





# Grass-derived carbon nanodots as a fluorescent-sensing platform for label-free detection of Cu (II) ions

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## ABSTRACT

Herein, carbon nanodots (CNDs) were facilely synthesized using grass as a carbon source by the reflux method. The as-synthesized CNDs were well characterized by UV–Vis spectroscopy, Fluorescence spectroscopy, XRD, FTIR, and SEM. The characterization results revealed that grass-derived CNDs showed strong absorption and fluorescence emission peak and are amorphous with the surface rich in carboxyl and hydroxyl groups. The average particle size is 20 nm with a narrow size distribution. The CNDs showed excellent fluorescent sensitivity and selectivity for the detection of Cu<sup>2+</sup> ions with a limit of detection as low as 0.89 μM with a linear detection range of 1–20 μM offering a novel sensing platform for Cu<sup>2+</sup> ion.

## 1 Introduction

Copper (Cu) is an abundant transition element that plays a significant role in the physiological process, dioxygen transport, and energy production [1]. However, an excessive amount of copper, most often Cu<sup>2+</sup> ions, in living organisms results in serious disorders, such as Parkinson's, prion, and Alzheimer's disease, gastrointestinal upset, kidney, and liver damage. It is listed as a major pollutant by the

US Environmental Protection Agency (EPA) due to its environmental polluting ability [2, 3]. Therefore, to reduce the toxic effect and pollution induced by Cu<sup>2+</sup> ions, it is of prime importance to develop an efficient, reliable, and green method for the detection of Cu<sup>2+</sup> ions [4].

Numerous analytical techniques have been applied for the detection of Cu<sup>2+</sup> ions, such as atomic absorption spectroscopy, electrochemical analysis, liquid chromatography, inductive-coupled plasma mass spectroscopy, and chromogenic sensor. However, these methods have some drawbacks, such as

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high cost, sensitivity, and time lasting. Therefore, there is a great demand for an effective and simple method for the detection of  $\text{Cu}^{2+}$  ions. Recently, fluorescence quenching has greatly attained extensive research attention as an analytical method for the detection of toxic metal ions [5–8].

In the fluorescence quenching method, semiconductor quantum dots have been widely investigated as fluorescent investigators for optical sensing and bioimaging applications due to their excellent photochemical stability, optical properties, and high brightness [9–13]. However, the toxic nature and poor water dispersibility of semiconductor quantum dots have limited their uses [14–17]. As of late, carbon nanodots (CNDs) are carbon-based fluorescent nanomaterials that have gained enormous attention from researchers due to their high chemical stability, excellent water dispersibility, low toxicity, and synthesis cost [14–16]. Due to these excellent properties, CNDs are the novel-type fluorescent investigators for the detection of metal ions than semiconductor quantum dots.

Generally, CNDs synthesized from the carbonization of carbon sources and quantum confinement of carbon materials. The natural carbon sources, such as fruits, fruit juices, vegetables, plants, cow manure, and grass, have attained much attention for the synthesis of CNDs due to its wide availability, eco-friendly, cheapness, and various surface groups [18]. For example, Liu et al. synthesized CQDs from pear juice by hydrothermal method for the detection of  $\text{Cu}^{2+}$  ions [19]. Boobalan et al. synthesized carbon dots by hydrothermal method from mushroom for the detection of toxic metal ions and as anticancer and antibacterial agents [20]. Chaudhary et al. prepared highly fluorescent CQDs from banana juice by one-step hydrothermal treatment for the detection of copper ion [18]. Based on the aforementioned results, it is very important to develop a simple and green strategy to produce CNDs from natural resources.

In this work, natural, low cost, and abundant grass is used as a carbon source for easy and green synthesis of water-soluble CNDs by the reflux method. The grass is selected as a carbon source due to its easy availability, cheapness, and multiple chemical components. The obtained CNDs showed excellent fluorescent sensitivity and selectivity for  $\text{Cu}^{2+}$  ions due to the presence of a large number of carboxyl and hydroxyl groups on the surface of CNDs. These

results provide a new route for the detection of  $\text{Cu}^{2+}$  ions by a facile and efficient approach.

## 2 Experimental

### 2.1 Materials and reagents

The fresh grass was collected from the garden of the Institute of Chemical Sciences, University of Peshawar, Pakistan. Metal salts used were copper(II) sulfate, iron(II) chloride, cadmium(II) chloride, mercury chloride(II), silver chloride, calcium chloride(II), lead(II) chloride, and zinc chloride(II). Deionized (DI, 18 M $\Omega$ ) water is produced by a water purification system (Q-Grad®1, Millipore) and is used in all experiments.

### 2.2 Instruments and methods

The absorption and fluorescence spectra of C-dots were acquired with a UV–Visible double-beam spectrophotometer (UV-1602) and the fluorescence spectra are recorded with a spectrofluorometer (Perkin-Elmer LS55), respectively. The crystallinity and phase purity of CNDs are investigated by X-ray diffraction (XRD JEOL JDX-9C-XRD Japan) spectrometer with  $\lambda = 1.5418 \text{ \AA}$  Cu-K $\alpha$  radiation. The functional groups on CNDs were analyzed using a Fourier transform infrared spectroscopy (Thermo scientific Nicolet 6700 FTIR spectrometer). The morphology and particle size of CNDs were examined by the field emission scanning electron microscopy (SEM) technique using (JEOL, JSM-5910 Japan) electron microscope.

### 2.3 Synthesis of CNDs

Briefly, 5 g of fresh grass was crushed with a mortar and pestle with 25 mL deionized water. The mixture was heated at 180 °C in a reflux condenser for 3 h. The CNDs were collected by centrifuging at 14,000 rpm for 10 min. The CNDs were dispersed in water for further use. The synthetic procedure of grass-derived CNDs is illustrated in Scheme 1.

### 2.4 Metal ion detection

Different metal ions were used  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ag}^{+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Zn}^{2+}$  along with  $\text{Cu}^{2+}$  ion. An aqueous solution of 100  $\mu\text{L}$  of each metal ion (stock

concentration of 1 mM) was mixed thoroughly with 50  $\mu\text{L}$  of CNDs solution (1 mg/mL). The fluorescence of CNDs with different metal ions was recorded. The specific detection of  $\text{Cu}^{2+}$  ions using CNDs was established by analyzing thoroughly the linear plots of absorbance versus concentration for the estimation of the limit of detection (LOD) and the quenching effect has been thoroughly explained using the Stern–Volmer equation.

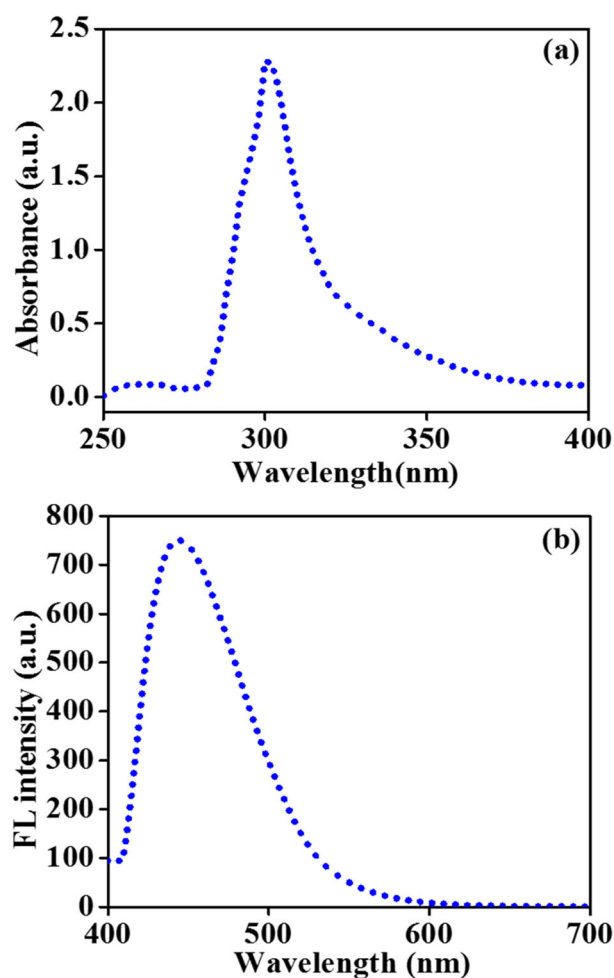
## 2.5 Specific detection of $\text{Cu}^{2+}$ ions by CNDs

The CNDs have been applied specifically for the detection of  $\text{Cu}^{2+}$  ions. In brief, the stock solution of  $\text{Cu}^{2+}$  ion (0.1 M) was prepared by their sulfate salt and the stock solution of CNDs (1 mg/mL) was prepared by dissolving the CNDs in distilled water. The CNDs (3 mL) were thoroughly mixed with a known concentration of  $\text{Cu}^{2+}$  ion solution, left undisturbed for 10 min, and then the fluorescence spectra are recorded.

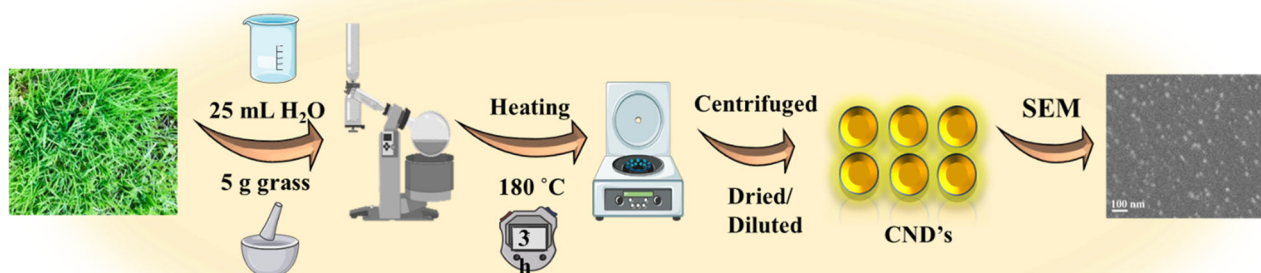
## 3 Results and discussion

### 3.1 Characterization of CNDs

The optical properties of CNDs were characterized by UV–Vis and fluorescence spectroscopy. Figure 1a shows a broad absorption peak around 300 nm, while the fluorescence property of CNDs is confirmed by FL emission intensity as shown in Fig. 1b. The CNDs



**Fig. 1** UV–Visible absorbance spectra (a) and fluorescence emission spectra (b) of CNDs



**Scheme 1** Synthetic scheme of CNDs prepared from grass using reflux method

were excited at 360 nm giving a strong emission peak at 444 nm indicating the synthesized CNDs exhibit fluorescent property.

The XRD analysis was used to determine the crystallinity and phase purity of CNDs and the result is depicted in Fig. 2a. It can be seen that CNDs are amorphous in nature with a slight diffraction peak detected at  $2\theta = 28^\circ$ .

The surface functional groups of CNDs are determined by FTIR analysis as shown in Fig. 2b. The broadband around  $3500\text{ cm}^{-1}$  confirms the asymmetric stretching vibration of the O–H group, while the bands at 2910, 2679, 2311, and  $2110\text{ cm}^{-1}$  can be assigned to C–H stretching vibration. The broadband at  $1727\text{ cm}^{-1}$  is due to the C=O stretching vibration of the carboxylic acid group, while the bands at 991 and  $567\text{ cm}^{-1}$  could also be attributed to the C–O

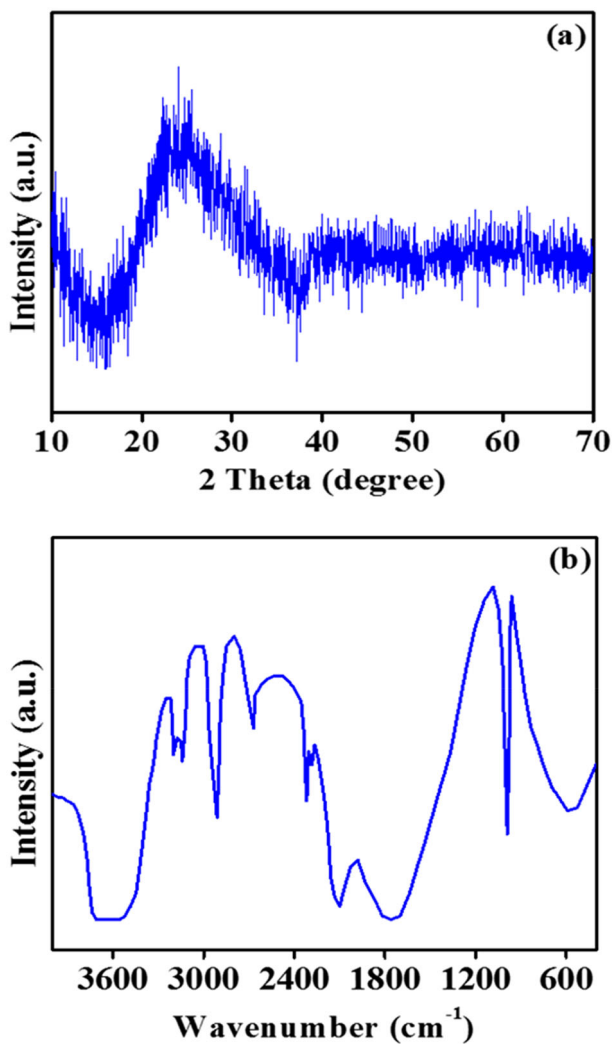


Fig. 2 XRD pattern (a) and FTIR spectrum (b) of CNDs

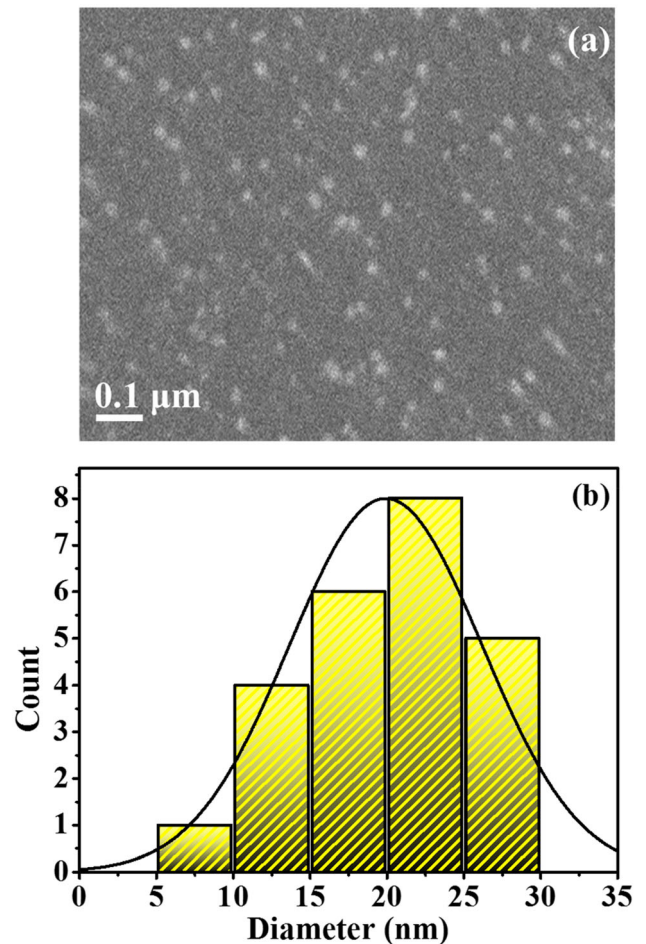


Fig. 3 SEM micrograph (a) and particle size distribution graph (b) of CNDs

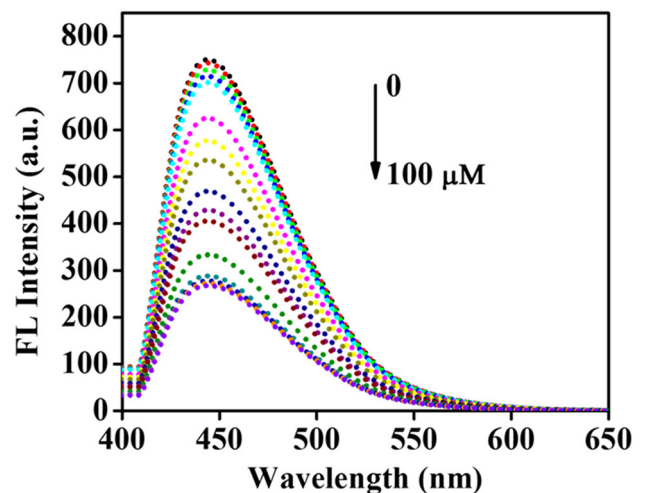
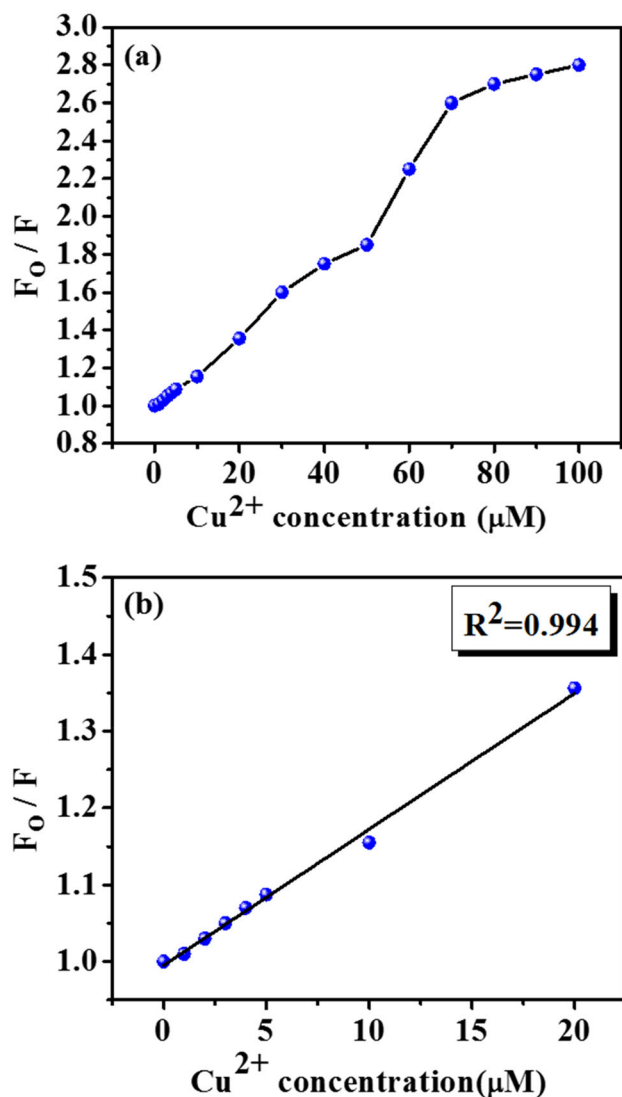


Fig. 4 Fluorescence emission spectra of CNDs (a) in presence of different  $\text{Cu}^{2+}$  ion concentrations (0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, and 100.0  $\mu\text{M}$ )

stretching vibration. The peak at  $3250\text{ cm}^{-1}$  is due to the N–H group's asymmetric stretching vibrations. Based on our findings, we conclude that our CNDs are composed of many hydroxyl, carbonyl, and carboxyl functional groups which match well with previous literature values [17, 21, 22].

The surface morphology and particle size are characterized using SEM analysis. Figure 3 shows typical SEM images of CNDs displaying near-uniform dot-shaped particles. The SEM micrograph confirms the successful formation of CNDs and the particle size distribution was found to be in between 15 and 25 nm range with an average particle size of 20 nm.

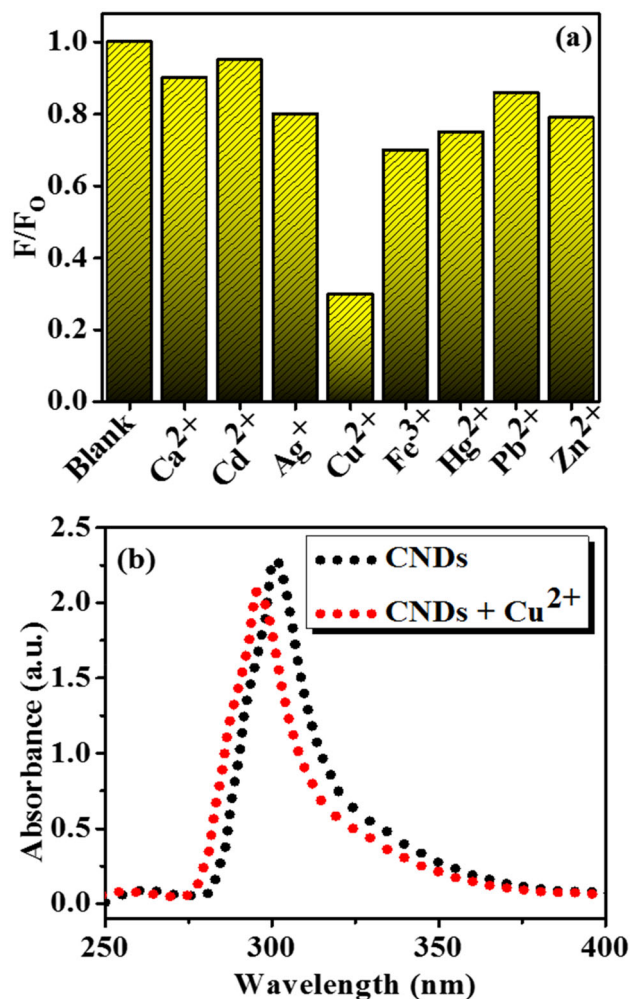


**Fig. 5** The plot between  $F_0/F$  versus different concentrations (a) of  $\text{Cu}^{2+}$  ions (0.0–100  $\mu\text{M}$ ) and (b) linear fitting of  $F_0/F$  versus  $\text{Cu}^{2+}$  ion concentration (0–20  $\mu\text{M}$ )

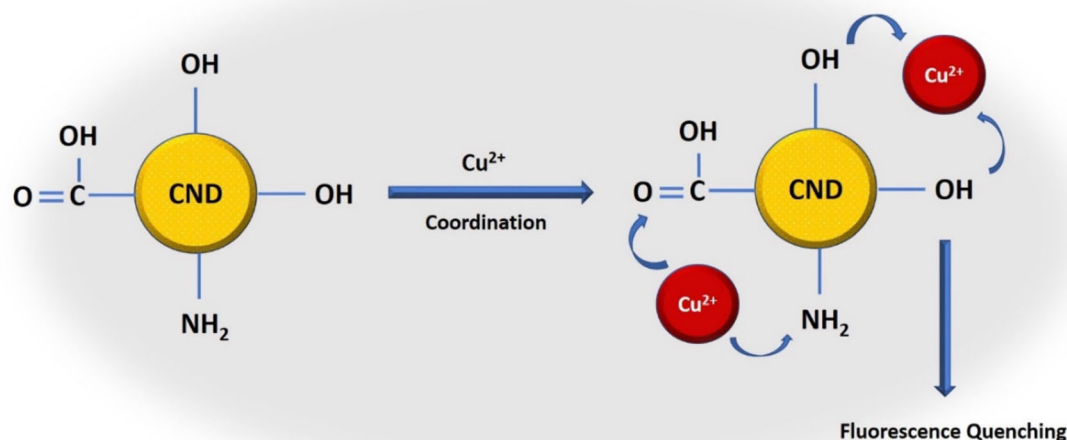
### 3.2 $\text{Cu}^{2+}$ ion sensing

The heavy metal ion,  $\text{Cu}^{2+}$  ion, was detected with the help of CND as a fluorescent probe. We tested the analytical system's capacity to detect  $\text{Cu}^{2+}$  ions. As shown in Fig. 4, lacking  $\text{Cu}^{2+}$  ions in the solution, the CND shows a strong emission peak at 444 nm. Nonetheless, the FL intensity of CNDs decreased gradually with the addition of  $\text{Cu}^{2+}$  ions. Due to the quenching effect of  $\text{Cu}^{2+}$  ions on the fluorescent CNDs, the intensity of the fluorescent emission synchronously turns weak due to the increase of the concentration of  $\text{Cu}^{2+}$  ions in the dispersion solution.

The effective fluorescent quenching is due to energy or electron transfer taking place between CNDs and  $\text{Cu}^{2+}$  ions [20]. It is believed that the



**Fig. 6** Fluorescence intensity difference of CNDs in the presence of different metal ion solutions (100.0  $\mu\text{M}$ ) (a) and UV–Vis absorbance spectra of CNDs and CNDs with  $\text{Cu}^{2+}$  ions (b)



**Scheme 2** A schematic representation of  $\text{Cu}^{2+}$  ions sensing mechanism using grass-functionalized CNDs. Fluorescence quenching occurs via the coordination between hydroxyl and carboxyl groups of CNDs and  $\text{Cu}^{2+}$  ions

decrease in fluorescence intensity takes place due to complex formation taking place between  $\text{Cu}^{2+}$  ions and CNDs. The concentration of  $\text{Cu}^{2+}$  ion was varied from 0 to 100  $\mu\text{M}$  and it can be seen (Fig. 4) that the emission intensity decreases with the increase in  $\text{Cu}^{2+}$  ion concentration.

The quenching effect can be thoroughly explained using the following Stern–Volmer equation:

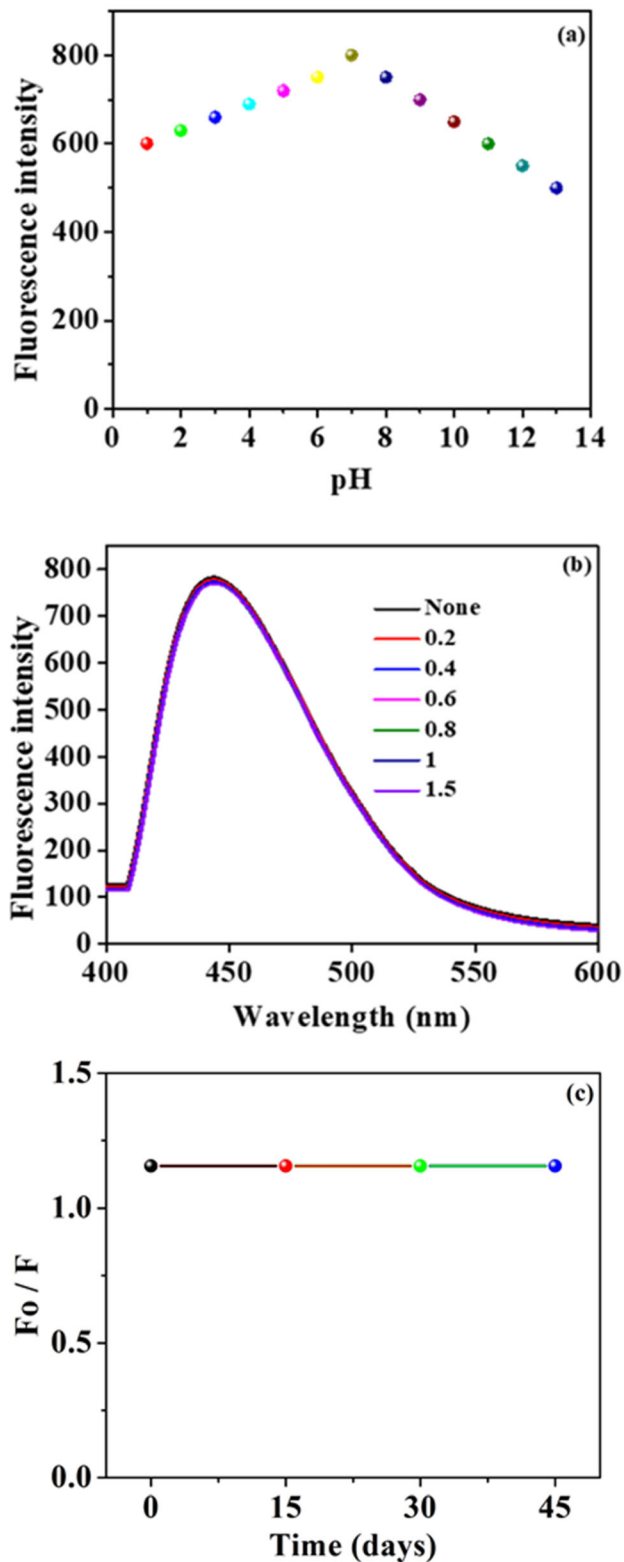
$$F_0/F = 1 + K[Q],$$

where  $F_0$  and  $F$  are the fluorescence intensities at 444 nm without and with  $\text{Cu}^{2+}$  ions concentration,  $K$  is the Stern–Volmer quenching constant, and  $Q$  is the  $\text{Cu}^{2+}$  ions concentration, respectively. Figure 5a shows the relationship between  $F_0/F$  versus the concentration of  $\text{Cu}^{2+}$  ions. It is noteworthy that the whole concentration range (0–100  $\mu\text{M}$ ) does not fit well in a linear relationship and does fit well in a concentration range of 0–20  $\mu\text{M}$ , as shown in Fig. 5b with  $R^2$  value 0.999. The detection limit is calculated based on  $3\sigma/k$  and is found to be 0.89  $\mu\text{M}$  which is much lower than the maximum concentration of  $\text{Cu}^{2+}$  ions in drinking water permitted by the US environmental protection agency [23].

To examine the selectivity of CNDs for  $\text{Cu}^{2+}$  ions detection and whether our system was specific, different metal ions such as  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Zn}^{2+}$  along with  $\text{Cu}^{2+}$  ions were tested for fluorescence change under the identical

conditions as a comparative study (Fig. 6a). It is noteworthy that the fluorescence change was highest for  $\text{Cu}^{2+}$  ions only, while other metal ions do not show remarkable fluorescence change by adding into the dispersion. These results suggest that the prepared CNDs are highly sensitive and selective toward  $\text{Cu}^{2+}$  ions than other metal ions. This is possibly due to the strong binding affinity of CNDs for  $\text{Cu}^{2+}$  ions because of the large number of carboxyl and hydroxyl groups on the surface of CNDs. The excellent specificity combined with high sensitivity and fast response of CNDs to  $\text{Cu}^{2+}$  ions suggested that our method might be directly applied for detecting  $\text{Cu}^{2+}$  ions in real samples [24]. It is also believed that  $\text{Cu}^{2+}$  has a faster chelation process and a higher thermodynamic affinity to N and O than other heavy metal ions [25–27].

The fluorescence quenching occur through electron transfer, charge diverting, and surface absorption. The fluorescence quenching mechanism can be static and dynamic. The grass contains polysaccharides and essential amino acids; upon the addition of  $\text{Cu}^{2+}$  ions to CNDs, it may form a complex with hydroxyl and carboxyl groups present on the surface of CNDs confirmed by FTIR analysis (Fig. 2b). This lead to fluorescence quenching of CNDs due to the formation of strong coordination bond between functional groups of CNDs and  $\text{Cu}^{2+}$  ions, as illustrated in Fig. 6a by fluorescence spectroscopy. The quenching



**Fig. 7** Effect of **a** pH, **b** ionic strength, and **c** storage time on the fluorescence intensity of CNDs

mechanism was investigated by UV–Vis spectrophotometer by studying the relationship between CNDs and  $\text{Cu}^{2+}$  ions. As can be seen in Fig. 6b, there is a decrease in absorbance and blue shift in wavelength of CNDs, which attribute to the formation of a non-fluorescent ground state complex between CNDs and  $\text{Cu}^{2+}$  ions, suggesting static quenching occurs [32]. Also besides, the selective sensing of  $\text{Cu}^{2+}$  ions with CNDs is due to  $\text{Cu}^{2+}$  ions unfilled d orbitals. The quenching mechanism would be mainly associated with the PET process from excited CNDs to the unfilled d orbitals of  $\text{Cu}^{2+}$  ions. The  $\text{Cu}^{2+}$  ions considerably quench the fluorescence of CNDs and hence the impact of other examined metal ions can be dismissed (Fig. 6a). The schematic representation of formation of coordination bond between  $\text{Cu}^{2+}$  ions and CNDs is illustrated in Scheme 2.

### 3.3 Stability of CNDs

The stability of CNDs is investigated in terms of effect of pH, effect of ionic strength, and effect of storage time. The effect of pH was investigated by varying the pH from 1 to 13 and it was found that the CNDs showed the maximum fluorescence intensity for pH 7 as shown in Fig. 7a. The effect of ionic strength was also investigated by varying the strength of KCl (0–1.5 M) and it was found that there is no change in the fluorescence intensity of CNDs in the presence of KCl (Fig. 7b). Furthermore, the effect of storage time of the CNDs was investigated by storing the CNDs for 45 days and it was found there is no change in the fluorescence intensity of CNDs after 45 days as shown in Fig. 7c, suggesting the good photostability of CNDs.

### 3.4 Comparison of the present work with reported literatures

The detection limit for  $\text{Cu}^{2+}$  ions is compared with the previously reported works of CNDs and nanoparticles for detection of  $\text{Cu}^{2+}$  ions as shown in Table 1 [28–35]. The comparison is made on the basis of carbon sources used for the preparation of CNDs, synthetic approach, and concentration range. It can be seen in Table 1 that the present work offers much lower detection limit as compared to other CNDs and nanoparticles derived through several methods with

**Table 1** Comparison of detection limit for Cu<sup>2+</sup> ions over different fluorescent sensors

Sample	Carbon Source	Method	Metal ions	Concentration linear range (μM)	Detection limit (μM)	References
CaP/CDs	Citric acid	Biomimetic	Cu (II)	10–20	9.82	[28]
CDs	Sago waste	Thermal pyrolysis	Cu (II)	–	7.78	[29]
FCDs	Peanut shells	One pot pyrolysis	Cu (II)	0–5	4.8	[30]
CuNPs	–	–	Cu (II)	15–35	5.6	[31]
Cys-CdS	–	Quantum confinement	Cu (II)	2–10	1.5	[32]
CdTe QDs	–	Reflux method	Cu (II)	1.6–63	0.94	[33]
H-CDs	Amino acids	Hydrothermal method	Cu(II)	0–550	2.67	[34]
CDs	Activated carbon	–	Cu(II)	0–100	2.4	[35]
CNDs	Grass	Reflux method	Cu (II)	0–20	0.89	Current work

different carbon sources, which suggests the sensitivity of the present CNDs for the detection of Cu<sup>2+</sup> ions. Thus it can be deduced that the present work offers several advantages over other methods in the detection of Cu<sup>2+</sup> ions by CNDs and nanoparticles.

## 4 Conclusion

In summary, the CNDs were prepared by reflux method using grass as a carbon source for the first time with high sensitivity and selectivity and this method has proven to be effective. The CNDs show a strong affinity for Cu<sup>2+</sup> ions over other metal ions due to the presence of a large amount of carboxyl and hydroxyl groups on the surface of CNDs. The method relies on the fact that Cu<sup>2+</sup> ions can quench the FL intensity of the CNDs. The response of CNDs for fluorescence intensity versus concentration of Cu<sup>2+</sup> ions showed a good linear relationship within the range of 0–20 μM and the detection limit is found to be 0.89 μM which is much lower than the maximum concentration of Cu<sup>2+</sup> ions in drinking water. Therefore, it is proposed that facilely green-synthesized CNDs might be used in a variety of innovative applications, including biological and optoelectronics, owing to their low cytotoxicity, high sensitivity, and high dispensability.

## Author contributions

Writing—original draft preparation: HK; Formal analysis and investigation: MFK; Conceptualization and methodology: AI; Writing—review and editing: MZ; Software: BA; Resources: MI; Supervision: AI and MZ.

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## Data availability

All data generated or analyzed during this study are included in this published article.

## Code availability

Not applicable.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.



**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

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