

# Carbon Dots as Selective Fluorescent Probes for Metal Ions-Influence of *Moringa Oleifera* Leaf as a Precursor

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## Abstract

The work presents a one-step green synthesis of green luminescent C-dots using *Moringa Oleifera* leaves as precursor which has not been explored as a source or precursor for the fabrication of the lowest dimensional carbon material. The presence of calcium ion in the bulk structure and the surface passivated functional groups of the C-dot with a size of 1-2 nm were well correlated using extensive spectroscopic tools. The as-prepared C-dots were investigated as a fluorescent probe for detection of heavy metal ions such as Fe(III), Hg(II), Cu(II), Pb(II), Cr(VI) and As(III). The C-dots were extrapolated as a potent turn-off sensor for Cr(VI) ion in the range of 0.05-10 mM. The study provided a new approach to the application of *Moringa Oleifera* leaves for nanotechnology.

**Keywords:** Carbon quantum dots, Green fluorescence, *Moringa Oleifera*, Sensing metal ions, Turn-off fluorescence and Water-soluble.

## 1. INTRODUCTION

Carbon based nanomaterials are a great field of interest in recent days of research. Based on their dimension, CBNs are classified as Carbon nanotube (CNT) (1D), Graphene oxide (GO) (2D), and Graphene quantum dots (GQD), Carbon nanodots (CD) and Fullerene (0D). Among these, Carbon quantum dots, a zero-dimensional Carbon-based nanomaterial has gained attention due to its size tunable surface properties such as photoluminescence behaviour, conductivity, excellent biocompatibility, aqueous solubility, and its functionalization ability [1] and multi-dimensional applications [2].

The CDs are generally synthesized via either top-down or bottom-up approach methods [3]. The green synthesis of CD by choosing a biomass as a source of Carbon is booming in the field of research because of its abundance, easily accessibility, low-cost and environmental friendliness. The use of plant materials such as coriander leaves [4], *Syzygium Cumini* fruit [5],

*Manilkara Zapota* fruit [6], Mango peel [7], *Mangifera indica* leaves [8], pomegranate peel [9], groundnut shells [10], *Hibiscus Sabdariffa* leaves [11] and several biowastes [12] have been reported by the researchers.

In this study we report a one-step thermal Carbonization of plant leaves extract for the synthesis of CDs [13]. *Moringa Oleifera* (MO) leaves is chosen as an innocuous (benign) source of Carbon as the material is rich in content of Carbon along with several micro- and macro nutrients (Calcium, Magnesium, Phosphorus, Sulphur, Sodium, Cobalt, Potassium, Manganese, Iron, Copper, Zinc and Selenium) [14].

The salient features of the leaves of the *Moringa Oleifera* are many in terms of their antioxidant activity. In fact, all parts of the tree are explored for medicinal applications. In today's context, the conversion of active biomass materials rich in Carbon and Nitrogen into quantum dots

is gaining importance for multidimensional applications. It is observed that the leaf of *Moringa Oleifera* has not been tapped for its potential source or a precursor for the preparation of Carbon-based nanomaterials especially Carbon dots. Upon consideration of the above factors, the present study explored the process of converting the leaves into thermodynamically, photo stable C-dots well suitable for sensing heavy metal ions and bio-imaging studies.

The results of the study will add value to the conversion of biomass to C-dots for the removal of heavy metal toxicity and for the environmental protection. The presence of heavy metal ions such as  $\text{Fe}^{3+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Co}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$  in the environment is a major threat to the ecological system and it also imposes health hazards. The permissible limit of some heavy metal ions in drinking water according to World Health Organization (WHO) report of 2004 is  $\text{As}^{3+}$  (0.05 ppm),  $\text{Pb}^{2+}$  (0.05 ppm),  $\text{Cr}^{6+}$  (0.05 ppm),  $\text{Cu}^{2+}$  (1.5 ppm),  $\text{Hg}^{2+}$  (0.001 ppm) and  $\text{Cd}^{2+}$  (0.005 ppm) respectively [15, 16]. Hence, there is an urgent need for developing a low cost, effective and biologically safe sensors for detecting the heavy metal ions in aqua systems, human body, and blood-plasma level within permissible limits. The fluorescent behaviour of the Carbon dots enables its use as a probe in the field of sensing and this sensing ability of CDs makes it as a heavy metal sensor. The selectivity and sensitivity of the C-dots towards target ions and molecules largely depend on the surface functionality of C-dots [17].

In today's context, various electroplating, textile and leather industries discharges hexavalent Chromium ( $\text{Cr}^{6+}$ ) ion as an effluent which is one of the major threatening metal ions causing land and water pollution. Due to its high solubility,  $\text{Cr}^{6+}$  causes adverse effects in living systems [17]. Besides many conventional techniques of detection and removal methods of heavy metal ions, the CDs are being used as fluorescent probe for the detection and removal of heavy metal ion

because of their excellent solubility and hydrophilicity properties. Among them, the CDs derived from green precursors such as groundnut [10], *Hibiscus Sabdariffa* [11], jute caddies [18] and denatured milk [19] were used as fluorescent sensor for the detection of Chromium ion because of their surface functionalities. In the present study, highly soluble self-passivated CDs were derived from plant biomass which is further explored as a potent fluoroprobe for the selective and sensitive detection of hexavalent Chromium.

## 2. MATERIALS AND MATERIALS

### 2.1. Materials Required

The fresh *Moringa Oleifera* leaves were collected from the local area of Gummidipoondi, Tiruvallur district of Tamil Nadu. The chemicals used for the synthesis and applications of Carbon quantum dots were Potassium dichromate (Nice chemicals), Sodium hydroxide (Isochem) and Glacial acetic acid ( $\text{CH}_3\text{COOH}$ ). The chemicals were used without further purification.

### 2.2. Preparation of Aqueous Leaf Extract

The collected leaves were washed to remove dirt, shade dried and ground to fine powder. The water extract was prepared by stirring the dried powder with water in the ratio of 1/10 (w/vol) on a magnetic stirrer for 15 minutes. The extract was filtered through a Whatman no.1 filter paper and the filtrate used for further experimental procedures.

### 2.3. Green Synthesis of Carbon-Nano Dots

The C-dots were prepared by concentrating (thermal Carbonization) 10 ml of the prepared extract (pH=5.6) at  $150^\circ\text{C}$  for 4 hours in a Hot air Oven. The reaction resulted in black solid powder which was completely soluble in water. The reaction conditions such as temperature, time and pH conditions were optimized for the synthesis of C-dots by carrying out the

reaction at different time, temperature and pH. The optimized pH to carry out the synthesis was found to be 5.6. On changing the reaction to acidic/alkaline conditions, it tends to result in liquified product and this change in nature of the product is due to the presence of amino acids (AA) in the precursor. Since all the AA exist as Zwitter ions (neutral form) in the pH of around 5-6, the decrease/increase in the pH of the extract tends to change the AA form into a positive/negative ion which greatly affected the nature of interactions with other moieties in the precursor in the formation of the CDs.

The as-synthesized Carbon dots were analysed for the presence of heteroatoms and their nature of binding using Fourier-Transform Infrared analysis (IR Affinity-1). Further X-ray Photoelectron Spectroscopic (XPS) (Ulvac-Phi, Inc.; Model: PHI5000 Version Probe III) study was done to confirm the presence of surface functional groups. The structural morphology and its size were analysed through Transmission Electron Microscopic (Joel) image. The interlayer spacing of the Carbon dots were calculated from the Powder X-ray Diffractogram (PXRD) data using a Cu-K $\alpha$  radiation (PAN analytical model Xpert Pro). The size of the prepared CDs was calculated using the Debye-Scherrer equation. The absorption (UV-SHIMADZU 1601) and fluorescence (Agilent, Cary Eclipse) spectra was obtained to study the luminescent behaviour of the CDs. The Raman Spectra for the prepared Carbon dots were recorded on a Micro-Raman spectrometer (Bruker, RFS). The lifetime analysis of the aqueous CD solution in the presence and absence of metal ion was done using the Horiba Jobin Yvon-Fluorolog F3-111. The thermal stability of the prepared C-dots was studied by performing thermal analysis using a NETZSCH STA 2500 TGA-DTA setup. The  $^{13}\text{C}$  NMR was recorded for the reaction intermediate isolated after 3 hours

of reaction time using Bruker 400 MHz EXT41122.

#### 2.4. Fluorescent Studies of the C-Dots

The emission behaviour of the prepared C-dots was evaluated by carrying out the fluorescence spectral studies at various excitation wavelength ( $\lambda_{\text{ex}} = 330\text{-}400\text{ nm}$ ), different pH conditions (pH=3,5,7,9 and 11) and various concentration of CD solutions (250 ppm, 500 ppm, 750 ppm and 1000 ppm). The photostability of C-dots which is a significant factor was also investigated by recording the PL spectra of the prepared CD solution before and after continuously irradiating the solution under UV source for 30 minutes. The aqueous solution of the CD was stored for 21 days and its PL spectra were recorded at 7 days interval to study its aqueous stability nature.

#### 2.5. Heavy Metal Ion Sensing

The metal ion sensing ability of the prepared CDs was estimated by monitoring the emission intensity of the CD solution in presence of different metal ions ( $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cr}^{6+}$ ,  $\text{As}^{3+}$ , and  $\text{Hg}^{2+}$ ) in the ratio of 1ml CD:3ml metal ion solution at an excitation wavelength of 350 nm. The selectivity of CD towards hexavalent Chromium was further studied to evaluate the sensing ability of CDs. About 1 ml of  $\text{Cr}^{6+}$  ion solution of various concentration (0.05 mM-10 mM) was added to 3 ml of the prepared CD solution (500 ppm) and its fluorescence intensity was recorded at an excitation wavelength of 350 nm. The sensing experiment was performed at three different pH conditions (pH=3,7 and 11) to find out the influence of pH in the sensing study.

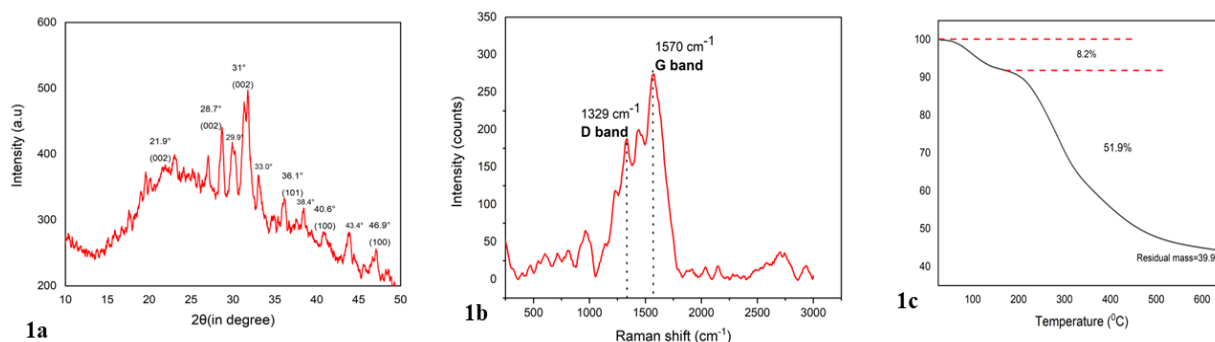
### 3. RESULTS AND DISCUSSION

The PXRD pattern of synthesized CDs (Fig.1a) shows a broad diffraction peak corresponding to  $2\theta$  centred at  $21.9^\circ$ ,  $28.7^\circ$  and  $31^\circ$  relative to (002) plane of carbon which attributes the presence of graphitic lattice [3]. The d-spacing was determined

to be 0.335 nm which is found analogous to graphite inter-layer spacing [20, 21]. The sharp peak at  $2\theta=29.9^\circ$  and  $43.4^\circ$ , and  $2\theta=33^\circ$  and  $38.4^\circ$  represents the presence of calcium in the form of Carbonate and oxide [22, 23] which can be further evidenced using EDS data. The average crystallite size of the CDs is 1-2 nm, calculated using Scherer equation assuming that the shape of the crystallites to be spherical.

Micro-Raman spectroscopy for Carbon based nanomaterials is extensively used in material recognition and to study lattice dynamics and vibrational characteristics of  $sp^2$  hybridized carbons [24]. The Micro-Raman spectrum of CDs (Fig.1b) shows a D band (disorder) at  $1329\text{ cm}^{-1}$  and a G band (graphitic) at  $1570\text{ cm}^{-1}$ . The intensity of G band is higher than that of D

band indicating the presence of more  $sp^2$  Carbon atoms than  $sp^3$ . Thus, the CDs are composed of  $sp^2$  graphitic and  $sp^2$  carbonyl groups (COOH, COO, CONH etc.,) with the mixture of  $sp^3$  Carbon defects including  $sp^3$  Carbon-based functionalities such as CHO, CHOH, etc., [25] This type of more  $sp^2$  Carbon atoms and graphitic structure is due to the richness of amino acids in the precursor which is well proven by the formation of Carbon dots from amino acids individually. The absence of 2D peak in the obtained spectra eliminates the choice of graphene quantum dots [3] and confirms the presence of Carbon dots [26]. The  $I_D/I_G$  ratio was found to be 0.68, indicating the amount of defect density which arises due to the presence of heteroatoms and Oxygen rich functional groups.



**Figure 1.** a, b, c) X-ray diffractogram, Micro-Raman spectrum, and TGA of the as-synthesized Carbon dots.

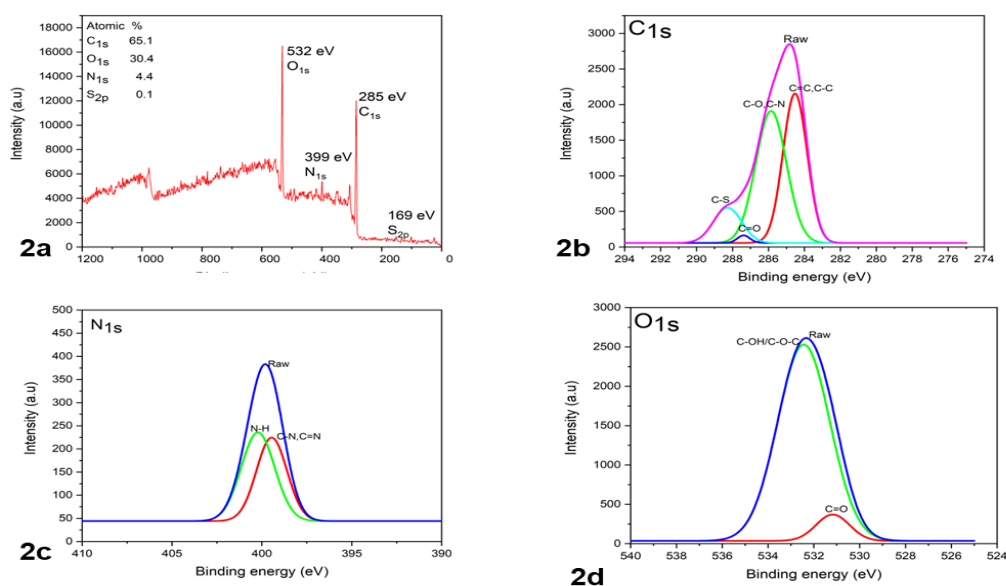
The functional groups of the synthesized Carbon dots were identified through the FT-IR spectrum which was then compared with the FT-IR spectrum of dried MO leaves powder. The appearance of broad band at  $3277\text{ cm}^{-1}$  is attributed to the stretching vibration of -OH and -NH which indicates the presence of a hydrophilic environment on the surface [10]. The peak observed at  $2917\text{ cm}^{-1}$  in the precursor arises due to the stretching vibration of C-H bond. The appearance of strong absorption peak at  $1629\text{ cm}^{-1}$  in the precursor is due the vibrational absorption of C=C bond. The absence of C-H bond peak and shift in C=C bond peak to  $1589$

$\text{cm}^{-1}$  in the prepared C-dots arising due to cyclic alkenes indicates the occurrence of aromatization process in the formation of C-dots. The appearance of strong peak at  $1407\text{ cm}^{-1}$  in the precursor which is shifted to  $1386\text{ cm}^{-1}$  in the synthesized CDs is ascribed to S=O bond. The peak at  $1000\text{--}1100\text{ cm}^{-1}$  indicates the stretching mode of C-O and C-N group.

The chemical states of the elements in the prepared dots were analyzed through the XPS spectra. From the wide scan spectrum (Fig.2a), the presence of peaks at the binding energy of 283.4 eV, 399.8 eV, 531 eV and 169 eV confirm the presence of Carbon, Nitrogen, Oxygen and Sulfur at

a percentage of 65.1, 4.4, 30.4 and 0.1 respectively. The high-resolution spectrum of C<sub>1s</sub> (Fig.2b) shows the presence of COOH, C=O, C-O/C-N/C-S, and C=C bonds at the binding energies of 288.3, 287.3, 285.8 and 284.5 eV respectively. The N<sub>1s</sub> spectrum (Fig.2c) consists of two peaks at 400.2 and 399.4 corresponding to N-H and C=N or C-N bonds. The peaks at 532.4 and 531.1 in the O<sub>1s</sub> spectrum (Fig.2d) corresponds to C-OH/C-O-C and C=O bonds respectively. Thus, the XPS

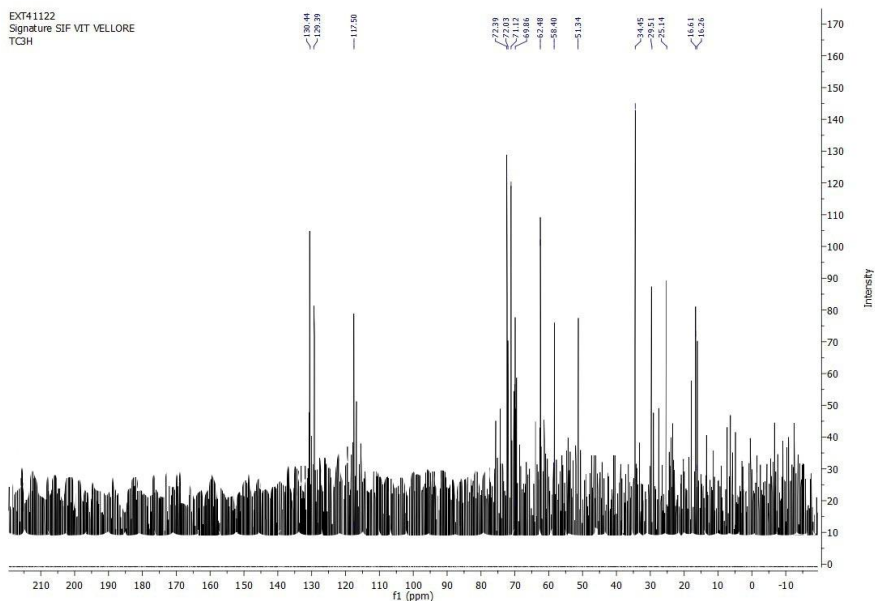
spectra clearly revealed that the CDs contains Carbon, nitrogen, sulfur and Oxygen on its surface. The presence of C=C peak strongly indicates that CDs are composed of graphitic domains [25] which is in accordance with the Micro-Raman spectrum. The presence of Oxygen functionalities creates more defects on the surface of CD as confirmed by the XPS which also correlates well with the FT-IR reports.



**Figure 2.** a) Wide scan XPS spectrum and b, c, d) High resolution spectra of C<sub>1s</sub>, N<sub>1s</sub> and O<sub>1s</sub> of the prepared CDs.

The content (in percentage) of Carbon, Hydrogen, Nitrogen, Sulfur and Oxygen (calculated) in the precursor and C-dots were found to be 40.785 and 36.858, 4.3 and 3.509, 6.154 and 5.645, 0.999 and 1.587, and 47.762 and 52.401 respectively from the elemental analysis. The <sup>13</sup>C NMR spectra of the reaction intermediate which had been isolated after three hours of reaction time (Fig.3) shows three different regions *viz.* **0-50 ppm (for sp<sup>3</sup> Carbons)**, 50-100 ppm for Carbons attached with Oxygen and Nitrogen groups and **100-150 ppm (for sp<sup>2</sup> Carbons or C=C aromatic)**. The absence of C=O peak in the FT-IR of

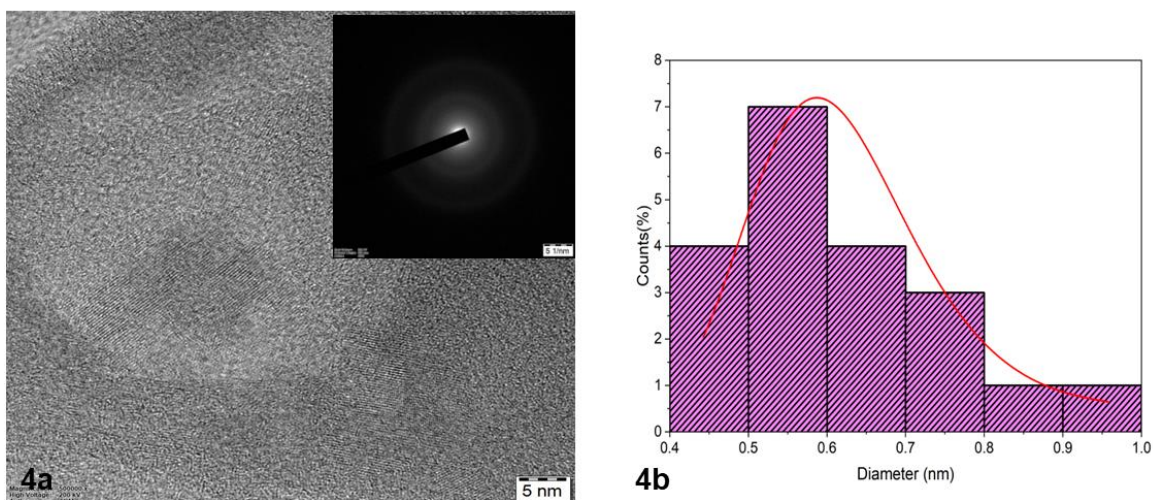
the prepared C-dots is well supported by the NMR data. The peaks at 50-100 ppm were mostly due to C-O than C-N which is evident from the elemental analysis of the CDs on comparison with the precursor. The presence of peaks at 117.5, 129 and 130 ppm confirm the formation of C-dots through aromatization which also correlates well with the FT-IR (vibrational absorption peak at 1589 cm<sup>-1</sup>) and Raman spectrum (high intense G band indicates that the CDs possess more amount of sp<sup>2</sup> Carbons).



**Figure 3.**  $^{13}\text{C}$  NMR of the reaction intermediate.

The surface morphology of the prepared CDs was assessed from TEM analysis. The image (Fig.4a) shows that the CDs are quasi-spherical in shape and the average size of the CDs is found to be 0.6 nm distributed in the range of 0.4-1 nm (Fig.4b). Selected area electron diffraction (SAED) pattern of the CDs (Fig.4a inset) comprises a diffused halo devoid of rings with diffraction spots, indicating the graphitic areas of the synthesized CDs.

The Energy Dispersive X-ray Spectrum (EDS) was used to identify the elemental abundance and composition of the CDs [20]. EDS elemental mapping distribution shows the presence of Carbon, Oxygen, nitrogen, sulphur, magnesium, calcium, and phosphorus in CDs which correlates well with the distribution of various functional groups on the surface of CDs and with the elemental analysis data.



**Figure 4.** a) HRTEM image with its Selected Area Electron Diffraction (SAED) pattern (inset) of MO derived CDs and b) Size distribution curve of the C-dots.

The CDs exhibit excellent thermal stability which is evident from its TGA

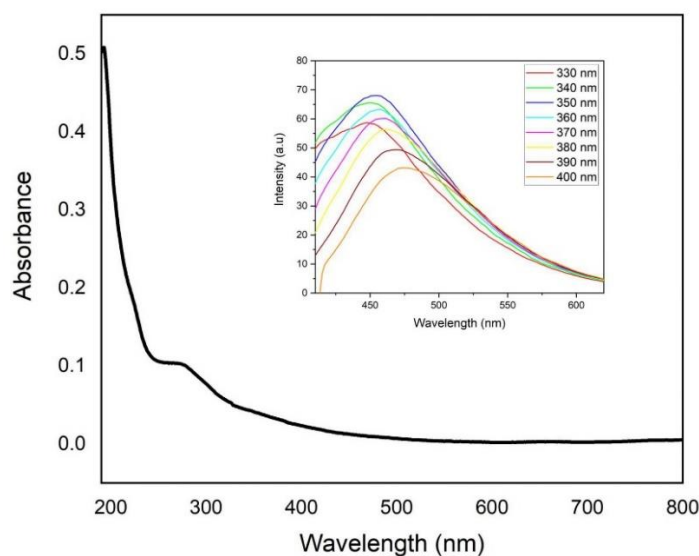
graph. From the TGA graph (Fig.1c) it is evident that the weight loss below 200°C

corresponds to dehydration and evolution of pyro-gases such as Carbon dioxide and Carbon mono-oxide from the CDs surface. The weight losses in the range of 200-350°C may be due to the evolution of gasification products from different functional groups (COOH, CONH, NH<sub>2</sub>) from the CDs. The major decomposition occurred in 300-450°C range which is due to the degradation of phenyl-like aromatic rings and pyridine-like and pyrrole-like rings.

### 3.1. Photo Luminescent Behavior of the CDs

The UV-Visible spectra (Fig.5) show an absorption band at 193 nm and a small hump at 271nm. Since the raw material is

made up of a lot of flavonoids, these two absorption peaks are due to  $\pi$ - $\pi^*$  electronic transition of C=C bond in the aromatic domains and n- $\pi^*$  electronic transition of C=O existing on CDs surface [27]. The absorption band around 260-270 nm corresponds to amino acids present in the precursor which is not observed in case on non-amino acid mediated Carbon dots. It shows that the amino acids are efficiently involved in CD formation. The direct optical band gap energy of the prepared CDs was found to be 4.57 eV [28]. This high value of band gap energy was due to the electronic states with well-defined conjugate aromatic structure and surface passivation.



**Figure 5.** UV-Absorption spectrum and excitation dependent PL behavior (inset) of the prepared Carbon dots.

The present study also investigated the tunable emission property of CDs based on the excitation wavelength. The maximum emission wavelength ( $\lambda_{em}$ ) for the prepared CDs was found to be 454 nm while exciting at 350 nm ( $\lambda_{ex}$ ). With increase in excitation wavelength, the emission peak was shifted to higher wavelength (red shift) which is clearly shown in Fig.5 inset. This excitation dependent behavior of the CDs is due to their size effects or surface

defects. The size effect (variation in size of the CDs resulting in distribution of various particle size) affects the number of particles being excited at particular wavelength which in turn affects the position and intensity of emission peak [29]. The presence of more Oxygen containing functional groups on the surface of synthesized CDs (surface defects) causes interaction between surface functionality and  $sp^2$  Carbon core which

results in surface emissive traps. On exciting the CDs at a particular wavelength, a surface emissive trap becomes dominant. Thus, on changing the excitation wavelength another corresponding surface emissive trap will become dominant [29].

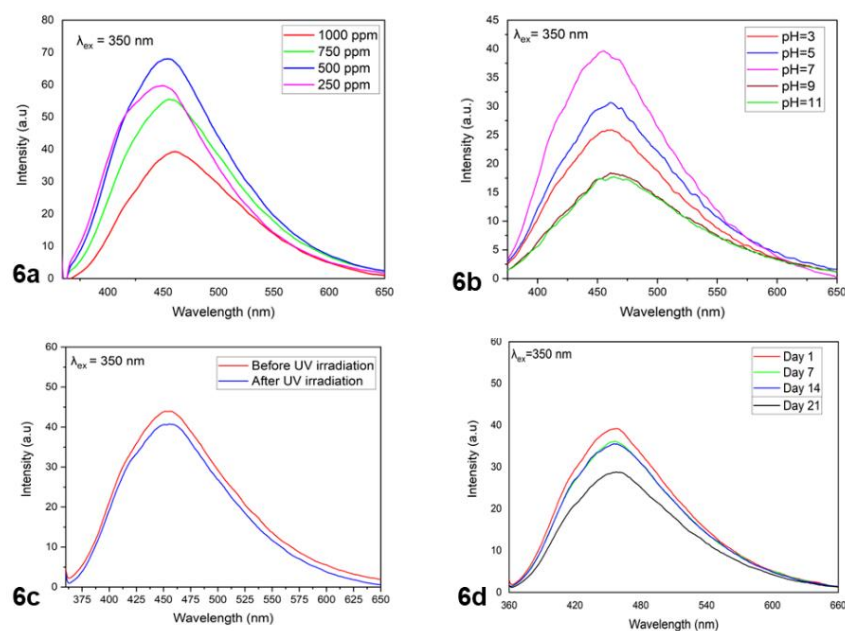
The tunable fluorescence of the C-dots in the green region arises due to the formation of additional inter-band (defect induced or interstate emission) between HOMO-LUMO which is evidenced by the calculation of the direct band gap energy from maximum excitation wavelength [30]. From the maximum excitation wavelength ( $\lambda_{ex}$ ), the lowest direct absorption edge of CDs was found to be 3.5 eV. It is noted that the value of optical band gap energy of CD is greater than its excitation energy indicating the formation of an inter-band between HOMO and LUMO. Thus, on increasing excitation wavelength, the red shift occurs because the excitation energy is decreased and the transition is from HOMO to inter-band relating to excitation dependent PL behavior. In the present study, as the excitation wavelength is lower than the maximum excitation wavelength (350 nm) (Fig.6 inset) higher excitation energy is applied hence the transition occurs between band to band (HOMO-LUMO) which was not an excitation dependent PL behavior as reported by Julin Joseph et.al. who prepared C-dots from tannic acid [26].

The intensity of the PL spectrum of the prepared CDs is concentration dependent (Fig.6a) with 0.5 mg/ml (500 ppm) solution showing maximum emission intensity. The intensity of the PL spectra decreases with increase in concentration of the CDs which may be due to the agglomeration of polar functional groups on the surface of CDs at higher concentrations. At lower concentration, the interaction between the polar functional groups on the surface of the CDs is decreased which results in higher intensity of the PL spectrum [24].

As seen in the Fig.6b, the intensity of the PL is weak under both acidic and alkaline conditions. The pH sensitive behavior of the CDs explicitly arises due to the protonation and deprotonation of the Oxygen-containing groups on its surface [31]. The PL intensity of the prepared CDs increases when the pH is increased from 3 to 7 and decreases at higher pH accompanying a little red shift. The protonation of the carboxyl and hydroxyl groups leads to a broadening in the energy level (changes in  $\pi-\pi^*$  and  $n-\pi^*$  electronic transition) of the CDs hence the fluorescent intensity increases without a shift [32]. When the pH is increased from 7 to 9, the deprotonation of the functional groups happens which results in decrease in the intensity and red shift of the emission peaks. The maximum PL emission intensity is recorded at neutral pH (pH=7) hence the prepared CDs is safe at the physiological pH [29] which extends its use in the field of biomedical applications such as bio-imaging and pH sensors.

The photostability property of the C-dots is an important factor for their catalytic and biomedical application such as drug development and bioimaging applications [4]. In the study, the as prepared samples after continuous irradiation with UV light for 30 minutes, retained about 94% emission intensity (Fig.6c) which establishes its nature of high photostability. The excellent photostability of the prepared CDs can be further extended for its use in intracellular deep tissue penetration bioimaging studies. The stability and the shelf life of the CDs are also evidenced by measuring its fluorescent intensity (Fig.6d) in aqueous solution. The retention of about 90% of its photoluminescent intensity of the CDs after 14 days and 50% after 21 days. The results are indicative of the superior quality of the CDs sample in terms of stability for further multidimensional applications.





**Figure 6.** a, b) Concentration dependent and pH sensitive fluorescent behavior of the prepared CDs and c, d) Photostability and Aqueous stability of the synthesized CDs.

### 3.2. Sensing of Cr<sup>6+</sup> Ion

The conventional techniques viz:- ultraviolet–visible spectrometry, atomic absorption/emission spectroscopy, X-ray absorption spectroscopy, inductively coupled plasma mass spectrometry, Auger electron spectroscopy, stripping voltammetry and polarography for heavy metal sensing require sophisticated instrumentation, use of toxic chemicals, tedious process restrict their usage in practical applications [12]. Hence, the development of CDs as fluorescent probes for the detection of heavy metal ions, organic and inorganic contaminants, dye molecules, pharmaceuticals as an alternative method have been developed by various research groups [33] in recent days. It is due to the fact that CDs show strong photoluminescent behavior, easy surface-functionalization, good aqueous solubility, biocompatibility, low-cost and environmentally friendly nature.

In the study, the ability of the prepared CDs as fluorescent probe in sensing application is evaluated by their response in their fluorescent intensity with respect to the presence of different heavy metal ions. Fig. 7a shows the variation of fluorescent intensity of CDs (0.5mg/ml) with different

metal ions 6 metal ions (Cu<sup>2+</sup>, Pb<sup>2+</sup>, Hg<sup>2+</sup>, Fe<sup>3+</sup>, Cr<sup>6+</sup> and As<sup>3+</sup> (each at a concentration of 10mM). Among the metal ions considered in the study, there is significant quenching with respect to Fe<sup>3+</sup> and Cr<sup>6+</sup> ions. It may be attributed to the coordination between the metal ions and the functional groups on CD surface similar to previous reports [34,35]. Also, there is higher selectivity of the CDs towards Cr<sup>6+</sup> ions than other metal ions.

The enormous use of highly toxic and carcinogenic Chromium in leather tanning, textile and metallurgical industries imposes a major threat to the environment. Very few studies have been reported for the optical sensing of Chromium using biomass derived C-dots [12, 17]. Even the reported studies involve either tedious synthesis procedure or involves adding surface passivating agents [35,36]. Hence, the present study is unique in its way as the preparation of the CDs involves a very simple single step process without any additional agents. Also, the prepared CDs exhibit excellent concentration and pH dependent fluorescent behavior. Hence, they can be used as a selective and sensitive fluoroprobe for Chromium sensing studies by observing the change in

emission intensity at an excitation wavelength of 350 nm.

At neutral pH, the emission intensity of the aqueous CD solution (absence of Cr<sup>6+</sup>) and the change in fluorescent intensity of CDs on adding different concentration of Cr<sup>6+</sup> in the range of (0.05-10mM) is recorded. The fluorescence intensity of the C-dots was quenched in a concentration-dependent manner upon addition of Cr(VI) ions (Fig.7b). This fluorescence quenching of the C-dots occurs by either electron donor-acceptor phenomena [34], as the prepared CDs contain more electron donating groups that may donate electrons to the electron deficient metal ion where the electron-hole recombination results or by Inner Filter Effect, as the absorption spectrum of chromate ion solution overlaps with the excitation and emission spectrum of the C-dots.

Even though the prepared CDs effectively sense the metal ion at extremely acidic, neutral and alkaline conditions (pH=3, 7 and 11) it was found that the neutral pH was favorable which correlates with our pH variation reports. Since most of the sensing experiments were reported at neutral conditions, the prepared CDs were able to detect the metal ion at all different situations which is of greater advantage in sensing technology.

The quenching efficiency of the CDs can be further described using the Stern-Volmer (SV) relationship [4]. The SV equation is as follows:

$$I_0/I = 1 + K_{SV}[Q]$$

where I<sub>0</sub> and I are the fluorescence intensities of the CDs in the absence and presence of quencher (metal ion), K<sub>SV</sub> is the Stern-Volmer constant/quenching constant/slope of the linear fit and [Q] is the concentration of quencher (metal ion).

The plot between (I<sub>0</sub>/I)-1 versus concentration of Cr<sup>6+</sup> is shown in Fig.7c in which the prepared CDs exhibited a good linearity in the range of 0.05-1 mM with a correlation coefficient of 0.9985. The limit of detection (LOD) is of 1.28 mM

(0.001M) which is calculated via the following equation,

$$3\sigma/m$$

where σ is the standard deviation of the CD solution without Cr<sup>6+</sup> (n=5) and m is the slope of the linear fit.

The present work detects Chromium to an extent of 0.001M using self-passivated CDs derived from a very simple one-step synthesis. The detection limit of the as-synthesized CDs is comparable with the CD derived from *Hibiscus Sabdariffa* leaves [11]. Being self-passivated C-dots, the sensing limit for Cr(VI) is to an extent of 0.001 M which is a unique feature of the current study because the CDs derived from other sources such as groundnut [10], *Ocimum sanctum* leaves [33] and *Carica Papaya* waste pulp [34] involves surface passivation using external agents. Hence, with additional/external doping process there is a possibility to enhance the detection limit of the heavy metal ion. This sensing ability of the CDs can be further extended for detecting Chromium in industrial waste waters and other than organic/inorganic moieties too.

### 3.2. Quenching Mechanism of the Prepared CDs with Cr<sup>6+</sup>

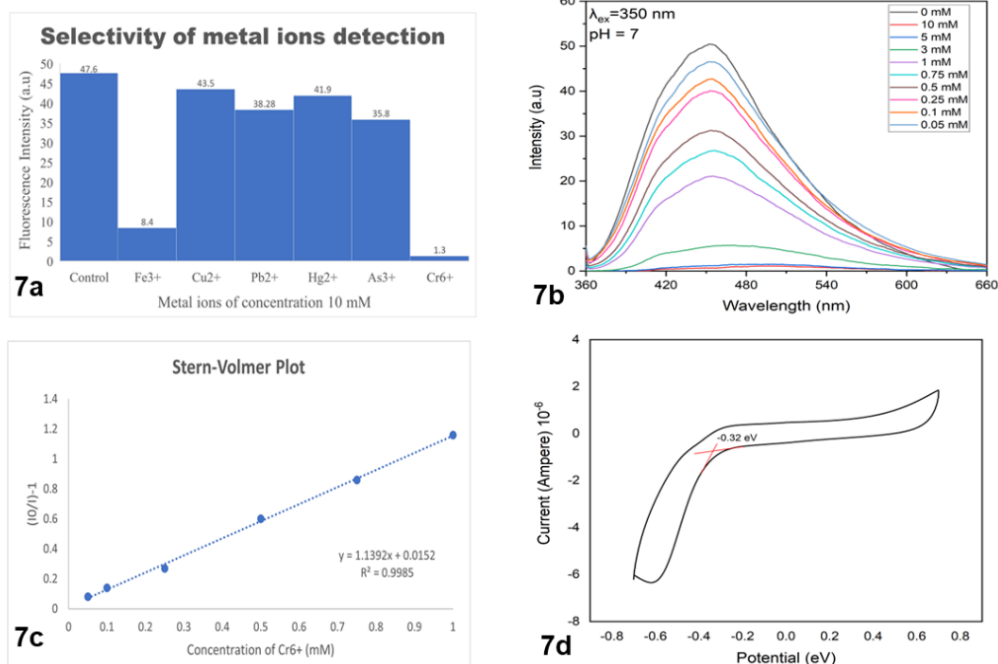
The lifetime of the fluorophore (CDs) in the absence and presence of quencher (Cr<sup>6+</sup>) was found to be 5.1 ns and 4.7 ns respectively. The change in lifetime rules out the possibility of static quenching and IFE mechanism. The linear Stern-Volmer plot of the prepared CDs confirms that the mechanism followed while sensing Chromium is dynamic quenching. To further investigate about the quenching mechanism, the lowest occupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO) energy levels of the prepared CDs are calculated using the following formula [25,31],

$$E_{HOMO} = -(E_{ox} + 4.4) \text{ eV} \quad (1)$$

$$E_{LUMO} = -(E_{red} + 4.4) \text{ eV} \quad (2)$$

$$E_{HOMO} = E_{LUMO} - E_g \quad (3)$$

Where  $E_{ox}$  and  $E_{red}$  are the oxidation and reduction potential of the CDs



**Figure 7.** a, b) Selectivity and Sensitivity of CDs towards Cr(VI) and c, d) Stern-Volmer plot of the CD solution on adding Cr(VI) and Cyclic voltammogram of the prepared C-dots.

The reduction potential of the prepared CDs is found to be -0.32 eV from the cyclic voltammograms (Fig.7d). The value of direct band gap energy ( $E_g$ ) is found to be 4.57 eV from the UV-absorption spectrum. Then using the above equations, the  $E_{LUMO}$  and  $E_{HOMO}$  were found to be -4.08 eV and -8.65 eV respectively. Based on the electrode potential values of metal ions from the Electrochemical series, it is observed that the HOMO/LUMO band gap of the as-prepared CDs overlaps with the electrode potential of ions of Hg<sup>2+</sup>, Cu<sup>2+</sup>, Pb<sup>2+</sup>, Co<sup>2+</sup>, Fe<sup>3+</sup>, Ni<sup>2+</sup> and Cr<sup>6+</sup> indicating that the electron-transfer between the CDs with the metal ions is energetically allowed and hence can be further explored.

The factors also confirm the occurrence of the photo-induced electron transfer from LUMO of as synthesized CDs to the orbitals of the Cr<sup>6+</sup> ions with comparable energy (possibility of  $d^2sp^3$  hybridisation involving 3d ( $e_g$  orbitals), 4s and 4p orbitals of Chromium). The electron transfer is facilitated through the coordination between the metal ion (Cr<sup>6+</sup>)

respectively.

and the functional groups on the surface of prepared CDs (non-radiative electron-hole recombination between the CD and metal ion) [36].

#### 4. CONCLUSION

The present study reported the use of a simple and inexpensive source as a starting material for the synthesis of highly water-soluble Carbon dots. The C-dots exhibits excitation wavelength dependent and concentration dependent fluorescent behavior in the 450 nm range. The CDs as a fluorescent probe for the detection of hexavalent Chromium ion via strong coordination and electron transfer has been developed which provides an easier way to quantitatively sense the heavy metal ion. The mechanism of quenching is well studied through the energy level calculation and the lifetime analysis. The pH dependent luminescent behavior of the CDs can be explored its use in biomedical applications such as bio-imaging, biosensors and drug delivery. The excellent photostability, aqueous solubility and

stability of the prepared C-dots can be further extended for its wider application in biomedical fields.

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#### CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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