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Carbon Nanodots

A Biocompatible Fluorescent Ink Based on Water-Soluble Luminescent Carbon Nanodots**

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Carbon nanodots (C-dots) are fascinating carbon materials that are attracting increasing interest because they possess distinct benefits, such as chemical inertness, a lack of optical blinking, low photobleaching, low cytotoxicity, and excellent biocompatibility, compared with organic dyes and other semiconductor nanodots with heavy metal cores.[1] C-dots are versatile and can be used in a wide range of technologies, such as bioimaging,[2] photocatalysis,[3] sensing,[4] lasers,[5] LED,[6] and energy conversion/storage devices.[7] However, with the exception of promising biological applications, the performance of C-dot-based solid-state luminescent devices is still not satisfactory because strong fluorescence quenching occurs in dry and aggregate states. Only a few cases of high fluorescence in solid-state materials based on C-dots have been reported.[8] At present a lot more research into C-dots is required before they can be used for commercial applications.

C-dots can be prepared by several existing techniques, such as laser ablation,[9] pyrolysis,[10] wet oxidation,[11] ultrasound-[12] and microwave-assisted[13] synthesis, hydrothermal synthesis,[14] and electrochemical etching.[15] Among these methods, microwave synthesis has become popular because of the lower associated costs than for the other methods and synthesis is achieved in one step. To achieve luminescent C-dots, surface-passivation reagents are usually required, and the reported quantum yields for C-dots without surface passivation are relatively low (approximately 1%). Qu et al. reported a microwave-assisted one-step method, involving the addition of a tiny amount of an inorganic ion, for the synthesis of luminescent C-dots without surface passivation.[13a] However, the quantum yields of these microwave-synthesized luminescent C-dots are often less than 10%.

Herein, we report a simple, low-cost, one-step microwave synthesis route towards water-soluble luminescent C-dots, and their application as a new biocompatible fluorescent ink. First, citric acid (3 g) and urea (3 g) were added to distilled water (10 mL) to form a transparent solution. The solution was then heated in a domestic 750 W microwave oven for 4–5 mins, during which the solution changed from being a colorless liquid to a brown and finally dark-brown clustered solid, indicating the formation of C-dots. This solid was then transferred to a vacuum oven and heated at 60°C for 1 h to remove the residual small molecules. An aqueous solution of the C-dots was purified in a centrifuge (3000 r min^{-1}, 20 min) to remove large or agglomerated particles. The resulting colored (brown) aqueous solution remained indefinitely stable at various concentrations.

The morphology of the C-dots was characterized using transmission electron microscopy (TEM) and atomic force microscopy (AFM). Drops of a dilute aqueous solution of the C-dots were deposited on a carbon-coated copper grid for TEM and on glass and silicon substrates for AFM. The TEM image (Figure 1a) and AFM images with associated height analyses (see the Supporting Information, Figures S6 and S7) illustrate that the C-dots are spherical and well dispersed, and range between 1–5 nm in diameter. The XRD patterns of the C-dots (Figure 1b) displayed two broad peaks centered at 6.8 Å and 3.4 Å, which are attributed to highly disordered carbon atoms, similar to the graphite lattice spacing.[16] Elemental analysis revealed the composition of the C-dots to be C 41.54 wt%, H 4.41 wt%, N 20.79 wt%, and O (calculated) 33.12 wt%, thus indicating these dots are carbon-rich nanodots. The Raman spectra of the C-dots (Figure 1c) display two broad peaks at around 1365 cm^{-1} and 1575 cm^{-1}, which are attributed to the D band (sp3-hybridized) and G band (sp2-hybridized), respectively. The D band is associated with vibrations of carbon atoms with dangling bonds in the termination plane of disordered graphite or glassy carbon. The G band corresponds to the E_{2g} mode of graphite and is related to the vibration of sp2-hybridized carbon atoms in a two-dimensional hexagonal lattice.[17] The relative intensity of the disordered D band and crystalline G band (I_D/I_G) for the C-dots is around 0.86, thus indicating that they have a similar structure to graphite. ^{13}C NMR spectroscopy is an effective technique to distinguish sp3-hybridized carbon atoms from sp2-hybridized carbon atoms. The existence of sp2- and sp3-hybridized carbon atoms in C-dots is confirmed from the ^{13}C NMR spectrum of the C-dots in D_2O (see the Supporting Information, Figure S8); in this spectrum signals in the range 30–45 ppm correspond to aliphatic (sp3-hybridized) carbon atoms, and signals from 90–185 ppm indicate sp2-hybridized carbon atoms.[18] The signals in the range 170–185 ppm are most correspond to carboxyl/amide groups. The surface functional groups of the C-dots are detected by FTIR (Figure 1d). Broad absorption bands at 3100–3500 cm^{-1} are assigned to ν(O-H) and ν(N-H). These functional groups

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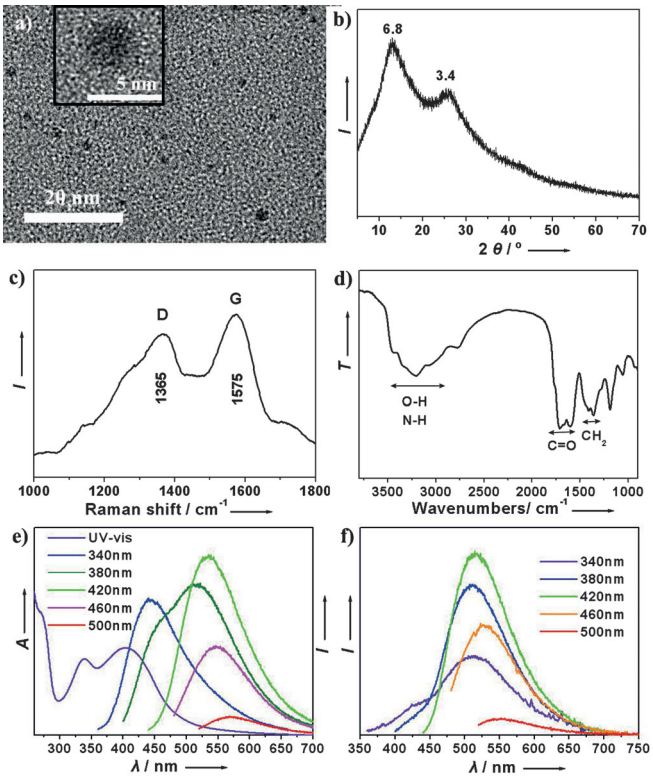

Figure 1. a) TEM image of synthesized C-dots; scale bar: 20 nm (inset: high-resolution TEM image of a C-dot; scale bar: 5 nm). b) XRD patterns, c) Raman spectrum, and d) FTIR spectrum of C-dots in the dry state. e) UV/Vis absorption and PL spectra of a dilute aqueous solution of C-dots at various excitation wavelengths. f) PL spectra of C-dot-coated commercially available lens paper at various excitation wavelengths.

improve the hydrophilicity and stability of the C-dots in aqueous system. Absorption bands at 1600–1770 cm^{-1} are assigned to ν(C=O). Absorption bands from 1350–1460 cm^{-1} are assigned to $\delta(CH_2)$. The zeta potential of the C-dots in the aqueous solution was +88.1 mV, owing to the presence of abundant N-containing groups on the surface, thus indicating the C-dots are highly dispersed and stable in the aqueous system.

The dilute (0.2 mg mL^{-1}) aqueous solution of C-dots has a broad absorption spectrum with peaks centered at 270 nm, 340 nm, and 405 nm, which represent the typical absorptions of an aromatic pi system (Figure 1e). In contrast to the UV absorption spectra of citric acid and urea (see the Supporting Information, Figure S2), these absorption peaks indicate extended conjugation in the C-dot structure. The high-energy tail in the visible region of the absorption spectrum of the C-dots, is attributed to Mie scattering caused by nanosized particles.[19] The dilute aqueous solution of the C-dots exhibits excitation-wavelength-dependent photoluminescence (PL) properties with emission peaks ranging from 440 nm (blue) to 570 nm (yellow) at excitation from 340 nm to 500 nm (Figure 1e). The strongest fluorescence emission band, located at 540 nm, is observed under 420 nm excitation and has a high fluorescent quantum yield of 14%. Since neither citric acid nor urea emit in the visible or near-UV range, the fluorescence is attributed to the C-dots; this conclusion was further confirmed by fluorescent imaging experiments (see the Supporting Information, Figure S9). These fluorescence properties 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.[20] At this stage, the mechanism underpinning the excitation-wavelength-dependent PL properties of C-dots remains elusive. We proposed that these PL properties are due to different nanoparticle compositions and structures as well as nanoparticles of different sizes. Further investigations are necessary and will be undertaken.

The fluorescence is strongly quenched when the aqueous solutions of C-dots are deposited on glass, metal, silicon, or plastic substrates owing to the formation of aggregates. In contrast, enhanced fluorescent emissions are observed when the C-dots are coated on paper. The paper was coated with an aqueous solution of C-dots and then dried in air. Excitation-wavelength-dependent fluorescence was also observed in this C-dot-coated paper. The PL spectra of C-dot-coated commercially available lens paper are shown in Figure 1f. The green emission bands under ultraviolet excitation are significantly increased. The strongest fluorescence emission is observed centered at 515 nm under 420 nm excitation with an enhanced fluorescent quantum yield of 40%. This result indicated these aqueous solutions can be used as a new type of fluorescent ink. The excitation-wavelength-dependent PL properties endow C-dot-based fluorescent ink distinctly different features to other fluorescent inks (Table 1). Doubly encrypted characters can be composed from the aqueous solution of C-dots and commercially available green fluorescent ink (Figure 2a and b). On commercially available paper, these characters are invisible in daylight. Under blue-light excitation, green fluorescent characters 8 8 8 are observed. Under green-light excitation, the lines written in commercially available green fluorescent ink are invisible, and only the red fluorescent characters 3 9 5 from the aqueous solution of C-dots appear. This finding indicates that the C-dot-based fluorescent ink can be used in anti-counterfeiting and information encryption. The C-dots not only exhibit excitation-wavelength-dependent fluorescence when coated on paper but also when coated on vegetable fibers, animal fur, feathers, and skin (see the Supporting Information). However, nearly no fluorescence was observed after coating C-dots on synthetic fibers. Adjacent cotton threads and nylon fibers barely fluoresce under blue- and

Table 1: PL properties of the C-dots in dilute aqueous solution and as a coating on commercially available lens paper.

States	Dilute aqueous solution			Coating on commercially available lens paper		
λ_{ex} [nm]	340	420	500	340	420	500
$\lambda_{em,max}$ [nm]	440	540	570	512	515	552
τ [ns]	2.57 (14.0%)	2.11 (15.9%)	1.27 (26.0%)	1.94 (20.8%)	2.81 (15.3%)	1.11 (31.1%)
	10.0 (86.0%)	5.97 (84.1%)	6.77 (74.0%)	10.33 (79.2%)	10.70 (84.7%)	7.59 (68.9%)
χ^2	1.02	1.06	0.85	0.93	1.01	0.83
Φ_F	0.10	0.14	0.08	0.28	0.40	0.24

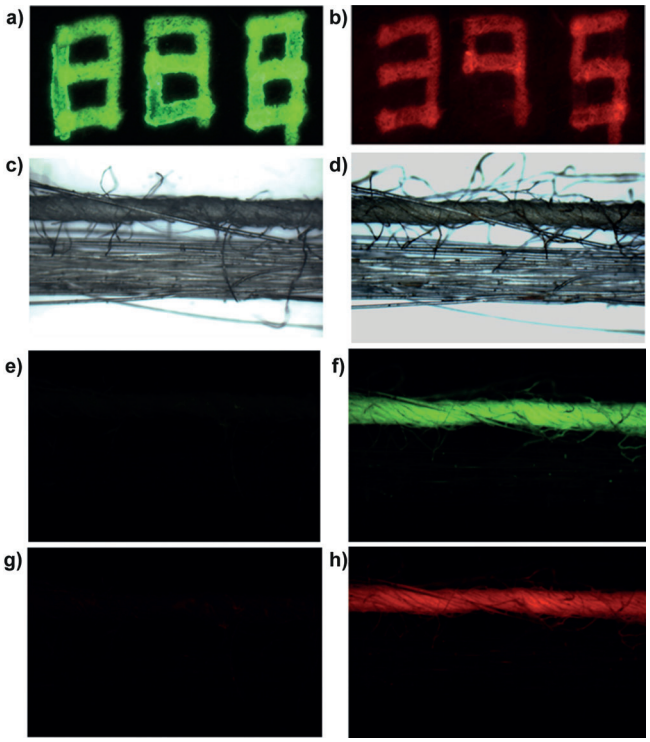

Figure 2. Fluorescent images on commercially available paper of characters composed from C-dot aqueous solutions and commercially available green fluorescent ink captured in exciter filter BP 450–480 nm, BA 515 nm, exposure time 50 ms (a) and exciter filter BP 510–550 nm, BA 590 nm, exposure time 150 ms (b). Optical image (c), and fluorescent images captured in exciter filter BP 450–480 nm, BA 515 nm, exposure time 50 ms (e) and exciter filter BP 510–550 nm, BA 590 nm, exposure time 150 ms (g) of adjacent cotton threads and nylon fibers that do not have a C-dot coating. Optical image (d), and fluorescent images captured with exciter filter BP 450–480 nm, BA 515 nm, exposure time 50 ms (f) and exciter filter BP 510–550 nm, BA 590 nm, exposure time 150 ms (h) of adjacent cotton threads and nylon fibers that have a C-dot coating.

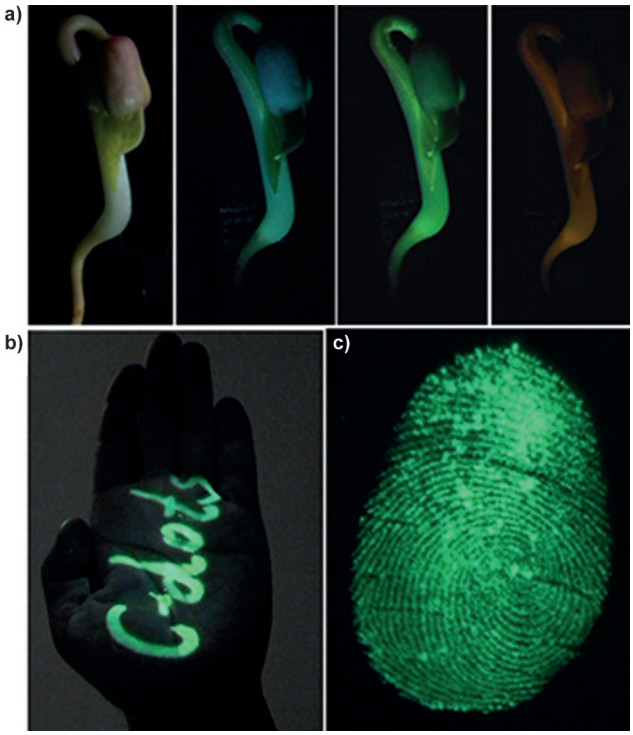

Figure 3. a) From left to right, optical and fluorescent images of a bean sprout grown with C-dot aqueous solution (1.5 mg mL^{-1}) under daylight, 340 nm excitation (BA 395 nm), 420 nm excitation (BA 450 nm), and 500 nm excitation (BA 550 nm). b) C-dot-marked fluorescent characters on human skin captured under 420 nm excitation (BA 450 nm). c) A C-dot-formed fluorescent fingerprint on commercially available filter paper captured under 420 nm excitation (BA 450 nm).

green-light excitation (Figure 2e and g). When the cotton threads and nylon fibers are coated with a drop of the aqueous solution of C-dots and then dried under air, clear excitation-wavelength-dependent fluorescence is observed in the cotton threads, whereas nearly no fluorescence is observed in the nylon fibers in the same exposure time (Figure 2 f and h). This result indicated the C-dots were biocompatible and could be used in distinguishing biological products from synthetic products.

Toxicity studies of the C-dots were performed with both plants and animals. Bean sprouts could be grown in a aqueous solution of C-dots (1.5 mg mL^{-1}) and showed excitation-wavelength-dependent fluorescence (Figure 3a). This fluorescence property of the sprouts indicated that C-dots could permeate throughout the plant cells, but were nontoxic and did not hinder plant growth. Animal toxicity studies were carried out on mice. Ten mice, on an otherwise normal food intake, were given the aqueous solution of C-dots (0.7 mg mL^{-1}) to drink for five weeks. All the mice survived the five-week duration period of the experiment, and none exhibited any symptom of anorexia, or other clinical symptoms, such as hair loss, scabs, vomiting, or diarrhea. The mice acted normally, without any violent or lethargic behavior, over the five-week period and were similar to a control group of mice given normal water. The excitation-wavelength-dependent fluorescence phenomena could be observed in the urine of mice given the aqueous solution of C-dots, whereas no excitation-wavelength-dependent fluorescence was observed in blood taken from the ophthalmic artery of each mouse. The 530 nm emission bands under 380–480 nm excitation indicated the C-dots could pass through the urinary tract (see the Supporting Information, Figure S15). After the test mice had been given normal water for four weeks, the PL spectra of their urine returned to normal, that is, similar to the control group. These results indicate that C-dots exhibit low or zero toxicity to both plants and animals. Toxicity studies of C-dots have been conducted by various research groups, and all results suggest that C-dots have low or no toxicity.[21] These results mean that C-dots could possibly be used as a new type of biocompatible fluorescent ink. Figure 3b shows C-dot-marked fluorescent characters on human skin that can be easily washed off with water. Furthermore, the water-soluble C-dot-based fluorescent ink could replace traditional inks to safely form clear, permanent, adelomorphic fluorescent fingerprints and would no longer contaminate fingers. A C-dot-formed fluorescent fingerprint on commercially available filter paper is shown in Figure 3c.

In summary, we have demonstrated an easy, economic, one-step microwave synthesis route to water-soluble lumi-

nescent C-dots, which exhibited stable, excitation-wavelength-dependent PL properties in aqueous solutions (maximum quantum yield about 14%). Enhanced excitation-wavelength-dependent fluorescent emissions are observed from the C-dot coating on biological products, whereas C-dots fluorescence was strongly quenched by inorganic or plastic substrates or synthetic fibers. The toxicity studies indicate that the C-dots display zero or low toxicity to both plants and animals. The low cost, biocompatibilty, and low-toxicity of C-dots and their distinct PL properties indicate that these C-dots could potentially be synthesized on an industrial scale and could be used as a new type of biocompatible fluorescent ink for versatile applications, such as anticounterfeiting, information encryption, and information storage. Investigations on the structure and luminescent mechanisms of the C-dots in solutions and on biological products are in progress.

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