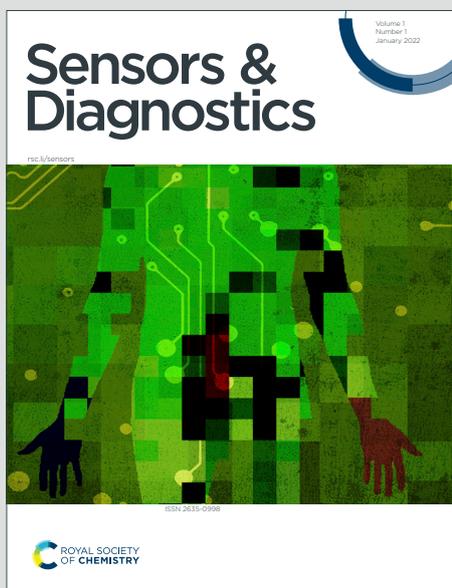


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ARTICLE

Selective sensing of heavy metal ions using carbon dots synthesized from *Azadirachta indica* seedsSomedutta Maity^b, Sathosh Kumar^b, Gurmeet Singh^a, Sukanya Patra^a, Divya Pareek^a, Pradip Paik^{*a}Received 00th January 20xx,
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There are notable advancements in the technology associated with using waste resources to create novel and beneficial products. Herein, it is demonstrated that the Kernel part of *Azadirachta indica* (Neem) seeds can be used in a sustainable way for the similar purpose. Carbon dots (CDs) of size ca. 4-8 nm were made from Kernel part *Azadirachta indica* seeds by calcinations followed by modification of their surface using diethylamine, sodium methoxide, and alcohol to produce waste seed-derived luminous surface-quaternized CDs (Ai-CDs). They were used as a fluorescent nano probe to detect the extent of inorganic contaminants from low (5 μM) to high concentration (120 μM) due to their strong photostability, excitation-dependent emission in aqueous solutions. Ai-CDs was used to measure extent of Cd^{2+} and As^{3+} in solution through the quenching of luminescence intensity ("turn-off"), and when compared to other metal ions, cupric ions (Cu^{2+}) selectively overpower fluorescence ("turn-on") for sensing. The current method of synthesis CDs offers the benefits of quick reaction times as well as great selectivity and sensitivity. The CDs can absorb Cd^{2+} and As^{3+} preferably which would cause a sharp dimming of fluorescence intensity by 27% and 30%, respectively, whereas for Cu^{2+} and Cu^+ the fluorescent intensity enhanced by . Consequently, this unique characteristic was utilized to exclude and identify these Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} and Cu^+ ions, with detection limits of 5 μM to 120 μM correspondingly. Further, we demonstrated the heavy metal ion sensing activity of CDs from their salt solutions to project CDs as environmental friendly metal ion detecting agent. The cell viability assay was carried out and reveals that the CDs are not toxic.

1. Introduction

In the present day, heavy metal ion has become a serious issue in rapidly developing countries.¹ Recently, there has been a lot of focus on the need for extremely effective methods to identify heavy metals. Cadmium (Cd^{2+}) and arsenic ions (As^{3+}) are two main causes of worry, among many others due to their high toxicity. The environment and ecological balance are diversely threatened by the heavy metal ions due to toxicity.² Henceforth, the identification of ions of heavy metals becomes a major focus for researchers.³ The heavy metals are with atomic weight greater than 20 with density⁴ greater than 5.0g cm^{-3} . Initial time it is believed that the heavy metal ions present in environment naturally and do not threaten the human body and ecology.¹ Due to the recent industrial revolution, the limit of heavy metals increased in rivers, lakes, and soils. Cd(II) is one of the such most hazardous heavy metal ions poisoning soil and water, mostly through industrial

waste and entering our food chain directly or indirectly.⁵ Bioaccumulation of heavy metals in the human body causes various acute illnesses, including neurological system and digestive system dysfunction, renal failure, bone weakness, degeneration of testicular tissues and red blood cells, and even creates genetic abnormalities.⁶ Once heavy metal accumulates in water sources or animals and plants, it enters the food chain which leads to its presence inside the human body.⁷ As soon as heavy metal ions enter the body they interact with enzymes and proteins, however it never metabolized by the human body,⁸ subsequently they lose biological activity. In continuation with this, heavy metal ions can accumulate inside the body and cause acute kidney failure (AKD)⁹, memory loss,¹⁰ breathing difficulties,¹¹ and possibly even cancer.¹² It has been stipulated by The US Environmental Protection Agency (USEPA) that the highest permissible concentration of heavy metals in drinking water supposed to be less than 1.3 ppm for Cu, 2 ppb for Hg, 15 ppb for Pb, 10 ppb for As, 5 ppb for Cd and 100 ppb for Cr.¹³ Keeping this in mind the detection and elimination of heavy metal ions is essential. Extensive studies have been conducted on unconventional ways of Cd(II) removal from water, particularly adsorption, due to its simplicity, cost-effectiveness, diverse adsorbent material selection, and adsorbent renewal.¹⁴ Nanomaterials are potential materials for the removal of heavy metal specifically Cd(II) from water. Carbon-based

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nanomaterials, specifically carbon nanotubes (CNTs) and carbon dots (CDs) based composites, have got a lot of attention because of the functional groupings that include oxygen on CDs, such as epoxy groups, carbonyl groups on the basal plane, and carboxylic groups on the edges.^{15,16} In the last few years, the identification and detection of the process of fluorescence quenching is the process where quantum yield decreases in the form of fluorescence induced by a variety of molecular interactions with quencher molecules. There are various methods that can be used for the detection of heavy metal ions, among them ICP emission spectroscopy, fluorescence spectroscopy, UV-visible spectroscopy, atomic absorption, etc.¹⁷ Among them, fluorescence spectroscopy is one of the options for the detection of heavy metals and is a more appealing technique due to its precise sensitivity, facile operation, and wide range of detection level. To overcome these concerns, numerous CD modifications utilizing other materials have been investigated. One of the problems associated with the detection of heavy metal ions that fluorescence is the ability among some metal ions to reduce emissions resulting in intense quenching in fluorescence. It is known that the quenching refers to the process where the fluorescence intensity decreases in a given substance. The approach involved in this process is excited-state reactions. Because of their high specific surface area, which gives a large number of reactive sites for heavy metals, carbon dots have recently attracted substantial attention among researchers for detecting heavy metals. As a result, carbon nanoparticles are ideal for heavy metal detection. CDs are useful for wastewater treatment due to their widespread availability, low cost, and anti-bacterial properties.¹⁸ Furthermore, CDs functionalized with hydroxyl groups, which aid in the detection of heavy metal ions from effluents.

In the above line, this research is focused on the heavy metal detection using carbon quantum dot (Ai-CDs) synthesized from *Azadirachta indica* seeds using solid-state synthesis method and characterized. It is necessary to assess the levels of hazardous components in the Ai-CDs, such as As and Cd. The amounts of heavy metal elements are often at the $\mu\text{g mL}^{-1}$ level, and the fluorescent quenching and ICP-MS analysis were performed to determine their amount present. Heavy metal ions have the ability to produce charge transfer complexes. The Ai-CDs that emit fluorescent is quenched by a transfer of energy from the metal's lowest excited singlet state to another electronic state, resulting in fluorescence loss. The interaction of two light-sensitive components Ai-CDs and heavy metal ions, a donor and an acceptor, causes dynamic quenching. The benefits of high sensitivity and simultaneous multielement analytical capacity are provided by ICP-MS. This study assessed the ICP-MS's capacity to quantify the amounts of healthy elements and

heavy metals in Ai-CDs. Several heavy metals and lower amounts of elements, such as As (III) and Cd (II), are detected using ICP-MS. Finally, the biocompatibility of the Ai-CDs was confirmed through the MTT assay to confirm that they are not toxic to cells and can be used to detect the analytes of the physiological medium.

2. Experimental

2.1. Materials and reagents

The unprocessed *Azadirachta indica* seeds were collected from the farm house of the institute, washed, dried, and stored under a hot plate at 60 °C for 24 hrs. The chemicals required for the synthesis of carbon dots i.e., sodium methoxide (CH_3ONa) (98%, SRL), diethyl amine [$(\text{CH}_3\text{-CH}_2)_2\text{NH}$] (99%, SRL), and methanol (CH_3OH) (99.5%, SRL) were used without further purification. During every experiment, ultrapure water was utilized. Details synthesis method has been filed for Indian patents (Ref No: 202111060434, Dated: 23rd December 2022).

2.2. Synthesis of Carbon dots

By using solid-state reaction method, Ai-CDs were produced using a fairly easy procedure. i.e., a tube furnace was used to directly heat a crucible holding 1.0 g of neem seed kernel portion to 400° C (Ai-CDs-1) and 450° C (Ai-CDs-2) while maintaining a constant argon flow at a rate of 5 °C min⁻¹ for two hours. It produced a black-brown powder that was dissolved in clean water once it had cooled to room temperature. Following that, diethyl amine, sodium methoxide, and methanol were used to modify their surface to create waste seed-derived luminous surface-quaternized CDs (Ai-CDs). The solid phase approach produced Ai-CDs with an 83% yield. It can be noted that the details synthesis method has been filed for Indian patents (Ref No: 202111060434, Dated: 23rd December 2022).

2.3 Characterization

TEM studies were carried out using a FEI Technai G2S-Twin TEM equipment with a 200 kV acceleration voltage. A solid-state X-Ray powder diffraction (XRD) experiment was carried out using a Bruker D8 Advance diffractometer with nonmonochromatic Cu K α X-Ray radiation. A PerkinElmer LS 55 spectrofluorometer was used for the photoluminescence (PL) investigation. UV-Vis spectroscopy experiments were carried out in the 180-900 nm range (model: Lambda 750 spectrometer, PerkinElmer). Solid samples were studied using Raman spectroscopy (model: WITech Alpha 300 Raman spectrometer). Thermal stability was investigated using a thermogravimetry analyser (TGA) in an N₂ environment from 30° to 1000 °C at a heating rate of 10 °C min⁻¹. FTIR (Nicolet model Impact-410) was used to identify the chemical functionality of the Ai-CDs. Several heavy metals and lower amounts of elements, such as 'As' and 'Cd', were detected using ICP-MS. The Agilent 7800 ICP-MS was used for the heavy metal analysis. The most important phase in analytical investigations was sample preparation, which needs to be thoroughly examined to reduce mistakes. For our sample



preparation, we have used 2% HNO₃ for 24 hr for the digestion of unwanted components. Solid samples were studied using Raman spectroscopy (model: WITech Alpha 300 Raman spectrometer). Calculation of Quantum Yields (QY): CDs solution with maximum absorption of 0.1 at absorption maxima was prepared. Solution of CDs with absorption maxima was set to excitation wavelength ($\lambda = 270\text{--}310\text{ nm}$) and subsequently emission spectra was acquired. The absorbance spectra were acquired on Eppendorf bio spectrophotometer and Fluorescence spectra was measured using Hitachi Spectrofluorometer (Model no. F 7000). Relative quantum yield was calculated according to the reported literature¹⁹ assuming the quantum yield (QY) of coumarin standard as 0.34 %.²⁰

2.4 Metal Quenching Studies

The main focus of this work was to determine the effect of various parameters on the ability to absorb and remove the heavy metal, specifically Cd (II) ions and As(III). Metals have the ability to produce charge transfer complexes with CDs. The molecule that should glow, is quenched by a transfer of energy from the metal's lowest excited singlet state to another electronic state, resulting in fluorescence loss. The interaction of two light-sensitive molecules, a donor and an acceptor, causes dynamic quenching. The donor fluorophore sends energy to the acceptor, which may subsequently produce light or absorb the energy entirely. Electron excitation occurs before the quenching phase in dynamic quenching.

2.5 Cytotoxicity test

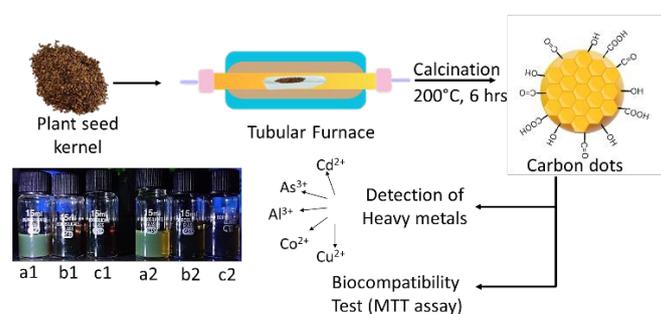
To assess the cytotoxicity of Ai-CDs MTT assay, normal spleenocytes were used. Normally, 104 cells/150 mL of Spleen cells were cultivated in a 96-well plate for 24 hrs at 37 °C in a humidified incubator with 5% CO₂. After that, 100 mL of Dulbecco's modified Eagle's medium (DMEM) with different doses of Ai-CDs (0, 20, 50, 100, 200, and 500 mg mL⁻¹) was added and kept for another two hours. The supernatant was removed, and the spleen cells were gently cleaned in a PBS solution. After adding 150 mL of DMEM and incubating for 4 hours, 20 mL of the MTT solution (5 mg mL⁻¹) was added. The culture media containing MTT was then removed. After that, 150 mL of DMSO was added to the mixture, and it was shaken for ten minutes at room temperature (25°C). Spleen cell-free versions of the identical trials served as the control. Finally, an enzyme-linked immunometric meter was used to detect the average optical density (OD) at 490 nm. The cell viability was then determined using the formula as below,

$$\text{Cell viability} = \text{OD sample} / \text{OD control}^{21}$$

Similar experimental process has been followed to find out the cytotoxicity of the MDA-MB-231 cells with different concentrations of CDs and the to study the cellular morphology after 24 hr of incubation of the cells under MTT assay, optical microscopy was used.

3.Results:

Seeds of Azadirachta indica kernel are the only carbon source to synthesize Ai-CDs using calcination process, as shown in Schematic-1. The carbonized temperature and heating duration are crucial in the formation of Ai-CDs. These two reaction parameters were tuned to increase the Ai-CDs quantum yield and emission wavelength. The carbon core of Ai-CDs generate fluorescence due to the presence of surface functional groups and defects. Additionally, to produce high-performance Ai-CDs, an appropriate calcination period is essential. As a result, 400° C and 2 hrs of calcination time are chosen as the optimal parameters, and the resultant Ai-CDs showed red emission with a high quantum yield. The morphology and size distribution of Ai-CDs were characterized through HRTEM (Fig. 1b-d). As shown in Fig. 1(a-d), the obtained Ai-CDs are monodispersed without aggregation. Their particle size ranges from 4-8 nm with an average size of ~ 6 nm for (Ai-CDs-1) and 7 nm for (Ai-CDs-2) in diameter. Fig. 1b and Fig.1d shown the HRTEM images of Ai-CDs-1 and Ai-CDs -2, respectively exhibiting fringes comprising the crystalline nature of the CDs. The hydrodynamic diameter of Ai-CDs-1 was found to be 13.56 nm and for Ai-CDs-2 to be 19.78 nm (Fig.1e). It can be noted that the hydrodynamic diameter in a solvent is greater than that of the size of Ai-CDs obtained from HRTEM, due to the high surface zeta potential (ζ) that interact the water molecules. The zeta potential analysis was carried out to quantify the charges on the surface of CDs and their colloidal stability. Ai-CDs have ζ of -35.12 mV for (Ai-CDs-1) and -33.72 mV (Ai-CDs -2) (Fig. 1f), indicating the presence of hydroxyl (-OH) and carboxyl (-COOH) functional groups. This high ζ provided CDs more colloidal stability.



Schematic-1. Represents the synthesis approach of CDs from *Azadirachta indica* kernel part. Emission obtained from Ai-CDs-1 treated with: a1) only CDs (untreated), b1) with Cd²⁺ and c1) with As³⁺. Emission obtained from Ai-CDs-2 treated with: a2) only CDs (untreated), b2) with Cd²⁺ and c2) with As³⁺.



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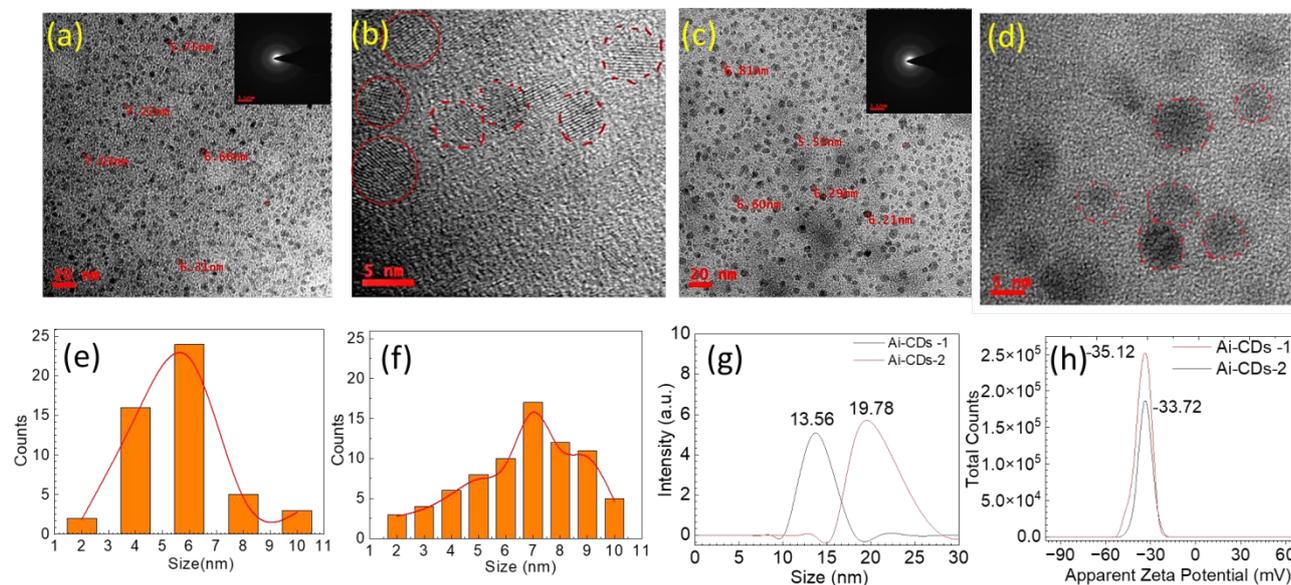


Figure 1 (a-b) TEM images of Ai-CDs-1 at lower and higher magnification, respectively and (c-d) TEM images for Ai-CDs-2 at low and high magnifications, respectively. In set of Fig. (a) shows the SAED pattern of Ai-CDs-1 and inset of (c) shows the SAED pattern of Ai-CDs-2. (e) Size distribution of Ai-CDs-1 and Ai-CDs-2 obtained from TEM images considering 500 number of particles, Fig. (f) particle size distribution for the Ai-CDs-1 and Ai-CDs-2 measured through DLS in water medium, and Fig. (g) the zeta potential profiles for Ai-CDs-1 and Ai-CDs-2 measured in water medium exhibiting negatively high surface zeta potential and colloidal stability.

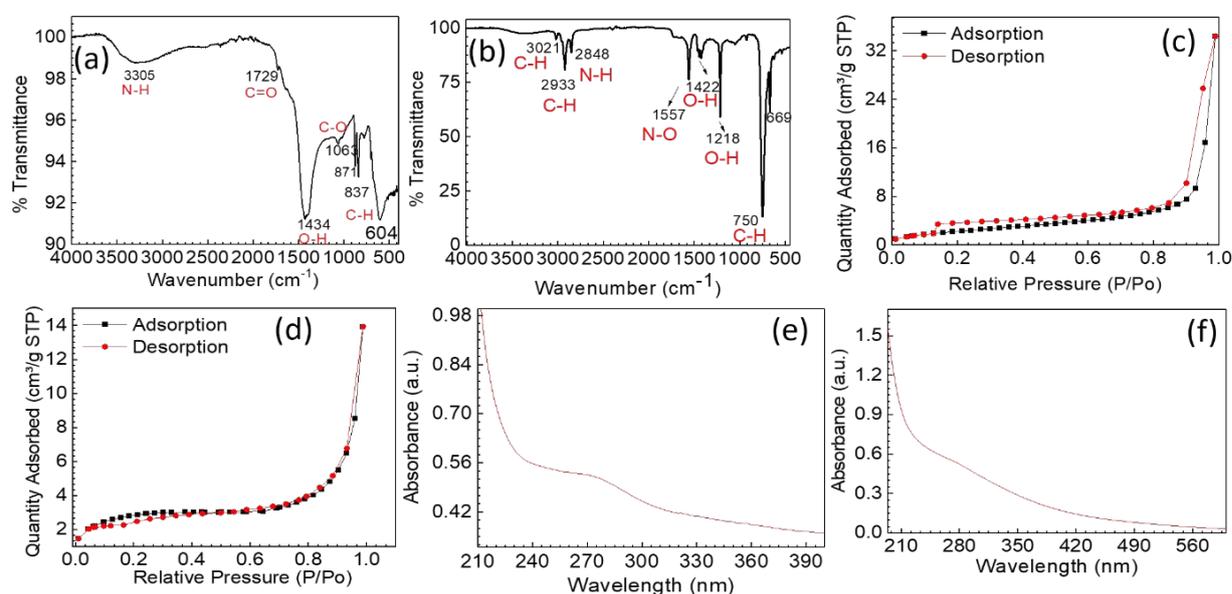


Figure 2. (a-b) FTIR spectra of Ai-CDs-1 and Ai-CDs-2, (c-d) BET N_2 -adsorption-desorption isotherms for Ai-CDs-1 and Ai-CDs-2, (e-f) UV-Vis spectra of the Ai-CDs-1 and Ai-CDs-2, respectively.



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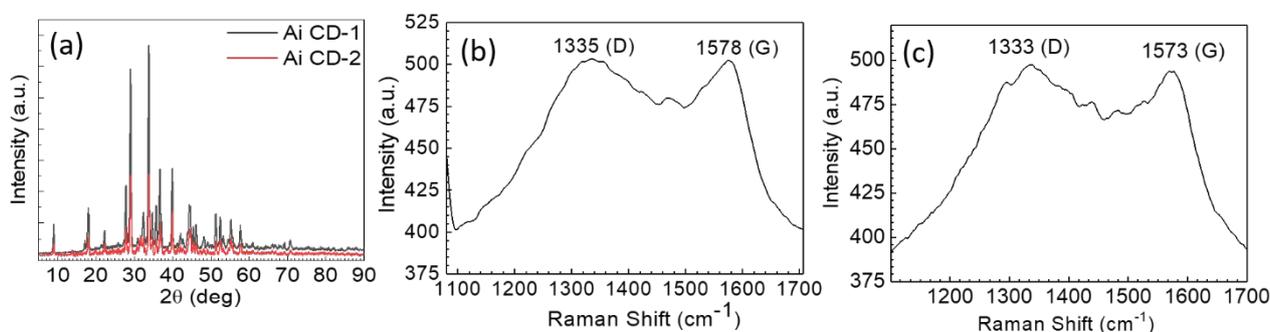


Figure 3. (a) XRD pattern of synthesized carbon dots Ai-CDs -1 and Ai-CDs -2, (b-c) Raman spectroscopic analysis of Ai-CDs -1 and Ai-CDs -2, respectively.

FTIR analysis helps to ascertain the chemical functionality attached on the surface of Ai-CDs. The FTIR spectrum of kernel part of neem seeds and as prepared Ai-CDs are shown in Fig. 2, respectively. The characteristic bands of functional groups for Ai-CDs-1 present as 3305 cm^{-1} (N-H/O-H, stretch), 1729 cm^{-1} (C=O, stretch), 1434 cm^{-1} (H-O, bending), 1063 cm^{-1} (O-C, stretch), 871-837 cm^{-1} (C-H, bending) (Fig. 2a) and 3021 cm^{-1} (C-H), 2933 (C-H, stretching), 2848 cm^{-1} (N-H, stretching), 1557 cm^{-1} (N-O, stretching), 1422 cm^{-1} (O-H, bending), 1218 cm^{-1} (O-H, stretching), 750 cm^{-1} (C-H, bending) and 669 cm^{-1} (C=C, bending) for Ai-CDs-2 (Figure 2b). The BET adsorption-desorption isotherms of Ai-CDs found to be of a type-IV isotherm with a hysteresis loop (Figure 2 c-d) for Ai-CDs-1 and Ai-CDs-2, respectively, representing porous structure of CDs. The degassing process was performed at 150 °C for 4 hr. Further, the BET surface area of Ai-CDs-1 is calculated to be 9.6862 $\text{m}^2 \text{g}^{-1}$, whereas 8.7338 $\text{m}^2 \text{g}^{-1}$ was obtained for Ai-CDs-2. The high surface area of CDs with chemical functionalities allows them for sufficient interactions with the metal ions, which can enhance quenching performance of fluorescence.

The ocular image of CDs dispersed into water (Fig. S1) and absorbance measured in the presence of UV light for Ai-CDs-1 and Ai-CDs-2 (Fig.2 (e-f)). The synthesized CDs suspended in water exhibit UV radiation causing a significant emission (at 273 nm and 268nm). The UV-Vis absorption spectrum of the as-synthesized CDs is shown in Fig.2 (e-f). The absorption bands obtained are related to the C=C bonds and due to the $n-\pi^*$ transition of C=O bonds.

Fig. 3a represents the powder XRD patterns of Ai-CDs-1 and Ai-CDs-1. The XRD pattern shows crystalline nature of the CDs. The sharp peaks are caused by the crystalline nature and the same is confirmed through the SAED pattern of HRTEM (Fig. 1c and e). From Fig. 3a, it is further concluded that the diffraction peaks appeared at $2\theta = 22-28^\circ$, 41° , 50° and 60° due to the (002), (100), (102) and (103) diffraction planes²². The thermal stability of CDs has been confirmed through TGA (Fig.S2). From TGA (Fig S2), the weight loss of Ai-CDs-1 is found

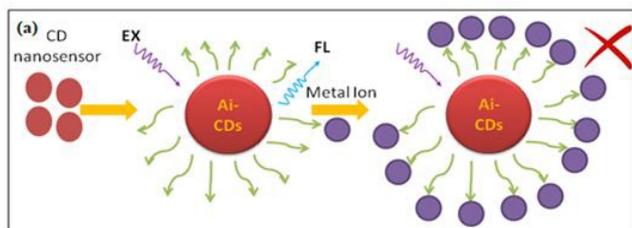
to be ~20 % at 40–150 °C, due to loss of moisture. In the temperature range 151–620 °C, the mass loss was attributed to the decomposition of organic components were present in CDs. While there was almost no weight loss occurred beyond 720°C. Ai-CDs-2 shows 20% weight loss around 40-130 °C followed by a weight loss range of 130- 390 °C and 580 °C due to the decompositions of carbon residuals Ai-CDs -1 and Ai-CDs-2 have been conducted (Fig. S2). DSC for both the samples confirmed that they showed exothermic picks due to decomposition (Fig. S3). The Raman spectroscopy analysis for CDs' for solid state structural analysis has been performed. Fig. 3(b) and Fig. 3(c) represent typical Raman spectra of Ai-CDs -1 and Ai-CDs -2, respectively, with two distinct bands representing D and G bands. The D bands at 1333 cm^{-1} for Ai-CDs-1 and at 1335 cm^{-1} for Ai-CDs-2 indicate the presence of disordered sp^2 hybridized carbon atoms, while the G band at 1573 cm^{-1} and at 1578 cm^{-1} for Ai-CDs -1 and Ai-CDs -2, respectively, correspond to the in-plane stretching vibration mode (E_{2g}) of crystalline graphite carbon atoms. The graphitization degrees for Ai-CDs-1 and Ai-CDs-2 are found to be 0.847 and 0.846, respectively.

Further, the quantum yields (QY) of CDs samples were calculated. As discussed in the method section, CDs solutions with maximum absorption of 0.1 at absorption maxima were prepared. Solution of CDs with absorption maxima was set to excitation wavelength ($\lambda = 270-310 \text{ nm}$) and subsequently emission spectra was acquired. The relative quantum yield was calculated according to the reported literature,¹⁹ assuming the quantum yield (QY) of coumarin standard as 0.34 %.²⁰ Quantum yields of Ai-CDs-1 and Ai-CDs-2 were calculated to be 4.20 % and 3.03 %, respectively.

Fluorescence spectroscopy studies of Ai-CDs for heavy metal detection

The previous report on CDs have made footprints for their non-toxic and hydrophilicity characteristic of fluorescence sensing. As a result, the newly designed facile synthesis approach with





Schematic 2. Diagrammatic representation of the heavy metal ion-CDs nano sensor quenching process,

attractive sustainable carbon sources has caused the researchers to focus on CDs. Considerably, present synthesis approach of CDs can reduce production costs and make a possible large-scale production. However, the fluorescence quenching properties of the CDs are changed when they react with the metal ions in the presence of surface functional groups of the CDs. Usually, the suitable plane of a CDs sensor can optically detect heavy metal analytes (metal ions).²³ The selection of a carbon source is necessary for this and synthesis of the CDs is the primary condition. Similarly, in optical sensing, the existence of CDs with metal ions can change the dot's optical signal, which causes a turndown or change in the intensity of emission spectra or the absorption spectrum.²⁴ All the solutions were prepared in double distilled water immediately before performing the quenching assay. Concentrated CDs dispersion was sonicated and used to produce 3 mL of solutions. Fluorescence measurements of CDs samples were carried out with increasing concentrations of 0, 10, 20, 40, 60, 80, and 120 μM of metal ions (Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} , Cu^+ , and As^{3+}). Before spectroscopic experiments, the samples were stirred using a magnetic stir for approximately 20s (RPM 300) following the addition of metal ion solutions. From these

experiments, we observed that the fluorescent intensity of CD's changed when metal ions were added gradually as compared to without heavy metal ions. Broadly, the surface oxygen moieties on CDs' surfaces, which are in charge of coordinating with metal ions, are effective colorimetric and fluorometric detectors. The camera image acquired AI-CDs-1 treated with: a1) only CDs (untreated), b1) with Cd^{2+} and c1) with As^{3+} and AI-CDs-2 treated with a2) only CDs, b2) with Cd^{2+} and c2) As^{3+} (Figure shown in supporting file Fig. S1). It is clearly evident that fluorescent signal changes with the treatment of heavy metal ions.

These results indicate how the specific metal ion is interacting with CDs. It cannot be utilized as a fluorescence sensor if the metal ion does not affect the fluoresce signal of CDs. As a result, among the criteria depicted in the sensing mechanism, we test the detection system's selectivity by capturing fluorescence spectra, when several metal ions are present (Schematic 2). One of the key output characteristics here was fluorescence properties. As a result, we evaluated the fluorescence intensity quenching or enhancing effects of several metal ions on AI-CDs in deionized water (Fig.4 and Fig.5). The influence of metal ions (e.g., Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} , Cu^+ , and As^{3+}) is significant for pollution and health hazards. The fluorescent intensity of CD solvents were measured in the presence of various metals. Then the metal-induced fluorescence was investigated using Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} , Cu^+ and As^{3+} metal ions containing solutions. When compared with the emission of Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} and As^{3+} significantly reduced fluorescence emission, whereas for Cu^{2+} and Cu^+ showed a significant enhancement of fluorescence²⁵ (Fig.4 and Fig. 5).

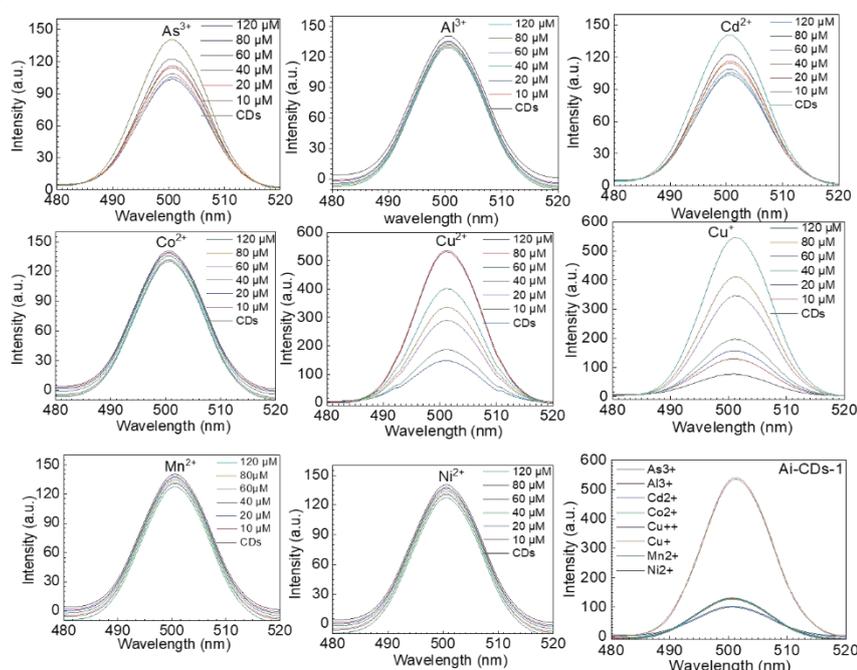


Figure 4 Fluorescence spectra of AI-CDs-1 with different concentrations of metal ions such as Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} , Cu^+ and As^{3+} at different concentrations such as 0, 10, 20, 40, 60, 80, and 120 μM .



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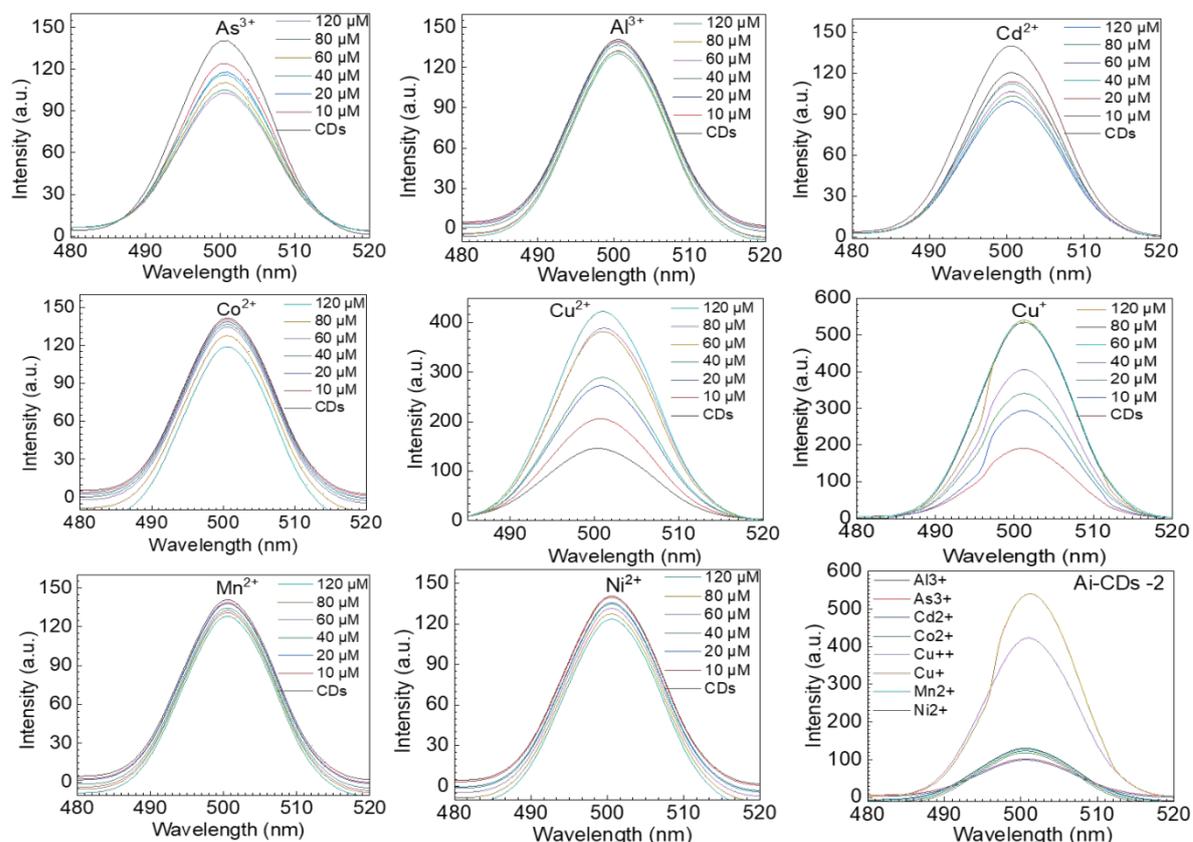


Figure 5 Fluorescence spectra of Ai-CDs-2 with different concentrations of metal ions such as Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} , Cu^+ and As^{3+} at concentrations of 0, 10, 20, 40, 60, 80, and 120 μM .

Notably, the fluorescence response of our Ai-CDs in the presence of various metal ions such as Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} , Cu^+ , and As^{3+} in different concentrations (from top to bottom 0, 10, 20, 40, 60, 80, and 120 μM , respectively), was measured. The decrease in intensity of fluorescence spectra for Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , and As^{3+} is illustrated with a percentage of decrease. The fluorescence intensity diminishes as the concentration of Al^{3+} , Cd^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , and As^{3+} increases. Furthermore, the fluorescence quenching impact on Cd^{2+} (-30%) and As^{3+} (27%) Ai-CDs-1 (Fig. 6a) are significantly higher than that of other ions such as Al^{3+} , Mn^{2+} , Ni^{2+} , Co^{2+} , etc. Furthermore, we have further examined the detection capabilities of Cu^{2+} , and Cu^+ by CDs. Our CDs exhibit an increase in fluorescence emission for Cu^{2+} and Cu^+ as added to the CDs solution.

The intensity of the fluorescence increases as the concentration of Cu^{2+} , and Cu^+ ions increases. The metal ions quenching capacities for Ai-CDs-2 (Fig. 6b) resembles with Ai-CDs-1 (Fig. 6a), however the intensity deferrers with increase and decrease in the concentrations of various metal ions, i.e., Cd^{2+} (29.7 %), As^{3+} (27.7 %). This fluorescence amplification is caused by the affinity of the functional groups for Cu^{2+} and Cu^+ ions. The fluorescence emission of CDs in the presence of copper with different concentrations, such as from top to bottom: 0, 10, 20, 40, 60, 80, 120, and 140 μM , respectively, is seen (Fig. 6a-b). Then, the presence of Cd^{2+} and As^{3+} metal ions were detected on the CD surface by ICP-MS (table of Fig.6).



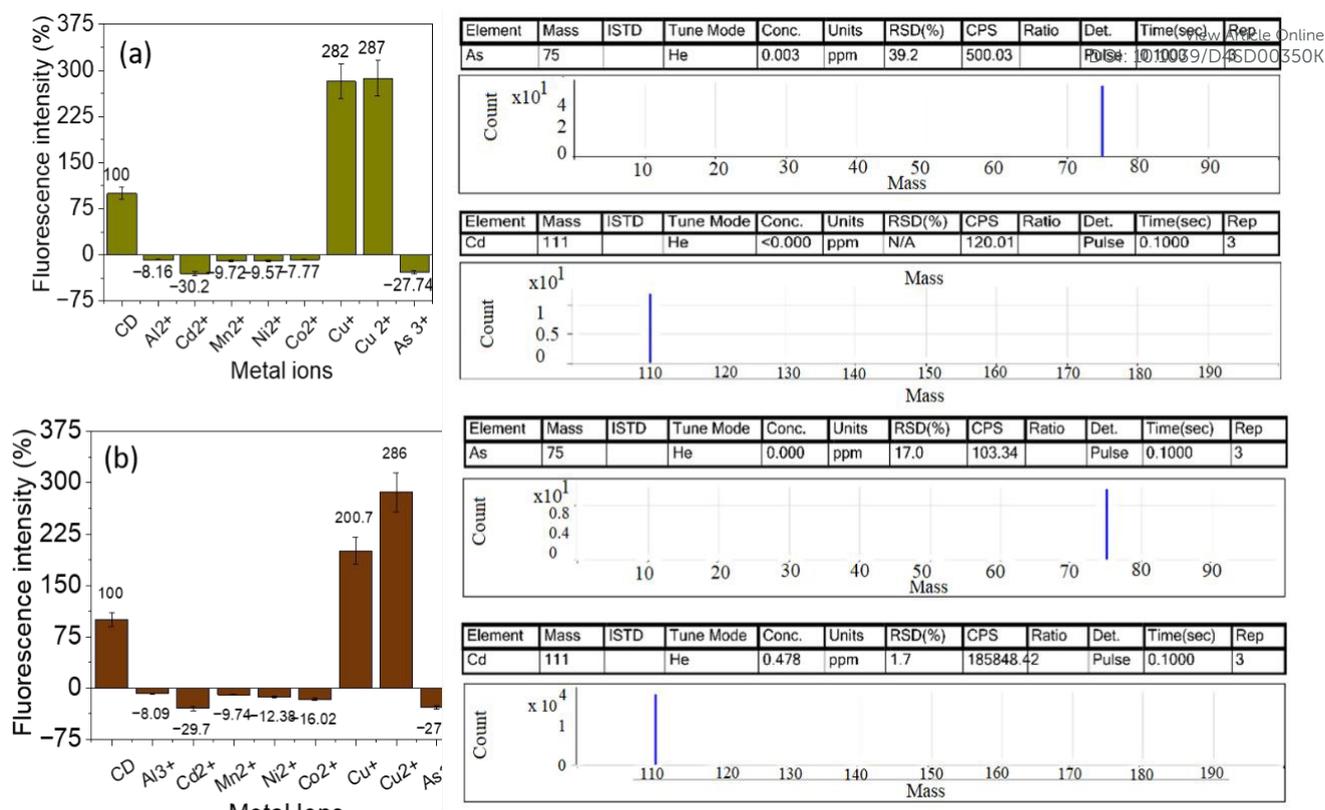


Figure 6: (a-b) Ai-CDs-1 and Ai-CDs-2, the fluorescence intensity percentage ratios (F-F₀)/F₀ with different ions (Al³⁺, Cd²⁺, Mn²⁺, Ni²⁺, Co²⁺, Cu²⁺, Cu⁺, and As³⁺) and table shows the ICP-MS data of As³⁺ and Cd²⁺ ion on the surface of CDs.

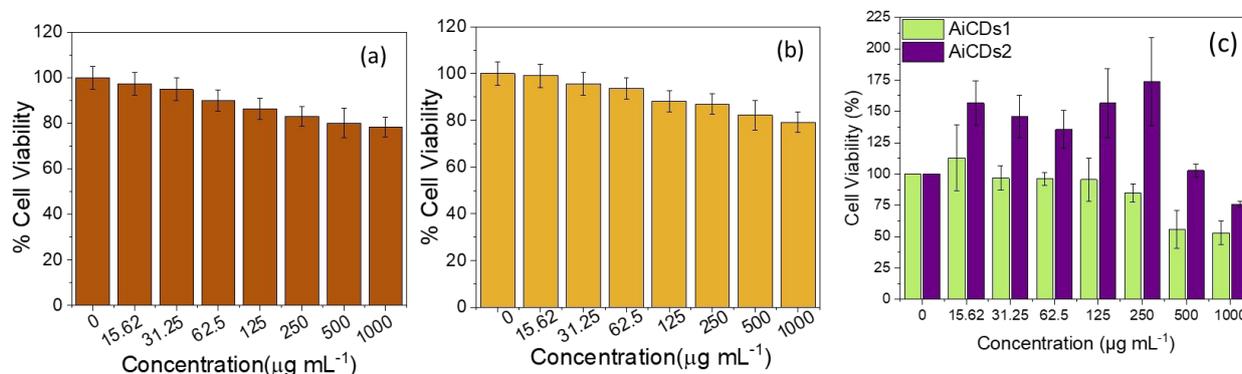


Figure 7: Illustrate the cell viability (%) estimated by MTT assay performed with varying concentration of: (a) Ai-CDs-1 and (b) Ai-CDs-2 at different concentrations (0, 15, 62, 31, 25, 62, 5, 125, 250, 500, 1000 μg mL⁻¹) at 37°C for 24 h in (a) normal splenocytes. 7(c) Illustrate the cell viability (%) estimated by MTT assay with different concentrations of Ai-CDs-1 and Ai-CDs-2 using MDA-MB-231 cells.

The effect of Ai-CDs on cell proliferation test using the MTT assay was examined to check the biocompatibility of Ai-CDs *in vitro* on normal splenocytes (Fig. 7a and 7b) and MDA-MB-231 cells (Fig. 7c). To study whether CDs affect cell proliferation or not, the MTT assay (Fig.7) was performed for Ai-CDs-1 and Ai-CDs-2. Cells were treated with 0, 15, 62, 31, 25, 62, 5, 125, 250, 500, and 1000 μg mL⁻¹ concentrations of CDs for 24 h. During the MTT assay for evaluation of biocompatibility, after 24 h of CDs treatment, normal cells showed more than 90 %

viability up to 125 μM and 250 μM concentrations of CDs. These results directed that CDs are biocompatible up to 125 μM and 250 μM concentrations of CDs until 24 h of incubation. Further, the Fig. 8 are the microscopy images showing the morphology of the cells after 24 h of MTT assay performed at 125 μg mL⁻¹. It is clearly evident that there is no change in the morphology of the cells after incubation with different concentrations of CDs.



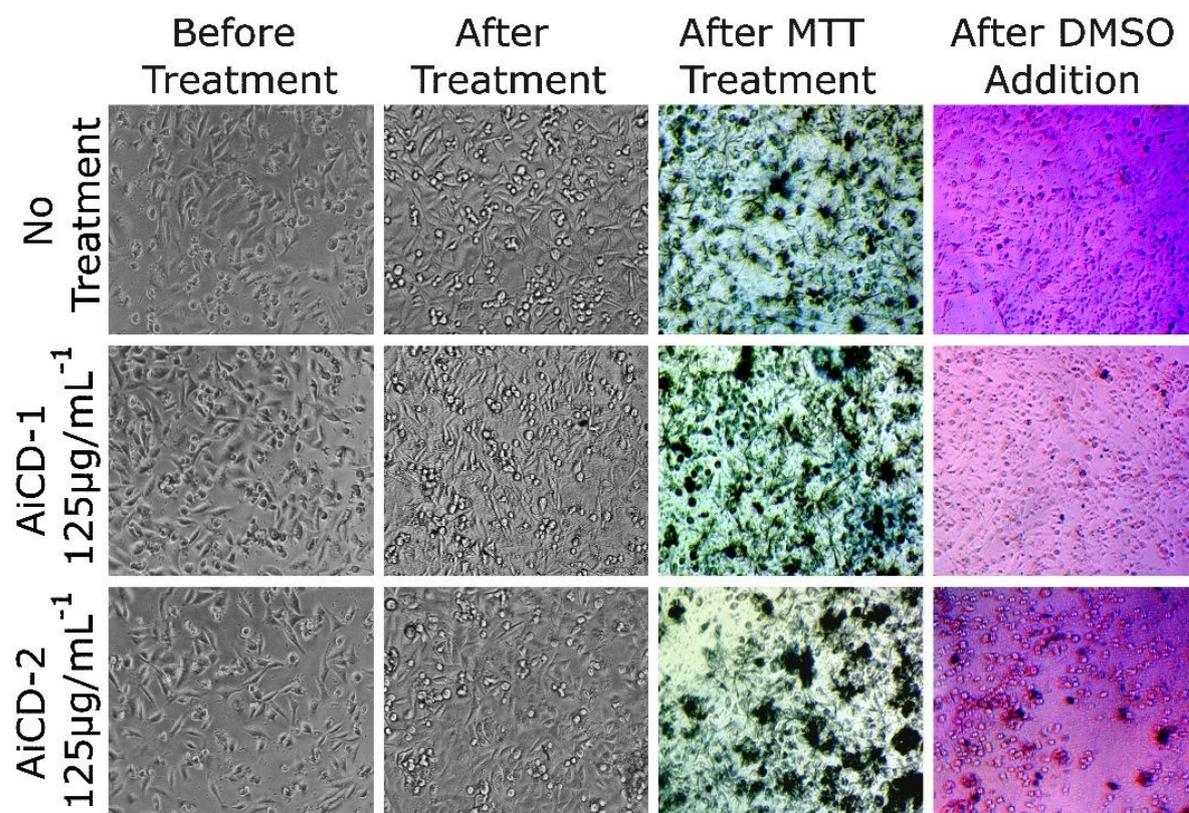


Figure 8. Representative microscopy images, showing the morphology of the cells after MTT assay performed at $125 \mu\text{g mL}^{-1}$. For acquiring the microscopy images MDA-MB-231 cells were used.

4. Conclusions

In the past few decades, a lot of studies on carbon dots have been reported. Studies on these fluorescent materials have mostly concentrated on their usage in bio-imaging. Therefore, recent developments using fluorescent properties of CDs have made it possible to create portable heavy metal detectors. The results summarized on CDs that facilitate the creation of heavy metal sensors. Even though several synthetic techniques have been reported, however the comparatively poor solubility of CDs in water remains a hurdle, and low cost for manufacturing devices is another prerequisite for the application of CDs in many industries. Ai-CDs measured the concentrations of Cd^{2+} and As^{3+} by quenching luminescence, resulting in a "turn-off" effect. Compared to other metal ions, cupric and cuprous ions uniquely enhance fluorescence, creating a "turn-on" effect for sensing. Currently, this method provides the advantages of rapid reaction times along with high selectivity and sensitivity. The CDs can effectively absorb Cd^{2+} and As^{3+} , leading to a significant dimming of fluorescence. Our study demonstrates that Ai-CD's sensitivity in detecting heavy metal ions is as low as a low of $10 \mu\text{M}$. Ai-CDs have been synthesized using a green synthesis process in this work and are biocompatible in nature. The synthesized Ai-CDs are spherical with sizes of 6-7 nm in diameter and emit light. Quantum yields of Ai-CDs-1 and Ai-CDs-2 were found to be 4.20 % and 3.03 %, respectively. These CDs are capable of

detecting metal ions such as Al^{3+} , Mn^{2+} , Ni^{2+} , Co^{+2} , Cu^{2+} , Cu^{+} , Cd^{2+} , and As^{+3} fluorescence quenching. The detection ability depends on the concentration of heavy metal ions. Additionally, the study demonstrates an efficient, environmentally friendly, and straightforward method for detecting heavy metal ions specially Cd^{2+} , and As^{+3} ions. Furthermore, better clarification is needed on the precise mechanism behind the various photoluminescent effects, which are dependent on the synthetic process and unprocessed carbon sources. However, carbon-based quantum dots for heavy metal sensing, which might result in the creation of more economical and environmentally friendly synthetic techniques for more applications.

Author contributions

P. Paik is the main project investigator (PI). Ideation, experimental design, experimental results analysis, and manuscript writing performed by Samedutta, Santhosh and P. Paik. Characterization helped by Sukanya, Divya and Gurmeet. All the authors checked and approved manuscript for publication.

Conflicts of interest



The authors have no conflicts of interest to declare.

Data availability

All the original data will be available with the communicating author of this article and they will be provided as required.

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