Carbon Dots | Hot Paper

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Red, Green, and Blue Luminescence by Carbon Dots: Full-Color Emission Tuning and Multicolor Cellular Imaging**

*Kai Jiang, Shan Sun, Ling Zhang, Yue Lu, Aiguo Wu, Congzhong Cai, and Hengwei Lin**

Abstract: *A facile approach for preparation of photoluminescent (PL) carbon dots (CDs) is reported. The three resulting CDs emit bright and stable red, green and blue (RGB) colors of luminescence, under a single ultraviolet-light excitation. Alterations of PL emission of these CDs are tentatively proposed to result from the difference in their particle size and nitrogen content. Interestingly, up-conversion (UC)PL of these CDs is also observed. Moreover, flexible full-color emissive PVA films can be achieved through mixing two or three CDs in the appropriate ratios. These CDs also show low cytotoxicity and excellent cellular imaging capability. The facile preparation and unique optical features make these CDs potentially useful in numerous applications such as light-emitting diodes, full-color displays, and multiplexed (UC)PL bioimaging.*

Multicolor photoluminescent (PL) materials, which can be excited by a single wavelength, continue to fascinate mankind because of their potential applications in sensing, bioimaging, light-emitting diodes, full-color displays, and optoelectronic devices.[1] Many multicolor emissive materials have been developed in the past decades, and include semiconductor quantum dots (QDs),[2] rare-earth based nanoparticles,[3] polymer dots,[4] molecular nanomaterials,[5] and organic fluorescent dyes,[6] but the potentially high toxicity, low-emission quantum yields (QYs), potential for photobleaching, poor water solubility, and complicated preparation procedures have hampered their practical applications.[2–6] Recently, carbon-based fluorescent nanomaterials, such as carbon dots (CDs), have attracted much attention because of their unique optical and low/nontoxic features.[7] This new class of nanomaterials is considered to be a potential alternative to semiconductor QDs. However, some of the major disadvantages of these nanomaterials stem from the difficulty in preparing long-wavelength (e.g. red-light region) and multicolor (capable of single wavelength excitation) emissive products.[8] Therefore, the development of facile methods for the preparation of water-soluble, biocompatible, and photostable, multicolor PL materials, particularly carbon-based ones, is still highly desirable.

Phenylenediamines [three isomers: *o*-phenylenediamine (oPD), *m*-phenylenediamine (mPD), and *p*-phenylenediamine (pPD)] are significant precursors for producing many heterocyclic compounds and polymers.[9] To the best of our knowledge, however, only oPD has been used to prepare PL nanoparticles or CDs.[10] In this current study, we report on the preparation of multicolor PL CDs, having red, green, and blue (RGB; the three primary colors) emissions by using the three different phenylenediamines isomers, through a facile solvothermal method. The as-prepared CDs emit strong and stable luminescence in solution and in a polymer matrix under a single-wavelength ultraviolet-light excitation. Interestingly, up-conversion (UC)PL emissions of these CDs are also observed in solution and in polymer films [e.g. poly(vinyl alcohol) (PVA)] with a femtosecond pulse laser ($\lambda = 800$ nm). Moreover, flexible full-color emissive PVA films are achieved through mixing two or three of these CDs in appropriate ratios. Finally, these CDs were confirmed to be of low cytotoxicity and to have excellent multicolor cellular imaging capability by a single excitation wavelength.

The preparation of RGB PL CDs (named *o*-CDs, *m*-CDs and *p*-CDs from oPD, mPD and pPD, respectively) can be readily accomplished, that is, heating oPD (mPD or pPD) in an ethanol solution at 180 °C for 12 hours in an autoclave and then purification using a silica gel column chromatography (see Figure 1 a and the Supporting Information). The resulting CDs can be dispersed within a variety of common solvents to form clear solutions. For example, as shown in Figure 1 b (left), clear colorless, yellow, and pink solutions (from *m*-CDs, *o*-CDs and *p*-CDs, respectively) are observed when they are dispersed in ethanol. Very interestingly, the each solution displays an emission for one of the primary colors (RGB) under single-wavelength UV irradiation (e.g. $\lambda = 365$ nm; Figure 1 b, right).

The ultraviolet-visible (UV/Vis) absorption spectra of the three CDs display analogous characteristics (Figure 2 a–c). In comparison to their corresponding starting materials, the higher- and lower-energy absorption bands of the three CDs red-shift, that is, from $\lambda = 212$ to 247 nm and $\lambda = 295$ to 355 nm for *m*-CDs, $\lambda = 209$ to 260 nm and $\lambda = 294$ to 426 nm for *o*-CDs, and $\lambda = 243$ to 286 nm and $\lambda = 309$ to 512 nm for *p*-CDs. These red-shifts of the electronic absorption transitions

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[*] K. Jiang, Prof. C. Cai
Department of Applied Physics, Chongqing University
Chongqing 401331 (P. R. China)
K. Jiang, S. Sun, Dr. L. Zhang, Y. Lu, Prof. A. Wu, Prof. H. Lin
Division of Functional Materials and Nano-Devices, Ningbo Institute of Materials Technology & Engineering (NIMTE)
Chinese Academy Sciences, Ningbo 315201 (P. R. China)
E-mail: linhengwei@nimte.ac.cn

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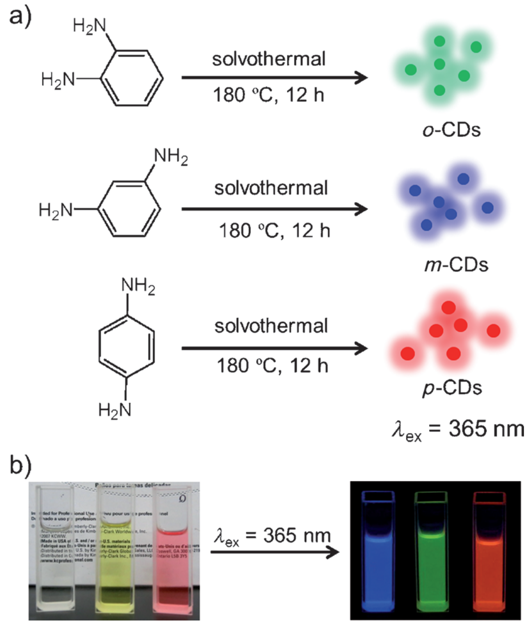

Figure 1. a) Preparation of the RGB PL CDs from three different phenylenediamine isomers (i.e., oPD, mPD and pPD). b) Photographs of *m*-CDs, *o*-CDs, and *p*-CDs dispersed in ethanol in daylight (left), and under $\lambda = 365$ nm UV irradiation (right).

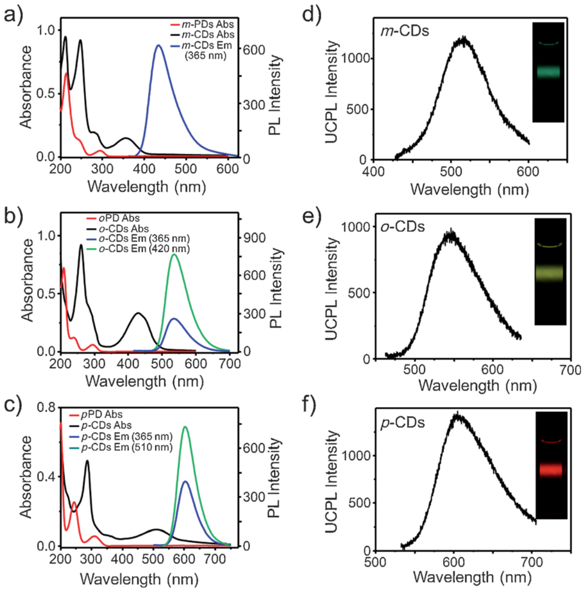

Figure 2. a–c) UV/Vis absorption spectra of mPD, oPD, and pPD (red line), *m*-CDs, *o*-CDs, and *p*-CDs (black line), and PL emission spectra of *m*-CDs, *o*-CDs, and *p*-CDs (blue and green lines). d–f) UCPL spectra and photographs (insets) of *m*-CDs, *o*-CDs, and *p*-CDs in ethanol under a $\lambda = 800$ nm pulsed laser excitation.

demonstrate that these CDs should contain smaller electronic bandgaps than phenylenediamines. Then, the PL properties of the three CDs were thoroughly investigated. First, their PL spectra were measured. As shown in Figure 2a–c, the emission maxima of *m*-CDs, *o*-CDs, and *p*-CDs are $\lambda = 435$, 535, and 604 nm, respectively, in an ethanol solution under a single excitation wavelength (i.e., $\lambda = 365$ nm). These PL spectra correlate well with the observed RGB emission as shown in Figure 1b. Actually, more intense emissions for *o*-CDs and *p*-CDs can be obtained if optimal excitation wavelengths are employed, that is, $\lambda \approx 420$ nm for *o*-CDs and $\lambda \approx 510$ nm for *p*-CDs (based on their PL excitation spectra; see Figure S1 in the Supporting Information). Second, the PL QYs were determined to be 10.4, 4.8, and 20.6 % for *o*-CDs, *m*-CDs and *p*-CDs, respectively, under $\lambda = 365$ nm excitation in ethanol solution (see Experimental and Figures S2–S4 in the Supporting Information).[11] The QYs reach 17.6 and 26.1 % for *o*-CDs and *p*-CDs, respectively, if the optimal excitation wavelengths are applied (i.e., $\lambda = 420$ nm and $\lambda = 510$ nm, respectively). Third, the PL lifetimes of these CDs were measured. As shown in Figure S5 and Table S1, the PL decays of *o*-CDs and *p*-CDs in ethanol solution are mono-exponential with lifetimes of 4.44 and 9.39 ns, respectively, but the PL decay of the *m*-CDs has to be fitted with a bi-exponential function and a much shorter averaged lifetime of 0.99 ns was found. Fourth, the photostability of these CDs in solution was investigated. As seen in Figure S6a–c, all of the three CDs are highly stable, and only slight (< 5 %) decays are found under continuous illumination with a $\lambda = 365$ nm UV light for one hour. Finally, UCPL emissions of these CDs were investigated. The ethanol solutions of these CDs emit bright UCPL under an 800 nm femtosecond pulsed laser (Figure 2d–f). The quadratic dependence between the laser powers and PL intensities demonstrate that the present UCPL emission is a two-photon excitation process (see Figure S7).[12]

Subsequently, transmission electron microscopy (TEM) and atomic force microscopy (AFM) characterizations were performed to examine the morphologies of the three luminescent CDs. As shown in Figure S8a–c, all three materials show uniform nanoparticles with average sizes of about 6.0 nm (*m*-CDs), 8.2 nm (*o*-CDs), and 10.0 nm (*p*-CDs). AFM images show that all of the three CDs are monodispersed and exhibit similar particle heights of approximately 2–3 nm (see Figure S8d–f). Fourier transform infrared (FT-IR) and X-ray photoelectron spectroscopy (XPS) spectra were carried out to characterize the chemical bonds and functional groups on these CDs. As shown in Figure S9a, the three CDs exhibit similar IR spectra, thus revealing the existence of similar chemical compositions. Notably, some new characteristic peaks emerged at about 2848–2920 cm^{-1}, 1336–1384 cm^{-1}, and 1225–1234 cm^{-1} for all the three CDs in comparison to the starting materials (see Figure S9b), and can be attributed to aliphatic C−H, C-N=, and C−O stretching vibrations, respectively,[13] and thus imply decomposition, intermolecular cyclization, and condensation reactions occurred during the formation of CDs. Although these reaction processes were suggested in the formation of similar CDs prepared from oPD,[10a] the actual mechanism remains unclear. The XPS surveys of these CDs further confirm the IR data. For example, the data in Figure S10a demonstrate that all three CDs have the same elemental composition (i.e. C, N and O) and their deconvoluted spectra indicate that they contain the same chemical bonds (see Figure S11). Through careful examination of the characterization data, only the particle sizes and nitrogen content (3.69 %, 7.32 %, and 15.57 % for *m*-CDs, *o*-CDs, and *p*-CDs, respectively; see Figure S10b) of the three CDs were found to be distinctly different. These variations are believed, at least partly, to be responsible for the different PL characteristics of the three CDs, but more in-depth studies are still required to address this issue.

Considering the interesting RGB emission of the CDs in solution, preparation of full-color emissive films was attempted. After screening, PVA was found to be the best candidate for preparing composite films. As shown in Figure 3 and Figure S12, films with emissions for each of the three primary colors can be obtained if the three CDs are doped in PVA (emission maxima at $\lambda = 426$, 529, and 603 nm; Figure 3c). Then, mixtures with various ratios of *m*-CDs, *o*-CDs, and *p*-CDs were employed to prepare full-color PVA composite films. As shown in Figure S13, all of the obtained films are uniform and clearly transparent. Excitingly, these films show a broad range of PL emission colors under a single UV light excitation ($\lambda = 365$ nm; Figure 3a). The CIE coordinates of all these PVA composite films display a triangular distribution, that is, the three pure CDs are located at the vertices of a triangle and their mixtures are located along the edges (Figure 3b). Note that a white-light emission film could also be achieved if appropriate ratios of all the three materials were used. For example, when *o*-CDs, *m*-CDs, and *p*-CDs were mixed in a ratio of 2:4:1 (weight), a white-light emission PVA film with CIE coordinates of (0.33, 0.34) was obtained (see IV in Figures 3a and b), and is very close to the pure white light (0.33, 0.33). The PL spectrum of this white-light emissive film shows three distinct emission bands (Figure 3d), which correlate well with the emissions of pure CDs PVA films (Figure 3c). This observation demonstrates that there is no significant interaction between the three CDs when they are encapsulated in the PVA matrix. Therefore, the Glassman color mixing law can be employed to achieve full-color emissions based on the three primary colors of the luminescent CDs.[14] Finally, the photostability and UCPL of these CDs PVA composite films were investigated and they demonstrated high stability (< 1 % variations; see Figure S6d–f) and possessed UCPL characteristics (see Figure S14).

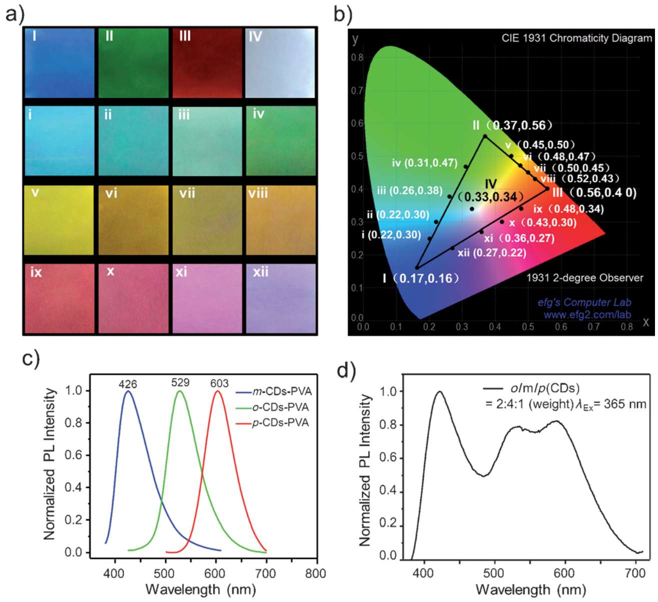

Figure 3. a) PL photographs of *m*-CDs, *o*-CDs, *p*-CDs, and their mixtures in PVA composite films under $\lambda = 365$ nm UV irradiation: I) *m*-CDs, II) *o*-CDs, III) *p*-CDs, IV) *o*-CDs/*m*-CDs/*p*-CDs = 2:4:1 (w/w/w); i–iv) *o*-CDs/*m*-CDs = 1:8, 1:4, 1:2, and 1:1; v–viii) *o*-CDs/*p*-CDs = 4:1, 2:1, 1:1, and 1:2; ix–xii) *m*-CDs/*p*-CDs = 1:4, 1:2, 1:1, and 4:1 (all ratios are w/w). b) Calculated CIE coordinates from the PL spectra of the PVA composite films shown in (a). c) Normalized PL spectra of *m*-CDs, *o*-CDs, and *p*-CDs PVA composite films under $\lambda = 365$ nm UV excitation. d) PL spectrum of a white-light emission PVA composite film (i.e. IV) under $\lambda = 365$ nm UV light excitation.

Since CDs have demonstrated superior optical properties and good biocompatibility, the as-prepared CDs were expected to potentially serve as multiplexed bioimaging reagents. The cytotoxicity of the three CDs was evaluated by using a standard MTT assay with MCF-7 cells. As shown in Figure S15, over 90 % cell viability is observed after incubation of MCF-7 cells with either of the three CDs at concentrations ranging from 10 to 50 $\mu g\,mL^{-1}$ for 24 hours, thus confirming the low cytotoxicity of these materials. To reveal potential applications of these CDs in multiplexed bioimaging, their cellular imaging capability was preliminarily investigated. After incubating MCF-7 cells with each of the three CDs for 4 hours, the live cells were imaged with a confocal microscope under a single 405 nm laser excitation. The confocal micrographs show different color emissions in the MCF-7 cells (Figure 4). It was also found from these images that the emissions mainly locate at cytoplasmic regions, thus suggesting that all three CDs can pass through cell membranes and enter into cells. Notably, all three CDs possess two-photon UCPL features (Figure 2d–f), and thus they should be applicable in two-photon deep-tissue PL imaging.[12]

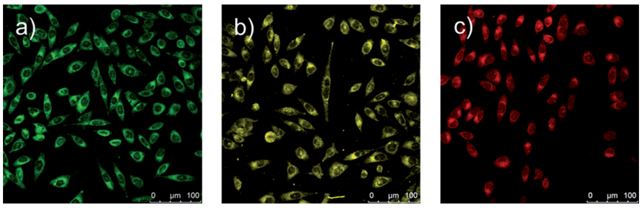

Figure 4. Confocal fluorescence images of *m*-CDs (a), *o*-CDs (b) and *p*-CDs (c) at a single $\lambda = 405$ nm laser excitation of MCF-7 cells.

In summary, we report the preparation of the three RGB PL CDs by using a facile approach. The resulting CDs emit bright and stable RGB luminescence in solution and in a polymer matrix under a single UV-light excitation (e.g., $\lambda = 365$ nm). The difference in the PL emission for the CDs are tentatively is proposed to result from the differences in particle size and nitrogen content. Importantly, the mixtures of these CDs in PVA films exhibit ensemble emissions of their individuals, thus demonstrating the potential for preparation of flexible full-color emissive films. In addition, these obtained CDs also exhibit UCPL characteristics in solution and PVA composite films under a femtosecond pulse laser ($\lambda = 800$ nm). Finally, the three as-prepared CDs demonstrate low cytotoxicity and exhibit excellent multicolor cellular imaging capability under single wavelength excitation. The UCPL characteristics of these CDs offer the added potential for applications in two-photon bioimaging for deep tissue. To the best of our knowledge, this is the first time that emissions of the three primary colors, with PL CDs excited at a single wavelength, have been achieved. The unique PL features make these CDs potentially useful in numerous applications, such as flexible full-color displays, optoelectronic devices, and multiplexed (UC)PL bioimaging.

Keywords: bioimaging · carbon dots · full-color emission · luminescence · materials science

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