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Article

# Phosphorus-Doped Carbon Quantum Dots as Fluorometric Probes for Iron Detection

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**ABSTRACT:** Carbon quantum dots (CQDs), a novel fluorescent nanomaterial, have been extensively employed/explored in various applications, that is, biosensors, bioimaging, nanomedicine, therapeutics, photocatalysis, electrocatalysis, energy storage system, and so forth. In this study, we report the synthesis, characterization, and the application of phosphorus-doped CQDs (PCQDs), synthesized using trisodium citrate and phosphoric acid by the hydrothermal method. The effect of phosphorus doping on optical features and the formation of PCQDs have been explored elaborately by controlling the concentrations of precursors, reaction time, and the temperature. The fluorescent quantum yield for PCQDs was determined to be 16.1% at an excitation/emission wavelength of 310/440 nm. Also, the optical and structural properties of PCQDs were determined by using various



spectroscopic and microscopic techniques. Static quenching of fluorescence was determined upon the addition of Fe<sup>3+</sup> to PCQDs because of the formation of the fluorescent inactive complex (PCQDs–Fe<sup>3+</sup>). Hence, this chemistry leads to the development of a new fluorometric assay for the detection of Fe<sup>3+</sup>. The lower limit of Fe<sup>3+</sup> detection is determined to be 9.5 nM ( $3\sigma$ /slope), with the linear fit from 20 nM to 3.0  $\mu$ M ( $R^2 = 0.99$ ). We have validated this new assay in the raw, ejected, and purified water samples of the RO plant by the standard addition method. These results suggest the possibility of developing a new commercial assay for Fe<sup>3+</sup> detection in blood, urine, and various industrial waste and sewage water samples. Furthermore, recycling the pollutant water into the freshwater using filters that consist of PCQDs offers a great deal.

# ■ INTRODUCTION

Fluorescent carbon nanomaterials can be classified into carbon quantum dots (CQDs), carbon nanodots, graphene quantum dots, and graphene oxide quantum dots based on their physicochemical properties.<sup>1</sup> These nanomaterials can be synthesized by hydrothermal, solvothermal, thermal decomposition, microwave, ultrasonic, arc-discharge, laser ablation, and electrochemical methods under the two significant approaches, such as (i) top-down and (ii) bottom-up methods.<sup>2</sup> Among them, the hydrothermal method is favorable because it is of low cost and is nontoxic and environmentally friendly.<sup>3</sup> Hydrothermally synthesized CQDs are widely reported for various applications such as biosensors, bioimaging, nanomedicine, drug carrier, chemical sensors, energy storage system, photocatalysis, electrocatalysis, and so forth.<sup>4</sup> However, a clear picture of the electronic and optical properties of CQDs is still unpredictable. For example, CQDs have an excitation-dependent fluorescence nature, widely because of fluorescence originating from surface states/defects.<sup>1</sup> However, few reports say that the CQDs have an excitation-independent nature because of their fluorescence, which usually originates from the central core. Researchers are doping various elements such as nitrogen,

boron,<sup>8</sup> sulfur,<sup>9</sup> and phosphorus<sup>10</sup> to CQDs to get an excitation-independent fluorescence nature via the passivation of surface states and defects. However, the fluorescence properties of CQDs are still not clearly understood and hence hold back their commercial application. For example, phosphorus-doped CQDs (PCQDs) show both excitation-dependent emission<sup>11</sup> and excitation-independent emission,<sup>10</sup> a controversial observation. Therefore, in this report, we have synthesized PCQDs at different doping fractions of phosphorus by regulating the concentrations of precursors, reaction time, and the temperature to get an answer to the question that "why PCQDs give excitation-dependent and -independent fluorescence?" Hence, to solve this tricky situation (vide supra), we have explored and investigated the optical properties of PCQDs at various amounts of phosphorus as

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# Scheme 1. Schematic Representation of the Formation of PCQDs



Figure 1. (a) Normalized UV-vis absorbance spectra of different PCQDs, (b) PL spectra of various PCQDs at an excitation wavelength of 310 nm, (c) and (d) PL spectra of PCQD 3 and PCQD 11 at different excitation wavelengths ranging from 240 to 440 nm with an interval of 10 nm.

an external dopant. Trisodium citrate (TSC) is known to undergo intermolecular dehydrolysis reaction, followed by the carbonization to produce undoped CQDs under the hydrothermal treatment.<sup>12</sup> The dehydrolysis reactions between phosphoric acid and sucrose<sup>13</sup> and between TSC and diethylenetriamine<sup>1</sup> during the formation of CQDs are reported. Hence, we have tried to prepare PCQDs using TSC and phosphoric acid.

The recent developments in nanomaterial chemistry, especially in the field of CQDs, provide a ground to explore their usage in the environment and health problems globally. Nowadays, environmental pollution is one of the significant issues all over the world. Various types of pollutants were added mainly to the earth's surface, water, and atmosphere, even beyond the lethal range of the living organism.<sup>14</sup> To overcome these issues, we need a scientific method to quantify and remove these pollutants to avoid various health issues and provide a world without pollution. Among them, iron is one of the essential metals, which is involved in oxygen transport via the metalloproteins such as hemoglobin, myoglobulin,<sup>15</sup> transcriptional regulation,<sup>16</sup> and oxygen metabolism.<sup>17</sup> At the same time, excess iron generates a wide range of harmful free radicals and enhanced metabolic acidity via oxidative phosphorylation rupture.<sup>18</sup> The iron overdose may induce gastrointestinal effects such as gastrointestinal bleeding, vomit, diarrhea, ulcerations, and the development of strictures.<sup>19</sup> Also,

an excess amount of iron possibly creates cancer through freeradical metabolism, oxidation of DNA molecules, Alzheimer's disease, and Parkinson's disease.<sup>20-22</sup> Therefore, sensitive and selective detection of iron has increasing attention worldwide. Many methods are reported for the detection of iron, including fluorescence,<sup>23–26</sup> colorimetry,<sup>27</sup> electrochemical methods,<sup>2</sup> ion chromatography with chemiluminescence,<sup>29</sup> and atomic absorption spectroscopy.<sup>30</sup> Among them, the fluorescence method is of the best choice to make a sensor/assay kit because of its high sensitivity toward analytes. Many fluorescent materials such as graphene quantum dots,<sup>31</sup> gold nanoclusters,<sup>32,33</sup> Fe<sub>3</sub>O<sub>4</sub>@ZnO nanoparticles,<sup>34</sup> Mg-Al layered double hydroxide intercalated with salicylic acid,<sup>35</sup> porous aromatic frameworks,<sup>36</sup> metal–organic frameworks,<sup>37</sup> and nanocomposites<sup>38</sup> were reported for the detection of iron. However, these probes have various limitations such as hard synthetic procedure, unstable in nature, photobleaching/ blinking effect, toxic, expensive, and deprived sensitivity or selectivity, which needs to be resolved toward developing a commercial sensor or assay kit.

In this report, we have synthesized phosphorus-doped CQDs (PCQDs) from a mixture of phosphoric acid and TSC using the hydrothermal method. The effect of reaction temperature, time, and the concentrations of precursors on the formation of PCQDs and the doping effect of phosphorus were efficiently discussed with the help of UV-vis and photo-

luminescence (PL) spectroscopy. Further, the PCQDs with high fluorescence quantum yield (QY) were characterized by several spectroscopic and microscopic techniques to investigate the structural, morphological, and crystalline properties. We have also investigated the changes in the fluorescence intensities of PCQDs upon the addition of  $Fe^{3+}$ , which is due to the formation of the stable fluorescent inactive complex (PCQDs– $Fe^{3+}$ ). Based on the observation, we have established a fluorometric assay for the detection of  $Fe^{3+}$  at very low concentrations, and the advantages of our proposed probe were validated in different water samples.

# RESULTS AND DISCUSSION

**Formation of PCQDs.** The PCQDs were synthesized from an aqueous mixture of TSC and phosphoric acid using the hydrothermal method. Similar to the previous reports, <sup>1,12,13</sup> the dehydrolysis reaction possibly occurs between TSC and phosphoric acid. The phosphorus atoms were covalently bound to the carbon and oxygen atoms of TSC molecules through a dehydrolysis reaction and form a polymer-like structure before the carbonization process upon hydrothermal treatment, as shown in Scheme 1. After completion of carbonization/aromatization, the PCQDs were formed as the final product.

**Optical Properties of PCQDs.** We have prepared 12 different types of PCQDs based on various experimental conditions such as the concentration ratios of precursors, reaction times, and the temperatures. The normalized UV–vis absorbance spectra and PL spectra of these PCQDs are shown in Figure 1a,b, and the experimental conditions of these PCQDs with fluorescence QY are tabulated in Table 1.

Table 1. Experimental Condition for the Synthesis ofPCQDs with Fluorescence QY

	cono tio	centra- n, M				
name	TSC	$H_3PO_4$	ratio	time, h	temperature, $^\circ C$	QY, %
PCQD 1	0.1	0.1	1:1	12	80	0.2
PCQD 2	0.1	0.1	1:1	12	110	0.7
PCQD 3	0.1	0.1	1:1	12	140	16.1
PCQD 4	0.1	0.1	1:1	12	170	6.6
PCQD 5	0.1	0.1	1:1	12	200	1.3
PCQD 6	0.1	0.1	1:1	6	140	1.0
PCQD 7	0.1	0.1	1:1	18	140	9.1
PCQD 8	0.1	0.1	1:1	24	140	2.5
PCQD 9	0.1	0.2	1:2	12	140	2.7
PCQD 10	0.2	0.1	2:1	12	140	2.1
PCQD 11	0.1	0.3	1:3	12	140	9.1
PCQD 12	0.3	0.1	3:1	12	140	1.0

To understand the effect of reaction temperature, we have prepared the PCQDs at five different temperatures, that is, 80, 110, 140, 170, and 200 °C at constant reaction time (12 h) and the concentration of precursors (0.1 M, 1:1 ratio). The PCQDs synthesized at 80 °C give a peak at 235 nm because of  $\pi$ - $\pi$ \* electronic transition of aromatic C=C domains in normalized (Figure 1a) and as-obtained (Figure S1) UV-vis absorbance spectra. We have experienced an increment in the absorbance with decreasing wavelength from 350 to 250 nm because of the n- $\pi$ \* electronic transition of carbonized functional groups such as C=O, C=P, and P=O groups.<sup>10</sup> The peak at 235 nm was shifted to a higher wavelength (242 nm, bathochromic shift) by increasing temperature up to 140 °C. Afterward, this peak was shifted to a lower wavelength (218 nm, hypsochromic shift) with increasing temperature up to 200 °C. In PL spectra, the fluorescence of PCQD 1 to PCOD 3 is independent of the excitation wavelength  $(\lambda_{ax})_{i}$ obtained by temperature from 80 to 140 °C, respectively (Figures S2, S3, and 1c). Beyond 140 °C up to 200 °C, the PCQDs were found to show the  $\lambda_{ex}$ -dependent fluorescence nature (PCQDs 4 and 5; Figures S4 and S5). We have observed the maximum fluorescence emission intensity at 440 nm with the  $\lambda_{ex}$  of 310 nm (Figure 1c) for PCQD 3, among the PCQDs (Figure 1b). Based on the UV-vis and PL experimental results, we can conclude that the amount of phosphorus doping on CQDs is increased with increasing temperature up to 140 °C and then decreased up to 200 °C. The QY versus reaction temperature graph is shown in Figure S6. From this graph, we have found that a suitable temperature for the formation of highly fluorescent PCQDs is 143 °C using polynomial curve fit. Phosphorus is one of the nitrogen group elements, and the bathochromic shift in the UV-vis absorbance band upon increasing the amount of nitrogen doping has been reported already.<sup>1,39</sup> Moreover, the amount of nitrogen content on nitrogen-doped CQDs is decreased at a higher temperature.<sup>12</sup> The observation of similar results shown in this paper indicates the ineffective phosphorus doping on PCQDs at a higher temperature. At higher temperatures, the  $\lambda_{ex}$ -dependent emission nature also proves the lack of phosphorus doping on CQDs. The amount of nitrogen content in PCQDs was quantitatively confirmed by X-ray photoelectron spectroscopy (XPS) measurement and discussed in the Structural Studies section. Based on the high fluorescence QY, the temperature, 140 °C, is better for effective phosphorus doping on the formation of PCQDs.

To explore the suitable time for the reaction completion with the highest yield of PCQDs, we have synthesized the PCQDs at different reaction times, that is, 6, 12, 18, and 24 h with constant temperature (140  $^{\circ}$ C) and for the fixed concentration of precursors (0.1 M, 1:1 ratio). In Figure 1a, the peak at 223 nm (PCQD 6 for 6 h) was shifted to 242 nm (PCQD 3 for 12 h), indicating that the phosphorus doping increased with increasing temperature. Then, the peak was shifted to around 230 nm for 18 h (PCQD 7) and 24 h (PCQD 8) heating time because of the lack of phosphorus doping. Also, we have observed the  $\lambda_{ex}$ -dependent emission in PCQDs prepared by 6 h reaction time, that is, similar to PCQDs synthesized at 18 and 24 h reaction times (Figures S7-S9, respectively). These results also revealed a lack of phosphorus doping on CQDs at lower or very higher temperatures. Hence, we have found that 12 h reaction time is most suitable for effective phosphorus doping on CQDs, which shows the highest QY, that is, 16.1%.

To understand the effect of concentration of precursors, we have prepared the PCQDs with different concentration ratios of TSC/phosphoric acid, that is, 1:2, 2:1, 1:3, and 3:1 and labeled as PCQDs 9–12, respectively. In UV–vis spectra, we have observed a peak around 243 nm for PCQDs 9 and 11 (Figure 1a) prepared by 1:2 and 1:3 ratios, respectively. This peak was shifted to lower wavelengths, that is, 235 and 229 nm for PCQDs 10 and 12, respectively, because of lower concentration of phosphoric acid. Based on these observations, we can propose that the phosphorus doping is ineffective at low concentration of phosphoric acid, obviously doping of phosphorus directly proportional to the concentration of



Figure 2. (a) UV-vis absorbance, (b) PL spectra of PCQDs at pH 2.0 and 12.0, (c) fluorescence decay curve of PCQDs before and after the addition of 5  $\mu$ M of Fe<sup>3+</sup>, (d) fluorescence intensity vs time plot for the stability of PCQDs at an excitation/emission wavelength of 310/440 nm.

phosphoric acid. These results contrast with the PL spectral measurements, as shown in Figures S10, S11, 1d, and S12, which are PCQDs prepared by the ratios of 1:2, 2:1, 1:3, and 3:1, respectively. The higher amount of phosphorus doping usually shows the  $\lambda_{ex}$ -independent emission nature. However, here, we have observed the  $\lambda_{ex}$ -dependent fluorescence nature with increasing phosphorus content in CQDs. In this context, we believe that the  $\lambda_{ex}$ -dependent emission nature of PCQDs is due to the reduced value of the ratio of doped phosphorus/ oxygen even though the phosphorus doping has been increased. Increasing concentration of oxygen content on CQDs creates more surface states, resulting in the  $\lambda_{ex}$ dependent fluorescence emission nature.<sup>40</sup> Therefore, under the given experimental conditions, we have observed a  $\lambda_{ex}$ dependent emission nature with increasing concentration of phosphoric acid. We also prepared undoped CQDs from 0.1 M TSC by hydrothermal treatment at 140 °C for 12 h. The PL spectra of undoped CQDs show the excitation wavelengthdependent fluorescence, as shown in Figure S13. Also, the PCQDs prepared from 0.1 M of a mixture of 1 equiv TSC and sodium phosphate by hydrothermal treatment at 140 °C for 12 h (labeled as PCQDs<sub>sp</sub>) show a  $\lambda_{ex}$ -independent fluorescence nature, as evident from Figure S14. Based on these results, we can clearly say that the doping of phosphorus prevents the surface-caused fluorescence. Among the various PCQDs (Table 1), we have selected the PCQD 3, which was synthesized by heating the mixture of TSC/phosphoric acid in 1:1 ratio at 140 °C for 12 h under the hydrothermal method, based on the high fluorescence QY of 16.1%. These PCQDs were used for further spectroscopic and microscopic characterization and analyses.

We have investigated the optical properties of PCQDs under acidic and basic pH conditions. The pH of as-prepared PCQDs is 5.4; however, the pH was adjusted to 2.0 and/or 12.0 using HCl and NaOH, wherever required. The UV-vis absorbance and PL emission spectra of PCQDs are shown in Figure 2a,b. The absorbance peak was shifted to lower wavelength (210 nm) under acidic pH condition, which is due to the protonation, and therefore led to discontinuation of the delocalization of  $\pi - \pi^*$  electrons. A similar behavior was also confirmed by the PL spectra, which were obtained at different excitation wavelengths (Figure S15). The excitation wavelength shifted from 310 to 250 nm, and the fluorescence spectra also showed a hypochromic shift; this confirms the weakening of  $\pi - \pi^*$  conjugation. The zeta potential of asprepared PCQDs (-1.7 mV) was found to shift from a negative value to a positive value (+0.4 mV) upon decreasing pH. At basic pH, the UV-vis spectrum shows that the absorbance value increased from 340 nm with decreasing wavelength. However, the excitation/emission wavelengths remain unchanged at 310/440 nm even after adjusting to basic pH (Figure S16); yet, the fluorescence intensity was found to decrease slightly compared to as-prepared PCQDs. Deprotonation was confirmed by the zeta potential measurement, that is, -27.1 mV for PCQDs at basic pH. We observed similar results as previously reported by Sun et al. that there is a weak fluorescence under acidic and basic pH conditions, whereas there is a strong fluorescence at neutral pH conditions for N, P co-doped CQDs.<sup>41</sup> The photoinduced electron transfer (PET) usually occurs in nitrogen-doped CQDs through lone pair electron of pyridinic nitrogen.<sup>6,42</sup> We believe that the PET is the possible reason for a weak fluorescence at basic pH via the interaction of lone pair of electrons available on the trivalent phosphorus atom of PCQDs. Based on UV-vis, PL, and zeta potential studies, it is very much clear that the PCQDs are sensitive toward the pH of the medium.

To understand the decay of fluorescence, we have analyzed the fluorescence lifetime measurement studies for PCQDs using a time-correlated single-photon counting technique. Figure 2c shows the fluorescence decay profile for PCQDs at an excitation/emission wavelength of 310/440 nm. The decay profile was fitted by the single exponential function, which indicates the existence of a single fluorescence domain and excitation-independent fluorescence emission nature. The lifetime of fluorescence is calculated to be 10.4 ns. Moreover, the fluorescence of PCQDs is found to be stable for more than



Figure 3. Deconvoluted high-resolution P(2p), C(1s), and O(1s) XP spectra of (a-c) PCQD 1, (d-f) PCQD 3, (g-i) PCQD 11, and (j-l) PCQDs<sub>sp</sub>.

5 h under continued exposure to the UV radiation (310 nm), and the emission intensity was measured at 440 nm wavelength (Figure 2d). We have studied the fluorescence stability of PCQDs in the presence of a high concentration of NaCl solution, and we do not observe any significant changes (Figure S17). From these results, we can conclude that the PCQDs have a very less photobleaching/blinking effect and give highly stable fluorescence.

**Structural Studies.** FT-IR spectral studies were performed to confirm the chemical composition and functional groups of PCQD 3. The FTIR spectrum of PCQDs gives four peaks at 956, 1080, 1161, and 2382 cm<sup>-1</sup>, as shown in Figure S18, which can be assigned to the stretching frequencies of P–O–C, P–O, P==O, and P–H functional groups, respectively.<sup>43</sup> These functional groups confirm the doping of phosphorus on CQDs. Further, we have observed the carbonized functional

groups stretching frequencies, such as C–O, C==O, and C==C at 1400, 1716, and 1651 cm<sup>-1</sup>, respectively. The peak appeared at 3583 cm<sup>-1</sup> is characteristic of the stretching frequency of O–H functional groups.<sup>44,45</sup> Thus, the PCQDs are aromatic phosphorus-doped carbon framework with many polar functional groups, which improve the aqueous solubility and stability.

These functional groups were quantified to estimate the doping amount of phosphorus in PCQDs 1, 3, and 11 and PCQDs<sub>sp</sub> by the X-ray photoelectron spectroscopic technique. The survey spectrum of PCQDs shows three significant peaks at 133, 284, and 532 eV (Figure S19) and can be assigned to P(2p), C(1s), and O(1s), respectively. The phosphorus contents in PCQDs 1 and 11 are very low (0.9 and 0.8%) compared to those in PCQD 3 and PCQDs<sub>sp</sub> (8.8%). These X-ray photoelectron survey spectra of all PCQDs are used to run

the high-resolution individual elemental and composition measurement and deconvoluted, as shown in Figure 3. The high-resolution P(2p) spectra of PCQD 3 (Figure 3d) have been deconvoluted into two peaks at 132.1 and 133.1 eV by the Gaussian function, which can be assigned to P-C (5.70%) and P–O (3.17%) groups, respectively.<sup>41</sup> The C(1s) spectrum (Figure 3e) can be deconvoluted into three peaks at 284.1, 285.4, and 288.4 eV by the Gaussian function that can be assigned to aromatic carbon (C-C/C=C, 18.89%), C-O/ C-P (7.35%), and C=O (2.42%), respectively.<sup>46,47</sup> We did not observe any carboxylic acid functional moiety in the C(1s)spectrum. Similarly, the Gaussian function was used to deconvolute the O(1s) spectrum into three peaks at 531.0, 532.4, and 536.0 eV (Figure 3f), which can be ascribed to C =O (16.76%), P=O (44.56%), and H<sub>2</sub>O (1.13%) groups, respectively.<sup>41,48</sup> The amounts of phosphorus, carbon, and oxygen in PCQDs are tabulated in Table S1. From the XPS, UV-vis, and fluorescence studies, we have confirmed that increasing concentration of the phosphorus dopant in PCQDs passivates the surface states, which leads to monochromatic  $(\lambda_{ex}$ -independent) fluorescence. Increasing concentration of oxygen in PCQDs enables the surface states, which leads to multiple chromatic ( $\lambda_{ex}$ -dependent) fluorescence. Based on the results of 12 different PCQDs, we have concluded that the PCQDs have a monochromatic emission nature at the dopant ratio of phosphorus and oxygen higher than 0.1 (P/O > 0.1) and a multiple chromatic emission nature at the ratio of phosphorus/oxygen lower than 0.1 (P/O < 0.1). Generally, phosphorous-doped materials are highly moisture-sensitive; thus, the water molecules cannot be obliterated.<sup>49</sup> Therefore, the synthesized material is hydrophilic, stable, phosphorusdoped CQDs, which enable further functionalization or applications.

**Morphological Studies.** The morphology and crystallinity of PCQDs were studied by high-resolution transmission electron microscopy (TEM) and selected area electron diffraction (SAED) analyses. The TEM image of PCQDs is shown in Figure 4a, which has uniformly distributed PCQDs.



**Figure 4.** (a) TEM image and (b) SAED pattern of PCQDs. The inset of (a) shows the normal distribution histogram for the sizes of PCQDs.

The average size of the PCQDs was found to be 2.7 nm, calculated from 17 dots using the standard distribution curve, and plotted as an inset of Figure 4a. We also calculated the size of PCQDs as 3.5 nm using a dynamic light scattering technique (Figure S20). The interplanar distances were calculated from the SAED pattern of PCQDs (Figure 4b) and found to be 3.19 and 2.29 Å that can be assigned to the (002) and (100) planes of the graphitic carbon framework,

respectively.<sup>48</sup> The phosphorous doping of CQDs decreases the interplanar distance in PCQDs from 3.34 to 3.19 Å, which is usually observed for the undoped graphitic carbon framework.<sup>1</sup>

As per the above results, we can conclude that phosphorus does not effectively dope on CQDs when prepared at higher temperatures and in longer reaction time. The excitationdependent fluorescence occurred because of the surface states of PCQDs at a high concentration of phosphoric acid. The independent excitation fluorescence was witnessed at an optimum amount of phosphoric acid and experimental conditions. Therefore, the synthesized material is highly fluorescent, highly stable, pH-sensitive, hydrophilic, phosphorus-doped graphitic CQDs.

**Determination of Fe<sup>3+</sup>**. Detection of Fe<sup>3+</sup> using PCQDs was performed by PL spectroscopy. We have added various concentrations of Fe<sup>3+</sup> ranging from 20 nM to 16  $\mu$ M in 3 mL of PCQDs (41 mg/mL). The PL spectra of PCQDs with different amounts of Fe<sup>3+</sup> are shown in Figure 5a. The



**Figure 5.** (a) PL spectra of PCQDs in the presence/absence of Fe<sup>3+</sup> ion concentration ranging from 20 nM to 16  $\mu$ M and its (b) Stern–Volmer plot, (c) PL intensities of PCQDs in the presence/absence of 500  $\mu$ M of various metal ions and 5  $\mu$ M of Fe<sup>3+</sup> ions at an excitation/ emission wavelength of 310/440 nm.

fluorescence intensity gradually decreased with an increasing concentration of Fe<sup>3+</sup> because of the formation of the ground-state Fe<sup>3+</sup> chelate complex. The formation of the complex was further confirmed by UV–vis absorbance spectroscopy. Figure S21 shows the UV–vis absorbance spectra of PCQDs, FeCl<sub>3</sub>, and a mixture of both. We have observed a gradual decay in the absorbance band intensity at 310 nm after the addition of Fe<sup>3+</sup> ions, which is due to the formation of the PCQDs–iron (PCQDs–Fe<sup>3+</sup>) complex.<sup>50</sup> The fluorescence static quenching rate constant can be calculated from the Stern–Volmer equation.<sup>51</sup>

$$\frac{F_0}{F} = 1 + K_{\rm SV}[Q] = 1 + k_{\rm q}\tau_0[Q] \tag{1}$$

where  $F_0$  and F are the fluorescence intensities of PCQDs before and after the addition of Fe<sup>3+</sup> ions.  $K_{SV}$ ,  $k_q$ ,  $\tau_0$ , and [Q] are the Stern–Volmer constant, the quenching rate constant, the fluorescence lifetime of CQDs, and the concentration of Fe<sup>3+</sup> ions, respectively. The Stern–Volmer plot is shown in

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for the Detection of Fe <sup>3+</sup>	disadvantages	indistinguishable, $\mathrm{Fe}^{3+}$ from $\mathrm{Fe}^{2+}$		relatively poor selectivity	use of plastic, toxic	sample pretreatment required	relatively poor sensitivity	relatively poor sensitivity, selectiv expensive probe	relatively poor sensitivity, expensi	fluorescence not stable beyond p	multistep fabrication, sample pretreatment required	short linearity, sensitivity	multiple fluorescence may affect detection	store with care due to hygroscopi
	advantages	high sensitivity, nontoxic		nontoxic	high sensitivity, sample pretreatment not required	inexpensive, rapid	inexpensive, selectivity	good recovery in spiked real samples	selectivity, good accuracy	high sensitivity, good recovery	selectivity, good recovery	selectivity	high sensitivity, good selectivity	high sensitivity, selectivity, nontoxic, inexpensive
	real sample analysis	blood serum, water		lake water	blood serum, water	polluted soil	tap water	tap, lake water, tablets	blood serum	blood serum, waste water	blood serum		water	various water samples
ole Analyses	LOD, (M)	$5.0 \times 10^{-9}$	$1.2 \times 10^{-8}$	$7.9 \times 10^{-8}$	$4.0 \times 10^{-8}$	$3.0 \times 10^{-9}$	$1.6 \times 10^{-6}$	$3.5 \times 10^{-6}$	$2.0 \times 10^{-6}$	$3.0 \times 10^{-9}$	$2.6 \times 10^{-8}$	$3.8 \times 10^{-5}$	$1.6 \times 10^{-8}$	$9.5 \times 10^{-9}$
2. List of Materials, Methods, Linearity, LOD, and Real Samp	linear range, (M)	$1.0 \times 10^{-8}$ to $1.0 \times 10^{-5}$	$5.0 \times 10^{-8}$ to $1.2 \times 10^{-5}$	$5.0 \times 10^{-7}$ to $1.0 \times 10^{-3}$		$1.0 \times 10^{-3}$ to $2.5 \times 10^{-2}$	$3.5 \times 10^{-6}$ to $6.7 \times 10^{-4}$	$5.0 \times 10^{-6}$ to $1.3 \times 10^{-3}$	$5.0 \times 10^{-6}$ to $2.0 \times 10^{-3}$	$1.0 \times 10^{-8}$ to $1.3 \times 10^{-4}$	$7.0 \times 10^{-8}$ to $1.0 \times 10^{-4}$	$5.0 \times 10^{-5}$ to $2.0 \times 10^{-4}$	$1.9 \times 10^{-9}$ to $1.1 \times 10^{-7}$	$2.0 \times 10^{-8}$ to $3.0 \times 10^{-6}$
	materials	nitrogen-doped carbon dots		nitrogen-doped carbon dots	ion-pair crystal violet cation/PVC	PtNPs/PANI/MWCNT/GCE	graphene quantum dots	gold nanoclusters	gold nanoclusters	Fe <sub>3</sub> O <sub>4</sub> @ZnO nanoparticles	Mg–Al layered double hydroxide	fluorescein-based aromatic framework	MBTBA-Fe <sub>3</sub> O <sub>4</sub> @SiO <sub>2</sub> nanocomposite	PCQDs
	method	fluorimetric	colorimetric	fluorimetric	colorimetric	amperometric	fluorimetric	fluorimetric	fluorimetric	fluorimetric	fluorimetric	fluorimetric	fluorimetric	fluorimetric
Table	sr. no.	1	5	Э	4	s	6	~	8	6	10	11	12	13

Figure 5b, and from this plot, we have calculated the Stern– Volmer constant ( $K_{SV} = 1.48 \times 10^6 \text{ M}^{-1}$ ) and the quenching rate constant ( $k_q = 1.423 \times 10^{14} \text{ M}^{-1} \text{ s}^{-1}$ ) using eq 1. We have calculated the limit of detection (LOD) for Fe<sup>3+</sup> ions to be 9.5 nM and the limit of quantification to be 31.6 nM with a linearity of 20 nM to 3.0  $\mu$ M ( $R^2 = 0.99$ ) better than those of N- and P-doped CQDs,<sup>52</sup> nitrogen-doped CQDs,<sup>26</sup> graphenequantum dots,<sup>31</sup> and gold nanoclusters.<sup>32,33</sup> Our proposed method for the detection of Fe<sup>3+</sup> ions is found to be better than the earlier reports, listed in Table 2, based on the sensitivity, selectivity, and the usage of the inexpensive, nontoxic probe.

Additionally, we have calculated the binding constant (*K*) to understand the binding affinity between PCQDs and Fe<sup>3+</sup> ions using the Lineweaver–Burk equation,<sup>4</sup> as shown below.

$$\frac{1}{F_0 - F} = \frac{1}{F_0} + \frac{1}{KF_0} \frac{1}{[Q]}$$
(2)

We have determined the binding constant value (2.256  $\times$  $10^6$  M<sup>-1</sup>), which was calculated from the Lineweaver-Burk equation using Figure S22. The quenching process is attributed to the static quenching because of the formation of the fluorescent inactive PCQDs-Fe3+ chelating complex. The static quenching was confirmed by fluorescence decay studies, which is shown in Figure 2c. The fluorescence lifetime of PCQDs was changed from 10.4 to 10.2 ns, as obtained by the single exponential function. Hence, negligible changes in the fluorescence lifetime of PCQDs after the addition of 5  $\mu$ M of  $Fe^{3+}$  in fluorescence decay curves confirm the static quenching caused by fluorescence inactive stable complex formation at the ground state. The selectivity of PCQDs was investigated using various transition, alkali, and alkali earth metal ions. Figure 5c shows the average of triplicate PL intensities of PCQDs before and after the addition of 5  $\mu$ M of Fe<sup>3+</sup> ions and 100 times higher concentration (500  $\mu$ M) of various metal ions at an excitation/emission wavelength of 310/440 nm. We did not observe any significant fluorescence changes for the metal ions except Fe<sup>3+</sup> ions. The fluorescence intensity was almost 90% quenched for 5  $\mu$ M of Fe<sup>3+</sup>. The reason for this quenching is ascribed to the strong affinity of PCQDs toward  $Fe^{3+}$  than other metal ions. In this regard, to confirm the weak interaction between the PCQDs and the other metal ions, we have calculated the binding constant of these metal ions toward PCQDs and it was found to be 4 orders lower than that of Fe<sup>3+</sup> ions (Figure S23). Additionally, we have added 0.5 mM of Fe<sup>2+</sup> in PCQDs to find the interaction between them using fluorescence studies (Figure S24). The fluorescence intensity is not decreased significantly; hence, the PCQDs are highly selective to Fe<sup>3+</sup> ions. Liu et al. reported an iron detection using nitrogen-doped CQDs.<sup>23</sup> However, they are unable to distinguish the  $Fe^{3+}$  and  $Fe^{2+}$  ions using their materials. We added 0.5 mM of Fe<sup>3+</sup> in undoped CQDs for PL spectra to find out the importance of P doping. The fluorescence intensity was decreased around 40% for 0.5 mM Fe<sup>3+</sup> (Figure S25). However, these PCQDs lost their fluorescence around 90% within 16  $\mu$ M of Fe<sup>3+</sup> addition. Therefore, the doping of phosphorus on CQDs greatly improves the sensitivity.

The strong interaction between PCQDs and Fe<sup>3+</sup> is explored by XPS studies. The survey spectrum of the PCQDs + Fe<sup>3+</sup> complex is shown in Figure S26 that shows five peaks at 133, 284, 532, 710, and 725 eV because of P(2p), C(1s), O(1s), Fe(2p)<sub>3/2</sub>, and Fe(2p)<sub>1/2</sub>, respectively. Each peak can be deconvoluted and fitted using a Gaussian function. Figure 6a



Figure 6. Deconvoluted high-resolution XPS spectra of (a) P(2p), (b) C(1s), (c) O(1s), and (d) Fe(2p) of the PCQDs-Fe<sup>3+</sup> complex.

shows the deconvoluted XPS spectrum of P(2p) peak that shows three peaks at 129.6, 132.0, and 132.9 eV because of P-Fe, P-C, and P-O, respectively.<sup>53</sup> C1s can be deconvoluted into three peaks at 284.3, 286.3, and 288.4 eV that can be assigned to aromatic C-C, C-C/C-P, and C=O, respectively (Figure 6b). O(1s) can be deconvoluted into three peaks at 530.2, 531.1, and 532.2 eV, which can be attributed to O-Fe, O=C, and O=P, respectively<sup>53</sup> (Figure 6c). The Fe(2p) peak can be deconvoluted into four peaks at 707.9, 713.4, 721.8, and 726.6 eV, which can be assigned to Fe–P and Fe–PO<sub>4</sub> of Fe(2p)<sub>3/2</sub> and Fe(2p)<sub>1/2</sub>, respectively<sup>53</sup> (Figure 6d). Hence,  $Fe^{3+}$  is coordinately connected to P and O of PCQDs and not connected to carbon. Because of complex formation, the size of the PCQDs is increased through aggregation, as seen from the TEM image (Figure 7a). We have investigated the PCQD interaction toward Fe<sup>3+</sup> using energy-dispersive X-ray spectrometry (EDXS) studies. Figure 7b-f shows the color mapping of the carbon, oxygen, phosphorus, iron, and overlap picture. Iron is bound with PCQDs, and we did not find any unbound  $Fe^{3+}$  in Figure 7e. However, carbon is spread over the picture because of not only carbon in PCQDs but also in carbon-coated copper grids. The high-angle annular dark-field TEM image of respective EDXS mapping is shown in Figure S27. These results confirm that our CQD assay is highly selective toward Fe<sup>3+</sup> ions.

Real Sample Analyses. The proposed assay was validated in various water samples such as raw, filtered, and ejected water of the RO system, collected at IISER, Tirupati. We have followed the standard addition method for the determination of Fe<sup>3+</sup> ions in all the water samples. We have added 0.5, 1.0, 1.5, 2.0, and 2.5  $\mu$ M of Fe<sup>3+</sup> as a standard solution to the mixture of 200  $\mu$ L of water samples and 2.8 mL of PCQDs. The PL spectra were obtained for the combination of water samples and PCQDs before and after the addition of the standard solution for the determination of an unknown amount of Fe<sup>3+</sup>. The PL spectra of PCQDs with raw (Figure S28a), filtered (Figure S28c), and ejected (Figure S28e) water samples in the presence of standard solution at the excitation wavelength of 310 nm are validated. The average of triplicate intensities at 440 nm was calculated to plot standard addition curves. Figure S28b,d,f shows the standard addition curves for raw, filtered, and ejected water samples, respectively. From the



Figure 7. (a) TEM image, EDXS color mapping of (b) carbon, (c) oxygen, (d) phosphorus, and (e) iron, (f) overlapped image of (b-e) of the PCQDs-Fe<sup>3+</sup> complex.

derivative plots, we have calculated the amounts of Fe<sup>3+</sup> in raw, filtered, and ejected water samples, which are found to be 2.2, 0.6, and 2.0  $\mu$ M, respectively. These values are less than the permitted values by US Environmental Protection Agency<sup>32</sup> and WHO guidelines.<sup>54</sup> Therefore, these PCQDs are a promising material for the detection of Fe<sup>3+</sup> in various samples such as sewage water, pollutant, and industrial wastewater samples. Researchers from different laboratories can use our new PCQD-based protocol for the determination of Fe<sup>3+</sup> even in the urine and blood serum samples.

# CONCLUSIONS

The pH-sensitive phosphorus-doped graphitic CQDs with a  $\lambda_{ex}$ -independent fluorescent QY of 16.1% were synthesized by the hydrothermal method. The phosphorus doping on CQDs is directly proportional to the concentration of phosphoric acid. However, high concentration of phosphoric acid increases the oxygen content instead of the phosphorus content in the PCQDs, which resulted in the formation of surface states, yielding  $\lambda_{ex}$ -dependent fluorescence. Additionally, we have optimized the reaction temperature and time to be 140 °C and 12 h, respectively, to get highly fluorescent  $\lambda_{ex}$ -independent PCQDs. The fluorescence intensity was selectively quenched by Fe<sup>3+</sup> because of the formation of the fluorescence-inactive PCQDs-Fe<sup>3+</sup> chelation complex. The fluorescence-quenching process follows the static quenching kinetics. Hence, an assay was formulated for the Fe<sup>3+</sup> detection with a linear range of  $20-3000 \text{ nM} (R^2 = 0.99)$  with a detection limit of 9.5 nM. Further, the assay was validated in various water samples, and the results are found to be satisfactory. Further, we will be focusing on the functionalization of PCQDs with charcoal carbon for the purification of industrial and sewage water as a futuristic industrial offer.

# EXPERIMENTAL SECTION

**Chemicals.** TSC, phosphoric acid, ferric chloride (FeCl<sub>3</sub>), ferrous chloride (FeCl<sub>2</sub>), cobaltous chloride (CoCl<sub>2</sub>), strontium chloride (SrCl<sub>2</sub>), magnesium chloride (MgCl<sub>2</sub>), potassium chloride (KCl), calcium chloride (CaCl<sub>2</sub>), cesium chloride (CsCl), lithium chloride (LiCl), mercurous chloride (HgCl), nickel chloride (NiCl<sub>2</sub>), cadmium chloride (CdCl<sub>2</sub>), sodium chloride (NaCl), manganese chloride (MnCl<sub>2</sub>), lead chloride (PbCl<sub>2</sub>), erbium chloride (ErCl<sub>3</sub>), ammonium chloride (NH<sub>4</sub>Cl), copper chloride (CuCl<sub>2</sub>), silver chloride (AgCl), bismuth chloride (BiCl<sub>3</sub>), mercuric chloride (HgCl<sub>2</sub>), fluorescein, and cellulose dialysis tubing (2 kDa) were purchased from Sigma-Aldrich. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from MERCK. All chemicals were of analytical grade and used without further purification. All solutions were prepared with Milli-Q water (18.2 M $\Omega$ ·cm).

Synthesis of PCQDs. Different ratios of TSC and phosphoric acid were taken and dissolved in 30 mL of Milli-Q water to synthesize the PCQDs. These mixtures were transferred to the 50 mL Teflon containers and kept in an autoclave. These autoclaves were heated at different temperatures by 3 °C/min rate for an appropriate time. The ratios of precursors, reaction temperatures, and reaction times are shown in Table 1. After finishing the reactions, the autoclaves were allowed to cool down to room temperature. After that, PCQDs were purified by a 0.2  $\mu$ m PTFE syringe filter to remove bigger particles and contaminates. Then, we have dialyzed the reaction mixture using a 2 kDa MWCO membrane to remove the salts and excess precursor. The pore size of the 2 kDa membrane is <0.1 nm. Therefore, all the small fragments and ions come out from the membrane and the PCQD remains exist in the tube. The purified PCQDs were taken for the UV-vis and PL measurements, followed by the QY calculations. Among the various PCQDs, the maximum QY was observed for PCQDs prepared from the 1:1 ratio of TSC with phosphoric acid and heated at 140 °C for 12 h. These PCQDs were characterized by different spectroscopic and microscopic techniques. The QY calculation and the experimental procedure for various spectroscopic and microscopic techniques are shown in Supporting Information, Section S1.

**Determination of Fe**<sup>3+</sup> **and Interference Studies.** The appropriate volumes of an aqueous solution of Fe<sup>3+</sup> ions (concentration ranges of Fe<sup>3+</sup>: 20 nM to 16  $\mu$ M) were added to 2.5 mL of CQDs dispersion. The net volume of the cuvettes was maintained to be 3 mL for all experiments by adding the appropriate amount of Milli-Q water. The excitation wave-

length was set to 310 nm to get the PL spectra for the determination of  $Fe^{3+}$ . The excitation/emission bandwidth and photomultiplier tube voltage were set to 5.0 nm and 400 V, respectively. The fluorescence intensities at the emission wavelength of 440 nm were taken to prepare the Stern–Volmer plots.

A multifunctional microplate reader BioTek Synergy H1 was used for interference studies. Interfering metal ions (150  $\mu$ L, taken from 1 mM) were added in 150  $\mu$ L of CQDs. The samples (300  $\mu$ L) were placed in black 96-well plates with a flat bottom for PL measurements. The fluorescence intensities were measured at an excitation/emission wavelength of 310/ 440 nm. For fluorescence measurements, each value is an average of three measurements.

## ASSOCIATED CONTENT

#### **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02627.

QY calculation; sample preparation for spectroscopic, microscopic, and zeta potential studies; UV-vis absorbance spectra of various PCQDs; PL spectra of PCQDs 1, 2, 4, 5, 6, 7, 8, 9, 10, and 12 at the various excitation wavelength; quantum yield versus reaction temperature graph derived from UV-vis/PL data; PL spectra of undoped CQDs and PCQDs<sub>sp</sub>; PL spectra of PCQDs at pH 2.0 and pH 12.0 with various excitation wavelengths; PL spectra of PCQDs in the presence of NaCl with various concentrations; FTIR spectrum of PCQDs 3; XPS survey spectra of PCQDs 1, 3, and 11 and PCQDs<sub>sp</sub>; DLS number distribution curve; UV-vis absorbance spectra of aqueous solution of PCQDs, 5  $\mu$ M of FeCl<sub>3</sub>, and a mixture of both; Lineweaver–Burk plot for the measurement of the binding constant; binding constant values of metal ions for phosphorusdoped CQDs; PL spectra of PCQDs before and after the addition of 0.5 mM of Fe<sup>2+</sup>; PL spectra of undoped CQDs before and after addition of 0.5 mM of  $Fe^{3+}$  ions; XPS survey spectrum of the PCQDs-Fe complex; HAADF EDS image of the PCQDs-Fe complex; PL spectra and its standard addition plots of PCQDs; UVvis and PL spectra of 0.1 M NaOH solution of fluorescein; amount of carbon, phosphorus, oxygen present in different PCQDs; and model calculation for QY, binding constant, and quenching rate constant (PDF)

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## Notes

The authors declare no competing financial interest.

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