost constant at about 430 nm. The resulting solution obtained at 31 h reaction time contains fluorescent carbon dots functionalized with PEG_{200} and MSS. Due to the physical characteristics of PEG_{200}, electron microscopy analysis could not be performed because the sample could not be dried. Alternatively, the size dispersion of the carbon dots was characterized by DLS.

Figure 1 shows the size dispersion of the nanoparticles as a function of the reaction time. The nanoparticles obtained by direct laser ablation (Fig. 1a) have two major size dispersions at average values of 63 and 373 nm. Accordingly to the laser ablation method used (without dispersing the nanoparticles) this size dispersion feature may be due to two factors: (i) the formation of clusters in an initial phase of the ablation and the subsequent ablation of these clusters, thereby leading to two size dispersions; (ii) the particles of 373 nm may be impurities since after functionalization these particles were no longer detected.

After activation and functionalization the size distribution becomes unimodal (Fig. 2b and c). Also, the analysis of the DLS shows that the carbon dots size grows up

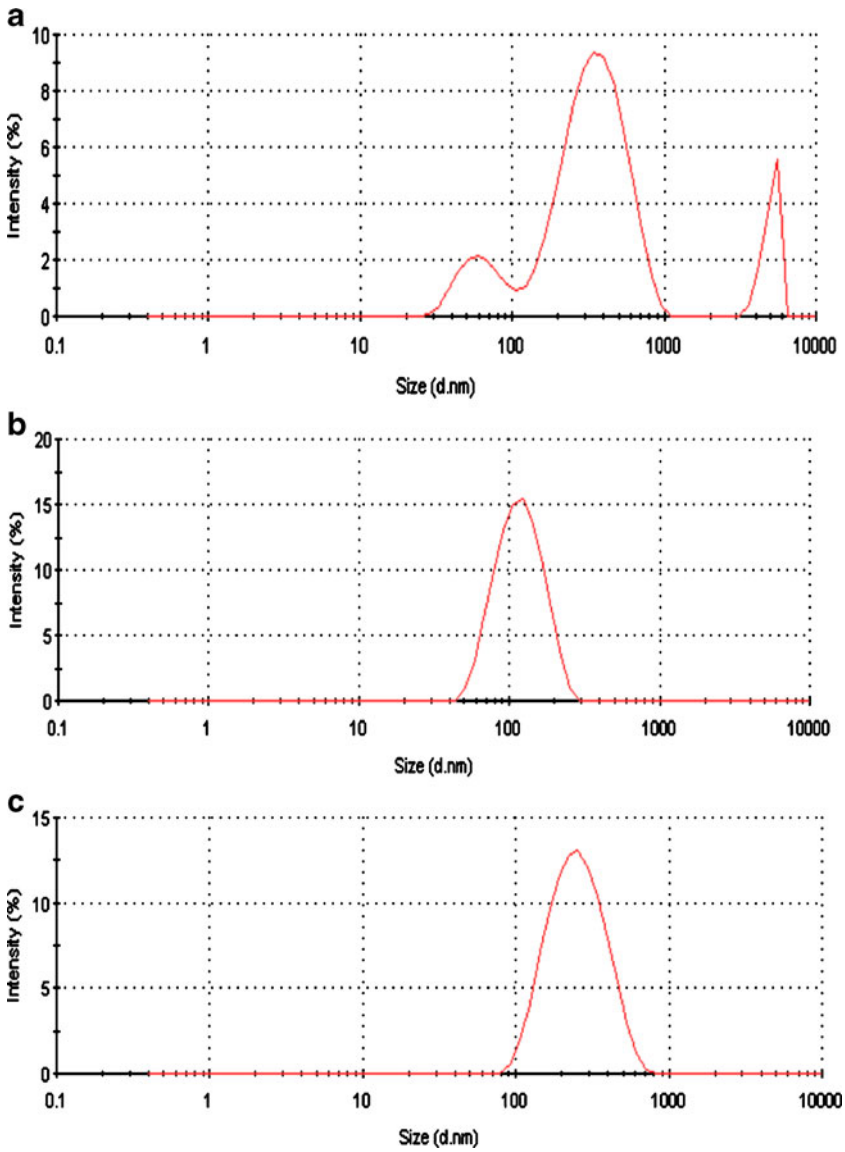

Fig. 1 DLS size dispersion of the **a** carbon nanoparticles obtained by direct laser ablation, **b** carbon dots functionalized with PEG_{200} at 31 h reaction time and, **c** carbon dots functionalized with PEG_{200} and MSS at 31 h reaction time

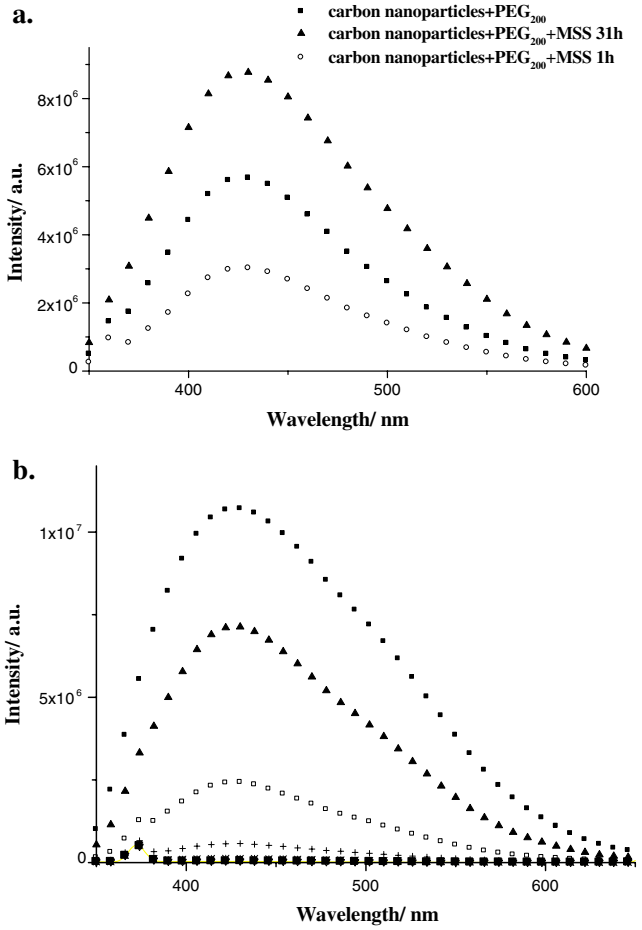

Fig. 2 **a** Fluorescence emission spectra of carbon dots functionalized with PEG_{200} at 31 h reaction, with PEG_{200} and MSS at 1 h reaction and with PEG_{200} and MSS at 31 h reaction time (excitation: 320 nm). **b** Variation of the fluorescence emission spectrum as function of the dilution of the aqueous carbon dots

accordingly with the reaction time: carbon dots + PEG_{200} 31 h - 122 nm (Fig. 1b); carbon dots + PEG_{200} + MSS 1 h - 193 nm; and, carbon dots + PEG_{200} + MSS 31 h - 267 nm (Fig. 1c).

Fluorescent properties

The emission spectra at maximum excitation (320 nm) of the synthesized carbon dots functionalized with PEG_{200} and MSS are shown in Fig. 2a. The fluorescence intensity increased with the reaction time but the maximum emission wavelength remained approximately the same at about 430 nm, which is an indication of a little variation of the quantum confinement. When the fluorescence intensity started to decrease with the reaction time, it was considered that the maximum particle size and quantum confinement was reached for that ligand and as such the reaction was stopped. The emission bands are relatively broad and the full with half maximum increases with the reaction time, namely: 87, 89 and 122 nm, respectively. Figure 2b shows that the decrease of the carbon dots concentration provokes a linear decrease of the fluorescence intensity without changing the emission wavelength.

The preliminary analysis of the decay time indicates that it is complex as it shows the presence of several lifetime ranges. Indeed, as shown in Table 1, only a three component decay time model originated a good fit for carbon dots functionalized with PEG_{200} (χ=1.08), and for carbon dots with PEG_{200} and MSS (χ=1.25) with the following lifetimes, respectively: τ_1=2.76 ns; τ_2=0.33 ns; τ_3=6.59 ns and τ_1=2.71 ns; τ_2=7.36 ns; τ_3=0.38 ns. These results show that the fluorescence lifetimes of the carbon dots were not affected after MSS functionalization. The results here obtained for carbon dots with PEG_{200} are comparable with the data reported by Sun et al. [7] for PEG_{1500}, indicating that the lifetime is also not affected by the length of the polymer in the dot surface.

Solvent, pH and ions effect on the carbon dots fluorescence

Figure 3 shows the effect of the solvent on the fluorescence properties of the carbon dots. As observed only the fluorescence intensity and not the emission wavelength is affected by solvents. This result shows that the solvent do not affect the quantum confinement of the carbon dots and only provokes the quenching of the fluorescence.

After functionalization with PEG_{200} and MSS it was possible to see a marked sensitivity of the fluorescence intensity as a function of the pH. Since both MSS and PEG_{200} are sensitive to the surrounding environmental pH, the sigmoid curve represented in Fig. 4 is broad. When we applied the Henderson-Hasselbalch equation, it was found an apparent pK_a of 7.4±0.2 and a slope of 2.1. This pH behavior is reversible. Also, as the slope is higher than 1 showing that a polyelectrolyte ionization is occurring.

However, the variation with the pH of the fluorescence intensity of the carbon dots was only observed when the titration of the sensing solution was performed with strong

Table 1 Lifetime intensity decays of carbon dots functionalized with PEG_{200} and MSS in water

Sample	N	τ_i (ns)	α_i	f_i	
Carbon dots with PEG_{200} 31 h	1	2.76(9)	0.0227(1)	42.9%	
	2	0.33(2)	0.0921(5)	20.7%	
	3	6.59(6)	0.00812(4)	36.4%	χ= 1.08
Carbon dots with PEG_{200} + MSS 31 h	1	2.71(8)	0.0227(1)	43.6%	
	2	7.36(9)	0.00634(4)	33.0%	
	3	0.38(1)	0.0870(5)	23.4%	χ= 1.25

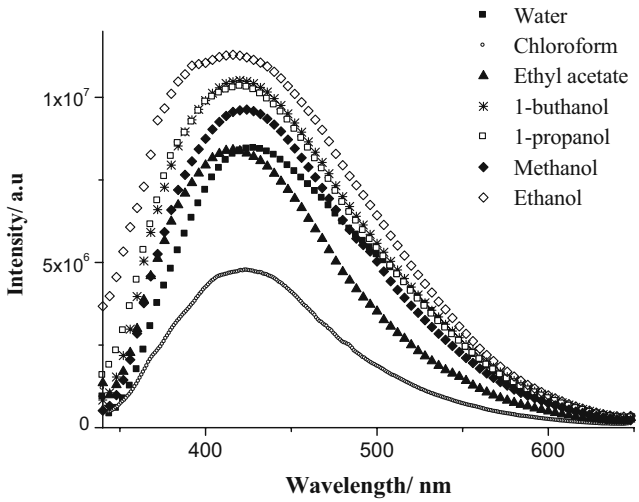

Fig. 3 Fluorescence emission spectra (excitation: 320 nm) of carbon dots functionalized with PEG_{200} and MSS at 31 h reaction time in different solvents

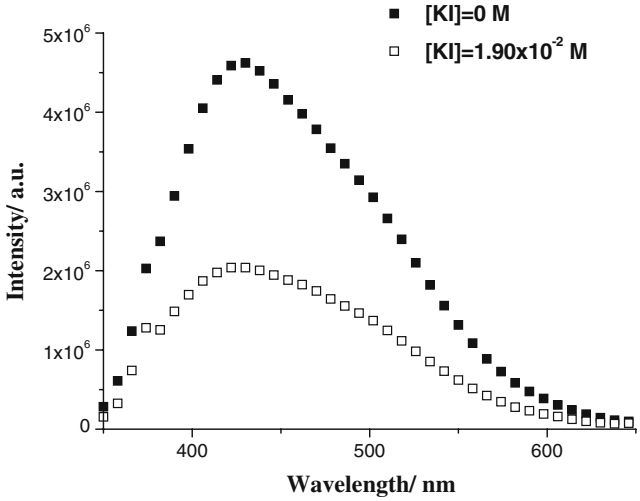

Fig. 5 Fluorescence emission spectrum (excitation: 320 nm) quenching of the carbon nanoparticles + PEG_{200} + MSS 31 h reaction time by iodide

acid and/or base. Indeed, when the same total phosphate buffer solutions with different pH values were used the fluorescence intensity did not change. This observation may be due to a stabilization effect on the dots surface charge promoted by the buffer solution.

In order to access if these carbon dots were sensitive to ions, several ion solutions of Hg(II), Cu(II), Cd(II), Ni(II), Zn(II), Ca(II) and iodide were tested.

Figure 5 shows the effect of iodide at milimolar concentration levels on the carbon dots fluorescence and it is possible to observe a marked quenching—the fluorescence signal decreases 55% upon addition of relatively high concentration of iodide (1.90×10^{-2} M). The analysis of typical Stern-Volmer plot of the I^- quenching on the carbon dots fluorescence shows that they follow a linear trend with $K_{sv}=78\pm2$ M^{-1} (Intercept = 0.92; $r=0.996$ with 12 points).

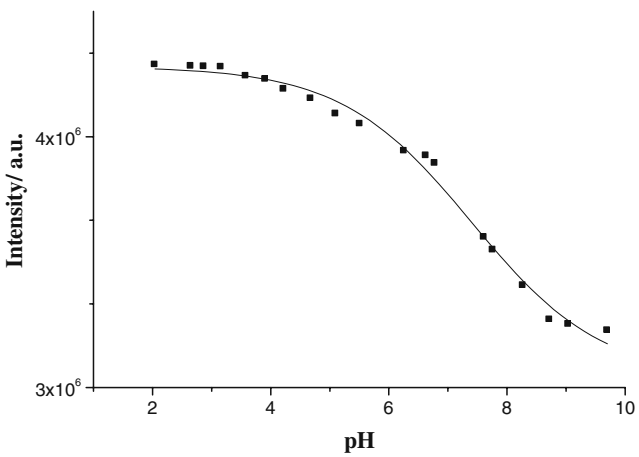

Fig. 4 Variation of the fluorescence intensity (excitation: 320 nm; emission: 430 nm) of aqueous carbon dots as function of the pH

This order of magnitude is compatible with a dynamic quenching mechanism.

The other metal ions analyzed, namely, Hg(II), Cu(II), Cd(II), Ni(II), Zn(II) and Ca(II) at micromolar concentration range show no measurable effect on the fluorescence of the carbon dots. The fact that these carbon dots remained stable in aqueous solutions and that they their fluorescence properties were not affected by the common interfering metals is an important step for the development of a non-toxic and stable nanosensor for bioimaging applications.

Conclusions

Fluorescent carbon nanoparticles (carbon dots) (with excitation at 320 and emission at 430 nm) with 267 nm dimension were easily synthesized in water and functionalized with PEG_{200} and MSS. The fluorescence intensity of the functionalized carbon dots remain stable in water and are solvent and pH sensitive. The lifetime decay of the carbon dots is complex and it is not affected by the size of the PEG chain as well as the presence of other capping agents. The fluorescence intensity of the carbon dots are not affected by the presence of micromolar quantities of metal ions but quenched (dynamic quenching) by the presence of the milimolar quantities of iodide.

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