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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 03

Res. Jr. of Agril. Sci. (2022) 13: 791–797

CARAS

Res. Jr. of Agril. Sci. (May-Jun 2022)
13(3): 791–797

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

Full Length Research Article

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Received: 23 Mar 2022 | Revised accepted: 30 May 2022 | Published online: 11 June 2022

ABSTRACT

The present work reports a simple, economical, and green preparative strategy toward water soluble, fluorescent carbon nanodots (wsCNDs) by a one-step pyrolysis method using low-cost wastes of bottle gourd peel *(Lagenaria siceraria)* also known as calabash as a carbon source. The synthesized carbon dots were well characterized with X ray Diffraction (XRD), Zeta Potential Measurement, Fourier Transformer Infra-Red spectroscopy (FTIR) and Fluorescence measurement. We further explore the use of such wsCNDs as fluorescent probes for a metal ion detection, which is based on metal induced fluorescence quenching of EDTA functionalized carbon nanoparticles. This sensing system exhibits good sensitivity towards metal ions, and in this sensor system, the minimum concentration that we determine is 2 µM. Thus, the fluorescent carbon nanodots can act as an optical probe for quantifying metal ions in aqueous solution.

***Key words*:** Carbon nanodots, Fluorescence, Chemo-sensor, Metal sensing, Biogenic carbon dots

Carbon nanodots (CNDs) are emerging as a new class of carbon-based fluorescent nanomaterials for their applications in wide areas, owing to numerous alluring properties [1,2] which include environmental friendliness, favourable biocompatibility, excellent chemical stability, broad excitation spectra, resistance to photo bleaching and ease of surface modification [3]. Among various other important applications, the use of such nanodots as a fluorescent sensor has occupied a central stage in research activities due to their photostability [4], low toxicity [5] easy processibility and highly water-soluble nature. Their versatile tunable properties along with solution processibility, endow CNDs for enormous potential applications in photo catalysis [6], photo- detectors [7], water remediation [8], sensors [9], drug delivery [10] and bio-imaging [11]. Carbon is commonly a black material and until recently was generally considered to have low solubility in water and weak/ no fluorescence [12]. The main reason why such tiny carbon nanodots have recently attracted wide attention is due to their strong fluorescence, for which they are also referred to as fluorescent carbon dot. Such fluorescent CNDs have sizes bellow 10 nm, are a collection of hexagonal lattice of *sp2* carbons incorporated with *sp*3 carbons and heteroatomic functional groups at a nanometer scale [13]. The large amounts of C-C bonding and pi-electronic conjugations that exist in CNDs are irregular and disrupted into small fragments. Hence CNDs could also be termed as highly defected composites of coexisting regions of aliphatic and aromatic carbons, which are usually modified with surface polar groups. CNDs also exhibits some properties as other allotropes of nanocarbons.

Defects and surface functionalities govern the properties of CNDs, which strongly depend upon the precursor material and synthetic conditions. Cost and availability of precursor material are the prime concerns for commercial productions of CNDs. Hence the uses of green sources are slowly taking over the other chemicals. For example, Tea leaves [14] Coriander leaves [15], Egg shell [16], Spinach [17] are some of the precursor materials already reported for synthesis of CNDs.

Sensing of different chemicals has long been occupied a central stage owing to its importance in diagnosis of disease, controlling the vital parameter of living organism, controlling pollution, detecting explosives etc. Nanomaterials, offering a much higher sensitivity as well as selectivity for a sensor design, are slowly replacing the other conventional materials in an important way. Carbon nanodots are one of the latest contributions in the field of sensing. Although, initially carbon nanodots were derive from chemical sources have been reported

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to for several important in vitro as well as in vivo sensing process, research has now been shifted towards the use of carbon dots derived from biogenic/ waste materials [18] to be used in highly efficient sensing process, giving the process a more sustainable and environmentally compatible approach.

In this paper we describe such an approach to synthesize water soluble carbon nanodots (wsCND) from the peels of bottle guard and functionalized with EDTA and demonstrate its sensing properties against a few divalent metal ions. Metals are important ingredients in life just as the organic molecules. For example, the divalent calcium and magnesium ions play important regulatory roles in cells. However, the concentrations of such metals ions also play a determining role in various vital functions of a living organism. The concentrations required for a health life lies within a narrow limit. Presence of metal ions both over and below this range may results in various types of diseases and sometimes may be fatal too. Presence of toxic metal ions in our food chain is very dangerous and raises serious environmental and health concerns [19]. At certain times they may even interfere with metabolic processes.

Therefore, developing effective analytical methods for the sensitive and selective detection of trace amounts of metal ions is especially important. Traditional techniques including atomic absorption/emission spectroscopy, auger electron spectroscopy, inductively coupled plasma mass spectroscopy and polarography require sophisticated instrumentation and special sample preparation techniques, which limit their practical applications [20-21]. Fluorescence has several advantages, such as high sensitivity, fast analysis and being non-sample-destructing or less cell-damaging and have been proven to be alternative method for metal detection [22-23]. So far, many fluorescent probes including organic molecule, metal nanoclusters, semiconductor quantum dots (QDs) etc. have been developed for fluorescent detection of metal ions [24-26]. However, the above fluorescent materials suffer from complex synthesis routes with the involvement of toxic or expensive reagents, and hence are not recognized in later time period as sustainable materials. Accordingly, the development of a simple, economical and green preparative strategy toward fluorescent materials is highly desired. A facile synthesis of aqueous stable CNDs using cheap and natural precursor having high quantum yield is a current need of time. Herein, we present a simple, facile, and economic approach for the synthesis of fluorescent carbon nanodots based on pyrolytic carbonization of precursor bottle gourd peels followed by microwave assisted method. Such synthesized water-soluble CNDs are in the range of 10 nm in particle size with good aqueous solubility, stability in solution and high photo-stability. On the basis of their fluorescent property, here we use the synthesized CNDs as a sensing agent for the detection of a few bivalent metal ions. Development of such fluorescent based chemo-sensor using cheap, environment friendly and sustainable material will lead to cheap sensor system for bivalent metal ions.

MATERIALS AND METHODS

Bottle gourd (Lagenaria siceraria) peels were collected from as waste materials from hostel kitchen. All the chemicals such as calcium chloride, magnesium sulphate heptahydrate, copper sulphate pentahydrate, EDTA, HCl, used are of analytical grade from MERCK, India. Double Distilled water was used in all the experiments.

Synthesis of water-soluble carbon nanodots (wsCNDs)

The wsCNDs were synthesized by the pyrolysis of bottle gourd peels as a green precursor. Prior to pyrolysis, bottle gourd peels were washed several times with deionised water to remove any contamination and dried in oven at 190 to 200°C for nearly 24 hours. Dried peels were finely powdered in mortar and pestle. The finely powdered substance was heated again for two hours at 200°C, allowed to cool to room temperature and solid CNDs were collected.

The synthesized CNDs are partially dispersed in water as well as in acetic acid. The solution was then centrifuged to remove residues and to get an aqueous solution of the carbon dots. The solution was then subjected to microwave radiation at power level 180W of which resulted in intense yellow coloured solution. The resulting yellow solution showed blue fluorescence and this was used in the subsequent experiments as aqueous carbon nanodot solution. The EDTA functionalized carbon nanodots were synthesized by adding Na_2EDTA solution (1 mM) to the aqueous CND solution with constant stirring. The solutions were then further centrifuged to remove any suspended bigger particles.

Detection of metal ions

The detection of metal ion was performed at room temperature in EDTA functionalized carbon dot solution. In a typical run, to the CND dispersion (3 mL) a calculated amount of metal ion solution (Ca^{2+}, Mg^{2+}, Cu^{2+}) of different concentration in the range were added gradually. The mixture was then allowed to stand for 5 minutes at room temperature and the photoluminescence (PL) spectra were recorded.

Photoluminescence measurements

Photoluminescence measurements in aqueous solution at room temperature were performed using a Horiba fluoremax-4c fluorescence spectrometer. For this study the as prepared solution was excited with a wavelength range from (300nm-350nm) to observe the wavelength dependent fluorescence. For the sensing applications 340nm excitation was chosen and the resulted emission after addition of the respective metal ions was recorded.

Fourier transform infrared (FTIR)

FT-IR spectra were recorded in both solid (partially carbonised product) and water-soluble carbon dots (wsCNDs) derived from the solid were recorded in a Bruker FTIR spectrometer in the range of 600 to 4000 cm^{-1}.

The uv-vis absorption spectra

To study the absorbance properties of the synthesized water-soluble carbon nano dots, the aqueous solutions of the nanodots were scanned under uv-vis light in the wavelength range from (190nm-1100nm) on a LABINDIA 3200 UV-VIS spectrometer.

Dynamic light scattering and surface charge analysis

The surface charge of the carbon nanodots, zeta potential measurements were in a colloidal solution were performed with Melvern ZS 90 zeta potential analyzer.

X-ray diffraction spectra

X-ray diffraction measurements were performed to confirm the crystalline or amorphous nature of the carbon nanodots. The measurement was carried out by drop casting the dispersion onto a glass plate. X-ray diffraction pattern were recorded on a Rigaku Ultima IV instrument, in a slow scan mode.

RESULTS AND DISCUSSION

Here we demonstrate successful synthesis of biogenic carbon nanodots from bottle gourd peel (also known as

calabash) having a comparatively high quantum yield. The synthetic procedure requires the temperature of 200°C which is low as compared to other reported methods. The synthetic protocol in simple and the use of waste material like bottle gourd peel make the process viable for scale –up. Figure 1 depicts the scheme for the preparation of the nanodots. After partial pyrolysis at 200°C in air a greenish black powder is obtained which might contain a few bigger particles. To obtain nanodots typically 20mg of the powder is dispersed in water/acetic acid and then solution is put under microwave irradiation at a power level of 180W for 5 mins. This results in a yellow-coloured solution. The solution is then centrifuged at a 2000 rpm speed to remove the suspended greenish back particles. The supernatant solution is taken for further use. The wsCNDs solution is fluorescent when shined with UV light (Fig 1). The wsCNDs was then functionalized with EDTA (Ethylenediaminetetraacetic acid) to obtain EDTA capped wsCNDs.

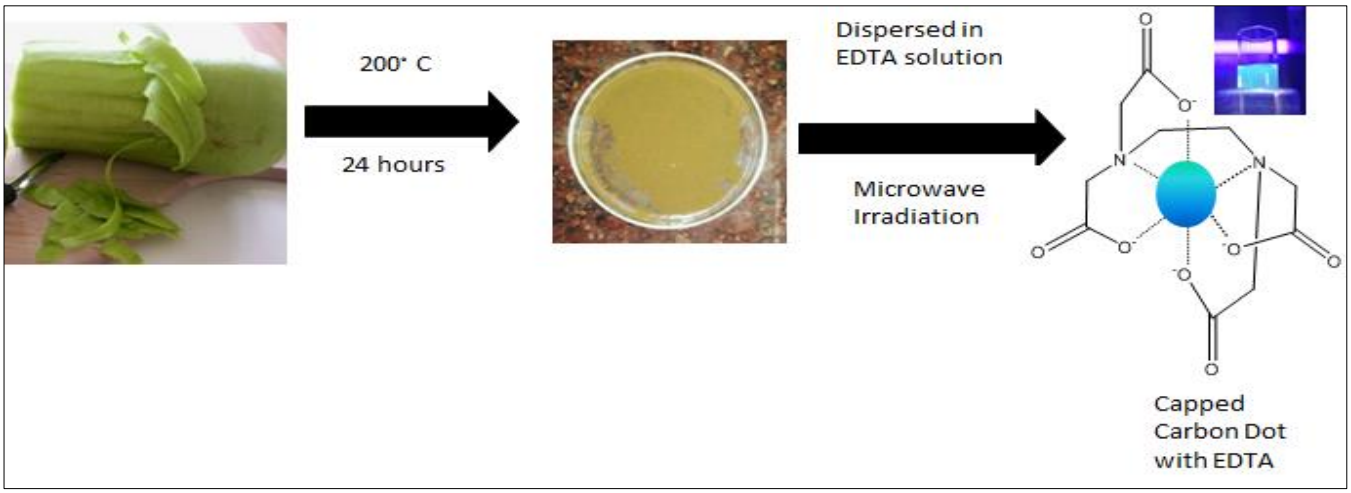

Fig 1 Schematic of preparation of wsCNDs. Also shown fluorescent solution of wsCNDs after irradiation with UV light

Dynamic light scattering (DLS) of the synthesized carbon nanodots shows that majority of the particles are between 7nm-8nm, which is also consistent with the blue emission as reported earlier. The DLS measurement also shows negative zeta potential value of –20.3 mV for the wsCNDs, suggesting high surface charge accumulation due to presence of COO^- group. This may also have attributed due to functionalization of EDTA on carbon nanodots surface (Fig 2).

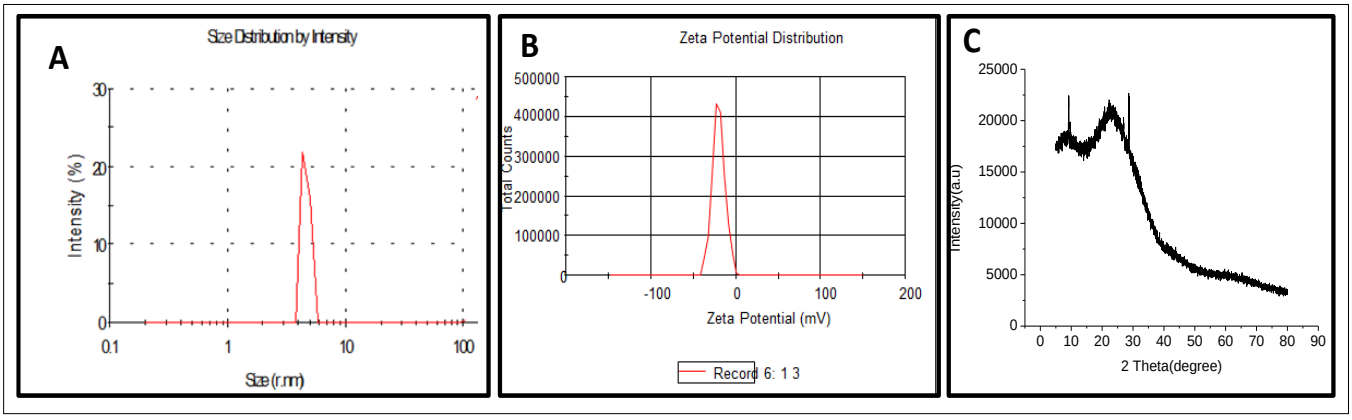

Fig 2 (A) Particle size distribution (B) Zeta potential measurement (C) X-ray diffractogram of wsCNDs

The X-ray powder diffraction (XRD) pattern were used to investigate the crystallinity of the synthesized wsCNDs. The XRD pattern of the wsCNDs displays a slightly broad and high intensity diffraction peak (Fig 2C) centered at a 2θ value of 23.6° corresponding to (002) plane, which indicates carbon-based materials with abundant sp^3 disorder [24], [33].

Further to identify the nature of the functional groups present on the surface of carbon nanodots, FTIR spectra were recorded. The FTIR studies were conducted of the greenish black powder, and carbon nanodots and the EDTA capped carbon nanodot. It has been found that in the as synthesized greenish black product mostly gives sharp bands at 1024 cm^{-1} and 1537 cm^{-1} which are indicative of C – O and C ═ C linkages [25]. The stretching vibrations of C = O are also observed at 1640 cm^{-1} with asymmetric and symmetric stretching vibrations of C – O – C (at 1365 and 1226 cm^{-1}). The broad band around 3300 cm^{-1} indicates the presence of hydrogen bonded O – H while the bands at 2897 cm^{-1} can be assigned to C – H linkage as shown in (Fig 3A).

The FTIR spectrum of carbon nanodots is shown in figure 3B. The carbon nanodots show bands due to C = O and hydrogen bonded O – H with a broad signal around 2200 cm^{-1} which may be due to C = O and C – O stretching vibrations, indicating that the surfaces of carbon nanodots are partially oxidized [26]. Further the bands at 730 and 1480 cm^{-1} suggest the presence of COO^- in carbon nanodots. After functionalization of the carbon dots with disodium salt ethylenediaminetetraacetic acid (Na_2EDTA), the EDTA (Ethylenediaminetetraacetic acid) functionalised carbon dots were confirmed by the two additional bands on the FTIR spectrum at bands at 1050 and 1377 cm^{-1} appear which suggest the presence of COOH group while the bands at 1089 $cm^{-1}$and 1228 cm^{-1} indicates the presence of C – N and C – O moieties as shown in the (Fig 3C).

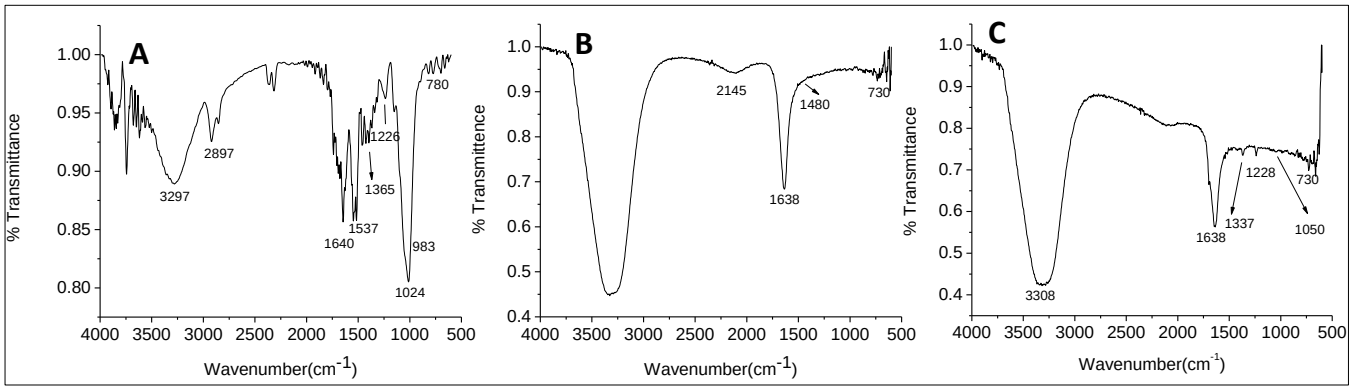

Fig 3 (A) FTIR spectrum of greenish black powder (B) FTIR spectrum of wsCNDs (C) FTIR spectra of EDTA functionalised wsCNDs

The absorption spectrum of wsCNDs is shown in (Fig 4). The spectrum shows a broad spectrum with continual increase from 600 nm to 200 nm attributed to the π - π^* and n - π^* transitions of the C=C and C=O bonds, respectively. The uv-vis spectrum does not indicate any other morphology of nanocarbons or amorphous carbon, as resulting from partial carbonization of precursor, since there is no sign of background absorbance in the visible region [27]. Aqueous solution of wsCNDs after several weeks of storage at room temperature did not result any visible aggregation, demonstrating excellent stability of wsCNDs in solution.

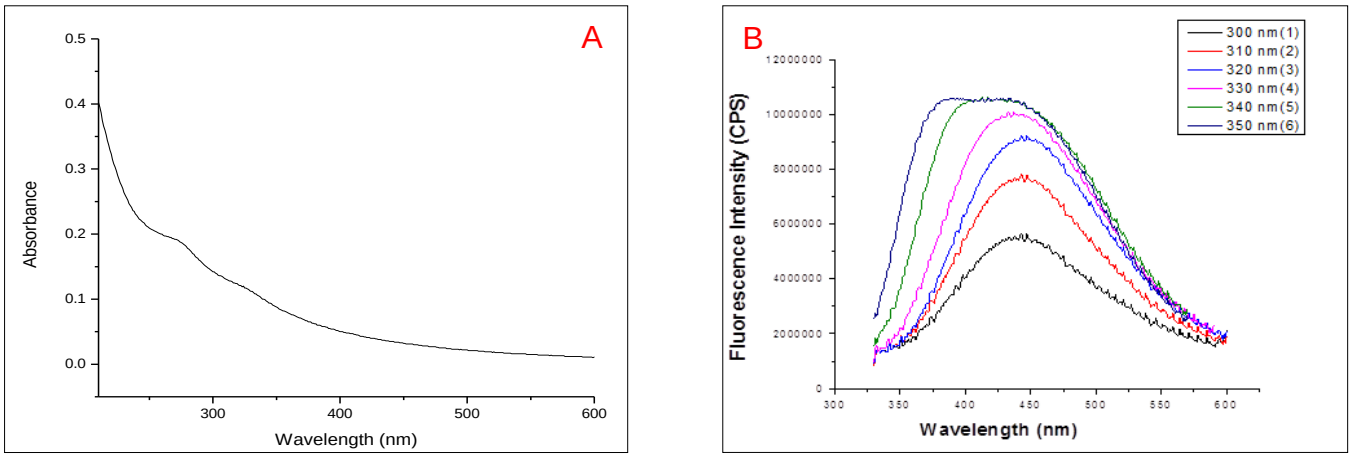

Fig 4 (A) Uv-visible spectrum of wsCNDs (B) Photoluminescence emission of wsCNDs at different excitation from 300- 350 nm

A detailed Photoluminescence (PL) study with different excitation wavelengths (λ_{ex}) ranging from 300 to 350 nm was done to explore the optical properties of the wsCNDs. The emission spectrum of the wsCNDs is excitation dependent as shown in figure 4B and is characterized as a generic feature of nanoparticles possessing carbogenic core [28]. On changing the excitation wavelength from 300 to 350 nm, with a continuous increase of 10 nm, emission wavelength also changes slightly. There is a nominal change in the intensity as well as emission spectra broadened. It is observed that, the PL emission of the wsCNDs ranges throughout the visible region of 340 to 600 nm with maximum intensity occurring at 436 nm against the excitation wavelength of 330 nm as shown in (Fig 5). The origin of PL emissions in CNDs still remains a big mystery and highly controversial with different explanations due to various synthetic approaches and numerous precursor ingredients along with multiple complicated components and structures. Tuneable emissions of wsCNDs reflect the effect from different surface emissive traps and particle size. Previous reports indicated that the polar surface functionalities such as hydroxyl and carboxylic groups dominated the "giant red-edge effect" and hence attributed the excitation dependent emission behavior of CNDs [29]. Zhang and coworkers proposed that oxygenous functional groups lead to the defects and furthermore their respective energy emissions in the wide regions of spectra [30]. In another report by Demchenko and Dekaliuk the CND was proposed as an assembly of integral quantum emitters (anisotropic arrangements of aromatic and aliphatic carbon domains leading to generation of excitonic H-type aggregates) and tuneable emissions attributed to summation of their individual contributions [11]. No perceptible change in fluorescence in fluorescence emission intensity was observed after 3 hours under continuous irradiation of UV light. Significantly, the aqueous solution of the wsCNDs exhibited good photostability, indicating good luminescence properties, and their appearance remains unaffected, when kept in aqueous solution no agglomeration was observed and also fluorescence intensity remains unaffected.

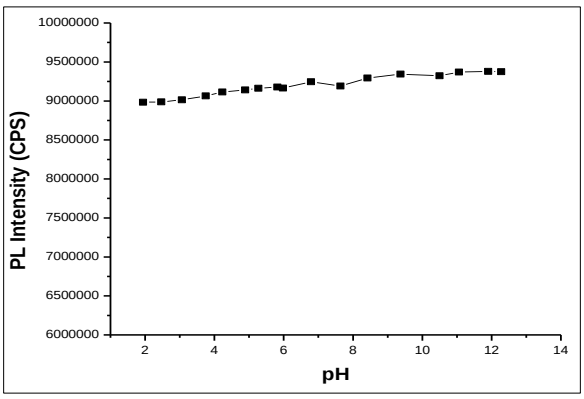

Fig 5 Variation of PL intensity with pH of wsCNDs

(Fig 5) shows the fluorescence curves of the wsCNDs at different pH values. It is seen that an increase in pH from 1.5 to 12 results only a slight change in PL intensity. This indicates that the CNDs are stable within a wide range of acidic as well as basic conditions.

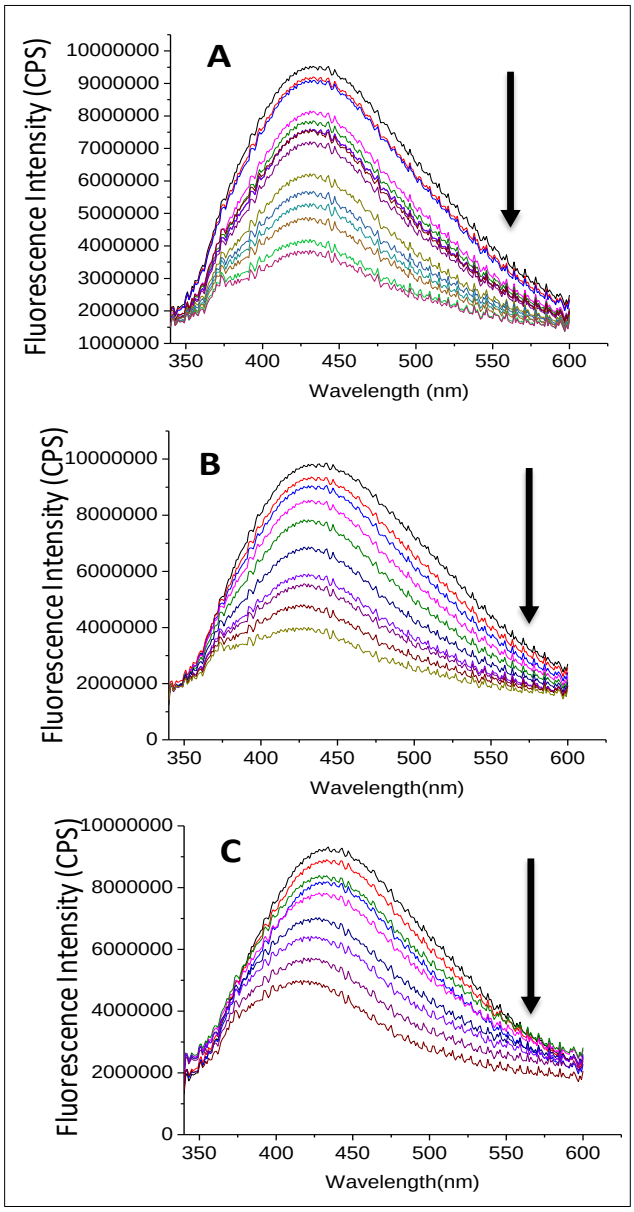

Fig 6 PL spectra of EDTA capped wsCNDs in the presence of different metal ion concentration (A) Ca^{2+} (B) Mg^{2+} and (C) Cu^{2+}

Detection of metal ions

The feasibility of using these wsCNDs for metal ion detection has been explored. It is seen that wsCNDs exhibits a strong fluorescence emission peak at 436 nm. We have selected some of the bivalent meal ions such as Ca^{+2}, Mg^{+2}, Cu^{+2} these metals are among some of the important components having active roles in many biological processes. At the same time Ca and Mg are also known to have important roles in causing hardness and softness of water. For this study as mentioned in EDTA functionalized carbon dot solution, metal ions solution such as Cu^{2+}, Ca^{2+} and Mg^{2+} is added gradually in the concentration range of 0µM to 1.6 µM. It was observed that the addition if metal ions leas to quenching of fluorescence intensity as shown in the (Fig 6A-C) respectively. Hence these three metal ions could be easily detected by the sensor system developed.

The fluorescence quenching data follows the Stern-Volmer equation, via either a dynamic or a static mechanism:

$$F_0/F - 1 = K_{SV}C$$

Where; K_{SV} is the Stern-Volmer quenching constant, C is the analyte (metal) concentration and F_0 and F are the PL intensities of EDTA functionalized wsCNDs at 436 nm in the absence and presence of metal ions.

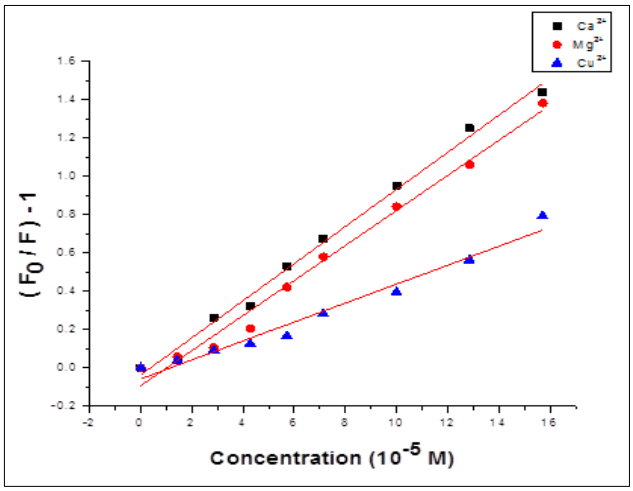

Fig 7 Linear fitting of Stern – Volmer plot for the quenching of EDTA functionalized wsCNDs PL by different metal ions (excitation at 360 nm; F_0 and F are wsCNDs fluorescence intensities at 436 nm in the absence and present of metal ions, respectively)

The linear fitting of the Stern-Volmer plot has been shown in (Fig 7). The correlation coefficients (R^2) are 0.99, 0.98, 0.96 over different linear concentration ranges for Ca^{2+}, Mg^{2+} and Cu^{2+} ion respectively. From the plot (Fig 12), the slopes of linear fits gave the Stern –Volmer quenching constants of 0.96×10^4 M^{-1}, 0.91×10^4 M^{-1} and 0.49×10^4 M^{-1} for Ca^{2+}, Mg^{2+} and Cu^{2+} respectively. These quenching constants are the binding constants for interaction of metal ions with the EDTA functionalized wsCNDs. The higher valued constants suggest that a much stronger interaction or complex is formed between the wsCNDs and metal ions.

Mechanistic interpretation

We also tried to look into the mechanistic interpretation of the observation. This observation can be attributed to that the metal ion can quench the fluorescence of the wsCNDs presumably via electron or energy transfer. In case of Cu^{2+} is a paramagnetic ion with an unfilled d shell and as a result, quench the fluorescence of wsCNDs via electron or energy transfer [31]. Most probably the mechanism of quenching is followed by photoinduced electron transfer (PET) due to presence of EDTA in the sensor system [32].

CONCLUSION

In this work we demonstrate for the first time that pyrolysis assisted treatment of bottle gourd peel is an effective strategy for producing fluorescent carbon nanodots (wsCNDs). Furthermore, this synthesis process offers the advantages of simple, cost efficient and sustainable as it uses a waste to synthesize carbon nanodots. The CNDs have average particle size of 7 nm. Such CNDs have been further modified by functionalized with EDTA and used as a novel sensing probe for sensitive detection of bivalent metal ions Ca^{2+}, Mg^{2+} and Cu^{2+}. The minimum detectable limit is ~ 1.6 µM. Development of such cheap sensor system will lead to scalability in terms of green production of fluorescent and biocompatible nanocarbons which could be applied in bioimaging and other applications.

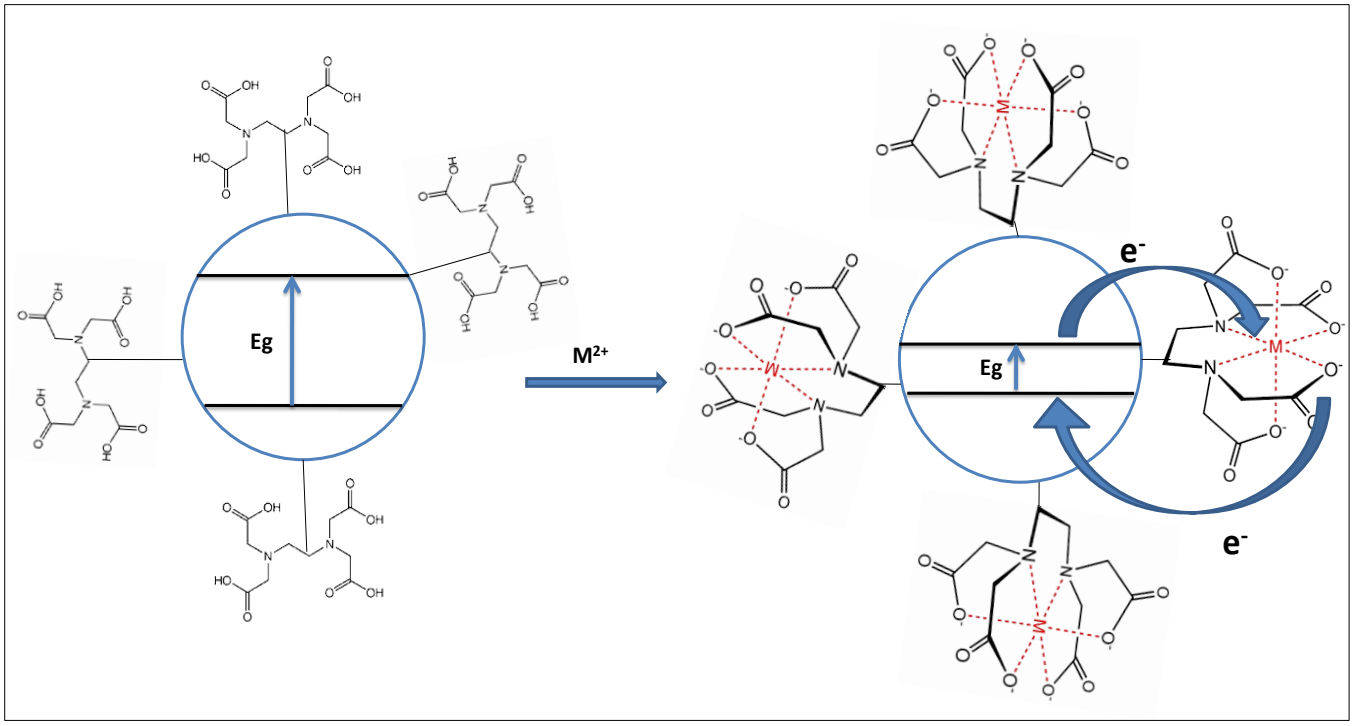

Fig 8 Mechanistic insight of EDTA functionalized wsCNDs for sensing of metal ions

Acknowledgement

We acknowledge TEQIP project No. ASTU/TEQIP-III/Collaborative Research/2019/3605) and No. ASTU/TEQIP-III/Collaborative Research/2019/2417) for research grants. We also thank Dr. Sonit Kumar Gogoi of Gauhati University for XRD measurement and Dr. Sristi Majumdar and Jayanta Sarma Baruah of IASST (Institute of Advanced Study in Science and Technology) for DLS data.

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