Fluorescence properties of yellow light emitting carbon quantum dots and their application for effective recognition of heavy metal ions in aqueous medium

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Abstract
Water-dispersible and fluorescence-stable carbon quantum dots (CQDs) have been synthesized at gram scale using pyrene as carbon precursor by employing the bottom-up approach. X-ray diffraction, FTIR, Raman spectroscopy and UV visible spectroscopy confirm the formation of CQDs. TEM images shows that size of the CQDs ranges from 2 to 5 nm. A detailed study of their optical and fluorescence characteristics was performed. Their aqueous dispersion radiates bright yellow light under UV lamp (365 nm). The research work includes derivation of their excitation wavelength, observation of influence of dilution on fluorescence and monitoring of emission intensity for about 4 months. The CQDs can effectively recognize the presence of heavy metal ions in aqueous medium at neutral pH via fluorescence quenching.

KEYWORDS
carbon quantum dots, chromium ions, copper, fluorescence, iron, quenching

1 INTRODUCTION

Industries, biological processes and environment are associated with metal ions. Metal ions are found in nature and have also been indiscriminately discharged into the atmosphere by anthropogenic activities leading to the deterioration of the quality of water bodies and water resources. [1–3] Quantification and continuous monitoring of metal ions in individual products, processes, environment and so on are required in many instances. An excess of some common ions such as Fe$^{3+}$, Cr$^{3+}$, and Cu$^{2+}$ influences the quality of water resources. On one hand, these metal ions are biologically important to a certain level, while their excess in the environment causes deterioration of water resources. Fe$^{3+}$ is an essential mineral for human health and it is associated with the transport of oxygen to tissues. However, its excess availability can cause disorders. [4–6] Cr$^{3+}$ is essential for protein metabolism in a moderate amount. Cu$^{2+}$ involves in the maintenance of healthy bones, blood vessels and immune function. [7,8] Therefore, sensing and continuous monitoring of these metal ions from the environmental, biological and industrial perspective is highly essential. Advanced analytical techniques such as ICP-MS, ICP-OES, XRF and so on are generally used to quantify the metal ions. However, these analytical technologies are quite expensive, their maintenance/
2 | EXPERIMENTAL SECTION

Pyrene was purchased from Sigma Aldrich. Sodium hydroxide (NaOH) was purchased from Himedia laboratories private limited. In all the experiments, we have used de-ionized water. All the metal perchlorates were purchased from Sigma Aldrich and were used as such.

Pyrene was used as a precursor for the synthesis of CQDs. Pyrene (2 g) was mixed with HNO₃ (160 mL) to form a solution (Scheme 1). Then it was refluxed at 120°C for 10 hours to form 1, 3, 6 trinitropyrene. This solution is then diluted with deionized water (1 L) after it gets cooled to room temperature, followed by filtration using a 0.22 µm filter membrane to remove larger unreacted species and acid. The subsequent yellow 1, 3, 6 trinitropyrene solution was dispersed in NaOH solution of deionized water (0.2 M in 0.6 L) and ultra-sonication for 2–3 hours was done. The above solution was transferred to a Teflon-line autoclave and heated at 200°C for 10 hours. After cooling to room temperature, the resultant was filtered through a 0.22 micro-porous membrane to remove unreacted moieties. Then it is further dialyzed in a dialysis bag (3500 Da MWCO) for 2 days to remove unreacted small molecules and sodium salt. The resultant black CQDs were dried at 80°C–90°C for further characterization and other measurements. The aqueous dispersion of the CQDs emit yellow light under UV lamp (365 nm).

Transmission electron microscopy (TEM) images were recorded with JOEL, JEM 2100 microscope. After ultrasonication the sample was deposited on grid. Images were captured after air-drying of the sample. Fourier transformation infrared microscopy (FTIR) spectrum was recorded using Perkin Elmer spectrometer (model G-FTIR). UV-Visible spectra were recorded using Varian make UV-Vis-NIR spectrophotometer (model Cary 500). Fluorescence spectral recording was done with Edinburg spectrofluorometer (model F900).

All emission spectra of CQDs were recorded after their dispersal in deionized water. Spectrofluorometer’s operational parameters such as bandwidth, step, dwell time and scan range were kept uniform throughout the study. Stock solution (0.2 M) of each metal ion (Ba²⁺, Ca²⁺, Cr³⁺, Cu²⁺, Fe³⁺, Li⁺, Mg²⁺, Na⁺) was prepared in deionized water. All the experiments were performed at room temperature. In case of sensing application, recording of emission spectra was done after 2 hours of addition metal ions to aqueous dispersion of CQDs.

3 | RESULT AND DISCUSSION

The schematic diagram showing the synthesis and application of the CQDs is given in Scheme 1. Characterization
The synthesis of CQDs was performed using standard microscopic and spectroscopic techniques. TEM images of the synthesized CQDs were recorded at different magnifications as shown in Figure 1. The TEM images are clearly showing the spherical morphology of the CQDs. These CQDs are well dispersed and well separated from each other and therefore, it can be said that these are not present in aggregation form. The size of the CQDs statistically determined was <5 nm (inset image Figure 1). The lattice spacing observed using TEM was 0.19 nm. The cross-section in a plane dividing for CQDs ranges from 0.18 to 0.25 nm, while the interlayer separation of graphite is in the range of 0.32-0.34 nm or higher. It follows the literature report of such carbonaceous dots, which is falling in the reported range of 0.18-0.25 nm.[25,26] CQDs have plenty of oxygen-containing groups on their surface. The FT-IR spectrum of the CQDs is shown in Figure 2. It shows the broad vibrational band at 3393 cm–1, confirming the presence of O-H group at their surface. The characteristic band at 1582 cm–1 represents the C = C bond in these CQDs. The bands for C-OH and C-H vibrations are exhibited at 1027 and 520 cm–1. The synthesized CQDs are dispersible in water and the hydrophilicity is attributed to the presence of –OH functionality at their surface, as revealed by FT-IR spectrum. The graphitization brought by the synthetic route is confirmed by the Raman spectrum as shown in Figure 3 (inset). The spectrum is clearly showing the G-band at 1572 cm–1 while the D-band at 1354 cm–1. The intensity of G to D band ratio is calculated as 1.2 which corroborates CQDs formation.[27] As depicted in Figure 3, the X-ray spectroscopy (XRD) pattern shows a peak at 2θ of about 23.4°. It can fall between 20° and 26° as per the reported literature.[18] The aqueous dispersion of CQDs obtained after purification (dialysis) appears dark brown by bare eyes, as shown in Scheme 1. However, it emits yellow light when it was irradiated with 365 nm of light under UV lamp. Their UV-Vis absorption spectrum exhibit three excitons at 216, 298, and 355 nm. Tail of the spectrum extends to the whole visible region as shown in Figure 4. It also shows an optical absorption edge at 455 nm. The absorption peaks are due to π to π* of the phenyl rings and C = C bonds or from n to π* of C = O bonds or belonging moieties. Such type of absorption spectrum is commonly found in the literature of CQDs.[28] The CQDs were excited at different excitation wavelengths (λex) and the corresponding emission spectra were recorded. The tested excitation wavelength ranges from 300 nm to 600 nm with increment of 30 nm. This strategy was adopted to obtain an emission spectrum having the highest intensity. Figure 5 depicts the emission spectra recorded with different excitation. The excitation wavelength of 450 nm exhibited the emission band at 545 nm with the highest emission intensity. Thus, the excitation wavelength derived by this strategy for the synthesized CQDs is found as 450 nm. Therefore, 450 nm is considered as excitation wavelength (λex) and 545 nm as emission wavelength for the study. The effect of dilution on fluorescence intensity was recorded to observe whether intensity remains persistent with dilution. The emission spectra recorded as a function of the concentration of CQDs upon the addition of ultrapure water is shown in Figure 6. It suggests that the aqueous dispersion of CQDs remains emissive in nature even on dilution.

Additional studies were conducted to explore the fluorescent properties of CQDs under different conditions. The effect of pH on the intensities was observed (Figure 7). Fluorescent intensity of CQDs remains nearly same for pH 2 to 6, however, it increases when the pH rises from 8 to 13. This experiment suggests that they remain emissive in nature under the tested pH range. Other studies were performed to observe the effect of NaCl concentration on the fluorescence intensity of CQDs (Figure 7 inset). The intensities...
observed were relatively steady with the increase in NaCl concentration. It suggests that these can be explored for certain applications in presence of saline medium also.

The feasibility of using the aqueous dispersion of CQDs as fluorescent nanoprobe for sensing of metal ions is undertaken in this study. It is based on perturbation of
fluorescence characteristics of aqueous dispersion of CQDs on addition of aqueous solution of metal ions. For this, comparison of fluorescence spectrum without any metal ion (that is control) is done with that of addition of metal ion. Aqueous solution of common metal ions (Ba$^{2+}$, Ca$^{2+}$, Cr$^{3+}$, Cu$^{2+}$, Fe$^{3+}$, Li$^{+}$, Mg$^{2+}$, Na$^{+}$) were added to the aqueous dispersion of CQDs and were allowed to get interacted for about 2 hours. The fluorescence spectra recorded after 2 hours of addition of metal ion is shown in Figure 8. The effective concentration of the metal ion in the dispersion is 50 mM. It shows that Cr$^{3+}$, Fe$^{3+}$ and Cu$^{2+}$ exhibited selective interaction with CQDs, as they can switch “OFF” the fluorescence while the other metal ions are unable to do so. Fluorescence quenching effect was observed with Cr$^{3+}$, Fe$^{3+}$ and Cu$^{2+}$ whereas rest of the metal ions displayed negligible influence on fluorescence intensity of CQDs. This selective interaction is further confirmed by the progressive addition of Cr$^{3+}$, Fe$^{3+}$, and Cu$^{2+}$ to aqueous dispersion
FIGURE 9  Effect of progressive addition of Cr\(^{3+}\), Fe\(^{3+}\), and Cu\(^{2+}\) on fluorescence of aqueous dispersion of CQDs (\(\lambda_{\text{ex}} 450\) nm). Inset shows the linearity plots of CQDs that is fluorescence titration experiment was conducted for the metal ions. This experiment was helpful to know the linearity between quenching of the fluorescence and concentration range of the metal ion. Monitoring of the emission intensity at 545 nm with the incremental concentration of the metal ion was done. There is a linear response between these two parameters as revealed by the excellent correlation coefficient (R\(^2\)) shown in Figure 9 (inset). The R\(^2\) for Cr\(^{3+}\), Fe\(^{3+}\) and Cu\(^{2+}\) thus obtained is 0.96, 0.98 and 0.97 respectively. The fluorescence emission intensity of CQDs with the addition of Cr\(^{3+}\) (1 nM to 100 mM), Fe\(^{3+}\) (10 nM to 50 mM), and Cu\(^{2+}\) (10 nM to 50 mM) decreases linearly as shown in Figure 9A, 9B and 9C. The linearity plot between the concentration of these metal ions and fluorescence quenching, as shown in Figure 9 (Inset). 100 mM of Cr\(^{3+}\), while 50 mM of Fe\(^{3+}\) and Cu\(^{2+}\) each cause switch “OFF” the fluorescence of CQDs. The results show that CQDs have an excellent capacity to detect Cr\(^{3+}\), Fe\(^{3+}\), and Cu\(^{2+}\) at very lower concentrations. As clear from the figure, the CQDs shows high fluorescence under UV light of 365 nm, which disappears in the presence of Cr\(^{3+}\), Fe\(^{3+}\), and Cu\(^{2+}\). Fluorescence spectral recording were conducted at lower level of concentration of the metal ion for determination of limit of detection (LOD). LOD is determined from the obtained data by plotting I\(_0\)-I/I\(_0\) as a function of concentration of the metal ion. I\(_0\) is the emission intensity of the aqueous dispersion of CQDs at 545 nm without addition of the metal ion while I is the emission intensity upon addition of certain concentration of the metal ion. The concentration where appreciable

| Source               | Metal ion | LOD [\mu M] | References |
|----------------------|-----------|-------------|------------|
| Banana juice         | Cu\(^{2+}\) | 0.30        | 29         |
| O-Phenylenediamine   | Cu\(^{2+}\) | 0.28        | 30         |
| Pyrene               | Cu\(^{2+}\) | 0.10        | This work  |
| Denatured milk       | Cr\(^{3+}\) | 14          | 31         |
| Degrease cotton      | Cr\(^{3+}\) | 0.12        | 32         |
| Pyrene               | Cr\(^{3+}\) | 0.10        | This work  |
| Sodium citrate, glutathione | Fe\(^{3+}\) | 0.28        | 33         |
| Food waste           | Fe\(^{3+}\) | 32          | 34         |
| Pyrene               | Fe\(^{3+}\) | 0.10        | This work  |
enhancement in the value of $I_0 - I$ observed, has taken as LOD. The limit of detection (LOD) for $\text{Cr}^{3+}$, $\text{Fe}^{3+}$, and $\text{Cu}^{2+}$ is calculated and found as $100 \, \text{nM}$ of each of these metal ions (Figure 10A, 10B and 10C). The LOD obtained in this work is also compared with other quantum dots synthesized from different carbon sources as shown in Table 1. Examination of stability of fluorescence intensity is quite essential for a fluorescent nanoprobe. Thus, the fluorescence stability of aqueous dispersions of CQDs was randomly monitored by observing emission intensity at $\lambda_{\text{em}}$ (545 nm) for about 4 months (Figure 11). No significant fluctuation in fluorescence intensity was noticed for the tested duration. Apparently, there was no sedimentation or aggregation of the CQDs observed. It suggests that synthesized CQDs have stable fluorescence for a long time and therefore termed as fluorescence-stable in this work.

The FT-IR spectrum reveals the presence of $\text{-O-H}$ and $\text{C=C}$ functionalities at the surface of CQDs (Figure 2). These are presumed functionalities which are interacting with the positively charged metal ions ($\text{Cr}^{3+}$, $\text{Fe}^{3+}$ and $\text{Cu}^{2+}$) and contributing for their sensing. There is a tendency of the metal ion complexation due to the interaction. The O-H groups from different CQDs can assemble to form cluster/aggregation that tends to satisfy the coordination of the metal ions. The metal ion-induced clustering/aggregation of CQDs leads to quench their fluorescence presumably by electron transfer from electron-rich surface of CQDs to positively charged metal ions. The sensing mechanism of carbon dots involves photoluminescence quenching and non-radiative relaxation phenomena. The excitation starts in CQDs by absorbing an adequate amount of light energy. In the absence of heavy metal ions, the electron relaxes in the ground state, with light emission (radiative relaxation process), which gives emission spectra in PL. In the presence of heavy metal ions, the electrons (excited) interact with these ions, and non-radiative relaxation phenomena occur. The photoluminescence quenching can be seen in PL spectra (Figure 12), followed by the declination in the intensity peak with the increase in the concentration of these metal ions. By this way the CQDs acquire the fluorescence OFF state.

4 | CONCLUSION

We have obtained water-dispersible and fluorescence-stable CQDs at gram scale using pyrene as a precursor via bottom-up approach. These synthesized CQDs were characterized by TEM, FT-IR, FT-Raman, XRD, UV-Visible, and fluorescence technique. The CQDs are spherical with sizes ranges from 1 to 5 nm. The conditions required to synthesize CQDs were optimized. Also, environment factors (like concentration of CQDs, reaction time, pH and effect of NaCl) and their effects on the fluorescence intensity of CQDs were investigated. These have acted as fluorescent nano-probes for $\text{Cr}^{3+}$, $\text{Fe}^{3+}$ and $\text{Cu}^{2+}$ ions in the aqueous medium. These CQDs are capable of exhibiting LOD of $100 \, \text{nM}$ for each of these ions. These fluorescence-stable
CQDs are simply synthesized, and stored for a period of long time for the sensing-based applications.

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CONFLICT OF INTEREST
The authors have no any conflict of interest to declare.

DATA AVAILABILITY STATEMENT
Data openly available in a public repository that issues datasets with DOIs. All data is given in the manuscript.

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