Blue-fluorescent and biocompatible carbon dots derived from abundant low-quality coals

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ABSTRACT

Coal is one of the most abundant natural carbonaceous materials. This paper reports a novel oxidative chemical method for the synthesis of high-value carbon dots (CDs) from cheap and abundant low-quality high-sulfur coals for use in high-end applications. These CDs were synthesized by using wet-chemical ultrasonic stimulation-induced process which is environmentally facile and less drastic compared to other chemical methods of production of CDs. The sizes of the synthesized CDs from different types of coal samples were estimated to be in the range of 1–4 nm, 1–6 nm, 2–5 nm, and 10–30 nm. The quantum yield (QY) of the CDs was determined and it was found to be around 3–14%. For high-end field application, the CDs were further tested for toxicity and were reported to be safe for environmental and biological applications. The cell image analysis under the fluorescence microscope further indicated that the synthesized CDs could be used as a promising bio-compatible material for optical-imaging as well as bio-imaging. The CDs showed promising fluorescent sensing property and can be utilized as a good probe for silver ion detection/sensing. The CDs is also found to be a promising reagent for silver nanoparticles synthesis. The results provide a new avenue for large-scale synthesis of CDs.

1. Introduction

Carbon dots (CDs) are newly rising carbon nanomaterials among the nanocarbon members [1]. Carbon dots with ≤10 nm size are termed as carbon quantum dots (CQDs) and graphene nanosheets with a plane size < 100 nm are termed graphene quantum dots (GQDs) [2]. CQDs were discovered in 2004 in the purification of single-walled carbon nanotubes (SWCNTs) [3]. Since then, CQDs have emerged as an intensive research activity due to their broad range of application in extraordinary fields of drug delivery, bioimaging, biosensing, photocatalysis, photovoltaic devices, and optoelectronics [1–4]. In comparison to the traditional semiconductor quantum dots, the unique characteristics of the CQDs are low toxicity, chemical inertness, bio-compatibility, cost effective, and have the similar fluorescence properties of semiconductor quantum dots [5].

CQDs are defined in terms of a surface-functionalized carbogenic core. CQDs are quasi-circular carbon nanoparticles consisting of amorphous or nanocrystalline centres with dominantly turbostratic or graphitic carbon (sp\textsuperscript{2} carbon) or graphene and graphene-oxide sheets blended by using diamond-like sp\textsuperscript{3} hybridized carbon additions [1,4,5]. Simultaneously, CQDs are commonly terminated with the aid of oxygenic moieties on their surface. These moieties facilitate CQDs to impart high solubility in all aqueous solutions and provide the potential for additional functionalization compared to different forms of carbon nano-materials. Due to the above-mentioned specific structures, CQDs possess properties like effective absorbance of photovoltaic light, infrared-responsive up-converted photoluminescence (UCPL), tunable photoluminescence (PL), and distinctive photo-induced electron transfer [1,4,5]. Specific synthesis routes determine the surface states of CQDs. The routes can be modified to accomplish tuneable light emissions, rendering environmental feasibility for various applications in optical-science research [5]. The physical properties of these materials are governed by size, surface-area, and the functional groups present on their surface [1,4,5]. The fluorescence properties of CQDs can be ascribed to their size (quantum confinement effect) or functional groups on their surface [1,4,5].

CQDs have been synthesized from macroscopic carbon-structures like carbon nano-tubes, graphite, fullerences, graphene oxide, and

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activated carbon by adopting top-down approaches like arc-discharge, electrochemical oxidation, chemical oxidation, laser ablation, and ultrasonic synthesis [1,4,5]. The bottom-up approaches have been used to produce CQDs from the molecular precursors like citric-acid/urea [6], ascorbic acids [7], ethanol, sucrose, and glucose [5]. Both the pathways are effective in synthesizing CQDs of varied size and properties. However, top-down pathways are reported to be preferable for large-scale production [5]. CQDs can be synthesized through various methods; however, there is always an interest for a “golden standard” to create CQDs from low-priced materials for its versatile applications [8]. Coal has been found to be superior and inexpensive among currently available the carbon sources [8].

Over the years researchers have noted a few methods for the synthesis of CQDs from coal by utilizing distinctive types of physical, chemical, and hydrothermal methods [8–23]. Most of the reported synthetic methods were multi-step, time consuming, and involved drastic treatments by using strong acids like H₂SO₄ and HNO₃ for oxidation of synthetic methods were multi-step, time consuming, and involved drastic treatments by using strong acids like H₂SO₄ and HNO₃ for oxidation of synthetized CQDs show remarkable Ag⁺ ion sensitivity, hence, making these CQDs an ideal probe for detection of metal ions.

2. Materials and methods

2.1. Materials

The coal samples were obtained from the North-eastern coalfield (Cenozoic age) India (samples are denoted as: Coal-NK, Coal-NG, Coal-T60, and Coal-T20). The raw coals (~1 kg) were first ground to about 0.211-mm fineness by using a standard method (ASTM 2010). H₂O₂ (30%; Merck) was used as received for exfoliation of coal. For the purification of the products, fresh 0.22-μm polytetrafluoroethylene membranes (Sartorius, lot number 11806-47-N) and dialysis bags (Sigma-Aldrich) were used.

2.2. Synthesis of carbon dots (CDS)

12 g of coal samples was mixed with 200 mL of hydrogen peroxide (30%) in an ice-cold condition. The reaction mixture was then ultrasonicated (frequency: 40 kHz) in a microprocessor-based ultrasonicator (Model-Power Sonic 520) for about 6 h at an atmospheric pressure and temperature. The resultant mixture was then cooled to room temperature and followed by the addition of ammonium solution until the pH of the mixture was found to be neutral. This mixture was then filtered by using a 0.22-μm polytetrafluoroethylene membrane and further purified with the help of dialysis technique (1 kDa dialysis bags). After dialysis, the solution was concentrated in a rotary evaporator to obtain the purified carbon dots (CDS). The synthesized carbon dots from each coal sample were denoted as Coal-T60-CDs, Coal-T20-CDs, Coal-NK-CDs, and Coal-NG-CDs in the subsequent discussion.

2.3. Characterization techniques of raw coals and CDS

The proximate and ultimate analyses were carried out in a Thermogravimetric Analyzer (Leco, TGA-701) and Truspec CHN Macro Determinator (Leco, 630-100-300). Sulfur analysis was carried out in a Dual Range Sulfur Analyzer (Leco S-144DR). The scanning electron microscope (SEM) analysis was done with a scanning electron microscope (Model No. JSM-6390LV; JEOL) with the help of an Auto Fine Coater (JFC-1600; JEOL, Tokyo, Japan). HRTEM analysis was also performed by using High Resolution-Transmittance Electron Microscope (HRTEM; Joel JEM-2100). The SEM and HRTEM images were further developed by using “Image J” program (software version 1.47). The FTIR spectra were recorded by using Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan). The XPS spectra were recorded in X-Ray Photoelectron Spectrometer (model: ESCALAB Xi+). A Laser micro Raman system (Make- Horiba Jobin Yvon, Model Lab Ram HR) was used for performing Raman analysis. UV-Vis (Ultraviolet-visible) spectroscopy was recorded in an Ultraviolet visible spectrophotometer (Analytikjena, SPECORD 200, Germany) and Fluorescence (FL) spectra were recorded in F-2700 Fluorescence spectrophotometer (2423-008). Time-resolved single-photon counting spectroscopy studies were performed by using an FAST Version 1.9.1. Edinburgh Instruments Ltd. The surface charge analysis of CDs was determined by using ZETASIZER (Model- Nano ZS, Malvern, UK).

2.4. Quantum yield (QY) of the CDs

The quantum yield of the CDs was determined by using the following formula [8–9]:

\[ \Phi = \frac{\int \tau I d \lambda}{\int \eta A d \lambda} \]

wherein, “\( \Phi \)” is the relative quantum yield with respect to the reference/standard. In the present study, 0.1 mg L⁻¹ quinine sulphate in 0.1 M H₂SO₄ solution (literature [8] QY of 0.54 at 340 nm) was used as a standard. The quantum yields were determined by comparing the integrated FL intensity (excited at 340 nm) absorbance value (at 340 nm) of the standard with each of the coal derived CDs, based on the above equation. Where, “I” stands for measured integrated emission intensity, \( \eta \) refractive index of solvent and A-optical density (absorbance). The subscript “r” alludes to standard index of the quinine sulphate. So as to limit the re-absorption effects, absorbance in a 10 nm fluorescence cuvette was held under 0.1 at an excitation wavelength of about 340 nm.

2.5. Assessing the safety of CDS for application

The as-synthesized CDs were tested for their antimicrobial, antifungal, cytotoxicity effect, and acute oral toxicity/pathogenicity for
their possible application in bio-labelling and environmental analysis.

2.6. Antimicrobial and antifungal test

The antimicrobial activity of the as-synthesized CDs was tested against five bacterial species [gram negative: *Pseudomonas aeruginosa* (MTCC2453), *Escherichia coli* (MTCC739) and gram positive: *Mycobacterium abscessus* (ATCC19977), *Staphylococcus aureus* (MTCC96), and *Bacillus subtilis* (MTCC441)], and two fungal species [*Candida albicans* (MTCC3017) and *Fusarium oxysporum* (NCIM1281)], respectively. For the activity assessment, 100 μL of the bacterial culture was injected in nutrient agar plates and the fungal culture in potato dextrose agar plates using a spread plate method. In each plate, four 6-mm wells were prepared using sterilized cork-borer and 50 μL of each test sample was inoculated in it. The as-prepared bacterial and fungal plates were then kept in an incubator at 35 °C for 24 h and 28 °C for five days, respectively.

2.7. Cytotoxicity test

The commercially available cell lines namely, Rat muscle cell line (L6), Human prostate cancer cell line (PC3), Human cervical cancer cell line (HeLa), and Human breast adenocarcinoma cell line (MDAMB 231) obtained from National Centre for Cell Science, Pune, were used for the cytotoxicity study using the methods reported elsewhere [13].

2.8. Acute oral toxicity/pathogenicity

The acute oral toxicity/pathogenicity test of the CDs was done in APT Testing and Research Private Limited, approved by the Food and Drug Administration-Maharashtra State, Pune, LIC No.-37-PD/TL/7 (Report No. MS124/1617/0975a).

2.9. Metal ion detection activity of CDs

The as-synthesized CDs were evaluated for their metal detection activity for their possible application as probes for environmental applications. Aqueous solution of Mg²⁺, Zn²⁺, Ca²⁺, K⁺, Hg²⁺, and Ni²⁺ were prepared from their chloride salts and that of Na⁺ and Ag⁺ from their nitrate salts. Aqueous solution of Cu²⁺, Cd²⁺, and Al³⁺ were also prepared from their sulfic acid salts. The utility of the coal-derived CDs as fluorescent sensor for metal ion detection was performed at a room temperature in PBS buffer (pH 7.0). Na⁺, Ag⁺, Mg²⁺, Zn²⁺, Cu²⁺, K⁺, Hg²⁺, Ni²⁺, Cu²⁺, Cd²⁺, and Al³⁺ solution were prepared with a concentration of 50 μmol L⁻¹. From each metal ion solution, 100 μL was taken and then added independently to 1000 μL PBS buffer. A fixed concentration of CDs (0.02 mg/mL) was added to each buffer-ion solution. For the purpose of comparison, 2 mL volume of CDs solution was fixed before mixing with each buffer-ion solution. The mixture was shaken for 5 s and the fluorescence emission spectrum was noted. All the measurements were performed at an excitation wavelength of 300 nm recording the emission between 320 nm and 700 nm, at room temperature. The Stern-Volmer equation was used:

\[ \frac{F}{F'} = 1 + Ksv (\text{ion}) \]

where, “F” is the fluorescence intensity in absence of the ion and “F’” is the fluorescence intensity in presence of the ion used to represent the quenching [11,14].

3. Results and discussions

3.1. Chemical characteristics of coal feedstock

The results of physico-chemical properties of the coal samples used in the investigation are shown in Table 1. The analysis clearly indicated that the coal samples have different composition of carbon (60–80%), hydrogen (5–8%), mineral matter (ash content 2–19%), and sulfur (1–4%) content. The volatile matter and sulfur contents are also found to be high. The SEM images of the feed coal samples without any treatment are shown in Fig. S1 (see Supporting information), which indicated that the coal particles have irregular size and shape distributions. The FTIR analysis (Fig. S2a–b) of the feed coal samples suggested the presence of C–O, C=O, H–C, and O–H functional group on the surface. The Raman spectra (Fig. S2c–d) of the feed coal samples showed D-bands and G-bands, suggesting the presence of graphitic-like stacking domains along with aliphatic carbon.

3.2. Chemical characteristics of CDs

3.2.1. Observations from electron beam analysis (SEM/HRTEM)

During the detailed electron beam analysis (TEM/HRTEM) the microstructure/nanostructure of the synthesized CDs were investigated. In Fig. 1(a–h) the formation of carbon nano-crystals with different size ranges can be observed. The diameters of these synthesized carbon nanostructure were found to be in the range of 1–6 nm, 2–5 nm, 10–30 nm, and 1–4 nm for Coal-T60-CDs (Fig. 1a–b), Coal-T20-CDs (Fig. 1c–d), Coal-NK-CDs (Fig. 1e–f), and Coal-NG-CDs (Fig. 1g–h), respectively. The electron beam analysis revealed the formation of carbon quantum dots (CQDs) as well as graphene quantum dots (GQDs) from the coal feedstock. The produced CQDs and GQDs have crystalline as well as amorphous carbon additions on their edges. It should be mentioned that the observed microstructure of the CQDs and GQDs are similar to the CDs structure derived from other types of carbon sources reported earlier [1–3,7,8,10–18,20–23]. The CDs nanostructure predominantly comprise of carbon, oxygen, nitrogen, and sulfur as indicated by the energy-dispersive spectroscopy of CDs (ΣIGMA-Field Emission Scanning Microscope, Carl Zeiss Microscopy) (Fig. S3 see in supporting information).

3.2.2. Observation from FTIR, XPS, and Raman analysis

FTIR characterization of the CDs was carried out so as to know the chemical composition and surface states. The FTIR spectra of the as-synthesized CDs showed the presence of C=C, C–O, C=O, H–C, and O–H vibration modes, as depicted in Fig. 2a. A wide absorption peak at about 3400 cm⁻¹, corresponding to the extending vibrations of O–H bonds is observed. The sharp absorption peaks at about 1600 and 1720 cm⁻¹ are mainly because of the C=C group and C=O group, respectively. The intensity of the C–O, C=O, and O–H vibration mode is found to be enhanced after treatment.

The chemical composition of the CDs was also determined by XPS (X-ray photo-electron spectroscopy). The XPS spectra of CDs mainly shows four distinct peaks centered at 285 eV, 400 eV, 531 eV, and 168 eV, corresponding to the percentages of C, N, O, and S, respectively, as depicted in Fig. 2b. At high-resolution deconvolution of C1s (Fig. 2c) and O1s signals (Fig. 2d) of CDs, three main peaks centered at 284 eV (sp² c-c), 285 eV (C-OH), and 288 eV (C=O) are observed for C1s and two main peaks centered at 531 (organic C=O) and 533 (organic C=O) for O1s are observed. From the FTIR and XPS study of CDs,

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proximate analysis (%)</th>
<th>Ultimate analysis (%)</th>
<th>TS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>Ash</td>
<td>VM</td>
</tr>
<tr>
<td>TD-T60</td>
<td>2.20</td>
<td>2.95</td>
<td>45.02</td>
</tr>
<tr>
<td>TD-T20</td>
<td>2.35</td>
<td>2.33</td>
<td>50.27</td>
</tr>
<tr>
<td>TD-NK</td>
<td>3.82</td>
<td>19.04</td>
<td>36.32</td>
</tr>
<tr>
<td>TD-NK</td>
<td>9.11</td>
<td>4.44</td>
<td>49.07</td>
</tr>
</tbody>
</table>

M-Moisture; Ash-Ash Matter; FC-Fixed Carbon; C-Carbon; H-Hydrogen; N-Nitrogen; TS-Total Sulfur.

M-Moisture; Ash-Ash Matter; FC-Fixed Carbon; C-Carbon; H-Hydrogen; N-Nitrogen; TS-Total Sulfur.

Table 1

Physico-chemical characterizations of the coal samples (as received basis, wt %).

Fig. 1. (a) HRTEM images of Coal-T60-CDs and (b) corresponding particle size distribution indicating the fabrication of 1–6 nm sizes carbon nanostructure, (c) HRTEM images of Coal-T20-CDs and (d) corresponding particle size distribution indicating the fabrication of 2–5 nm sizes of carbon nanostructure, (e) HRTEM images of Coal-NK-CDs and (f) corresponding particle size distribution indicating the fabrication of 10–30 nm sizes of carbon nanostructure, (g) HRTEM images of Coal-NG-CDs and (h) corresponding particle size distribution indicating the fabrication of 1–4 nm sizes of carbon nanostructure.
It is observed that the as-synthesized CDs is highly functionalized with oxygen-containing hydrophilic functional groups, which impart in high solubility of CDs in water, as shown in Fig. 2(i). The high solubility of the as-synthesized CDs in water system is advantageous for their applications.

The structural defects appeared in the CDs after exfoliation of coal was investigated by using Raman analysis. The Raman spectra of the CDs demonstrate principally two characteristic bands at 1616 cm$^{-1}$ (G-
band) and 1375 cm$^{-1}$ (D-band) for Coal-T60-CDs; 1553 cm$^{-1}$ (G-band) and 1381 cm$^{-1}$ (D-band) for Coal-T20-CDs; 1553 cm$^{-1}$ (G-band) and 1382 cm$^{-1}$ (D-band) for Coal-NK-CDs; and 1595 cm$^{-1}$ (G-band) and 1387 cm$^{-1}$ (D-band) for Coal-NG-CDs as shown in Fig. 2f. The ID/IG ratios after exfoliation of low-quality NER coals are found to increase noticeably, which indicates that the as-synthesized CDs have the defects.

Fig. 3. (a) UV-visible absorption spectra of synthesized CDs; (b) fluorescence spectra of as-synthesized CDs (excited at 320 nm); (c–f) excitation dependent (at an increment of 20 nm) fluorescence spectra of as-synthesized CDs (c: Coal-NG-CDs, d: Coal-NK-CDs, e: Coal-T20-CDs, f: Coal-T60-CDs).
states such as oxygenated functional groups in the lattice sites of CDs, as confirmed by the FTIR analysis.

The G-band evolved from the vibration of the sp\(^2\)-hybridized carbon framework within the two-dimensional hexagonal lattice of graphite cluster and D-band from a lattice defect inclusive of the sp\(^3\) hybridized carbon. The G-bands relate to the 1st-order scattering of the E\(_{2g}\) stretching mode of graphite. The D-band is a result of the remaining ill-organized graphite.

3.2.3. Observation from UV-Vis spectroscopy, FL spectroscopy, and time-resolved single-photon counting spectroscopy

A fascinating property of the as-synthesized CDs is that when illuminated with an UV ray of wavelength 365 nm, it shows a bright blue fluorescence and this can be easily observed in diluted colloidal solutions [see Fig. 2e (ii)]. The special feature of photo-physical properties of the as-synthesized CDs is to comprehend the presence of conjugated π-domains and optically active defect centers for practical applications, particularly biomedical imaging.

The photo-physical properties of the CDs were investigated by using ultraviolet (UV-Vis) spectroscopy, FL spectroscopy and time-resolved single-photon counting spectroscopy. The UV-visible spectrum of CDs shows a typical optical absorption band at about 250–350 nm because of the excitation of pi-electrons (π→π\(^*\)) of aromatic π system (Fig. 3a). The tailing band which showed up at 300 nm is attributed to the n–π\(^*\) transition of the functional groups containing oxygen (Fig. 3a). The molar absorption coefficient (\(ε\)) of the CDs are estimated to be about 5662 M\(^{-1}\)cm\(^{-1}\) (Coal-NG-CDs), 18,716 M\(^{-1}\)cm\(^{-1}\) (Coal-NK-CDs), 10,500 M\(^{-1}\)cm\(^{-1}\) (Coal-T60-CDs), and 22,500 M\(^{-1}\)cm\(^{-1}\) (Coal-T20-CDs).

The FL properties of CDs were observed to be excitation dependent and comparable to the reported data elsewhere [10–18,20–23]. At 460 nm, corresponding to blue regions, the maximum FL intensity was observed. With the increase of excitation wavelength, the FL spectra of CDs is seen to be red-shifted to green/yellow regions as numerous chromophore or fluorophore systems with aromatic and oxidation groups are present, as confirmed by the FTIR and XPS analysis. Also, when excitation wavelength is increased, the stokes shift trends is observed to be linearly to zero, which suggested that the

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Table 2
Life time calculated from the time-resolved decay profile of the synthesized carbon dots (CDs).

<table>
<thead>
<tr>
<th>Samples</th>
<th>(τ_1) (ns) ± (\sigma)</th>
<th>(f_1)</th>
<th>(τ_2) (ns) ± (\sigma)</th>
<th>(f_2)</th>
<th>(τ_3) (ns) ± (\sigma)</th>
<th>(f_3)</th>
<th>Avg (τ) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coal-T60-CDs</td>
<td>0.791 ± 0.088</td>
<td>15.84</td>
<td>2.747 ± 0.028</td>
<td>19.51</td>
<td>10.139 ± 8.7e-4</td>
<td>64.65</td>
<td>4.55</td>
</tr>
<tr>
<td>Coal-T20-CDs</td>
<td>0.992 ± 0.069</td>
<td>19.49</td>
<td>3.520 ± 0.017</td>
<td>31.60</td>
<td>9.982 ± 0.002</td>
<td>48.91</td>
<td>4.83</td>
</tr>
<tr>
<td>Coal-NK-CDs</td>
<td>0.870 ± 0.067</td>
<td>23.02</td>
<td>3.340 ± 0.021</td>
<td>34.00</td>
<td>8.873 ± 0.004</td>
<td>42.98</td>
<td>4.36</td>
</tr>
<tr>
<td>Coal-NG-CDs</td>
<td>0.626 ± 0.156</td>
<td>16.37</td>
<td>2.453 ± 0.018</td>
<td>39.32</td>
<td>8.105 ± 0.002</td>
<td>44.30</td>
<td>3.72</td>
</tr>
</tbody>
</table>

ns: nanosecond.
The observed \( \tau \) times are summarized in Table 2. The observed life times of the synthesized CDs at neutral pH is shown. The corresponding life times were found to be varied, which is due to \( \pi-\pi^* \) electronic transition of aromatic core (sp\(^2\) skeleton of the CDs) [27]. The observed \( \tau \) is found to be also varied for CDs and is due to the structural disorder in the synthesized CDs [28]. The life times \( \tau_1 \) (> 10 ns, > 9 ns, > 8 ns, and > 8 ns) for Coal-T60-CDs, Coal-T20-CDs, Coal-NK-CDs, and Coal-NG-CDs, respectively, are in comparison to the reported earlier [9, 11–15], which accounts for the higher FL emission.

The observed life time of the CDs suggests that the synthesized CDs can show potential in optoelectronics and biological applications. The fluorescence property of the as-synthesized CDs is because of the presence of oxygen-containing functional group, quantum size effect and defects states. The fluorescence properties of the CDs are excitation dependent which in turn makes them suitable for multi-colour imaging and sensing.

3.3. Quantum yield (QY) and concentrations of CDs

The relative quantum yields of the as-synthesized CDs were calculated to be about 3% (Coal-T20-CDs), 4% (Coal-NK-CDs), 8% (Coal-T60-CDs), and 14% (Coal-NG-CDs). These values are higher than the other reported values for CDs (Table S2 in Supporting information) [8, 9, 11–15]. The concentrations of the as-synthesized CDs were determined by using thermogravimetric analysis and are estimated to be about 7.4 mg/mL (Coal-T20-CDs), 4.4 mg/mL (Coal-NK-CDs), 4.2 mg/mL (Coal-NG-CDs), and 2.4 mg/mL (Coal-T60-CDs).

3.4. Plausible mechanism for the formation of CDs

The mechanism of formation of CDs was hypothesized as described in a previous study [28]. Similarly, in the present study, ultrasonication of low-quality coals in the presence of hydrogen peroxide may lead to the exfoliation of graphitized and non-graphitized polyaromatic hydrocarbons (PAHs) present in coal. The PAHs fragments were then further fragmented and, because of the polymorphic reaction of C\(_2\) carbon units, CDs were formed. The mechanism of formation of CDs is also hypothesized due to the phenomena of metastable phase nucleation at nanometer size [29]. The hydrogen present in raw coals also plays a vital role in CDs formation [30–32].

3.5. Antimicrobial, antifungal test, cytotoxicity, and acute toxicity test of CDs

During the course of the antimicrobial and antifungal tests study, it was observed that 50 μL of the CDs do not inhibit the growth of any bacterial and fungal strains (see Fig. S4 in Supporting information). L6 (Rat muscle cell line), HeLa (Human cervical cancer cell line), PC3 (Human prostate cancer cell line), and MDA MB 231 (Human breast adenocarcinoma cell line) cell lines were used to assess the cytocompatibility of the as-synthesized CDs. After treatment, all the cells were attached with the slide and no change in the morphology of the cells was detected against the control. Cell Viability (MTT) Assay of CDs in HeLa cell line induced moderate cell death in a dose-dependent manner (see Fig. S5a–b). After incubating for 3 h, the survival rate was assessed to be > 70%, regardless of the increase in concentration of the CDs up to 24-μg/mL (Coal-T60-CDs), 44-μg/mL (Coal-NK-CDs), 42-μg/mL (Coal-NG-CDs), and 74-μg/mL (Coal-T20-CDs) (see Fig. S5a–b in Supporting information), which indicated that the toxicity of the CDs is fairly low. In addition, cell imaging was performed on a fluorescence microscope after incubating the L6 cell line with the planned amount of CDs for 3 h. The bright-field image (see Fig. S5e in supporting information) showed that the treated cells held their actual fusiform morphology, which likewise affirmed the low toxicity of the CDs. The FITC image (see Fig. S5f in Supporting information), TRITC image (see Fig. S5g in Supporting information), and merged image (see Fig. S5h in supporting information) showed that the L6 cells labelled by the as-fabricated CDs shine under UV-radiation, further indicating that the synthesized CDs could be used as a promising material for optical-imaging/bio-imaging.

The acute oral toxicity/pathogenicity test of CDs in mice model revealed that the CDs are non-toxic and have the potential to use in vivo fluorescence imaging.

3.6. Cost of production analysis of CDs from low-quality coals

The cost analysis of the CDs produced on lab-scale was made on both recurring and non-recurring cost components. Considering 110 g of basic source materials as low-quality coals, about 580 mL of CDs was produced.
produced. It was estimated that the cost of CDs with purity level of 2.4–7.4 mg/mL will be US $0.80 per mL. However, the cost of the equivalent CDs available in the market is US $3.00–5.00 per mL, which signifies that the process currently developed is economically viable.

3.7. Metal ion sensing capacity of CDs

The low-quality coal-derived CDs were found to be of high fluorescence stability and having significant fluorescence properties, as confirmed from the photo-physical properties. One of the promising
The surface of the synthesized CDs is found to be functionalized with oxygen-containing functional groups like carboxylic, carbonyl, and phenolic groups as confirmed from the FTIR and XPS study. In the present investigation, the cationic Ag⁺ has a tendency to coordinate with the negatively charged functionalized CDs, as confirmed from the surface charge analysis. The oxygen containing surface functional groups of CDs reduce the Ag⁺ to Ag⁰ [42–44] and, as a result, silver nanoparticles are formed on the outer surface of CDs. The outer charges of the surrounding CDs help to stabilize the silver nanoparticles in aqueous solution and prevent their agglomeration.

Compared to the XPS spectrum of CDs (Fig. 2b), a new peak of Ag element is observed in the XPS survey spectrum of Ag/CDs composite (Fig. 6c). The Ag 3d spectrum revealed two peaks at binding energy value 367 eV (3d₅/₂) and 373 eV (3d₃/₂), which indicated the formation of metallic Ag (Fig. 6d). As compared to the high-resolution deconvolution of O1s signal of CDs (see Fig. 2d), the O1s signals of Ag/CDs gives a new peak with high intensity centered at 532 eV (Fig. 6e), which is due to the C=O group in the quinone-like structure of Ag/CDs [44]. This confirmed that the structure of CDs is changed after reduction of Ag⁺ to Ag⁰ [44].

In order to validate or assess the applicability of the as-synthesized CDs for detection of silver ions, river water samples were analyzed. But, no samples show positive value for Ag⁺ ions. Also, as per our literature survey, the concentration of silver ion in river water samples of Northeastern region of India is found to be negative [45–49]. However, the local river water samples will be tested in future research plans by using a standard addition method of Ag⁺ ions [33,37,40].

The present low cost coal-derived carbon dots (without any masking agent) are observed to be more promising and suitable for the detection of Ag⁺ ions as compared to the previously reported methods elsewhere (Table 3) [33,37,39,40,50–52].

4. Conclusions

The observations revealed that the water soluble CDs could be easily produced by using an ultrasonic-assisted wet-chemical method from low-quality coal feedstock. In present work, the coal-derived high-value CDs comprises of both carbon quantum dots (amorphous carbon structure) and graphene quantum dots (crystalline carbon structure). The CDs show stable bright-blue fluorescence and exhibited excitation-dependent fluorescence properties, which holds potential for application in optoelectronics and sensing. The as-synthesized CDs could also be used as a marker for detecting of Ag⁺ ions (detection limit as low as 0.19 μmol L⁻¹) in water, which exhibits great potential for utilization in high-end application like sensing of hazardous elements. The CDs are found to be non-toxic. The process presented in this paper will be an alternative simple way for the production of high-value CDs in large-scale from cheap coal feedstocks at a low cost.

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