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Carbon Quantum Dots: A Sustainable Approach to Bio-imaging and Environmental Remediation: A Review

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Abstract: Carbon quantum dots (CQDs) are zero-dimension carbon-based nanoparticles that have garnered attention recently because of their excellent properties. These zero-dimensional carbon nanostructures, typically less than 10 nm in size, display remarkable optical and electronic properties making them useful in various fields including photovoltaics, sensing of harmful substances, bio-imaging, drug delivery, and photocatalysis. The excellent features of CQDs, such as tuneable photoluminescence, high biocompatibility, low toxicity, and excellent photostability, make them quite useful in the bio-imaging field, where they are used as fluorescent probes for cellular imaging and tracking in living cells. Carbon quantum dots can absorb light across a wide range of spectrums including visible light allowing them to initiate photocatalytic reactions in different lighting conditions making them effective in environmental remediation. This review discusses the synthesis methods of CQDs including hydrothermal, microwave, and pyrolysis, highlighting green approaches that utilize renewable resources and waste materials, thus minimizing environmental impact, and also discussing the various characterization techniques including UV-Vis spectroscopy, FTIR, XRD, and TEM, which are essential for understanding CQDs properties. In this review, we also highlight the current advancement of carbon quantum dots in the photocatalytic and bio-imaging field along with existing problems and new insights.

Keywords: Carbon quantum dots, Synthesis, Characterization, Photocatalytic, Bio-imaging of cells

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Introduction

In the 21st century environmental deterioration, pollution, and resource depletion have become global concerns. Scientists worldwide are paying attention to environmental issues related to the

mixing of harmful and toxic contaminants in water and air that are affecting humans, plants, and animals alike. The uncontrolled extraction of resources from nature, the use of harmful

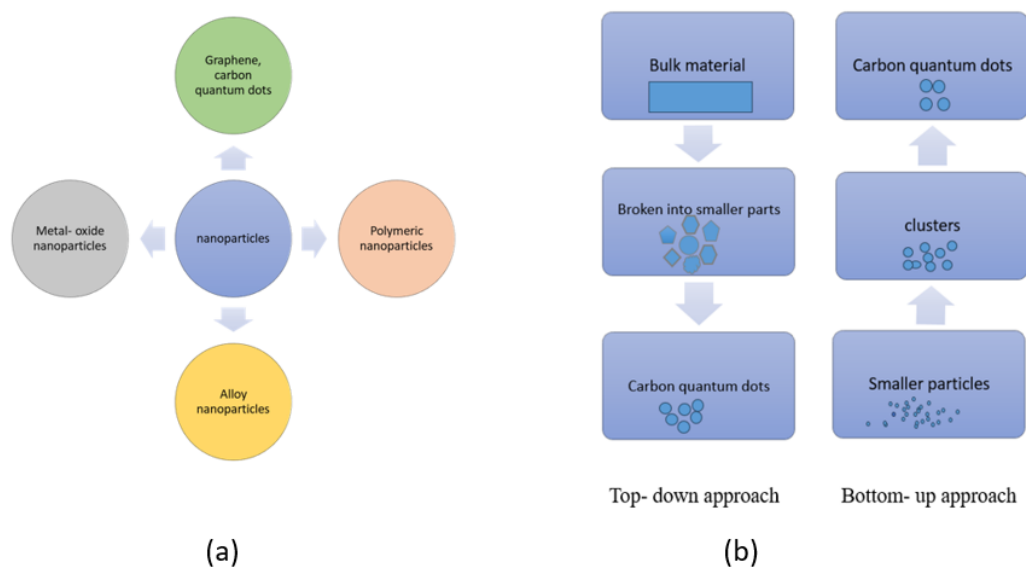


Fig.1: (a) Types of nanoparticles; (b) Schematic illustration of the synthesis approaches of CQDs.

chemicals to produce daily necessities, the production of various types of medicines, and the improper handling of their waste materials after production have led to water contamination in rivers, lakes, and groundwater. Many contaminants, including Surface-active agents, dyeing agents for textiles, toxic metallic elements, insecticides, and agricultural chemicals have been found in the aquatic environment particularly those in aquatic habitats, where contamination can lead to toxicity, behavioral changes, and mortality (Sharma *et al.*, 2019). Generally, these pollutants can be categorized into many types such as organic pollutants (e.g. dyes, polyaromatic hydrocarbons, pharmaceuticals) and also inorganic pollutants (e.g. heavy metals, radioactive waste, and halides) based on their constituents (Samanta *et al.*, 2019). These pollutants stay in water for a very long time, restricting light penetration, disrupting photosynthesis, and impairing the food chain, while heavy metals accumulate in animal tissues, producing bio-magnification and endangering higher-order species, including predators. The ecological and health implications of these pollutants on animals are critical, prompting the need for creative

methods to detect, monitor, and remove dangerous compounds from the environment. Different conventional methods are used to eliminate pollutants, including filtration, reverse osmosis, sedimentation, adsorption, coagulation, and advanced oxidation processes (AOPs). Among these techniques, AOPs based on the photocatalytic approach have attracted interest because of their environmental and green preservation capability. (Guo *et al.*, 2020). In recent years, carbon quantum dots (CQDs) have emerged as promising materials in the field of photocatalysis for environmental remediation. After being accidentally discovered in 2004, carbon quantum dots have been attracting the attention of scientists from all around the world because of their excellent optical and electrical properties. CQDs are the newest addition to the nanoparticle family (Fig. 1) consisting of nanoparticles like graphene, fullerene, nanodiamonds, and carbon nanotubes. In the beginning, they were just known as fluorescent carbon nanoparticles, it was Sun and his teammates named these particles as Carbon quantum dots when they synthesized nanoparticles with sizes smaller than 10 nanometers. The optical properties of CQDs,

characterized by their size-dependent absorption and emission, make them excellent candidates for photocatalytic applications (Lin *et al.*, 2018; Mochalin *et al.*, 2020; Saud *et al.*, 2022; Szczepankowska *et al.*, 2023). Their tunable bandgap allows for efficient light absorption in the UV and visible regions, facilitating the generation of electron-hole pairs essential for catalytic reactions (Martindale *et al.*, 2017).

CQDs also possess excellent electrical properties, including higher electron mobility and conductivity, enhancing their effectiveness in charge separation and charge transfer during the photocatalysis process, and improving overall performance in pollutant degradation. Integrating CQDs into photocatalytic systems can enhance the production of reactive oxygen, thus accelerating the decomposition of pollutants. CQDs also have high biocompatibility, and low toxicity, and can cross biological barriers making them suitable for various biomedical applications. CQDs have been employed for bioimaging, drug delivery, and photothermal therapy (Chen *et al.*, 2010; Cayuela *et al.*, 2016). The excellent fluorescence properties enable tracking and imaging of biological structures at the cellular and molecular levels. Carbon quantum dots are used in the sensing and detection of various metals (Pan *et al.*, 2020), as the surface of CQDs can be functionalized with different kinds of functional groups, making them effective sensors for heavy metals and poisonous substances. CQDs have been used in detecting heavy metal ions such as lead, mercury, cadmium, and various other pollutants, showcasing their potential in monitoring and early detection of environmental contaminants.

Synthesis Methods

There are several methods to synthesize carbon quantum dots, but in general, there are two main approaches to synthesizing carbon quantum dots: (i) top-down approach and (ii) bottom-up approach (Fig. 1) (Hutton *et al.*, 2017). The top-down approach involves breaking down a big carbon material into smaller parts through methods such as the Arc-discharge method,

chemical oxidation, and electrochemical methods (Chao-Mujica *et al.*, 2021). In the top-down approach, we can control the shape and size of the synthesized carbon dots but the top-down approach requires costly materials, involves complicated steps, and has a harsh reaction condition. On the other hand, in the bottom-up approach, the smaller particles assemble to form carbon quantum dots via a chemical reaction with methods like hydrothermal, microwave, and pyrolysis methods (Dao *et al.*, 2016). The benefit of the bottom-up method is that we can use plants, biomass, and waste materials as precursors to synthesize carbon quantum dots making this method green and cost-effective. Bottom-up methods are easy to perform, less costly, and do not have too many complicated steps compared to top-down methods. The CQDs synthesized by the bottom-up approach generally have uniform size compared to the top-down approach where particles have a wide range of size distribution.

The hydrothermal method can be considered the most used synthesis method to synthesize carbon quantum dots because it involves less complicated operational steps, is cost-effective, and requires less energy as mentioned in many research studies. CQDs were synthesized using the fish scale through a hydrothermal method at 180°C for 24 h and then let them cool at room temperature. From the TEM image of carbon quantum dots, the average particle size was found 6 nm and CQDs exhibited green colour emission at 406 nm by the excitation of 330 nm (Dhandapani *et al.*, 2020). Zhao *et al.* (2020) synthesized carbon quantum dots with high quantum yield by using renewable biomass as a precursor through the hydrothermal method. Likewise, Atchudan *et al.* (2021) derived ultra-high yield CQDs from banana peel waste by one-step hydrothermal method. The average diameter of CQDs was 5 nm and had a quantum yield of 20%. The CQDs were used as fluorescent probes in multicolour imaging of nematodes (Atchudan *et al.*, 2021). Microwave synthesis can be considered another famous method to synthesize carbon quantum dots because it requires less energy, is faster, and can

Table 1: Synthesis of CQDs by various methods using different precursors

Precursors	Synthesis method	Average size	Temp. and time	Reference
Urea and p-phenylenediamine	Hydrothermal	3 nm	160°C, 10 h	Ding <i>et al.</i> (2016)
Citric acid (CA), ammonia	Pyrolysis	10.8 nm	200°C, 3 h	Wang <i>et al.</i> (2016)
Fennel seed	Pyrolysis	3.9 nm	500°C, 3 h	Dager <i>et al.</i> (2019)
Graphite rod	Electrochemical carbonization	6nm	-	Ipte <i>et al.</i> (2020)
Ammonium citrate	Microwave irradiation	20	180°C, 2 h	Mistry <i>et al.</i> (2019)
Jaggery and urea	Hydrothermal	9.5 nm	140°C, 2.5 h	Gao <i>et al.</i> (2020)
Manilkara zapota fruits	Hydrothermal	1.9 ± 0.3nm, 2.9±0.7nm, 4.5± 1.25nm	100°C, 1 h, 80°C, ½ h	Bhamore <i>et al.</i> (2019)
Waste cotton linter	-	10.14 nm	-	Eskalen <i>et al.</i> (2020)
Roasted chickpeas	Microwave-assisted pyrolysis	8.7nm	-	Başoğlu <i>et al.</i> (2020)
Expired fruit shells	Hydrothermal	~5nm	180° C, 3 h	Yang <i>et al.</i> (2021)
Cambuci juice	Hydrothermal	3.7 nm	190°C, 6h	da Silva Júnior <i>et al.</i> (2021)
Citric acid	Microwave	3.81 nm	-	Romero <i>et al.</i> (2021)

be stopped anytime. Guo *et al.* (2018) synthesized N-CQDs from Glucose and m-phenylenediamine (MPD) as the precursors and ethylene glycol as the solvent. They heated a mixture of glucose and MPD by using the microwave method at 120° C for 5 min in a 240W microwave. The obtained CQDs had a size range of 15- 35 nm with a size of 22.23 nm and exhibited green colour fluorescence when irradiated with a wavelength of 365 nm (Guo *et al.*, 2018). In another work, N-CQDs were synthesized via an efficient and green microwave method using citric acid monohydrate, and urea in a molar ratio of 1:3. The HRTEM image of synthesized N-doped showed that CQDs had spherical shape with a size range of 7.29 ± 3.91 nm. The N-CQDs have a quantum yield of 14.8% with blue colour fluorescence at 443 nm with an excitation wavelength at 360 nm. These N-CQDs successfully detected free chlorine in ClO⁻ form with a LOD (limit of detection) of 0.4 mM and LOQ of 1.2 mM at pH 9 (Bebas *et al.*, 2022). The synthesis of

carbon quantum dots using different method are listed in Table 1.

Characterization of carbon quantum dots

Characterization of CQDs is essential in understanding the properties and optimizing their performance for various applications. There are several methods to characterize the CQDS such as the XRD, UV-Vis spectroscopy, XPS, transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and photoluminescence.

(i) **UV-Vis Absorption Spectroscopy:** UV-Vis spectroscopy is used to determine the absorption and emission characteristics of carbon quantum dots. UV-Vis spectroscopy helps in understanding the bandgap, and quantum yield of CQDs. Liu *et al.* (2023) synthesized blue, yellow, and red emissive carbon dots (CDs) by using o-phenylenediamine (OPD) with aniline, 1,8-diaminonaphthalene, and [1,1'-binaphthalene]-2,2'-diamine as precursors

via solvothermal method. They observed that the emission properties of the CDs could be adjusted by selecting carbon precursors with varying quantities of benzene rings. The UV-Vis absorption spectra of the CDs showed strong absorption bands at 360 nm, 430 nm, and 500 nm, corresponding to the $n-\pi^*$ transitions of the C=O and C=N bonds of the aromatic sp^2 system (Liu *et al.*, 2023). Zhang *et al.* (2018) added crushed ginkgo leaves (12g) to ultrapure water subjugated it to 30 min sonication and then heated the mixture in the hydrothermal furnace for 10 h. After centrifuging and filtering the mixture, fluorescent and water-soluble CQDs with a quantum yield of 11% were obtained. The UV-Vis spectroscopy of synthesized CQDs showed an absorption peak at 273 nm corresponding to the $\pi-\pi^*$ transition of C=C functional groups and $n-\pi^*$ transition of C=O functional groups respectively (Zhang *et al.*, 2019).

(ii) **Fourier Transform Infrared Spectroscopy (FTIR):** FTIR is used to study the functional group attached to the surface of carbon quantum dots. As carbon quantum dots mostly consist of carbon, oxygen, and hydrogen, they develop due to partial oxidation of several carbon precursors groups such as $-\text{COO}^-$ or carboxylic acid groups, and $-\text{OH}$ groups are attached to the surface. FTIR spectroscopy is useful in identifying these functional groups. CQDs were synthesized by placing 20 g of corn stalk in 500 ml Ultra-pure water in a reaction kettle at 270°C temperature and 5 MPa pressure for 10m hydrothermally in near-critical water treatment. The CQDs exhibited blue colour fluorescence with a quantum yield of 16%. The produced carbon quantum dots' FTIR revealed two prominent peaks: one at 1118 cm^{-1} due to the C-H deformation vibration and the other at 1609 cm^{-1} , corresponding to the stretching vibration of the C=C. In the FTIR spectrum, there are multiple additional peaks located at approximately 3355 cm^{-1} , 1707 cm^{-1} , 1517 cm^{-1} , and 1205 cm^{-1} , which verify the existence of function groups such as hydroxyl, C = O, C-N, and C-O in CQD, respectively (Li *et al.*, 2021). Carbon quantum was synthesized with the

size of $6.33 \pm 1.34\text{ nm}$ and a very high QY of 60.7% from leaves of eucalyptus tree using hydrothermal synthesis at 180°C for 24 h. FTIR image of synthesized CDs shows broad stretching vibrational Peaks at 3232 cm^{-1} and 2927 cm^{-1} corresponding to N-H (or O-H) and C-H bonds, respectively and also sharp absorbance peaks at 1555 cm^{-1} attributing to COO^- and at 1384 cm^{-1} corresponding to C-H group, respectively. The FTIR shows the presence of aquaphilic groups such as amino, hydroxylic, and carboxylic groups on the surface of carbon dots promoting the fluorescence and aqueous solubility of the synthesized CQDs (Johny *et al.*, 2024).

(iii) **XRD spectroscopy:** XRD spectroscopy is used to study the crystalline structure of carbon quantum dots. XRD provides information about the phase purity, size, and whether carbon quantum dots have amorphous or crystalline structures. Thambiraj and Shankaran (2016) synthesized carbon quantum dots from sugarcane via chemical oxidation. The synthesized carbon quantum dots have a size of $4.1 \pm 0.17\text{ nm}$ and had quantum yield of 18.7%. X-ray diffraction of synthesized carbon quantum dots showed an intense peak at $2\theta = 11.4^\circ$ and 20.6° due to presence of graphitic carbon (Thambiraj and Shankaran, 2016). In another work, carbon quantum dots were synthesized from *Tamarindus indica* via a simple one-step hydrothermal method. The CQDs showed excitation-dependent behavior in the wavelength range of 240-460 nm and had an excellent quantum yield of nearly 46.6%. the synthesized carbon quantum dots size ranges from 3 to 3.5 nm and the XRD-spectrum of CQDs showed a broad peak at $2\theta = 22^\circ$. These CQDs were employed as sensing probes as they detected Hg^{+2} with a LOD (limit of detection) as low as 6 nM in the 0 to 0.1 μM range (Bano *et al.*, 2018).

(iv) **Transmission Electron Microscopy (TEM):** Transmission Electron Microscopy is used to determine the size, morphology, and dispersion of carbon quantum dots. TEM is also used to confirm the presence of core-shell structure and the degree of aggregation. Carbon quantum dots were

synthesized by placing cabbage in a hydrothermal reactor for 5 h at a temperature 140° C. TEM spectroscopy of synthesized carbon quantum dots showed that they had narrow size distribution with sizes ranging from 2 to 4 nm, were well dispersed, and had spherical morphology. HRTEM showed that CQDs had a partially crystalline structure with a lattice parameter of 0.21 nm, referring to the possibility of the presence of the sp²-graphitic crystal phase of graphene. These CQDs were employed for cellular imaging (Bano *et al.*, 2018). Similarly in another work, CQDs were synthesized from coffee beans roasted for different periods. TEM image CQDs showed that synthesized carbon dots were spherical and evenly dispersed without apparent aggregation. The average sizes of CQDs roasted for 5, 10, and 20 min were 5.69 ± 1.10 nm, 2.44 ± 1.08 nm, and 1.58 ± 0.48 nm, respectively (Chu *et al.*, 2023).

Photoluminescence of Carbon Quantum Dots

Carbon quantum dots' photoluminescence is one of the most remarkable properties that make them useful in environmental applications. The origin of photoluminescence can be considered as the combination of several effects such as quantum confinement effects, defect states, and surface functional groups. When CQDs are irradiated with light (typically UV or visible light) the electrons in the valance band jump to the conduction band leaving behind a hole in the valance band. After that the radiative recombination of these electron-hole pairs leads to the emission of photons, resulting in photoluminescence. According to many research findings, CQD generally exhibits blue or green colour photoluminescence independent of the approach of synthesis. CQDs with different colours (blue to red) were synthesized by combining citric acid and diamminonaphthalene and treating them hydrothermally at 200°C. The synthesized carbon quantum dots exhibited different colours of fluorescence with different sizes 1.95 nm, and 2.41 nm which showed blue and green colour fluorescence, while CQDs with sizes 3.78 nm, 4.90 nm, and 6.68 nm exhibited yellow, orange, and red colour fluorescence when

irradiated with ultraviolet light of the wavelength of 365 nm (Yuan *et al.*, 2017). Li *et al.* (2021) synthesized multicoloured (blue, green, and orange) nitrogen-doped carbon nano-dots to study the effect of different concentrations of raw materials on CNDs properties (optical and structural) by using different concentrations of citric acid (CA) and L-glutamic acid by heating them in the microwave at 300° C. They observed that CNDs with different ratio of CA and glutamic acid as 1:1, 1:2, and 1:3 showed fluorescence of different fluorescence of blue, green and orange colours (Li *et al.*, 2021).

Applications

(i) *Photocatalytic application*: Carbon quantum dots (CQDs) offer an effective way of addressing issues related to pollution in the environment through photocatalytic applications. CQDs can be utilized for different environmental and energy applications due to their distinctive electrical and optical characteristics, which have shown substantial photocatalytic activity under light irradiation. In photocatalysis, CQDs speed up chemical reactions by acting as catalysts under the influence of light. Nitrogen-doped carbon quantum dots were synthesized by heating an aqueous glucose solution with ammonia hydroxide (NH₄OH, 25%) as a nitrogen doping agent. Glucose/NH₄OH aqueous solutions were heated in a 5:1 ratio in a microwave reactor at 100°C for 1 min to synthesize carbon quantum dots (Prekodravac *et al.*, 2019). Obtained N-CQDs had a size range of 7-30 nm and were tested for their photocatalytic activity for the removal of toxic organic dye which shows remarkable activity with a 93% degradation rate under visible light irradiation demonstrating the potential use of CQDs in environmental purification (Prekodravac *et al.*, 2019). To create silica-carbon quantum dots (Si-CQDs), Viona and her coresearcher heated rice husk hydrothermally in an oven for ten h at 150–250 °C. The photocatalytic effect of Si-CQDs on methylene blue (MB) was assessed and the results indicated that Si-CQDs exhibited exceptional photocatalytic capacity, decolourizing 100% of the

Table 2: Prior studies on the use of CQDs to filter out environmental contaminants

Catalyst	CQDs effect	Pollutant	Removal rate	Reference
CQDs/BiOBr	Charge separation, Light harvesting	Bisphenol A(BPA)	100%	Ji <i>et al.</i> (2018)
NCDs/DCN	Photocatalyst	Phenol rhodamine B	100%	Liu <i>et al.</i> (2020)
TiO ₂ -CQDs	Light harvesting, charge separation	Phenol rhodamine B	77%	Wang <i>et al.</i> (2015)
S-CQDs	Photocatalysts	Fuchsin	81%	Huang <i>et al.</i> (2018)
N-CQDs	Photocatalyst	Methylene blue	41%	Feng <i>et al.</i> (2024)
ZIS/CQDs	Photocatalyst	Phenol rhodamine B and MO	99%, 91%	Ramar <i>et al.</i> (2018)
CQDs/C ₃ N ₄	Electronic transfer, Light Conversion	tetracycline (TC)	82.1%	Xu <i>et al.</i> (2018)
N-CQDs	Photocatalyst	Malachite green (MG), methylene blue	98%, 97%	He <i>et al.</i> (2019)

MB within 120 min and demonstrating a ~ 99% MB degradation rate by 240 min (Wongso *et al.*, 2021). In a similar work, CQDs were hydrothermally synthesized using Pluronic F127 (a block polyether surfactant) as a carbon source and then modified the surface of CQDs with NH₃ and thionyl chloride to obtain nitrogen-doped-CQDs and chlorine-doped-CQDs, respectively. The average diameter of the CQDs was 2.3 nm while the average diameter of N-CQDs and Cl-CQDs was 2.8 and 3.5 nm, respectively. The surface modification increased the size of CQDs with the introduction of nitrogen and chlorine groups. These CQDs were used to degrade two organic dyes, indigo carmine (IC) and light green SF yellowish (LG SF), under natural light irradiation. The nitrogen-doped-CQDs degraded 99.13% of IC in 15 days, while chlorine-doped-CQDs achieved a degradation rate of 97.59% for LG SF within the same period (Liu *et al.*, 2023). Some studies related to photocatalytic activity of carbon quantum dots are listed in Table 2.

(ii) Bio-imaging of living cells: Bioimaging is an essential instrument in cell biology, enabling the viewing of cellular structures and activities with high precision and resolution. The use of carbon quantum dots in bio-imaging has emerged as an innovative and eco-friendly technology, giving

various advantages such as intense fluorescence, low cytotoxicity, and biocompatibility. The smaller size of carbon dots allows them to penetrate cells at a very small level enabling them to provide better imaging of cells, allowing the study of cellular mechanisms, physiological responses, and toxicological impacts, particularly in animal models. Jayanthi *et al.* (2019) synthesized carbon dots from carrots. The synthesized carbon quantum dots had a size distribution of 3-5 nm and a spherical shape with a quantum yield of 4.56%. They tested CDs for their bio-imaging capability using Gram-negative bacteria, *Escherichia coli*, and Gram-positive bacteria, *Staphylococcus aureus* as models using fluorescence microscopy. They observed the rod-shaped morphology of *E. coli* and the cocci-shaped morphology of *S. aureus* was visible through labeling with blue fluorescence CQDs confirming its bio-imaging capability (Jayanthi *et al.*, 2019). In another work, Xue *et al.* (2019) synthesized lignin hybridized CQDs with different molar ratios of citric acid and ethylenediamine via hydrothermal method. The L-CQDs were spherical and had a diameter of less than 10 nm. These synthesized L-CQDs showed multicolour fluorescence with an emission maximum ranging from 454 to 535 nm under the excitation at a wavelength of 375 to 460

nm. The quantum yield of synthesized L-CQDs was 43%. The L-CQDs displayed viability of more than 90%. They were incubated with HeLa cells at the concentration of 0.062 mg/ml and 0.031 mg/ml after 24 h of incubation. When they increased the concentration of L-CQDs to 0.50%, the viability of the cell was still more than 80%. The L-CQDs were also employed for bioimaging of HeLa cells at three different wavelengths where they showed green, blue, and red colour under UV light demonstrating cell imaging capability of carbon quantum dots (Xue *et al.*, 2019).

Conclusion

Since the first time of their synthesis, extensive work has been done on carbon quantum dots because of their excellent optical and electrical properties. This includes the synthesis of carbon quantum dots, modification, and various applications in fields such as photovoltaics, bio-medical, and photocatalysis. In this review, we portrayed recent progress in the field of carbon quantum dots, focusing on their synthesis methods, different methods of characterization of synthesized carbon quantum dots, and also their different application in the environment field. The carbon dots mainly have two forms crystalline or amorphous depending upon the synthesis parameters such as what kind of synthesis method is being used, nature of precursors, temperature, type of solvents, pressure, and the reaction time. In all synthesis methods, bottom-up methods are less costly and easy to perform, but the drawback of that they offer less control over the size of CQDs when compared to top-down methods that offer better control over size but are very expensive. The tunable band gap, size-dependent absorption and emission, and high electron mobility allow them to play different roles in photocatalysis and bio-imaging applications. Although CQDs have a lot of potential for use in environmental applications, issues still need to be resolved. One of the remaining obstacles is the synthesis methods' scalability, particularly for large-scale applications. Additionally, the full understanding of CQDs' toxicity and environmental impact needs

further investigation to ensure their safe application. Improving the synthesis procedure should be the main focus of future research to boost productivity and reduce costs. New opportunities could be opened up by extending the use of CQD features to other fields, such as energy storage and catalysis, and tailoring them to specific environmental contaminants.

Ethical Statement

This is a review article thus an ethical statement is not needed.

Author Contributions

All the authors have contributed equally to the completion of this paper and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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