Generalized One-Pot Strategy Enabling Different Surface Functionalizations of Carbon Nanodots to Produce Dual Emissions in Alcohol–Water Binary Systems

Dongya Liu,*‡∥ Fei Qu,*†∥ Xianen Zhao,*†∥ and Jinmao You*†∥

The Key Laboratory of Life-Organic Analysis, Qufu Normal University, Jingxuan Road 57, Qufu 273165, Shandong, China
Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, Qufu Normal University, Jingxuan Road 57, Qufu 273165, Shandong, China
Northwest Institute of Plateau Biology, Chinese Academy of Sciences, 23 Xinning Road, Xining 810001, China

Supporting Information

ABSTRACT: Fluorescent dual-emission carbon nanodots (DECNDs), which contained two different emitters, the blue emitters ($\lambda_{ex} = 315$ nm, $\lambda_{em} = 386$ nm) and the yellow emitters ($\lambda_{ex} = 365$ nm, $\lambda_{em} = 530$ nm), were first and simply synthesized by one-step hydrothermal treatment using ascorbic acid (AA) as a carbon source in alcohol–water binary systems. The two emitters could be easily separated through extraction with chloroform. A series of experiments demonstrated that the alcohol–water binary systems played a vital role in synthesis of DECNDs, and the dual emissions were ascribed to two types of carbon nanodots due to the different modified ligands on their surfaces, such as ascorbic acid capped on the blue emitters and alcohol molecules capped on the yellow emitters. By taking advantage of the unique dual-emission characteristic, some new applications were developed. For instance, on the basis of the independent responses of dual emissions to low pH values, the DECNDs could be used as a reliable and accurate pH sensor in acid medium, and both intensity of a single emission and intensity ratios of dual emissions could be employed to construct calibrations for pH measurement. Besides, the obtained DECNDs also exhibited excellent biocompatibility and low cytotoxicity, suggesting great potential for biomedical applications.

INTRODUCTION

In recent years, carbon nanodots, abbreviated as CNDs, have attracted considerable attention among the carbon-based materials because of their outstanding characteristics such as nonblinking behavior, water solubility, nontoxicity, and biocompatibility.¹⁻³ These CNDs primarily exhibit broad excitation spectra⁴ and demonstrate utility in a wide range of applications, including biomedical imaging,⁵⁻⁸ photocatalysis,⁹ and multifunctional sensors.¹⁰⁻¹²

Compared with the traditional nanomaterials with a single emission, recent advances in the development of new dual-emission fluorescent nanomaterials have provided promising candidates to construct novel optical sensors with improved properties. The shortcomings of traditional nanomaterials possessing only one emission are obvious, in which one emission-based fluorescent assay with signal turn-off and -on is usually interfered by multiple factors, such as the concentration of sensors, similar interferences, or environmental effects in complex samples, leading to “false positive”. Accordingly, from the viewpoint of practical application, the development of dual-emission nanomaterials based on ratiometric fluorescent sensors has been demonstrated to be an efficient resolution with high stability, anti-interference, and good reproducibility.

Up to now, three common strategies have been proposed for constructing dual-emission nanoparticles. The first method is combining two different fluorophores in one kind of non-fluorescent nanoparticles: Zong et al.¹³ developed an effective dual-emission fluorescent silica nanoparticle for rapid and ultrasensitive detection of Cu²⁺, in which the fluorescein isothiocyanate-doped silica nanoparticle was covalently grafted by Rhodamine B isothiocyanate, and the two dyes supplied the dual emissions (525 and 580 nm, respectively). The second strategy is modification of dye molecules on the surfaces of fluorescent nanoparticles. For instance, CNDs as one emission source were coated by Rhodamine B-doped silica nanoparticles, showing characteristic fluorescence emissions of Rhodamine B (red) and CNDs (blue) under a single excitation wavelength.¹⁴ The third way is incorporation of two different fluorescent nanomaterials into one kind of nanoparticles. For example, the CNDs, emitting blue fluorescence, were hybridized with CdSe/ZnS quantum dots with red fluorescence, leading to dual-emission properties.¹⁵

These strategies have revealed a common feature that the dual emissions come from hybrid nanoparticles incorporated with dyes or two different nanomaterials. Thus, all of these previously mentioned methods suffer from more or less

Received: June 17, 2015
Revised: July 15, 2015
Published: July 22, 2015
These exciting results indicate that to the best of our knowledge, the two emitters can be separated from nanoparticles of the same size. It suggests that the alcohol molecules capping on the yellow emitters, revealing the ligands efficiently affect the emission properties of CNDs.

Furthermore, on the basis of the independent responses of the two emitters to low pH values, both intensity of a single emission and intensity ratios of dual emissions could be used to construct calibrations in pH measurement, and thus the DECNDs would act as a reliable and accurate pH sensor in acid medium. In the meantime, the results of cell imaging indicate that the DECNDs could readily penetrate the cell membrane and provide useful candidates of biolabeling agents for living cells with excellent biocompatibility and low cytotoxicity.

**EXPERIMENTAL SECTION**

**Materials.** Ascorbic acid (AA), methanol, ethanol, n-propanol, and ethylene glycol were purchased from Aladdin (Shanghai, China). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) experiment kit was purchased from Nanjing KeyGEN Biotech (China). Ultrapure water was used throughout all of the experiments.

**Apparatus.** Fluorescence measurements were performed on a Hitachi F-7000 fluorescence spectrophotometer. The transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) measurements were obtained on a Tecnai G2 F20 electron microscope. The X-ray diffraction (XRD) pattern was performed on a MiniFlex600 X-ray powder diffractometer. The Fourier transform infrared (FT-IR) spectra of the samples were analyzed by a WQF-520A FTIR spectrophotometer using KBr pellets. The X-ray photoelectron spectroscopy (XPS) measurements were performed on a Thermo Scientific/k-Alpha photoelectron spectrometer. The ultraviolet-visible (UV–vis) absorption spectra were obtained on a Cary 300 Bio UV–vis spectrophotometer. The quantum yield (QY) of DECNDs was determined by FLSP920 fluorescence lifetime and steady-state spectrometer. The MTT assay was measured at 492 nm using a Synergy H1 hybrid Reader (BioTek). The HeLa cells images after uptaking of the DECNDs were recorded on Perkin–Elmer Ultraview Vox confocal microscope equipped with a spectral detection system.

**Synthesis of DECNDs.** In this paper, the DECNDs were synthesized via one-pot hydrothermal method using AA as carbon source in alcohol–water binary systems; however, although some similar methods have been reported to prepare CNDs,16,17 unfortunately certain reasons led to avoiding the detection of the dual-emission feature in recent literature.

Herein, we develop a simple, low-cost, and green preparative strategy for synthesizing dual-emission carbon nanodots (DECNDs) using ascorbic acid (AA) as carbon source in alcohol–water binary systems. The DECNDs contain two different emitters, the blue emitters (λ_em = 315 nm, λ_fl = 386 nm) and the yellow emitters (λ_em = 365 nm, λ_fl = 530 nm). Compared with the conventional dual-emission nanomaterials, no supported fluorophore is used in this one-pot synthesis. These exciting results indicate that to the best of our knowledge, the distinctive dual emissions from CNDs based on the alcohol–water binary systems are not available in any other nanoparticles of the same size. It suggests that the alcohol–water binary systems play a vital role in synthesis of DECNDs. Most importantly, the two emitters can be separated completely through extraction with chloroform, indicating the different polarities of the two emitters. A series of experiments have been designed to demonstrate that the two emissions are actually ascribed to two types of CNDs due to the different ligands on their surfaces, such as ascorbic acid capped on the blue emitters and alcohol molecules capped on the yellow emitters, revealing the ligands efficiently affect the emission properties of CNDs.

The total volume of the mixture was 20 mL. Subsequently, under vigorous stirring to form a homogeneous solution, the mixture was heated to 160 °C in a constant temperature drying oven for 70 min. During this process, the color of the mixture changed from colorless to dark yellow, indicating the formation of DECNDs. After cooling to room temperature, the mixture was centrifuged (12 000 rpm, for 10 min). Finally, a clear yellow aqueous dispersion containing DECNDs was obtained, and the DECNDs were stored at ambient environment. Using this method, the concentration of the synthesized DECNDs was ∼36 mg/mL and the mass yield of DECNDs reached ∼90%.

**Separation of DECNDs.** The DECNDs could be separated through extraction with chloroform. In brief, a certain amount of DECNDs were dispersed in water; then, chloroform was added for extraction. After strong shaking, the mixture was rested for 1 h, and a layered solution was obtained. The blue emitters were extracted in chloroform (lower layer), and the yellow emitters remained in water (upper layer).

**Detection of pH Values.** First, this assay was performed by detecting the fluorescence of DECNDs at different pH values adjusted by adding HCl or NaOH (pH 0.5–12). Subsequently, the pH-responsive properties of DECNDs were validated in HCl–NaAc buffers (pH 0.65–5.20). The fluorescence spectra were measured with the excitation wavelength of 315 nm for the blue emitters and 365 nm for the yellow emitters.

**Cell Culture and Fluorescence Imaging.** The vitro cytotoxicity of DECNDs was assessed through MTT experiment. HeLa cells were grown in 5% CO_2 at 37 °C in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were first seeded at a density of 8000 cells per well on 96-well plates for 24 h and then incubated for another 24 h with DECNDs at desired concentrations at 37 °C. The medium was removed and replaced by fresh DMEM/FBS after being washed with phosphate-buffered saline (PBS) (100 μL, three times). For the MTT assay, 10 μL of MTT (5 mg/mL in PBS) was added to each well and the cells were incubated for an additional 4 h in dark. Finally, all medium was removed and 100 μL of dimethyl sulfoxide (DMSO) was added, followed by shaking for 10 min. The absorbance was monitored using a Synergy H1 hybrid Microplate reader at 492 nm.

For the in vitro imaging, HeLa cells were seeded on the glass coverslip in a six-well plate. After being incubated with DECNDs for 24 h, the cells were washed by PBS for three times, and fluorescence imaging of the cell was performed under a confocal microscope and the emission was measured by using laser excitation at 403.5 nm.
RESULTS AND DISCUSSION

To introduce the optical properties of DECNDs and the effective synthetic conditions for DECNDs, we focused our discussions on the DECNDs synthesized in ethylene glycol–water as the reaction media. The properties of DECNDs synthesized in other alcohol–water binary systems, such as methanol–water, ethanol–water, and n-propanol-water, are displayed in the Supporting Information.

Synthesis and Characterization of DECNDs in Ethylene Glycol–Water Binary System. Figure 1a,b showed the UV–vis absorption and fluorescence spectra of DECNDs, which hosted two emitters: The blue emitters exhibited the maximum emission at 386 nm when excited at 315 nm with the corresponding absorption peak at 260 nm (strong); the fluorescence emission of the yellow emitters showed a maximum in 530 nm with the excitation wavelength of 365 nm; and the corresponding absorption peak was located at 370 nm (weak). Analogously to most of carbon dots,18,19 the DECNDs also displayed the excitation-dependent emission, which was related not only to the size distribution but also to the diversified surface states (Figure S1).17,18

The DECNDs were characterized by using TEM, HRTEM, XRD, FT-IR spectrum, and XPS. As shown in the TEM and HRTEM images of Figure 2a, the DECNDs exhibited an approximately spherical shape and were well-dispersed. The sizes of them ranged from 1 to 3 nm with an average diameter of ~1.81 nm. The quantum yield (QY) of the present DECNDs was determined to be 12% using fluorescence lifetime and steady-state spectroscopy. The XRD pattern of DECNDs displayed a broad diffraction peak located at 2θ = 20.37°, which further confirmed an amorphous nature of CNDs20 (Figure 2b). The ligands on the surfaces of DECNDs were determined by FT-IR spectrum (Figure 2c), where peaks at 3452, 2900, and 1740 cm\(^{-1}\) were attributed to the stretching vibrations of O–H, C–H, and C=O and the peaks at 1195 and 1076 cm\(^{-1}\) corresponded to the asymmetric and symmetric stretching vibrations of C–O–C, respectively. Besides, the XPS spectra displayed two peaks at 284.1 eV and 532.2 eV, which were attributed to C 1s and O 1s (Figure 2d–f). The deconvolution of the C 1s spectra of the CNDs indicated the presence of three types of carbon bonds: C≡C (284.4 eV), C–O (286.0 eV), and C=O (288.6 eV) (Figure 2e). In the expanded image of the O 1s peak (Figure 2d), the signals at
Spectra (Figure S5). The phenomena also appeared when 439 nm with low intensity and the corresponding absorption wavelength was still 365 nm, but a new emission peak existed at 539.1 and 533.4 eV were attributed to C=O and C–OH/C–O–C groups. The results identified by XPS analysis were in good agreement with those of FT-IR spectrum, which demonstrated the presence of oxygen-containing functional groups such as hydroxyl and carbonyl groups on the surfaces of the two emitters.

The DECNDs could also be prepared successfully in other alcohol–water binary systems, such as methanol–water, ethanol–water, and n-propanol–water. The different alcohol–water reaction media did not show appreciable effects on size, absorption, and emission wavelengths of DECNDs. The TEM images, UV–vis absorption spectra, fluorescence spectra, and FT-IR spectra of DECNDs prepared in other alcohol–water binary systems are displayed in the Supporting Information (Figures S2–S4). Depending on the alcohol species, the DECNDs exhibited strong fluorescence, which would be preferred in practice.

Influence of Alcohol–Water Ratios on Properties of DECNDs. To understand the formation mechanism of DECNDs, we investigated the influence of alcohol–water ratios on properties of DECNDs in detail. Alcohol–water ratios were represented by alcohol volume fractions in the mixture, which ranged from 0 to 100%. As shown in Table 1 and Figure 3c, with the increase in alcohol volume fractions, the intensities of blue emitters dropped dramatically and the fluorescence of yellow emitters first increased and then decreased under higher alcohol volume fractions. Three points could be supplied from these results: (1) The blue emitters were closely related to the fractions of water in the binary systems, and the main reason was also the declined solubility of AA with the decrease in the water volumes, which led to the decline of quantities of synthesized blue emitters. (2) Accompanied by the increase in alcohol percentage, the yellow emitters appeared and the fluorescence built up, so it was believable that adding alcohols as the reaction media was necessary for the formation of yellow emitters, and when the ratio of alcohol to water was 1:1, both of the dual emissions were strong. (3) However, when the percentage of alcohol was >50%, the fluorescence of the two emitters decreased, so the water fraction was also the main constraint on the yellow emitters, which illustrated that the two emitters of DECNDs possessed the same original source that was AA or the same cores thermally decomposed from AA.

Meanwhile, the colors of the synthesized CNDs gradually changed from yellow to colorless with increasing alcohol ratios under visible light (Figure 3d). As shown in Figure 3e, under 365 nm UV light, when the CNDs were prepared in pure water or in lower (<5%) or higher (>95%) alcohol fractions, the solutions exhibited blue fluorescence with one emission peak in fluorescence spectra (Figure 3a,b); however, the CNDs, synthesized in middle ranges of alcohol–water binary systems, showed greenish yellow fluorescence under 365 nm UV light in accordance with dual emissions in the fluorescence spectra (Figure 3a,b).

In addition, the alcohol fractions could also affect the diameters of CNDs. As depicted in Figure S7, the CNDs synthesized in 50 and 100% ethylene glycol fractions were much smaller than those prepared in only water medium (Table S1), indicating that the CNDs grew uniformly in alcohol–water binary systems where alcohols could cap the CNDs and inhibit their agglomeration resulting in a better dispersion. Meanwhile, XPS data illustrated that alcohol fractions also determined the carbon and oxygen contents on the surfaces of CNDs (Table S1 and Figure S8).

Similar phenomena would be observed in the DECNDs synthesized in other alcohol–water binary media with changing the alcohol–water ratios (Figures S9–S11). On the basis of the previous discussions, water as well as alcohols is equally important for DECNDs, and these binary systems play a vital role in the synthesis. Tunable fluorescence colors could be realized by adjusting alcohol volume fractions. Most importantly, the two emitters possessed the same cores thermally decomposed by AA, and alcohol volume fractions could influence the diameter and surface states of CNDs.

The influences of other reaction conditions on the optical properties of DECNDs, such as reaction temperature and peak appeared at 260 nm (Figure S6). These results illustrated that the blue emitters were attributed to CNDs prepared by AA in water but the yellow emitters were not ascribed to the CNDs synthesized by AA in alcohols. When the preparations were carried out in alcohols only, the reaction conditions were disadvantages on the formation of CNDs because of the slight solubility of AA in alcohols, so the emission wavelength appeared to blue shift with low intensity.

In the middle ranges of alcohol–water ratios, as shown in Table 1 and Figure 3c, with the increase in alcohol volume fractions, the intensities of blue emitters dropped dramatically and the fluorescence of yellow emitters first increased and then decreased under higher alcohol volume fractions. Three points could be supplied from these results: (1) The blue emitters were closely related to the fractions of water in the binary systems, and the main reason was also the declined solubility of AA with the decrease in the water volumes, which led to the decline of quantities of synthesized blue emitters. (2) Accompanied by the increase in alcohol percentage, the yellow emitters appeared and the fluorescence built up, so it was believable that adding alcohols as the reaction media was necessary for the formation of yellow emitters, and when the ratio of alcohol to water was 1:1, both of the dual emissions were strong. (3) However, when the percentage of alcohol was >50%, the fluorescence of the two emitters decreased, so the water fraction was also the main constraint on the yellow emitters, which illustrated that the two emitters of DECNDs possessed the same original source that was AA or the same cores thermally decomposed from AA.

Meanwhile, the colors of the synthesized CNDs gradually changed from yellow to colorless with increasing alcohol ratios under visible light (Figure 3d). As shown in Figure 3e, under 365 nm UV light, when the CNDs were prepared in pure water or in lower (<5%) or higher (>95%) alcohol fractions, the solutions exhibited blue fluorescence with one emission peak in fluorescence spectra (Figure 3a,b); however, the CNDs, synthesized in middle ranges of alcohol–water binary systems, showed greenish yellow fluorescence under 365 nm UV light in accordance with dual emissions in the fluorescence spectra (Figure 3a,b).

In addition, the alcohol fractions could also affect the diameters of CNDs. As depicted in Figure S7, the CNDs synthesized in 50 and 100% ethylene glycol fractions were much smaller than those prepared in only water medium (Table S1), indicating that the CNDs grew uniformly in alcohol–water binary systems where alcohols could cap the CNDs and inhibit their agglomeration resulting in a better dispersion. Meanwhile, XPS data illustrated that alcohol fractions also determined the carbon and oxygen contents on the surfaces of CNDs (Table S1 and Figure S8).

Similar phenomena would be observed in the DECNDs synthesized in other alcohol–water binary media with changing the alcohol–water ratios (Figures S9–S11). On the basis of the previous discussions, water as well as alcohols is equally important for DECNDs, and these binary systems play a vital role in the synthesis. Tunable fluorescence colors could be realized by adjusting alcohol volume fractions. Most importantly, the two emitters possessed the same cores thermally decomposed by AA, and alcohol volume fractions could influence the diameter and surface states of CNDs.

The influences of other reaction conditions on the optical properties of DECNDs, such as reaction temperature and

### Table 1. Fluorescence Performances of DECNDs Synthesized in Ethylene Glycol–Water Binary Systems with Different Ethylene Glycol Volume Fractions

<table>
<thead>
<tr>
<th>Ethylene glycol volume fraction/%</th>
<th>EX½/nm</th>
<th>EM½/nm</th>
<th>FL½</th>
<th>EX/d/nm</th>
<th>EM/d/nm</th>
<th>FL/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>315</td>
<td>386</td>
<td>6896</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>315</td>
<td>386</td>
<td>6420</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>315</td>
<td>388</td>
<td>6198</td>
<td>365</td>
<td>526</td>
<td>922</td>
</tr>
<tr>
<td>50</td>
<td>315</td>
<td>386</td>
<td>2745</td>
<td>365</td>
<td>530</td>
<td>1858</td>
</tr>
<tr>
<td>75</td>
<td>315</td>
<td>384</td>
<td>505</td>
<td>365</td>
<td>532</td>
<td>1522</td>
</tr>
<tr>
<td>95</td>
<td>345</td>
<td>427</td>
<td>73</td>
<td>365</td>
<td>528</td>
<td>589</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td>365</td>
<td>439</td>
<td>94</td>
</tr>
</tbody>
</table>

* a and d, the maximum excitation wavelength; b and e, the maximum emission wavelength; c, the data of the blue emitters; and f, the data of the yellow emitters.

* DOI: 10.1021/acs.jpcc.5b05786
heating time, are displayed in the Supporting Information (Figures S12 and S13).

CNDs Synthesized Using Other Carbon Sources in Alcohol–Water Binary Systems. In this work, we also replaced AA with other carbon sources, such as glucose, citric acid, and green grass. All of them were frequently employed to fabricate CNDs via hydrothermal method; however, using the carbon sources previously mentioned, the CNDs with only one emission rather than dual emissions could be obtained under the same reaction conditions. Therefore, AA is pivotal carbon source in the synthesis of novel fluorescent DECNDs.

Separation of DECNDs. The as-synthesized DECNDs were a mixture consisting of the two emitters, which could be separated completely through extraction with chloroform. As shown in Figure 4a, a layered solution was obtained through extraction with chloroform: The upper layer (water phase) showed yellow fluorescence and the lower layer (chloroform phase) emitted blue fluorescence under 365 nm UV light. When excited at 315 nm, the lower-layer solution displayed strong fluorescence at 386 nm, which was consistent with the blue emitters in DECNDs, and at the same time the upper-layer solution showed no fluorescence (Figure 4b). Accordingly, when excited at 365 nm, similar emission spectrum could be
observed from the upper layer in comparison with that of yellow emitters, and the lower layer exhibited no fluorescence (Figure 4c). Moreover, the UV–vis absorption spectra of the upper- and lower-layer solutions were also in accordance with those of DECNDs (Figure S14a,b). Hence, it was believed that the upper layer was mainly composed of yellow emitters and the lower one mainly consisted of blue emitters, demonstrating that the two types of CNDs possessed the different polarities, which were associated with the modified ligands. Subsequently, the separated emitters were characterized by FT-IR spectra (Figure S14c), which suggested that the surfaces of the two emitters were occupied by some similar groups, such as the absorption peak at 3500 cm\(^{-1}\) associated with O–H stretching, a strong peak at 1716 cm\(^{-1}\) associated with C=O, and the peak at 1195 cm\(^{-1}\) attributed to C–O–C, potentially because AA molecules were the same carbon source of the two emitters; however, the upper layer showed stronger and sharper absorption peaks at 3500 cm\(^{-1}\) than the lower layer, and these bands were O–H stretching vibration, which revealed that more hydroxyl groups were capped on the surfaces of the yellow emitters. Therefore, the yellow emitters were insoluble in chloroform.

Besides, a series of experiments were designed to explore more definite information on the two emitters. (1) Linear range: The relationships between fluorescence intensities and concentrations of DECNDs were exhibited in Figure S15a. In a certain range, the fluorescence became stronger with increasing concentrations of two emitters, and the yellow emitters had a wider linear range (0–50 \(\mu\)L/mL) than the blue emitters (0–15 \(\mu\)L/mL). (2) Stability: The fluorescence of blue emitters decreased significantly from 6000 to 2000 within 35 days, but no obvious change was observed for the yellow emitters (Figure S15b). This evidence could also suggest that the two emitters were ascribed to different kinds of CNDs.

**Formation Mechanism of DECNDs.** Herein, the influence of AA amount on the dual-emission properties was investigated to explain the formation mechanism of DECNDs. As shown in Figure S16a, with increasing the amounts of AA (from 0.05 to 0.8 g) in alcohol–water systems, the fluorescence of the two emitters grew gradually, but the intensities of blue emitters enhanced more apparently. If AA acted only as the carbon source, the fluorescence variations of two emitters would be exactly the same with increasing amounts of AA, so compared with the yellow emitters, AA showed a unique surface modification of blue emitters. When 0.05 g AA was used in the reaction, the fluorescence of yellow emitters was higher than that of blue ones (Figure S16b), which meant insufficient AA could only act as a carbon source rather than functional groups, and thus the modifications of alcohol molecules on the yellow emitters were more obvious under this condition, which could be proved by FT-IR spectra of the yellow emitters after separation (Figure S14c). Therefore, the blue emitters and the yellow emitters were ascribed to two different species of CNDs, which were produced due to the different modified ligands on the surfaces of CNDs, such as AA capped on the surfaces of blue emitters and alcohol molecules capped on the surfaces of yellow emitters, revealing the modifications could significantly influence the optical properties of CNDs.

It should be noticed that AA was hydrophilic, while blue emitters capped by AA were able to disperse in chloroform (Figure 4). To investigate the solubility of blue emitters, we carried out a supplementary experiment using excessive AA as carbon source in water phase (without alcohols), so only blue emitters were obtained. As shown in Figure S17, the blue emitters before extraction were soluble in water; however, through extraction with chloroform, most of the blue emitters were dissolved in the lower layer (chloroform phase), suggesting that the blue emitters showed better solubility in chloroform. The reason might be that both hydrophobic and hydrophilic functional groups were present on the surfaces of blue emitters, such as C–O–C, C–H, C=O, and O–H (Figures S8 and S14), and thus the blue emitters possessed excellent solubility in not only water but also chloroform.

Therefore, the formation progress of DECNDs could be described as follows (Figure 5): First, AA as carbon source dissolved in water and decomposed into small CNDs under hydrothermal treatment. Second, when the amount of AA was excessive, AA molecules would act as the functional groups on the surfaces of CNDs, leading to the formation of blue emitters. At the same time, other CNDs were capped by alcohol molecules, producing the yellow emitters in alcohol–water binary systems. Excessive AA and alcohol molecules competed for the CNDs in alcohol–water binary systems. In general, there were only blue emitters obtained in water medium, and the pure alcohol medium was unfavorable for the formation of CNDs because of the low solubility of AA in alcohols, so the DECNDs could only be synthesized successfully in alcohol–water binary systems. On the basis of this strategy, a novel and simple one-pot synthesis of dual-emission nanoparticles was obtained, which indicated the possibility that nanoparticles containing various emitters could be prepared based on two or more kinds of solvents as reaction media.

**APPLICATIONS**

**Fluorescent Sensors for pH Measurement.** Figure S18 showed the fluorescence variations of DECNDs at different pH values, which were controlled by the addition of NaOH or HCl. Under strong acidic conditions, the fluorescence intensities of two emitters were greatly weak, and the intensities reached the maximum in neutral pH values; however, when the pH was strongly alkaline, the fluorescence intensities of yellow emitters decreased sharply, and meanwhile, the stable fluorescence was observed for the blue emitters. This pH-dependent emission feature might be associated with the protonation and deprotonation of surface functional groups of CNDs.\(^{24}\) Subsequently, to validate the practicability of DECNDs as a pH sensor in acid media, the fluorescence of DECNDs was investigated in a series of HCl-NaAc buffer solutions from pH 0.65 to 5.20. The blue emitters and yellow emitters showed different linear relationships between fluorescence and pH values. For instance, the pH linear range for the blue emitters was 0.65 to 1.99 (Figure 6a,b) and that for the yellow emitters was 0.65–3.79 (Figure 6c,d), which were extremely different.
from other pH sensors of CNDs synthesized by using AA as carbon source.\textsuperscript{25} Moreover, the intensity ratios of dual emissions ($I_{386}/I_{530}$) could also be employed as pH calibration from 1.99 to 3.79 (Figure S19), and thus both a single emission and ratiometric of dual emissions could be available for the pH detection, which would enable the DECNDs to be a reliable and accurate pH sensor against common environmental interferences and measurement errors. By contrast, other ratiometric pH sensors based on nanoparticles usually incorporated at least one type of dyes to achieve ratiometric detection.\textsuperscript{14,26,27} Hence, the easily prepared DECNDs were more advantageous as a ratiometric pH sensor.

**Cell Imaging.** The merits of CNDs, such as their high brightness, good stability, and nontoxic features, are promising for biological applications.\textsuperscript{5−8} To assess the prospects of DECNDs as bioimaging materials, we determined the cell viability of DECNDs by an MTT assay. As shown in Figure S20, no significant difference between the DECND-treated groups and the control samples was observed after incubation for 24 h. These results illustrated that the DECNDs did not have significant cytotoxic effects on this cell line at maximal working concentrations.

The HeLa cells were used to explore the potential of DECNDs as a bioimaging agent. The cellular uptake of DECNDs by HeLa cells was tracked by the confocal microscopy with laser excitation at 403.5 nm. Figure 7a showed the bright-field image of HeLa cells. Obviously, after incubation with DECNDs, the cells showed bright blue and red photoluminescence at the test excitation conditions (Figure 7b,c). These results clearly indicated that the DECNDs could readily penetrate the cell membrane and provided useful candidates of biolabeling agents for living cells.

## CONCLUSIONS

In this assay, using AA as carbon source, we have developed a new and simple hydrothermal method to synthesize dual-emission fluorescent CNDs based on alcohol−water binary systems. The DECNDs possessed both blue emitters and yellow emitters, which could be easily separated through extraction with chloroform. Alcohol−water ratios and AA play a critical role in the synthesis of DECNDs, and the two emitters were ascribed to two types of CNDs due to the different modified ligands on their surfaces, such as ascorbic acid and alcohol molecules. By taking advantage of the unique dual-emission characteristic, the DECNDs could be used as a reliable and accurate pH sensor in acid medium based on the intensity of a single emission or the intensity ratios of the two emissions. Meanwhile, the obtained DECNDs also exhibited excellent biocompatibility, suggesting great potential for biomedical applications. Therefore, this assay is of interest not only

---

**Figure 6.** Fluorescence spectra of blue emitters (a) and yellow emitters (c) at different pH values in HCl-NaAc buffer solutions; the variations of fluorescence intensities of blue emitters (b) and yellow emitters (d) at different pH values from 0.65 to 5.20 in HCl-NaAc buffer solutions.

**Figure 7.** Bright-field microphotograph of HeLa cells (a). Confocal microscopy images of HeLa cells labeled with the DECNDs at 37 °C for 24 h, with detection by 450 nm (b) and with detection by 520 nm (c). Overlay of blue and red photoluminescence (d).
because it provides a simple synthetic method for the DECNDs with two different emitters but also because it exemplifies the utilization of binary reaction media to adjust the organic ligands on the nanomaterial surface to further improve the sensor performance, which can open a new design strategy for other sensor development.

**ASSOCIATED CONTENT**

- **Supporting Information**
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.5b05786.

Fluorescence emission spectra of DECNDs with different excitation wavelengths from 310 to 380 nm; TEM images, UV–vis absorption, fluorescence spectra, and FT-IR spectra of the DECNDs synthesized in other alcohol–water binary systems; UV–vis absorption and fluorescence spectra of CNDs synthesized in pure water and pure ethylene glycol as the only reaction medium; TEM images of CNDs synthesized in ethylene glycol–water binary systems with different ethylene glycol volume fractions; the influence of alcohol volume fractions on the diameters of CNDs, carbon contents, and oxygen contents on the surface of CNDs; XPS spectra and C 1s and O 1s spectra of CNDs synthesized in ethylene glycol–water binary systems with different ethylene glycol volume fractions; fluorescence spectra, the variation of fluorescence intensities, and the color changes of DECNDs synthesized in other alcohol–water binary systems with different alcohol volume fractions; reaction temperature-dependent and heating time-dependent fluorescence spectra of blue emitters and yellow emitters; UV–vis absorption and FT-IR spectra of the separated blue and yellow emitters; the concentration-dependent fluorescence intensities and the stabilities of DECNDs; the influence of AA amount on the dual-emission properties; photograph of blue emitters before and after extraction with chloroform under 365 nm UV light; fluorescence spectra of blue emitters before extraction, the upper- and lower-layer solution after extraction with chloroform; fluorescence intensity variations of DECNDs at different pH values adjusted by adding HCl or NaOH (pH 0.5–12); the ratiometric pH calibration plot of the fluorescence intensity ratio \(I_{530}/I_{500}\) in the pH range from 1.99 to 3.79; viability of HeLa cells after 24 h incubation with DECNDs (PDF).

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: qufei3323@163.com (F.Q.).
*E-mail: jmyou6304@163.com (J.Y.).

**Author Contributions**

Fei Qu and Dongya Liu contributed equally to this work.

**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by the National Natural Science Foundation of China (Youth Fund Project) (21405093) and the Scientific Research Foundation of Qufu Normal University (BSQD20130117).

**REFERENCES**


DOI: 10.1021/acs.jpcc.5b05786