

# Fluorescent Lignin Carbon Dots for Reversible Responses to High-Valence Metal Ions and Its Bioapplications

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A sensitive and reversible sensing platform based on fluorescent carbon dots (CDs) allowing capture and detection of metal ions is of significance for developing diagnostic probes and *in-situ* environmental-monitoring. Here, a simple and effective method for converting biomass lignin into fluorescent CDs is developed. The obtained CDs, with stable fluorescence and excellent water solubility, are fabricated as unique model biosensors to access the metal-induced quenching mechanism. It is demonstrated that selective fluorescence quenching behaviors of lignin CDs are firstly found related to the valence-state of metal ions. Moreover, a reversible control of CDs fluorescence is realized by introducing reducing agents. This novel biosensor and the approach to quenching mechanism may evoke new insight in designing ions-sensor and monitoring biologic redox signal.

**KEYWORDS:** Carbon Dots, Biosensing, Fluorescent Quenching, Metal Ions.

## INTRODUCTION

Fluorescent carbon quantum dots (CDs) are a new class of luminescent nanomaterials that have been intensively studied in recent years.<sup>1–5</sup> Compared to traditional quantum dots or organic fluorescent dyes, CDs are superior in green preparation, non-toxicity, environmental compatibility and biocompatibility, and resistance to photo leeching.<sup>6</sup> In earlier research, carbon resources from both inorganic (critic acid,<sup>7</sup> ascorbic acid<sup>8</sup>) or organic (chitosan,<sup>5</sup> PEG,<sup>9</sup> formamide<sup>10</sup>) simple components to complex matrix (hemicellulose,<sup>4</sup> sago<sup>11</sup>) have been investigated in a large scale to explore effective ways to tailor the dimension, structure, elements components and optical improvements of the CDs. Increasing acquaintance with the photoluminescent (PL) properties leads to applications in promising fields such as bio-imaging, biologic sensing and optoelectronic devices.<sup>12, 13</sup> Among them, bio-sensing carries forward as a promising field with non-ending perfection in improvements.

The trace element in human body such as Fe, Mn, and Cr play vital roles in numerous biological processes such as cellular metabolism, and enzyme catalysis.<sup>14–16</sup> In deficient or excess quantities, such essential ions are toxic leading to disorder or disease.<sup>17</sup> In addition, the accumulation of metal ions in the environment after their frequent use in diverse industry will result in potential threat to the public health. Therefore, it is quite meaningful to develop accurate methods of metal ion determination in biological systems or environment. Numerous analytical techniques including atomic absorption spectrometry,<sup>18</sup> electrochemistry,<sup>19</sup> differential pulse polarography,<sup>20</sup> and spectrophotometry have been developed. Nevertheless, the aforementioned analytical methods require sophisticated equipment and involve time-consuming and tedious handling, as well as difficult sample preparation processes.

The fluorescence method has obtained widespread attention because of its high sensitivity, quick response, and simplicity.<sup>21</sup> Due to the excellent chemical activity and instant response in fluorescence signal, CDs have becoming increasing useful in constructing selective and sensitive biological and environmental sensing platforms.<sup>16, 22</sup> In the early stages, Sun et al.<sup>23</sup> discovered that photo-excited CDs could linearly photo-reduce Ag<sup>+</sup> to Ag in aqueous

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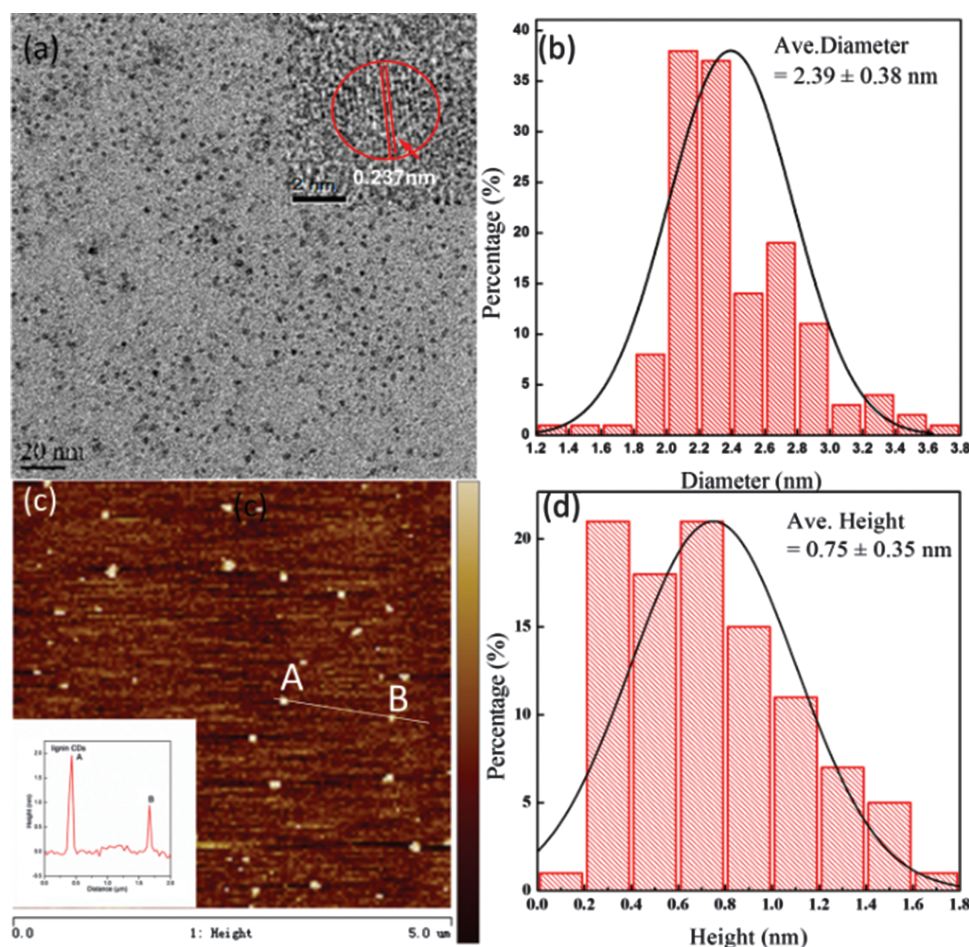
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**Table I.** Quantum Yield (QY) of lignin CDs obtained by different synthesis method.

| Method       | Ammonia concentration | Temperature (°C) | Time (h) | QY (%) |
|--------------|-----------------------|------------------|----------|--------|
| Hydrothermal | 0                     | 200              | 12       | 1.49   |
| Hydrothermal | 0.625%                | 200              | 12       | 6.48   |
| Hydrothermal | 1.25%                 | 200              | 12       | 6.28   |
| Hydrothermal | 2.5%                  | 200              | 12       | 10.40  |
| Hydrothermal | 5%                    | 200              | 12       | 14.24  |
| Hydrothermal | 10%                   | 200              | 12       | 6.68   |
| Hydrothermal | 20% NaOH              | 200              | 12       | 4.37   |
| Hydrothermal | 1%                    | 250              | 12       | 5.53   |
| Hydrothermal | 2.5%                  | 250              | 12       | 5.42   |
| Hydrothermal | 5%                    | 250              | 12       | 6.33   |
| Microwave    | 1%                    | 80               | 1        | 1.48   |
| Microwave    | 2.5%                  | 80               | 1        | 1.87   |
| Microwave    | 5%                    | 80               | 1        | 2.23   |

system with an interesting conclusion that the photo-excited CDs are both excellent electron acceptors and electron donors. Other literatures reveal that  $\text{Fe}^{3+}$ ,<sup>15,24</sup> and  $\text{Hg}^{2+}$ <sup>25</sup> can selectively quench the fluorescence of certain

CDs in specific sensing system. It was once considered CDs are only selective to one certain metal ion species. Sensitivity improvements in new strategies and complex designation such as ratiometric nanosensors based on CDs composite are also being developed for biosensing.<sup>26</sup> Meanwhile, selectivity-improvements towards CDs fluorescence quenching behaviours have been conducted hugely but merely concentrated on one certain metal ion. As the investigation goes deeper, interestingly but confusingly, various CDs are being utilized in sensing more and more metal ions such as  $\text{Cu}^{2+}$ ,<sup>8</sup>  $\text{Co}^{2+}$ ,<sup>9</sup>  $\text{Ni}^{2+}$ ,<sup>11</sup>  $\text{Pd}^{2+}$ ,<sup>27</sup>  $\text{Sn}^{2+}$ ,<sup>28</sup>  $\text{Fe}^{2+}$ ,<sup>29</sup>  $\text{Cr}^{3+}$ ,<sup>7</sup>  $\text{Bi}^{3+}$ ,<sup>30</sup>  $\text{Al}^{3+}$ ,<sup>11</sup>  $\text{Au}^{3+}$ <sup>31</sup> and  $\text{Pt}^{4+}$ .<sup>32</sup> Although the selective metal ions-based quencher pool is growing quickly with amazing diversity, there still lacks a dominant or detailed discussion on the quenching mechanism. In particular, explanations on an interesting phenomenon that high-valence metal ions dopants like  $\text{Fe}^{3+}$ ,<sup>15</sup>  $\text{Cr}^{6+}$ ,<sup>33</sup>  $\text{Mn}^{7+}$ <sup>22</sup> lead to more valid quenching of CDs compared to their low-valence forms are not available in literature. There is a significant need to develop a deep understanding of the phenomenological rules between the diverse quenching responses to



**Figure 1.** (a) HRTEM image of lignin CDs with an amplifying image as inset (b) statistic diameter distribution from TEM analysis (c) AFM image of lignin CDs with a height section (d) statistic height distribution from AFM analysis.

various metal ions. In current, limited understanding of CDs quenching behaviors remains a challenge to construct a simple but multi-sensible biosensor to meet the needs of medical visualization through instant capture and detection of target ions.

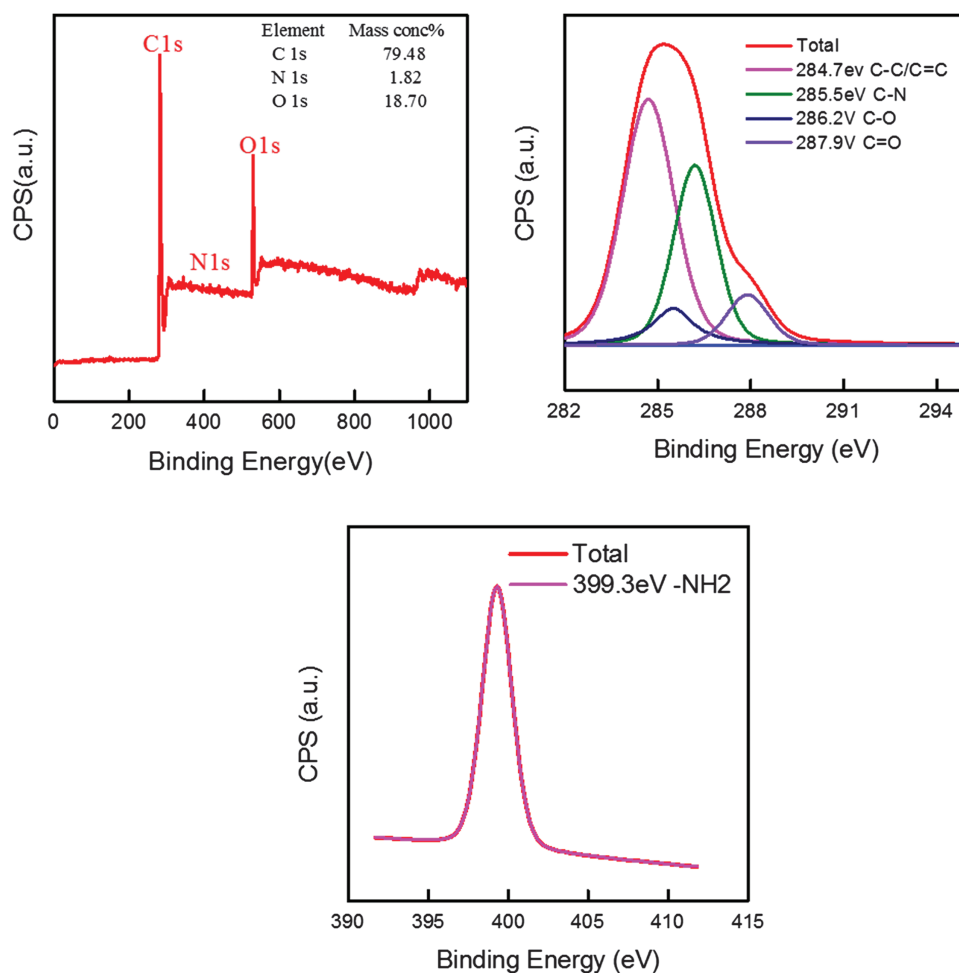
Here, we synthesized N-doped CDs via facile one-pot hydrothermal carbonization of biomass lignin<sup>34</sup>—a typical waste biomass produced in huge quantity annually by pulp industry. By introducing ammonia water as solvent and exterior nitrogen-doping agent, CDs with bright blue PL were obtained and exhibited good photostability against bleaching and various pH environments. The lignin-based CDs in aqueous system serve as a green and simple model of label-free fluorescent probe that responded selectively to  $\text{Fe}^{3+}$ ,  $\text{Cr}^{6+}$ , and  $\text{Mn}^{7+}$  by fluorescence quenching. To analyze the differentiated PL response of CDs, we initiated a fresh discussion on phenomenological mechanism illustrating valence-related electron-transfer quenching between CDs and metal ions. The valence-related fluorescence quenching behaviours of CDs could be reversibly recovered by green reducing agent, such as ascorbic acid. Using the selectively

quenching response to  $\text{Fe}^{3+}$  as an example, the CDs were successfully applied to bio-sensing of high-valence trace metal ions in fetal bovine serum. The reversible fluorescent sensing behaviour towards high-valence metal ions and ascorbic acid have been introduced into a logic gate operation. These bio-applications develop fluorescent analytic insights for potential bio-sensing and biological science purposes.

## MATERIALS AND METHODS

### Materials

Raw materials with 94.42% alkali lignin were provided by Beijing Key Laboratory of Lignocellulosic Chemistry. Different metal chloride including  $\text{SnCl}_4$ ,  $\text{FeCl}_3$ ,  $\text{FeCl}_2$ ,  $\text{CaCl}_2$ ,  $\text{CoCl}_2$ ,  $\text{KCl}$ ,  $\text{TiCl}_3$ ,  $\text{TiCl}_4$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{K}_2\text{CrO}_4$ ,  $\text{KMnO}_4$  etc. were purchased from Aladdin Chemistry Co. Ltd. Antioxidants such as ascorbic acid, hydroxylamine hydrochloride, GSH, pyrogallol acid were purchased from Macklin Reagent Biochemical Co. Ltd. Reagents above were all analytical grade and used as received. Deionized water was used throughout all the experiments.



**Figure 2.** The XPS analysis of lignin CDs with C1s and N1s analysis.

### Preparation of Lignin Carbon Dots (CDs)

Lignin carbon dots were prepared by hydrothermal treatments of lignin. Typically, 1 g purified lignin was dispersed in 2.5% ammonia water (19 mL) and continuously stirred overnight. Then the mixture was sealed into a 25 mL Teflon equipped stainless autoclave under 200 °C environment for 12 h hydrothermal treatment. After that, the obtained dark brown suspension was ultrasonic dispersed (100 W, 1 h) followed by centrifugation (15000 rpm, 15 min, three times) to remove the suspension. The transparent brown supernatant were neutralized with hydrochloride and then dialyzed against with ultrapure water using a 1000 Da cut-off dialysis bag for three days to remove the extra small molecular and ions. The dialyzate was then lyophilized to prepare solid-state CDs.

### Characterization of CDs

High-resolution transmission electron microscopy (HRTEM) images were taken on a TECNAIG20F20 (FEI, USA) electron microscope operating at 200 kV. Atomic force microscopy (AFM) images were taken on a Nano Surfaces Division Multimode 8 atomic force microscope (Bruker, Germany) under tapping mode by dropping the infinite dilute solution of CDs on mica substrate.  $\zeta$ -potential was measured on a Malvern Nano ZS instrument (Malvern, UK). Fourier transform infrared (FTIR) spectra were recorded on a Bruker TENSOR 27 spectrophotometer at frequencies ranging from 400 to 4000  $\text{cm}^{-1}$ . X-ray photoelectron spectroscopy (XPS) was investigated by using Axis Ultra DLD X-ray Photoelectron Spectroscopy/ESCA with a mono X-ray source Al K $\alpha$  excitation (1486.6 eV). Optical absorption was measured on a UV-3600 (Shimadzu, Japan) spectrometer. Fluorescence spectra were made on a FL-7000 fluorescence spectrometer (Hitachi, Japan) with slit width of 5 nm for excitation and emission at PMT voltage 400 V. The excitation increased by a 20 nm increment starting from 300 to 420 nm. All the optical spectra were recorded with a quartz cuvette of 1 cm path length. The quantum yield (QY) of the CDs was calculated by the equation:<sup>4</sup>

$$\Phi = \Phi_{\text{ref}}(F/F_{\text{ref}})(A_{\text{ref}}/A)(n^2/n_{\text{ref}}^2)$$

Where  $n$  is the refractive index of the solvent,  $A$  is the absorbance at corresponding excitation wavelength,  $F$  is the integrated intensity of the emission, and  $\Phi$  is the value of QY. Quinine sulfate in 0.1 M  $\text{H}_2\text{SO}_4$  was used as reference (literature QY 54% at 360 nm excitation).

### Fluorescence Quenching of CDs by Metal Ions

The solid-state CDs were dispersed in deionized water and diluted till 0.05 mg/mL as stock solution. For comparison, different metal chloride solutions with a final concentration of 1 mM were added into stock solution. After that, shake the mixture up and down for 30 s and then record the fluorescent spectra at an excitation wavelength of 320 nm and excitation/emission slits of 5 nm.

### Fluorescence Recovering Detection by Antioxidants

As described above, typically 1 mM ferric chloride solution was added into 4 mL stock solution to form an “on–off” sensing system. In the following assays, different kinds of antioxidants were added into the former “on–off” system. After shake for another 30 s, the fluorescence spectra were collected at the same condition as mentioned before. The effective recovery spectra were selected and the further linear relationship was investigated by addition of concentration gradient. All experiments were performed at room temperature.

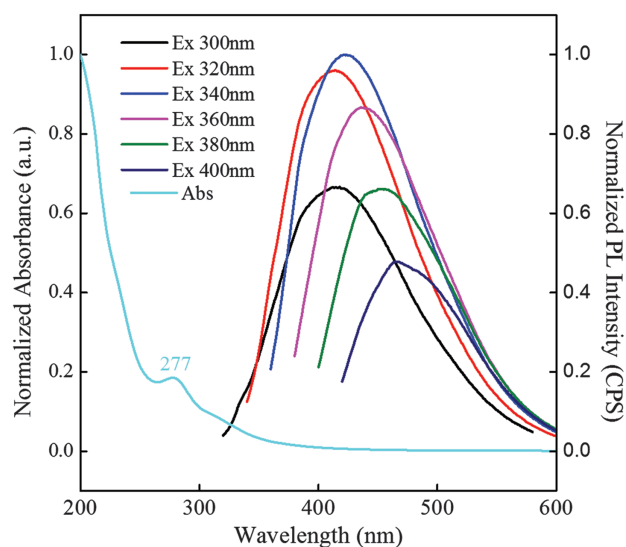
### Bio-Sensing Application in Real Serum Sample

To evaluate the capability of the proposed method in real sample analysis, the determination of  $\text{Fe}^{3+}$  in FBS fetal bovine serum samples was applied as a bio-sensing model. To precipitate protein, 2 mL of serum sample was added to 75% methanol aqueous. The mixture was centrifuged for 10 min at 12000 r/min to remove protein, and minimize nonspecific interactions of the CDs with proteins. Supernatant liquid was transferred to a fresh test tube, and then pH was adjusted to 6, and the resulting solution was spiked with different amounts of  $\text{Fe}^{3+}$ . The solution with a constant CDs concentration of 0.05 mg/mL was then analyzed by PL intensity under an excitation wavelength of 360 nm similar to the previous given procedure. The obtained results are given in Table III.

## RESULTS AND DISCUSSION

### Characterization of CDs

The blue emissive CDs were obtained by a facile hydrothermal route from lignin in ammonia water. The synthetic



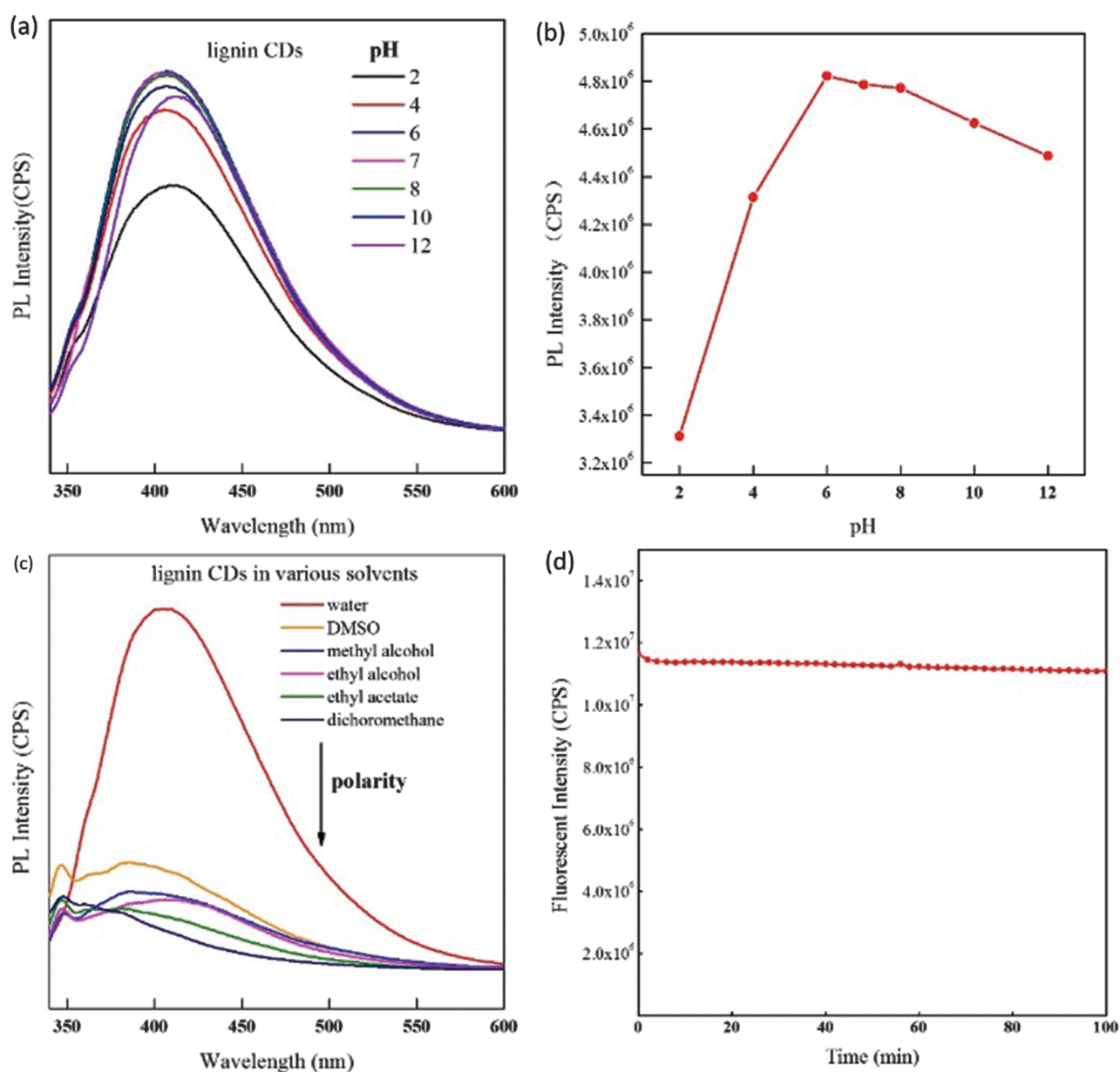
**Figure 3.** Normalized absorbance spectrum of lignin CDs and normalized photoluminescent of lignin CDs under various excitation wavelengths.



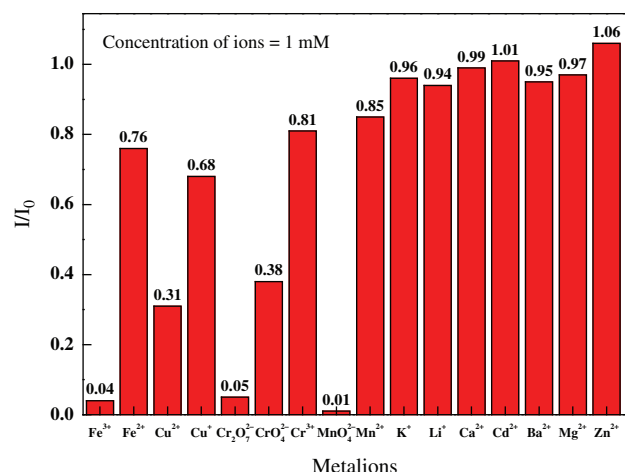
conditions of CDs were explored in detail, including the ammonia doping content, heating temperature and heating time. As shown in Table I, the concentration of introduced ammonia has a key effect on the fluorescence quantum yield (QY) of obtained lignin CDs. The nitrogen-doping introduced a new surface state. The electrons trapped by the new N-surface state promote a large amount of radiative recombination, and thus increase the fluorescent QY to as high as 14.24%, which is almost 10 times larger than that of the bare lignin carbon dots (1.49%). The heating temperature is another important factor that influences the fluorescent properties of lignin CDs. Overheating or low temperatures may lead to reduced QY, probably owing to the collapse of the carbon nanostructure or

invalid fluorescent carbon core with right configuration on surface.<sup>35</sup> Therefore, for the following experiments, further characterization and fluorescent response of lignin carbon dots were carried on samples as raw materials in the temperature of 200 °C, heating for 12 h with 2.5% ammonia water.

The structure, morphology, and optical properties of the as-prepared lignin CDs were investigated. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) observations in Figure 1 clearly confirmed the non-spherical morphology nanostructure of the products, which displayed a narrow diameter of  $2.39 \pm 0.38$  nm and an average height of 0.75 nm. The obtained CDs were smaller than those derived from hemicellulose and chitosan,<sup>4,5</sup>

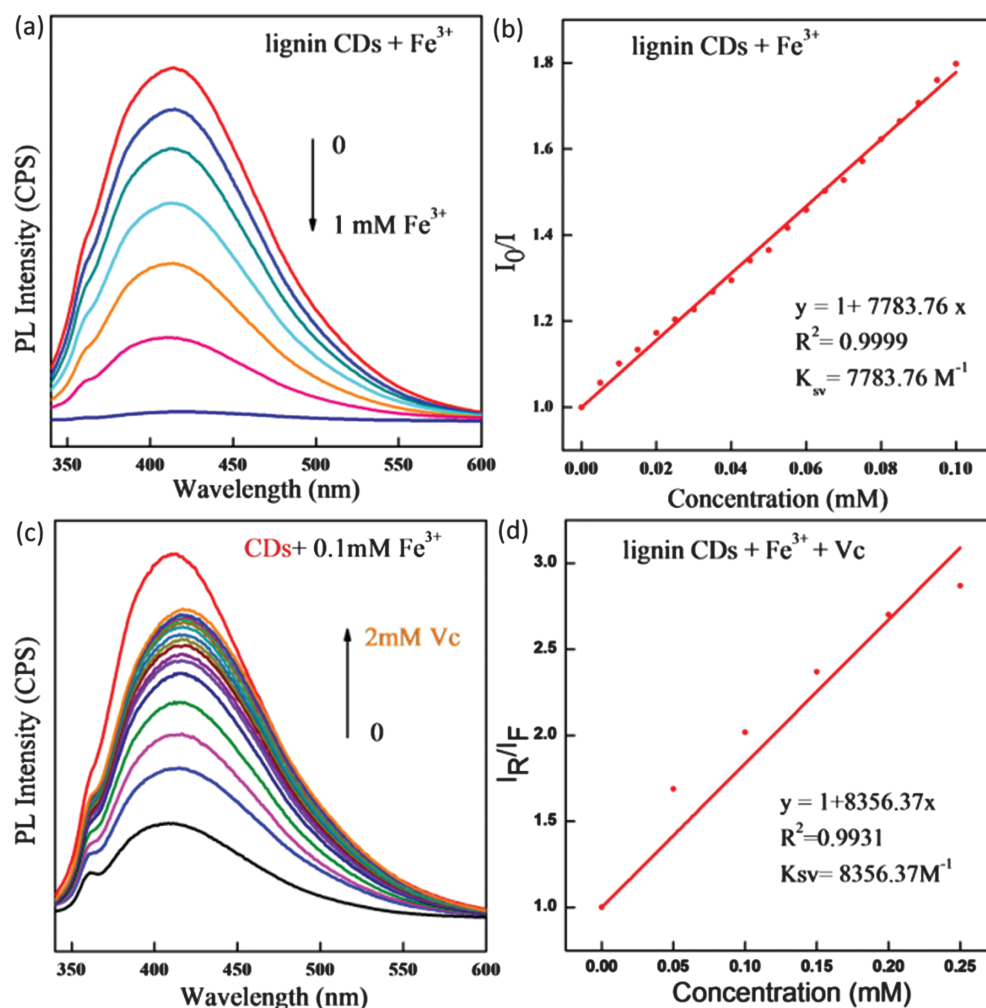


**Figure 4.** Influence of pH and various solvents on the fluorescence properties of lignin CDs.



**Figure 5.** Fluorescence quenching response of lignin CDs by various metal ions (1 mM) dispersed in CDs aqueous system (0.05 mg/mL).

suggesting a higher surface area with amorphous carbon core. The X-ray photoelectron spectroscopy (XPS) analysis (Fig. 2) of lignin CDs indicated that nitrogen was successfully introduced, and there were rich functional groups including  $-\text{OH}$ ,  $-\text{COO}^-$  and  $-\text{NH}_2$  on the CDs' surface. Since the surface potential of lignin CDs was measured to be  $-26.6$  mV, it can be concluded that the density of carboxyl groups were higher than that of amino groups. The UV-vis spectrum in Figure 3 shown the CDs had an absorption peak at around 277 nm, due to the  $\pi-\pi^*$  transition of  $\text{C}=\text{C}$  aromatic  $sp^2$  domains in the graphitic core of CDs.<sup>5</sup> In addition, there existed a narrowly recognized shoulder around 310–340 nm, which originates from  $n-\pi^*$  edge transition of  $\text{C}=\text{O}$  and is the main emissive center for the bright luminescence in the CDs. Similar to most CDs, lignin CDs have  $\lambda_{\text{ex}}$ -dependent PL emissions when excited at a wavelength ranging between 300 and 400 nm. The strongest emission is centered at 408 nm with bright



**Figure 6.** (a) Fluorescence quenching responses of the CDs (0.05 mg/mL) with  $\text{Fe}^{3+}$  (c) fluorescence recovery of  $\text{Fe}^{3+}$ -induced fluorescence quenching model by ascorbic acid. Plot of quenching efficiency versus concentrations of (b)  $\text{Fe}^{3+}$  and (d) ascorbic acid.

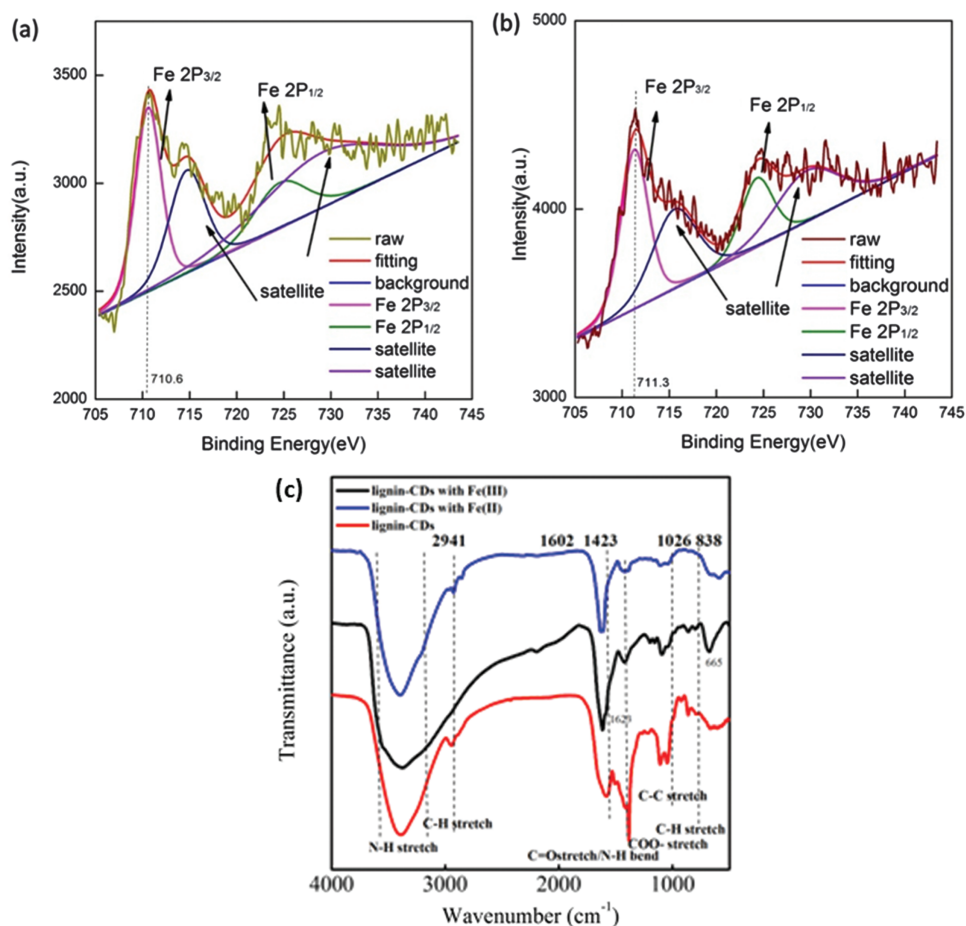
**Table II.** Fluorescent recovery of the  $\text{Fe}^{3+}$ -induced quenching model system by different reducing agents.  $I_R/I_F$  is defined as a recovery index calculated by the fluorescent intensity ratio of the system (1 mM  $\text{Fe}^{3+}$ -induced quenching) with and without 1 mM reductants.

| Reducing agents             | $I_R/I_F$ | Recovery rate (%) |
|-----------------------------|-----------|-------------------|
| Ascorbic acid               | 16.4      | 50.33             |
| Hydroxylamine hydrochloride | 7.2       | 22.05             |
| $\text{Ti}^{3+}$            | 4.3       | 13.1              |
| GSH                         | 2.1       | 6.37              |
| Pyrogallol acid             | 1.7       | 5.27              |

blue light under an excitation of 340 nm. As confirmed, the as-prepared lignin CDs exhibited very high fluorescence stability in a prolonged irradiation time and kept excellent fluorescence in a mild pH environment (pH = 6–10) when dispersed in water (Fig. 4). Such CDs are beneficial for applications in aqueous solution since they maintain the strongest and  $\lambda_{\text{ex}}$ -dependent fluorescence emission in water. Consequently, all these properties make the lignin CDs a qualified model material for a further research in biosensing of metal ions.

### Label-Free Detection of $\text{Fe}^{3+}$ and “Off–On” Bio-Sensing of Ascorbic Acid

The lignin CDs obtained by a facile one-pot hydrothermal carbonization in 2.5% ammonia water were used to fabricate the as-described model biosensor here. The fluorescence responses of lignin CDs towards different metal ions in aqueous solution were investigated by observing the changes in fluorescent intensity before and after the addition of the same amount of chlorides of metal ions.  $\text{Fe}^{3+}$  had a significant quenching effect on the fluorescence of lignin CDs with a sharp decrease of  $\sim 96\%$  intensity (Fig. 5). On the contrary, the lignin CDs showed negligible response towards most alkali metal ions ( $\text{K}^+$ ,  $\text{Li}^+$ ) and alkaline-earth metal ions ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ba}^{2+}$ ). Notably, here it was observed that other high-valence heavy metals and transition metals, such as  $\text{Cu}^{2+}$  and  $\text{CrO}_4^{2-}$  can also obviously quench the fluorescence of CDs. In the case of  $\text{Fe}^{3+}/\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}/\text{Cu}^+$  ions in pair can both quench the fluorescence of CDs. However,  $\text{Fe}^{2+}$  and  $\text{Cu}^+$  showed relatively lower efficiencies compared to their higher oxidation state forms. Similarly,  $\text{CrO}_4^{2-}$ ,  $\text{Cr}_2\text{O}_7^{2-}$ , and  $\text{MnO}_4^-$  can also serve as more efficient quenchers superior to  $\text{Cr}^{3+}$  and  $\text{Mn}^{2+}$ . Those multi-valence metal ions draw our attention



**Figure 7.** XPS spectra of CDs aggregations when interacted with (a)  $\text{Fe}^{3+}$  and (b)  $\text{Fe}^{2+}$  and FTIR spectrum (c) of bare lignin CDs, lignin CDs with  $\text{Fe}^{3+}$  and lignin CDs with  $\text{Fe}^{2+}$ .



and we come to hold a draft hypothesis that the selective quenching behaviours may be associated with high-valence metal ions.

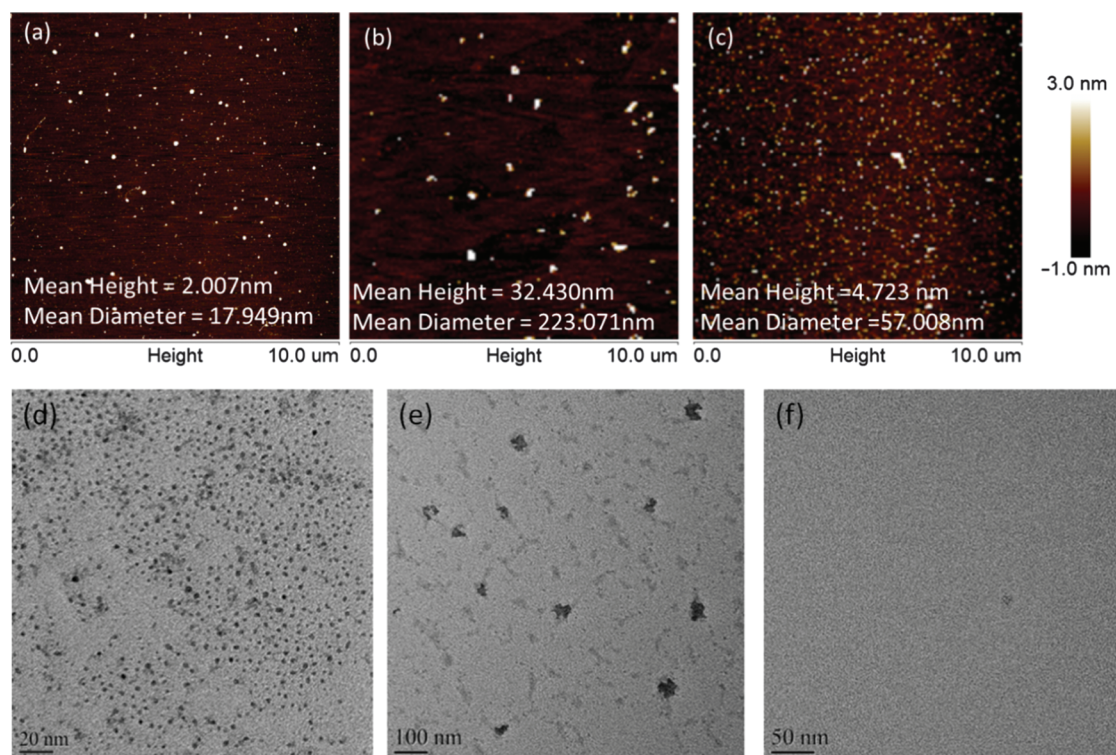
To achieve a better understanding of the valence-dependent quenching behaviours, an analytical model based on the  $\text{Fe}^{3+}$ -induced quenching system was built. The fluorescence intensity of CDs decreased stepwise till fully quenched as the concentration of  $\text{Fe}^{3+}$  increasing to 1 mM in Figure 6(a). Further analysis showed that this quenching follows a single mechanism of interaction, either static or dynamic. The Stern–Volmer<sup>5</sup> plots of  $I/I_0$  forms excellent linearity with a correlation coefficient  $R^2 = 0.9999$  in Figure 6(b) with a LOD (limit of detection) calculated as  $0.66 \mu\text{M}$ . The Stern–Volmer constant ( $K_{sv}$ ) derived from the plots of  $I/I_0$  is  $7783.76 \text{ M}^{-1}$ , indicating a high quenching efficiency. The result proves that CDs-based biosensor possesses high sensitivity toward  $\text{Fe}^{3+}$  ions and exhibits great superiority with simplicity, sensitivity and fast response.

An interesting “turn-on” fluorescence response was achieved when ascorbic acid ( $V_c$ ) was added to the system as a reducing agent, indicating reversible properties of a previously quenched system. As depicted in Figure 6(c), the quenched fluorescence of lignin CDs induced by  $\text{Fe}^{3+}$  was recovered instantly when adding  $V_c$  in, and the recovery reached a maximum of 89.36% with 2 mM  $V_c$  addition. A linear relationship (Fig. 6(d)) between  $I_R/I_F$  and

the concentration of  $V_c$  could be observed when the latter ranges from 0.05 to 0.25 mM, with a coefficient of determination ( $R^2$ ) for line fitting that is close to 1, i.e.,  $R^2 = 0.9931$ . Since ascorbic acid is an antioxidant and a crucial vitamin (vitamin C) in the human diet and has been directed at the precaution and therapy of the common cold, mental illness, cancer, and AIDS.<sup>7</sup> Hence, taking advantage of the above “off-on” biosensor, a fast and easy route for quantitative measurement of  $V_c$  is promising in the field of drug preparation, plus biological samples and systems.

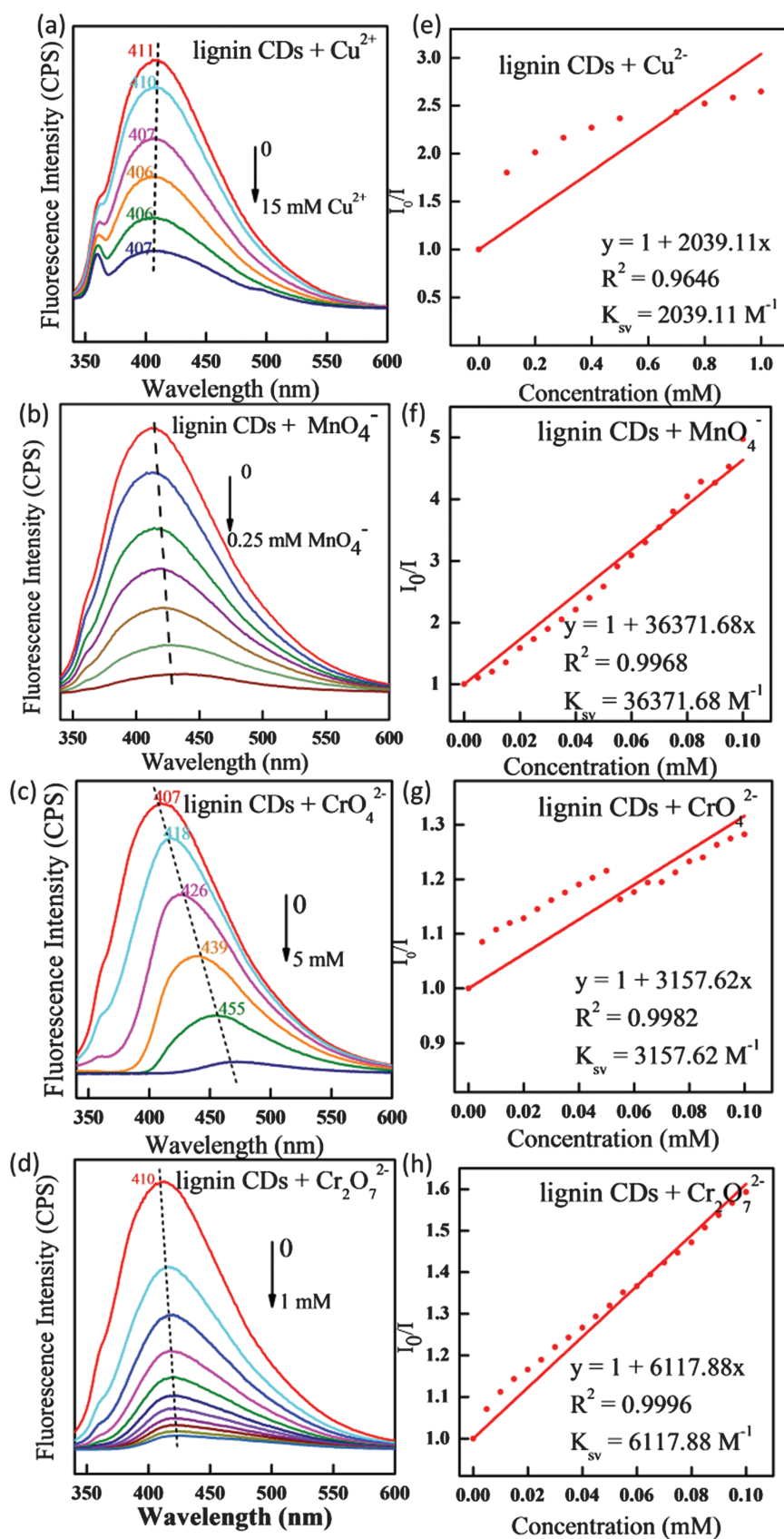
### Reversible Quenching Mechanism of Lignin-CDs

The reversible quenching mechanism of the above “on-off-on” biosensor was further explored. As determined by phenanthroline spectrophotometry,  $\text{Fe}^{2+}$  was hardly detected ( $0.09 \mu\text{g/mL}$ ) in the initial quenching system, indicating CDs cannot reduce  $\text{Fe}^{3+}$  ions by themselves. However, distinct release of  $\text{Fe}^{2+}$  ( $1.22 \mu\text{g/mL}$ ) was recorded after the introduction of  $V_c$ . The amount of detected iron matches the input dosage ( $1.12 \mu\text{g/mL}$ ), which indicates that the input  $\text{Fe}^{3+}$  ions existed in a form of  $\text{Fe(III)}$  and then was reduced to  $\text{Fe}^{2+}$  by  $V_c$ . To further confirm this assumption, other reducing agents including hydroxylamine hydrochloride, titanium trichloride, glutathione (GSH) and pyrogalllic acid were also applied and proven effective to reversibly change fluorescence of

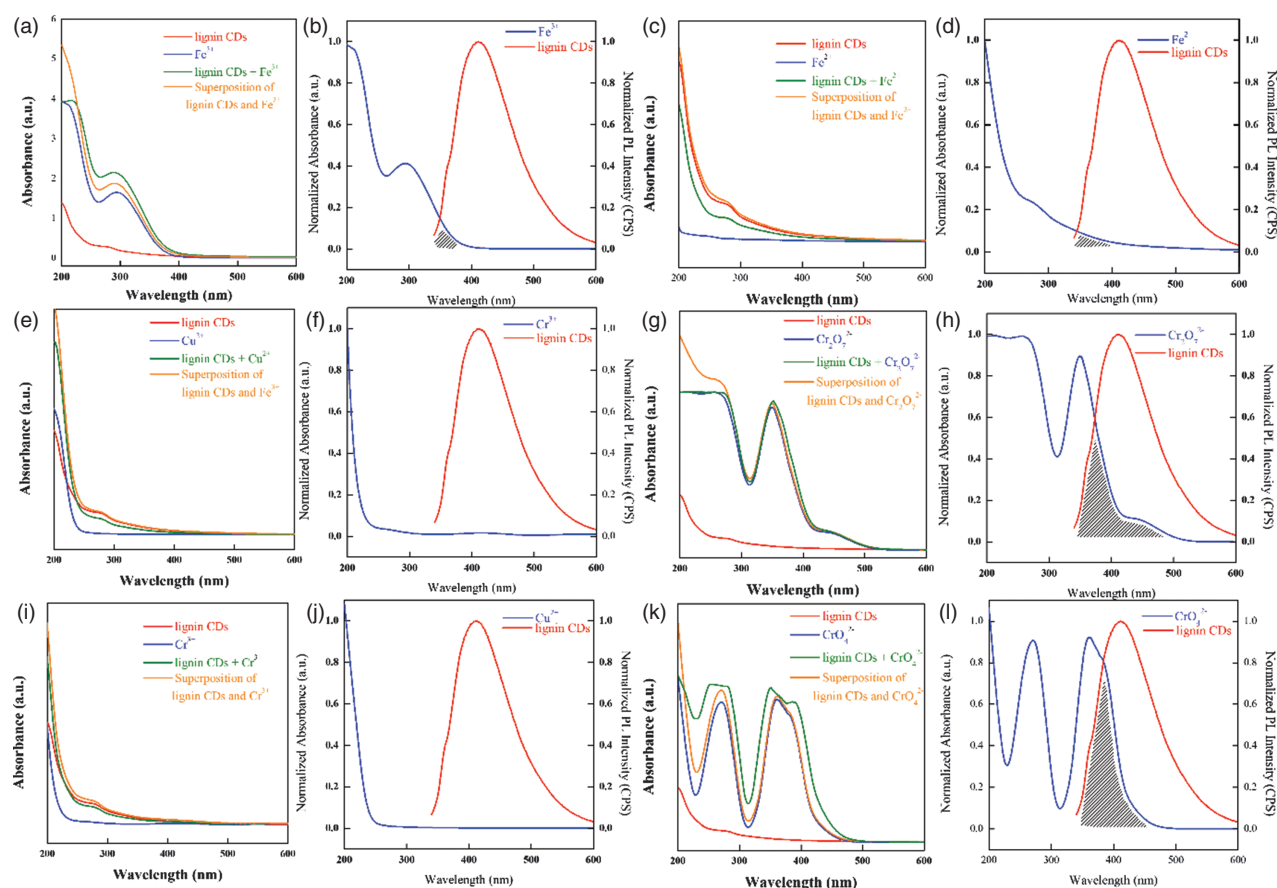


**Figure 8.** AFM and HRTEM images of CDs in the “on-off-on” model: tapping mode AFM images of (a) CDs alone; (b) CDs- $\text{Fe}^{3+}$  aggregation; (c) CDs- $\text{Fe}^{3+}$  aggregation in the presence of ascorbic acid on mica. (d) CDs alone (scale bar, 20 nm); (e) CDs- $\text{Fe}^{3+}$  aggregation (scale bar, 100 nm); (f) CDs- $\text{Fe}^{3+}$  aggregation in the presence of a scorbic acid (scale bar, 50 nm).





**Figure 9.** Fluorescence quenching behavior induced by high-valence metal ions.



**Figure 10.** UV spectrum change of lignin CDs before/after adding various metal ions and the respective spectra overlap between lignin CDs and respective ions: (a, b) for  $\text{Fe}^{3+}$ , (c, d) for  $\text{Fe}^{2+}$ , (e, f) for  $\text{Cu}^{2+}$  (g, h) for  $\text{Cr}^{3+}$ , (i, j) for  $\text{Cr}_2\text{O}_7^{2-}$ , (k, l) for  $\text{CrO}_4^{2-}$ .

CDs (Table II). The fluorescence recovery efficiency was associated with their redoxability,<sup>7,31</sup> indicating that the reduction reaction is the driving reason to recover the fluorescence.

Detailed information of the surface state of lignin CDs after reacting with  $\text{Fe}^{3+}$  or  $\text{Fe}^{2+}$ , respectively, was collected by XPS spectrum (Figs. 7(a and b)). The peak positions of Fe  $2p_{3/2}$  and Fe  $2p_{1/2}$  are located at 710.4 eV and 724.6 eV, respectively, which matches the presence of  $\text{Fe}^{2+}$ . For picture Figure 7(b), the peak of Fe  $2p_{3/2}$  and Fe  $2p_{1/2}$  appear at 711.3 eV and 724.3 eV respectively, suggesting the presence of  $\text{Fe}^{3+}$ . A higher binding energy is found between CDs/ $\text{Fe}^{3+}$  than that of between CDs/ $\text{Fe}^{2+}$ . This is possibly due to the formation of coordinate covalent bonds between an empty d orbital of  $\text{Fe}^{3+}$  and  $\pi$  electrons of the lignin CDs.<sup>29</sup> As for Fourier transform infrared spectroscopy (FTIR) spectra (Fig. 7(c)), stronger peaks at  $1423\text{ cm}^{-1}$  corresponding to  $\text{COO}^-$  absorption band and  $1250\text{ cm}^{-1}$  corresponding to phenolic hydroxyl group were observed in CDs/ $\text{Fe}^{3+}$  complex. Those observed characteristic peaks showed interaction taken place at oxygen functional groups on the surface of CDs.

The time-resolved fluorescence measurements presented additional evidence for the quenching mechanism by comparing the lifetime of CDs. As shown, the lifetime of CDs alone is tri-exponential with an average value of 4.57 ns, which is prolonged to 6.6 ns after the addition of  $\text{Fe}^{3+}$ . The prolonged lifetime of CDs can be explained by the disruption to the radiative recombination of the functional groups on the carbon surface, which is responsible for the trapping of photo-induced electrons and holes.<sup>36,37</sup> On the other hand, the lifetime of CDs in the presence of  $\text{Fe}^{2+}$  is 4.59 ns, which is almost the same with that of CDs alone, indicating non-/tiny interaction happening between  $\text{Fe}^{2+}$  and CDs.

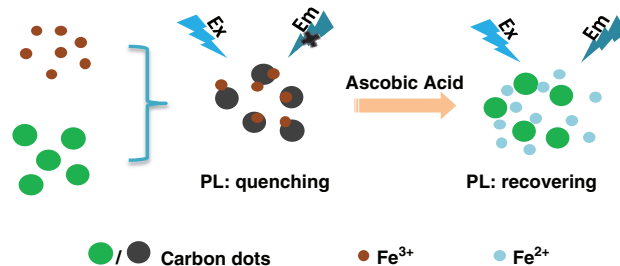
To obtain a straightforward view of the formation of the CDs/metal ions complex, AFM and TEM images (Fig. 8) were used to describe the morphology evolution with metal ion-induced aggregation ( $\sim 223\text{ nm}$ ) and  $V_c$ -induced deaggregation (57 nm). This result further confirmed our hypothesis. Herein, the doped  $\text{Fe}^{3+}$  ions tend to bond with phenolic hydroxyl group of lignin CDs and accumulate on the CDs' surface driven by chelation or/and electrostatic interactions, and thus quench (turn-off) the fluorescence

of CDs by scavenging the surface confined holes and electrons.

### Fluorescent “On–Off–On” Bio-Sensing of Cr(VI) and Mn(VII)

Similar “on–off–on” phenomena has been found among other high-valence ions based on our reversible sensing platform. By introducing the quenchers ( $\text{Mn}^{7+}$ ,  $\text{Cr}^{6+}$ ) to the CDs solution, the fluorescence was gradually weakened, and then previously quenching fluorescence could be recovered with increasing dosage of reducing agents ( $V_c$ ). As it clearly showed in Figure 9, the fluorescence decreased with a perfect correlation coefficient above 0.99 with a calculated  $K_{sv}$  as  $36371.68 \text{ M}^{-1}$  ( $\text{MnO}_4^-$ ),  $6117.88 \text{ M}^{-1}$  ( $\text{Cr}_2\text{O}_7^{2-}$ ),  $3157.62 \text{ M}^{-1}$  ( $\text{CrO}_4^{2-}$ ) and  $2039.11 \text{ M}^{-1}$  ( $\text{Cu}^{2+}$ ). These results indicated the potential of this system for quantitative analysis of those oxometallates. Due to a surface-defect states emission,<sup>38</sup> an obvious red-shift quenching is induced by  $\text{CrO}_4^{2-}$  (Fig. 9(c)), and there is tiny reversible control over the quenched fluorescence in the presence of  $V_c$  (Fig. 9(b)). Comparing the absorption spectrum in Figure 10, it was obvious that no new substances were generated since no new absorbance peak was observed. On the other hand, the tiny spectrum overlap between emission and absorbance indicates that the  $\text{Fe}^{3+}$ -induced quenching behaviour is definitely not by the energy transfer mechanism but by an electron transfer mechanism.<sup>5</sup> Specially, for  $\text{MnO}_4^-$ ,  $\text{Cr}_2\text{O}_7^{2-}$  and  $\text{CrO}_4^{2-}$ , the spectrum overlaps between the absorption of ions and the emission spectrum of CDs describe a possibly different quenching mechanism known as FRET<sup>13, 16</sup> or IFT,<sup>33</sup> which lay a foundation for a further sensor based on the quantitatively analysis of those mentioned ions by formation of donor–accepter pairs (CDs–high-valence ions). Taken together, all these results demonstrate that the fluorescence sensing of metal ions with lignin CDs is correlated with their valence-state. Similarly, a recovery control of the quenched fluorescence can also be obtained when  $V_c$  is introduced into the previously mentioned quenching system. In the case of  $\text{MnO}_4^-$ -induced quenching of lignin CDs, the maximal recovery rate is calculated to be 68.75% when 5 mM  $V_c$  is introduced. The recovery rate were rather smaller for  $\text{Cr}_2\text{O}_7^{2-}$  and  $\text{CrO}_4^{2-}$  calculated as 30.01% and 37.02%.

Taking the  $\text{Fe}^{3+}$ -induced reversible fluorescent response, here we draw a simple scheme to demonstrate the mechanism of sensing procedure. Scheme 1 illustrates our design rationale. A model system is built based on the aqueous dispersion of lignin CDs with  $\text{Fe}^{3+}$  as the typical metal quencher. When  $\text{Fe}^{3+}$  (or high-valence ions) ions are added, they tend to bond with lignin CDs and accumulate on the CDs' surface driven by chelation or/and electrostatic interactions between  $\text{Fe}^{3+}$  and the carboxyl group. As a consequence, the fluorescence gets quenched (turn-off) through energy or charge transfer. On the other hand,



**Scheme 1.** Schematic representation of  $\text{Fe}^{3+}$ -induced fluorescence quenching model based on lignin-based CDs and ascorbic acid-involved fluorescence recovery response model. The designed “on–off–on” model suits for other high-valence ions quenchers.

the quenched fluorescence can be restored in the presence of ascorbic acid due to a redox reaction, which departs the  $\text{Fe}^{3+}$  ions from the surface of CDs by reducing  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  (or reduced state). The different affinities were observed when studying metal ions in pairs with comparable valence. Similar recoveries were also achieved with other reductants. The reversible and valence-state related fluorescence response of lignin CDs to high-valence metal ions enables us to perform reversible control of the CDs' fluorescence through changing the valence-state of metal ions quenchers.

### Bioapplications

To utilize the CDs as fluorescent probes in biomedical applications, a standard addition method was applied for the detection of  $\text{Fe}^{3+}$  in FBS as a typical biosensing model. Iron is a ubiquitous metal in cells and plays a crucial role in a variety of vital cell functions, and both its deficiency and excess from the normal limit can induce serious disorders.<sup>29</sup> A series of mixtures of the diluted FBS serum supernatants (25  $\mu\text{L}$ ) and CDs (0.05 mg/mL) were spiked with the standard solution of  $\text{Fe}^{3+}$  (0–100 mM). Recovery experiments were performed to evaluate the accuracy of this method. The  $\text{Fe}^{3+}$  contents in FBS detected by the fabricated “on–off” probe were in the range of 93.98–114.93%, with relative standard deviations (RSDs) are around 3% ( $N = 3$ ) (Table III). On the basis of the observation that the fluorescence of CDs was efficiently quenched by  $\text{Fe}^{3+}$  even in rough FBS sample, which indicates the

**Table III.** Results of the determination of real samples by lignin CDs sensor.

| Sample      | Added ( $\mu\text{M}$ ) | Found ( $\mu\text{M}$ ) | Recovery (%) | RSD ( $n = 3$ ) |
|-------------|-------------------------|-------------------------|--------------|-----------------|
| Blood serum | 0.00                    | 4.42                    | –            | –               |
|             | 10.00                   | $13.55 \pm 2.60$        | 93.98        | 0.19            |
|             | 20.00                   | $27.20 \pm 2.50$        | 111.40       | 0.09            |
|             | 30.00                   | $37.80 \pm 2.47$        | 109.83       | 0.07            |
|             | 40.00                   | $45.04 \pm 2.19$        | 101.40       | 0.05            |
|             | 60.00                   | $74.04 \pm 2.10$        | 114.93       | 0.03            |





| AND LOGIC GATE                                                                                            |                                                                                                 |                       |
|-----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------------------|
| Input                                                                                                     |                                                                                                 | Output                |
|  high-valence metal ions |  Ascorbic acid | Fluorescence Recovery |
| 0                                                                                                         | 0                                                                                               | 0                     |
| 0                                                                                                         | 1                                                                                               | 0                     |
| 1                                                                                                         | 0                                                                                               | 0                     |
| 1                                                                                                         | 1                                                                                               | 1                     |

**Scheme 2.** Logic operation scheme of lignin-CDs toward high-valence metal ions and  $V_c$  and the simulation table for “AND” logic gate with a typical two-input logic circuit.

currently proposed biosensor has shown high analytical precision and average total recoveries. This may open up new possibilities in label-free monitoring of metal ions content in biological system.

### Logic Operation of Lignin-CDs Toward High-Valence Metal Ions and $V_c$

Based on the results presented above,  $V_c$  could induce the fluorescence recovery of CDs quenched by  $Fe^{3+}$ ,  $Mn^{7+}$ , or  $Cr^{6+}$ . Without high-valence metal ions,  $V_c$  itself has no interaction with lignin CDs, and cannot induce any fluorescence change. Thus, a fluorescence “AND” logic gate.<sup>14, 29</sup> could be realized by using high-valence metal ions and  $V_c$  as the input, and the fluorescence signal recovery could be realized by the reaction of CDs with high-valence metal ions or/and  $V_c$  as the output. For input, the absence and presence of high-valence metal ions or  $V_c$  were defined as 0 and 1, respectively. For output, fluorescence intensity recovery and none were defined as 1 and 0, respectively. In the absence of either (1/0 and 0/1) or both (0/0) inputs, no recovery of fluorescence was observed while in the presence of both (1/1) inputs; the system fluorescence was recuperative. Therefore, it could be performed as an AND logic operation (Scheme 2). Consequently, only the system receives the two inputs together (1/1), the fluorescence signal is enhanced visibly, and the gate is activated and exerts an output signal of 1. This exploration may largely enrich the analytical strategies in monitoring biological redox signal or environmental changes.

## CONCLUSIONS

In summary, we have successfully fabricated a novel reversible biosensing platform based on lignin CDs with a valence-dependent fluorescence response, and have developed a theoretical understanding of quenching mechanism by introducing  $Fe^{3+}$  as a model quencher. The CDs from

lignin are successfully obtained by a green hydrothermal treatment process. Selectively fluorescent quenching behaviours by a series of high-valence ions and recovering behaviors by antioxidants are compared. It is confirmed that valence-dependent fluorescence response is related to sensitive chelation and a formation of stable complex with CDs, thus inhibiting radical recombination. The novel sensing platform with instant fluorescence responses provides an insight in smart application for detection of a wide range of metal ions, biological or environmental redox signal monitoring, and a further exploration of complex composites.

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