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Review

Synthetic Methods and Applications of Carbon Nanodots

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Abstract: In the recent decade, carbon dots have drawn immense attention and prompted intense investigation. The latest form of nanocarbon, the carbon nanodot, is attracting intensive research efforts, similar to its earlier analogues, namely, fullerene, carbon nanotube, and graphene. One outstanding feature that distinguishes carbon nanodots from other known forms of carbon materials is its water solubility owing to extensive surface functionalization (the presence of polar surface functional groups). These carbonaceous quantum dots, or carbon nanodots, have several advantages over traditional semiconductor-based quantum dots. They possess outstanding photoluminescence, fluorescence, biocompatibility, biosensing and bioimaging, photostability, feedstock sustainability, extensive surface functionalization and bio-conjugation, excellent colloidal stability, eco-friendly synthesis (from organic matter such as glucose, coffee, tea, and grass to biomass waste-derived sources), low toxicity, and cost-effectiveness. Recent advances in the synthesis and characterization of carbon dots have been received and new insight is provided. Presently known applications of carbon dots in the fields of bioimaging, drug delivery, sensing, and diagnosis were highlighted and future applications of these astounding materials are speculated.

Keywords: carbon nanodots; synthesis; applications; surface functionality; biocompatibility; low toxicity; bioimaging; applications

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1. Introduction

Nanoparticles are microscopic particles with a size range of 1–100 nm. During the past decade, considerable research was conducted on the fabrication and application of nanoparticles in many fields. Based on their unique properties, nanoparticles have a substantial impact in various industries, including health, cosmetics, energy, pharmaceuticals, and food.

Enormous work was completed in recent years to design nanostructured materials with specific characteristics that will ultimately influence their function and application. In this era of carbon nanotechnology, special emphasis is laid on the organic functionality of nanomaterials or organic nanomaterials, including graphene, carbon nanotubes, and fullerenes. Because of their biocompatibility, ease of fabrication, and fascinating features, especially their water solubility fluorescence emission, carbon nanodots with a size in the range of 1–10 nm have taken the central stage of materials research. Carbon nanodots (CDs) are known to have zero dimension with almost spherical geometry. This material has become a rising star in the field of luminescent nanomaterials [1]. Due to their desirable qualities, such as hydrophilicity, ease of functionalization, outstanding biocompatibility, bright luminescence, good solubility, high chemical inertness, and low toxicity, they are potent candidates for various applications in solar cells, biosensors [2–10], bioimaging, and optoelectronic devices, etc. CNDs exhibit many remarkable properties including outstanding photoinduced electron transfer, stable chemical

inertness, low cytotoxicity [11–15], good biocompatibility, and efficient light harvesting. Dots made of carbon, such as carbon nanodots (CDs) and graphene quantum dots (GQDs), are a brand-new carbonaceous nanomaterial with zero dimensions [16–22]. Until now, a lot of work has been carried out, and substantial advances have been made in the synthesis and uses of carbon-based dots [23–27].

This class of carbon-based nanomaterials was initially found by the top-down approach of minimizing huge carbon nanomaterials, and has recently advanced at a startling rate. Their purity standards, classification, and fluorescence mechanisms have made an impact on the research community, as evident from the rapid pace of research and publications in this area. The emphasis of the review is mainly on the synthesis and applications of carbon nanodots. A schematic of the methods of synthesis of CNDs and their structure was depicted in Figures 1 and 2, respectively. They usually have the inner hybridization of sp^2 and outer hybridization of sp^3, and these hybridized structures tend to have functional groups containing oxygen atoms (such as -OH, -COOH, -CO, and many more).

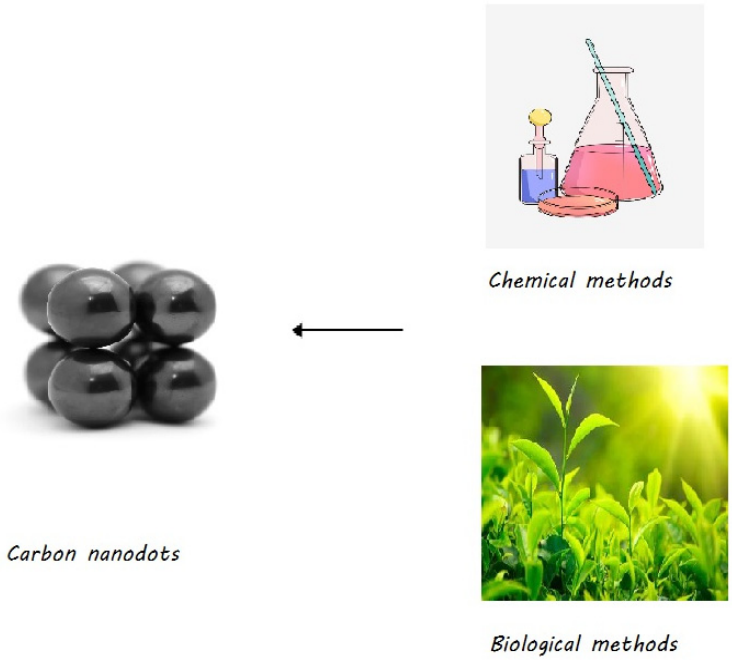


Figure 1. Methods used for the synthesis of Carbon nanodots (1–10 nm).

Due to their excellent characteristics, CNDs are prospective replacement probes for bioimaging and bioassay [28–33]. So far, a number of methods have been investigated to synthesize CNDs, including thermal oxidation, chemical oxidation, and arc discharge, laser ablation of graphite, electrochemical synthesis, microwave synthesis, and ultrasonic methods [34–40]. The use of most of these techniques are, however, constrained since they sometimes require an expensive carbon source, intricate reactions, lengthy process time, and post-treatment steps. Therefore, there is a significant demand for synthetic methods that are easy to use, sustainable, and ecologically friendly for the mass production of high-quality CNDs [41].

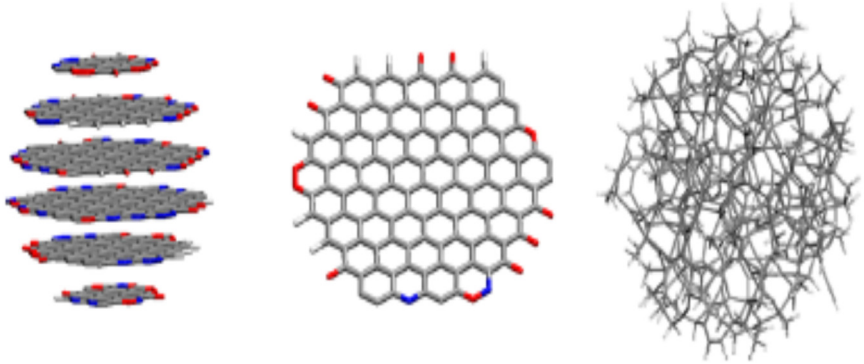


Figure 2. Commonly available carbon nanodot structures: spherical particles, nanosheets of graphene, and amorphous structures. Reproduced with the permission from Ref. [41], 2019, American Chemical Society.

There are not many reviews focused explicitly on the synthetic pathways, characteristics, and uses of the CDs, despite the fact that they have all been thoroughly summarized elsewhere [42–45]. For improved clarity and understanding, a general evaluation of the most recent developments in the synthetic methods of CNDs is presented. Moreover, the advances in the understanding of the properties of carbon dots and the resulting applications were highlighted, and a new insight is provided by correlating the synthetic strategy, property, and application [46–50]. The organization of the review comprises a discussion of the approaches for the fabrication of carbon nanodots for various sustainable resources (including biomass), with an emphasis on cost-effectiveness and eco-friendliness. Afterward, the major applications of CNDs were highlighted, with an emphasis on bioimaging and photocatalysis.

2. Novel Methods for the Synthesis of Carbon Nanodots

During the last ten years, namely 2013–2023, many methods were developed to synthesize carbon nanodots with attractive features and applications in a specific field. These well-known CD synthesis techniques are typically categorized into "top-down" and "bottom-up" categories. The top-down techniques involve the exfoliation of nanomaterials chemically, laser ablation, electrical and chemical oxidation, arc discharge, and ultrasonic synthesis. Graphene quantum dots, or two-dimensional nanomaterials, are often produced via a "top-down" technique by exfoliating and cutting the macroscale framework of carbon species, such as carbon rods, tubes, graphite powder, activated carbon, carbon black, carbon soot, and carbon fibers possessing the graphene lattices. Top-down strategies typically demand a lengthy processing time, challenging reaction environments, and expensive materials and machinery [51–53], and these methods work well for the mass production of CNDs. On the contrary, the bottom-up method is used for producing carbonized polymer dots and carbon quantum dots (3D nanoparticles with spherical centers) by polymerizing molecular precursors, including glucose, sucrose, and citric acid, using processes such as chemical vapor deposition, plasma treatment, microwave pyrolysis, and solvothermal reactions, with a high degree of controllability. Of course, these methods are not flawless altogether [54].

Hawrylak et al. [55] synthesized carbon nanodots (CNDs) for the first time by surprise in 2004 in their effort to purify single-walled carbon nanotubes. Arc-discharge soot was used in the experiment as a source of carbon nanotubes. The components of the soot suspension were separated during the procedure using gel-electrophoresis, which exposed a brand-new band of fluorescent material. Currently, CNDs can be synthesized by chemical or physical processes. Thermal therapy, electrochemistry, acidic or hydrothermal oxidation, or ultrasonic treatment are examples of chemical processes. Arc discharge, plasma therapy, and laser ablation are examples of physical approaches [56]. The processes for synthesizing CNDs can be roughly categorized as top-down or bottom-up syntheses, as mentioned earlier [57–66]. Top-down techniques often include etching, intercalation, hydrothermal

or solvothermal cutting, chemical oxidation, and laser ablation to break down a larger carbon structure into progressively smaller pieces. In bottom-up methods, CNDs are synthesized by carbonizing organic precursors. These include dehydration with sulfuric acid, microwave pyrolysis with solvent mediation, and refluxing pyrolysis. Using solvothermal or direct thermal breakdown, organic precursors such as allotropic carbon forms, natural gas, and carbohydrates are transformed into CNDs using direct thermal or solvothermal breakdown [67]. To achieve uniformity, the final product can be processed through electrophoresis, chromatography, centrifugation, dialysis, or through some other processes [68]. The carbon precursor, preparation technique, and experimental circumstances have a significant impact on the shape and structure of CNDs. For instance, depending on the parent material, CNDs made using top-down techniques have a different size and shape (coal, graphite powder, or graphene nanosheets). UV radiation or hydrothermal treatments can be used in a single preparation technique such as etching, and both would yield different outcomes. CNDs produced from the top-down approach would typically have dimensions below 10 nm, a spherical or sheet-like shape, and a size below 3 nm. Data indicate that bottom-up synthesized CNDs may be layered with a size of less than 10 nm.

Arc discharge and laser ablation are likely the most well-known top-down techniques for producing carbon-based nanomaterials. The term "arc discharge" refers to producing a current between two electrodes, often graphite rods, which causes them to vaporize. As a result, soot is formed, which may contain various nanoparticles of carbon. In contrast, the technique of laser ablation comprises applying a pulse of laser energy to a solid surface, resulting in carbon nanomaterials. Laser ablation in solution (LAS) has attracted interest as a top-down, single-step method for producing nanomaterials quickly and affordably, as shown in Figure 3 [69].

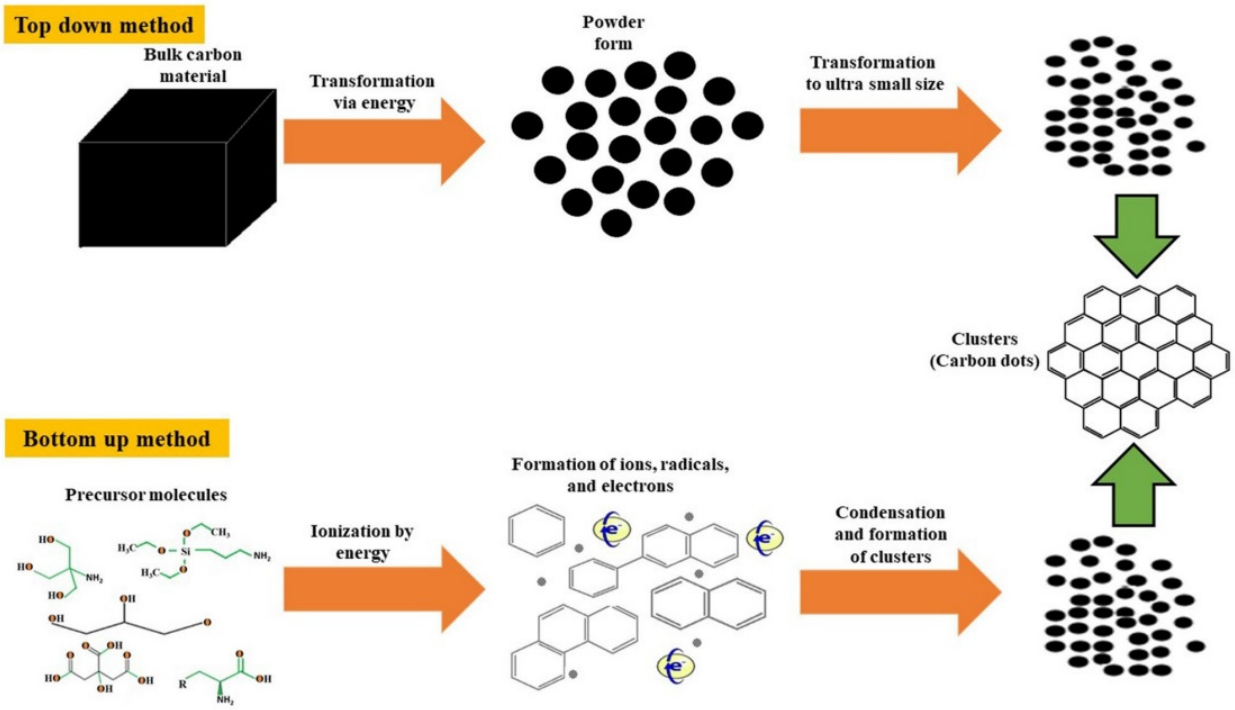


Figure 3. General methods of synthesis carbon nanodots (CNDs: Bottom-up approach: CNDs were synthesized from smaller carbon units (small organic molecules) by applying energy (electrochemical/chemical, thermal. laser, microwave, etc.). The source molecules will get ionized, dissociated, evaporated, or sublimated and then condensed to form CNDs; Top-down approach: CNDs are synthesized by transformation of larger carbon structures into ultra-small fragments by applying energy (thermal, mechanical, chemical, ultrasonic, etc.). Reproduced with the permission from Ref. [69], 2019, Springer Nature.

Amorphous (a) nanoparticles (a-CDs) are often produced at relatively low temperatures (<300 °C), whereas graphitized (g) structures are produced at higher temperatures (g-CDs). The surface functional groups of the resulting nanoparticles are significantly influenced by the precursors used. The most prevalent surface functional groups are amine and carboxylate, using precursors such as citric acid or polyamines. Such functionality can be generated via post-synthetic functionalization reactions. At the beginning of the research in this area, researchers found that proteins were the suitable precursors for the synthesis of carbon nanodots because they were readily available, affordable, and capable of undergoing dehydration and decarboxylation reactions to produce CNDs with heteroatom doping [70,71]. N-doping is accountable for better photoluminescence quantum yields, red-shifted absorption, and more favorable optoelectronic features. Citric acid in combination with amino acids (arginine, Arg) was also explored, leading to the formation of carbogenic nanoparticles' molecular precursor, which benefits from its distinct reactive behavior and capacity to serve as a "passivating agent" or "capping agent" for the outer surface [72,73].

The hydrothermal approach, aided by microwave heating, is one among numerous potential synthesis processes. It has been widely employed for manufacturing a variety of carbon materials. Hydrothermal synthesis offers a minimal toxicological impact on materials and processes [74]. The use of hydrothermal conditions causes the reagent's solubility to rise or change, enhances their chemical and physical interactions, and makes it easier for the carbonaceous structures to form. The production of nanomaterials with higher amounts of carbon, such as graphitic carbon compounds, and nanotubes at higher temperatures is a reliable process. Microwave-assisted methods have also grown in popularity as a method for synthesizing nanomaterials. Issues with the conventional heating process used for preparing nanomaterials, such as the tendency for insoluble compounds to cause heterogeneous heating, leading to an increase in the size of nanomaterials, is solved by microwave heating [75,76]. Due to its high energy consumption efficiency, MW irradiation offers a safe, inexpensive, and practical mode of heating, producing higher yields of the desired products [77]. As a result, the MW-assisted hydrothermal approach, which combines the benefits of both MW and hydrothermal processes, has become essential for the production of carbon dots.

The carbonization of small molecule precursors is achieved in the bottom-up fabrication of carbon dots. The most commonly available methods for the fabrication of carbon dots via the bottom-up method include a mixture of molecules having nitrogen atoms (such as urea) and citric acid [78–84]. The pyrolysis of these molecular precursors in an autoclave or microwave forms a black nanopowder of CDs. These CDs are easily dissolved in water and have exceptional fluorescent characteristics. These CDs are capable of emitting blue [85], green [86], and red emissions depending on their surface properties and circumstances (excitation source of radiation) [87,88], albeit a thorough purification is frequently required to separate the carbon dots [89]. When it comes to top-down methods, the starting materials include carbon structures such as amorphous activated carbon, carbon fibers, graphite, nanotubes, and fullerenes that are physically or chemically fragmented to produce very small carbon nanoparticles [90–93]. One such instance is graphitic oxidation in an extremely acidic environment [94,95], enabling the surface to be functionalized and the bulk-precursor to be broken up, resulting in the optical characteristics that are typical of CDs. Top-down synthetic methods frequently produce CDs with lower quantum yields of emission. These top-down synthetic methods of CDs are far more complex and time-consuming. However, they enable better structural control and end-product purity [96,97].

2.1. Sonochemical/Ultra-Sonic Fabrication of CDs

Sonochemistry is exploited for the synthesis of nanostructured materials. In this acoustic activation technique, severe physical and chemical conditions are generated as a result of the use of high-intensity ultrasound [98–106]. As the molecular dimensions are

smaller than acoustic wavelengths, the chemical features of the resulting materials are not a result of the interaction between the ultrasonic and chemical species in the liquid state. Sonochemical fabrication is the result of the high compression heating of gas and vapor, resulting in incredibly high temperature and pressure conditions [107].

For the first time, Zhuo et al. [108] described the synthesis of graphene quantum dots using the ultra-sonic exfoliation of graphene. A simple sonochemical approach for the production of extremely photoluminescent CDs was devised by Wei et al. [109] in 2014. High-intensity ultrasound forms collapsing bubbles that serve as microreactors and offer intense, momentary conditions ideal for the pyrolysis of carbon precursors. Sono-chemically produced CDs in the presence of surface passivation agents have a high quantum yield and outstanding photostability.

The ultrasonic technique has merits of being inexpensive and easy to operate for the synthesis of carbon dots. The ultrasound method involves alternate high-pressure and low-pressure waves, which cause small bubbles in liquid to form and break. Thus, by means of powerful hydrodynamic shear forces resulting from the cavitation of tiny bubbles, macroscopic carbon materials were reduced to nanoscale CDs. Generally, the ultrasonic power, reaction time, and solvent and carbon source ratio were altered to produce CDs with various properties.

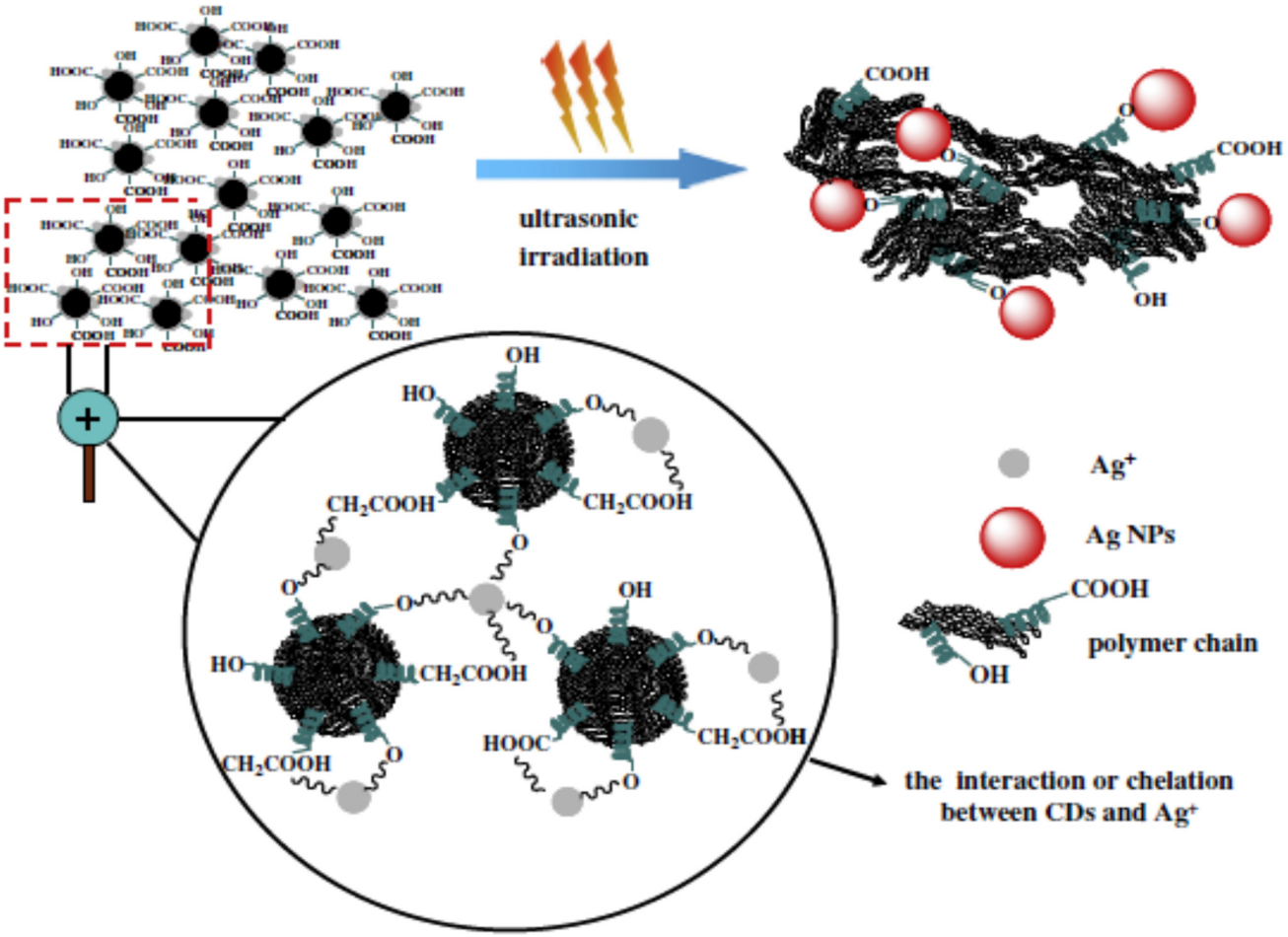
Huang et al. [110] used a direct ultrasonic exfoliation method to produce the chlorine-infused graphene quantum dots. Park et al. [111] conducted a typical experiment in which they first produced water-soluble CQDs from food waste. From ethanol and food waste mixture, approximately 120 g of carbon dots with an average diameter of 2–4 nm can be produced. The benefits of the as-prepared CDs for in vitro bioimaging include photostability, low cytotoxicity, and good PL characteristics.

Jiang et al. [112] reported a one-pot eco-friendly fabrication of silver nanoparticles supported on carbon from carbon and silver nitrate liquid solution without additional capping or reducing agents. Simply altering the molar ratio of carbon nanodots and $AgNO_3$ would change the size of the silver nanoparticles supported on carbon. The simple method used to create amorphous carbon-supported Ag NPs was significant because it is synthesized in the absence of reducing or capping agents. Moreover, the stability and electrical and catalytic activities of the silver nanoparticles for the electrocatalytic reduction of H_2O_2 were also enhanced. The great sensitivity and low detection limit of the Ag/C nanocomposites make them excellent non-enzymatic H_2O_2 sensors. The Ag/C nanocomposites acted as a non-enzymatic H_2O_2 sensor due to their high sensitivity and selectivity, as shown in Scheme 1.

Manoharan et al. [113] used an easy and affordable technique to turn coconut water into vivid eco-friendly fluorescent carbon nanodots. Coconut water, as an environmentally friendly and less expensive carbon precursor, is used to fabricate finely dispersible carbon nanodots with both the amorphous and nano-crystalline carbon-phase. High-resolution transmission electron microscopy was used to demonstrate the monodispersed CNDs' spherical shape, $(4 \pm 1$ nm) nm particle size. FT-IR measurements revealed extensive surface functionalization. Using UV-visible absorption and photoluminescence spectroscopic techniques, the eco-friendly luminescent properties of the carbon nanodots were assessed. The fluorescence quantum yield of the carbon nanodots having a core of carbon with extensive surface functionalization was found to be 60.18%. These CNDs, fabricated from tender coconut water, are more favorable than those from other resources due to their stability, high quality, fast reaction rate, and fine dispersion.

Currently, microalgae productivity has been increasing worldwide. To take advantage of the situation, Choi et al. [114] set out to show that the aqueous-type biofriendly luminescent carbon nanodots (C-paints) could be successfully applied for enhancing the growth rate of microalgae, *Haematococcus pluviali*. A straightforward procedure of ultrasonic irradiation with the passivating agent, polyethylene glycol, was used to prepare C-paints. The end product, called a C-paint, has a carbonyl-rich surface, outstanding

particle size homogeneity, high water-solubility, photo-stability, fluorescence efficacy, and biocompatibility.



Scheme 1. Schematic view of the formation of carbon-supported silver nanoparticles. Reproduced with permission from Ref. [112]. 2014, Elsevier.

Using polymer dots enclosed in NIR emissive hydrophobic carbon nanodots, Huang et al. [115] proposed the first endoplasmic reticulum (ER) focused nearinfrared (NIR) nanosensor for detecting Cu^{2+} in biosystems. With a detection limit of 13 nM, this nanosensor with stable fluorescence can be utilized to quantify Cu^{2+} in a linear range from 0.25 to 9.0 M. It responded quickly to Cu^{2+} (120 s). In addition, compared to other metal ions and amino acids, the nanosensor's fluorescence fluctuations are extremely selective to Cu^{2+}. Moreover, the developed nanosensor showed low cytotoxicity, superior biocompatibility, and ER targeting capability [116].

2.2. Hydrothermal Synthesis

The hydrothermal method for producing CDs is inexpensive and non-toxic. This is an easy method for creating carbon quantum dots compared to other synthetic approaches. Teflon lined stainless steel autoclaves are used as reaction vessels for the aqueous solution of the CD precursor and chemical agents. The autoclave is then placed in an air oven where the contents are hydrothermally reacted at a high pressure and high temperature to form the CDs [117–123]. Mehta et al. [124] developed a plant-based (sugarcane juice) source for producing luminescent carbon quantum dots, which are soluble in an aqueous medium with a size of less than 5 nm. These CQDs were used for the sensitive and specific detection of Cu^{2+}. In their proposal, Lu et al. [125] suggested the production of carbon quantum dots via this method from pomelo peel having the size of less than 5 nm. The fabricated carbon

quantum dots, which had remarkable yield, were used for the sensitive detection of Hg^{2+} at lower concentrations for the examination of water samples collected from the lake.

Li et al. [126] successfully manufactured CDs in 2018 using a one-step hydrothermal process that was ecologically friendly, easy, and affordable. Oxidation resistance, stability, excellent solubility, and high quantum yield (18.67%) were all features of the CDs. It was discovered that a charge transfer procedure could cause picric acid (PA) to quench the CDs' early fluorescence. These CDs worked well as fluorescent probes to identify PA in our study. The technique was successfully used on actual and laboratory-derived tap water samples, and it was found to have beneficial properties, such as an outstanding selective nature, excellent sensitive nature, and lower detection limit of 10 nM.

Gao et al. [127] proposed a simple and inexpensive technique in which they showed the coupling of graphene quantum dots with carbon nitride (hexagonal-structure) via freeze-drying. The result showed enhanced photocatalytic activity, improved absorption in the visible region, and the effective separation of photon-generated electron-hole pairs. We have successfully completed the simple synthesis of B/N co-doped, fluorescent surface passivated carbon nanodots with a high quantum yield at a low cost, as reported by Jahan et al. [128]. Further employing these carbon dots results in the production of supramolecular moieties, which turns off fluorescence before being turned on by SR III.

For the first time, Soni et al. [129] have shown the precise source of light absorption and its emission of carbon nanodots. They demonstrated that molecular fluorophore, which is generally found in the fabrication mixture as a by-product, is the true source of the emission in red emissive carbon nanodots.

Using p-phenylenediamine and urea, Ding et al. [130] developed multiple multicolor-emitting carbon nanodots via this process. After being purified using column chromatography, the carbon nanodots were obtained without excitation, causing fluorophores to show different colors. Along with a progressive increase in the red-shifted fluorescence emission, the oxidation on the surface of the carbon nanodots also became enhanced. The band width narrows as the oxygen content of the carbon nanodots' surface increases; as a result, the higher level of surface oxidation causes the red-shifted emission.

Bakier et al. [131] proposed a new turn-off fluorescent chemical sensor for the ultra-sensitive detection of aniline in the liquid phase, via the formation of colloidal carbon nanodots supported on nitrogen. They demonstrated a susceptible fluorescent aniline liquid sensor based on incredibly tiny carbon dots supported on nitrogen (Figure 4).

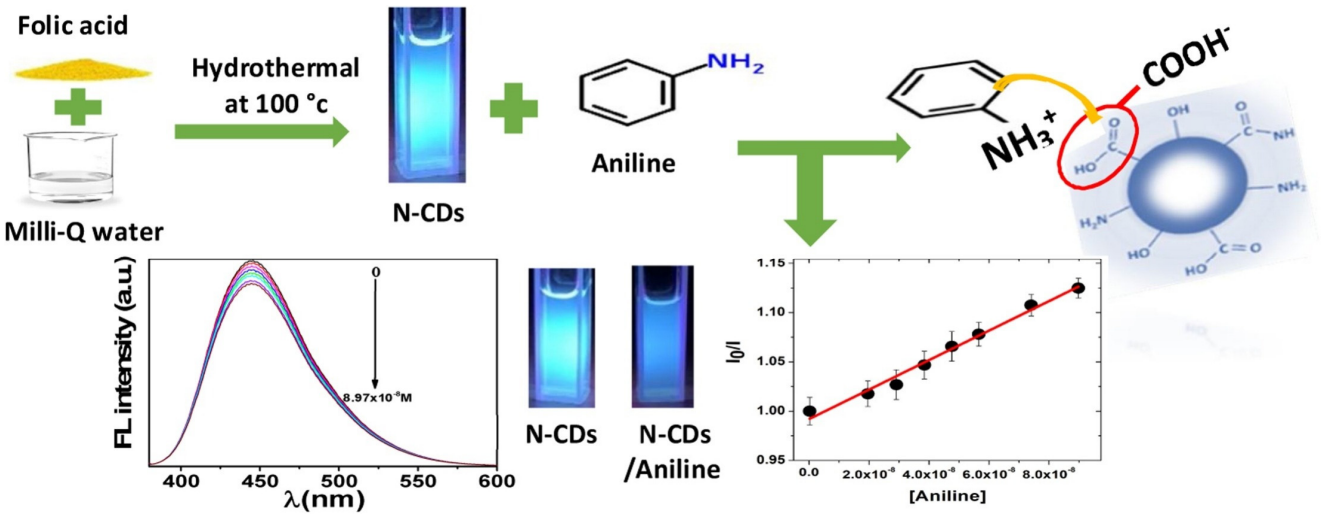


Figure 4. Fluorescent chemical sensor for ultra-sensitive detection of aniline in the liquid phase, via formation of colloidal carbon nanodots supported on nitrogen. Reproduced with permission from Ref. [131]. 2021, Elsevier.

The carbon dots supported on nitrogen were produced using folic acid that had undergone ultrasonic processing at lower temperatures. They further discovered that the sensor's operation followed the static N-CD fluorescence quenching caused by electrostatic contact with aniline. The detection limit for aniline via any method was 3.75 nM (0.332 ppb), which is the sensor's detection limit. Furthermore, real sample analysis was investigated using the N-CDs' nano-probe with real tap water, and excellent results were obtained with 99.7–101% recovery (Figure 5). Hence, this proposal could prove to be helpful in developing a simple and environmentally benign nano-sensing process with excellent sensitivity, outstanding selectivity, and good quantitative value to monitor harmful aniline against the degradation of the environment [131].

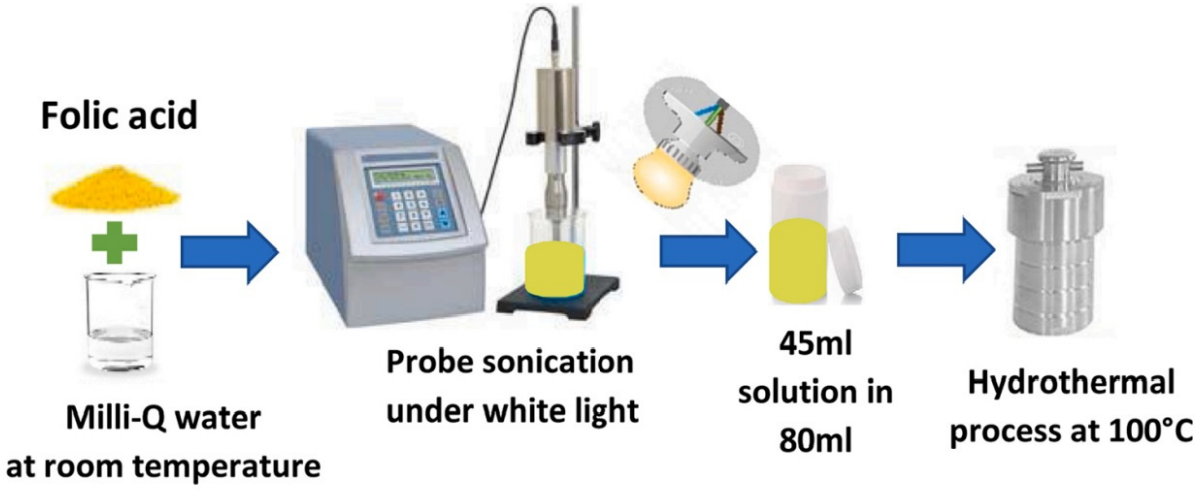


Figure 5. Schematic view of the experimental procedure. Reproduced with permission from Ref. [131]. 2021, Elsevier.

2.3. Carbonization/Pyrolysis

In recent years, pyrolysis has emerged as a potent technique for producing fluorescent CDs by using precursors that are microscopic carbon structures. Short reaction times, minimal costs, simple procedures, the absence of any solvent, and high quantum yields are all benefits of this technology. Under high temperatures, the following basic processes of heating, dehydrating, degrading, and carbonization are essential for converting the molecules with organic carbon into carbon quantum dots. During the pyrolysis process, strong concentrations of alkali perform the cleavage of carbon initiators into carbon nanoparticles.

Ma et al. [132] produced nitrogen-doped graphene quantum dots by directly carbonizing ethylene diamine tetra acetic acid at 260–280 °C, and this study also offered a growth mechanism for GQDs. It is important to note that ion doping has been found to produce a variety of CQD kinds. Li and colleagues created chlorine-doped graphene quantum dots by using HCl and fructose as precursors in a typical experiment. The average size of the quantum dots was found to be 5.4 nm. They altered the color of the emission by alternating the excitation wavelength from 300 to 600 nm, and the color changed from blue to red, respectively [133]. The fluorescent carbon quantum dots were also made by Praneerad et al. [134] by carbonizing the durian peel biomass waste. The produced CQDs were used to develop a composite-based electrode that displayed a significantly greater specific capacitance value as compared to the electrode made of pure carbon. According to 135.Zhang et al. [135], the quantum dots of carbon with increased sulphur and nitrogen contents were created by carbonizing hair fiber combined with H_2SO_4 through sonication.

Gunjal et al. [136] used a straightforward carbonization process to create waste tea residue carbon dots from surplus and inexpensive kitchen waste biomass, so that it is cheaper, greener, and more environmentally friendly than previous techniques. As soon as they are created, waste tea residue carbon dots exhibit excitation dependent emission and are very stable in ionic media. Furthermore, due to the oxidative nature of the ion,

it has demonstrated excellent fluorescence quenching for ClO^-. The fabricated sensor has the advantage in that it is highly sensitive and selective in comparison to the other 21 common interfering ions which were tested against it. Its detection limit is comparatively lower than other biomass-made carbon dots. Its quick rate of reaction enables easy and feasible ClO^- detection in real samples with excellent precision and reliability. A simple method to covalently immobilize nanoscale carbon dots upon conducting carbon surface for sensing purposes is reported by Gutiérrez-Sánchez et al. [137]. The carbon nanodots (N-CD) containing amine functionalization on the surface can be electro-grafted upon the electrodes of carbon, where they are then readily covalently immobilized. They were made using a carbonization approach with microwave aid and cost-effective, biocompatible initiators, such as D-fructose as the primary carbon source and urea as the N-donor reagent, to produce peripheral enhanced nitrogen CD. It has been determined through various methods of analysis that the synthesized nanomaterial comprises regular-sized amorphous structures that glow blue when exposed to UV light. Through the relatively stable immobilization of nitrogen carbon dots onto the electrode surfaces through electrografting, hybrid electrodes with higher relative surface areas and enhanced electron transfer capacities are generated, holding great potential for electrochemical sensing. Because of their conductive nature, electrical properties, abundant edges sites, and high catalytic activity, N-CDs that are immobilized on carbon electrodes efficiently amplify the electro-chemiluminiscence (ECL) signal from the luminophore $[Ru(bpy)_3]^{2+}$ in a taurine sensor.

2.4. Electrochemical Synthesis

The one-pot electrochemical method is used to controllably synthesize fluorescent or luminescent carbon nanodots (C-dots) from small molecular alcohols as a single carbon source for the first time. By adjusting the applied potential, it is possible to control the size of the resulting C-dots, which can then be used to image cells using luminescence microscopy.

A titanium tube cathode and a pure graphite loop electrode were assembled in the center, according to Pender et al. [138]. Distilled water was used for synthesizing both the electrolyte and the cathode while the anode was isolated from them by an insulating O-ring. Luminescent blue-colored carbon dots were broadly employed in pure water thanks to the use of electronic voltage and ultrasonic control, which eliminated the need for laborious cleaning. The amount yield was 8.9%, while the size of the synthesized C-dots was 2–3 nm. The C-dots offered good fluorescent properties and thermodynamic constancy in the aqueous phase. Fluorescent CDs were made from ethanol by electrochemical carbonization, according to Miao et al. [139]. The synthesizing procedure is easy, economical, and environmentally benign. The synthesized carbon dots were amorphous, spherical, and easily dispensable in water, making them ideal for analytical uses. A strong fluorescence intensity with a QY of 10.04% was attained in the absence of a surface passivation reagent. By identifying Fe^{3+} induced fluorescence quenching, carbon nanodots were successfully used for the Fe^{3+} test.

Keerthana and Ashraf [140] highlighted the hydrothermal carbonization approach for synthesizing carbon dots from chitosan. Chitosan was totally transformed into carbon dots, according to an analysis using UV-Visible spectroscopy. With one step microwave synthesis, Arvapalli et al. [141] were able to synthesize carbon nanodots that had remarkable selectivity and sensitivity for the detection of Fe (III) ions. The synthesized carbon nanodots exhibit excellent stability, high photoluminescence, and strong water solubility. Bright blue fluorescence from carbon nanodots was successfully internalized inside endothelial cells, and when the cells were nurtured with iron, the fluorescence quenching phenomenon was seen, demonstrating the possibility of sensing iron in living cells. The transfer of charge specifically between the carbon nanodots and iron was responsible for the fluorescence quenching of the carbon nanodots, and cyclic voltammetry experiments have further confirmed this.

Tyrosinase was immobilized on carbon-based nanoparticles and cysteamine (electrically active layer) covering the gold electrode in the small gold-epinephrine biosensor reported by Baluta et al. [142]. This sensor system made use of the differential pulse and cyclic voltammetry voltammetric methods to monitor the oxidation of norepinephrinetonorepinephrine-quinone via catalysis.

2.5. Microwave-Assisted Synthesis

The electromagnetic wave known as the microwave has a vast wavelength range of 1 mm to 1 m and is frequently employed in daily life and scientific study. Microwaves can also deliver high energy to breakdown the chemical bonds in a substrate, just like lasers can. It is believed that using a microwave to create CDs is an energy-efficient method. Additionally, the reaction time may be significantly reduced. The substrate is typically pyrolyzed and the surface functionalized during microwave-aided synthesis [143,144].

The CDs are synthesized more quickly using a green, economical microwave-aided method. For the creation of CDs, microwave irradiation can deliver consistent heat. For the first time, Li et al. [145] produced green-colored luminescent graphene quantum dots via the cleavage of graphene oxide sheets chemically in the presence of acids under microwave conditions. They have an emission peak of 500 nm when excited at 260 nm and 340 nm. For the first time, electrochemiluminescence has been observed from the graphene quantum dots and is highly applicable in imaging and bio-sensing.

Liu et al. [146] produced carbon dots under microwave conditions. They used glutaraldehyde as a cross-linking agent for the fluorescent system. The luminescent emissions of the carbon dots come in a range on the basis of the amount of glutaraldehyde used. The as-prepared carbon dots showed remarkable luminescent characteristics and were less toxic, highly stable, and water soluble.

A simple microwave-assisted hydrothermal was used to produce CDs from *Mangifera indica* leaves [147]. The resulting carbon dots were employed as temperature sensors inside the cells, had good biocompatibility, and strong photostability. To create carbon dots from raw cashew gum, Pires et al. [148] devised a heating method that is microwave-assisted and has dual steps. The carbon quantum dots have an average size of nearly 9 nm. The synthesis involves two steps: the first step is the partial depolymerization, i.e., autohydrolysis of the gum and production of 5-hydroxymethyl furfural, while the second step involves poly-condensation for the production of the polyfuranic structure, accompanied by carbonization and nucleation. The generated carbon quantum dots have been used in the cell imaging of live cells because they exhibit good biocompatibility and low cytotoxicity.

Simsek et al. [149] showed that under different physical conditions, a quick and one-step green synthesis of carbon nanodots from *Nerium oleander* leaves may be achieved using a household oven and a microwave-assisted hydro-thermal synthesizer (Figure 6). The effects of the synthesizer system, the kind of extract based on the plant extraction of the plant solvents, and the synthetic conditions, including the time of reaction, temperature of reaction, surface-passivation reagent inclusion into the reaction medium, physical and chemical properties, and optical characteristics of carbon dots, were examined.

Ren et al. [150] reported the synthesis of 5.6 nm-diameter N-doped graphene quantum dots using microwave-assisted heat. The resulting N-doped graphene quantum dots were used in metal ion detection and exhibit strong and constant blue fluorescence emission with an 8% quantum yield.

Sendao et al. [151] suggested a microwave method to synthesize blue-emitting carbon quantum dots and looked into photoluminescent emission features. They discovered that the synthesis technique created green-emitting molecular fluorophore that can hide the photoluminescent emission of the carbon dots. It is important to note that in the same solution, these fluorophores and the carbon dots do not function as different species with independent emissions. Instead, their interaction results in a hybrid luminescence which is seen. This method demonstrates that the reactive nature and the characteristics in the

excited-state are indistinct in comparison to their individual characteristics. The impurities of the fluorescence generated from its formation have formed a critical drawback in the investigation of the photoluminescent property of the carbon quantum dots (Tables 1 and 2).

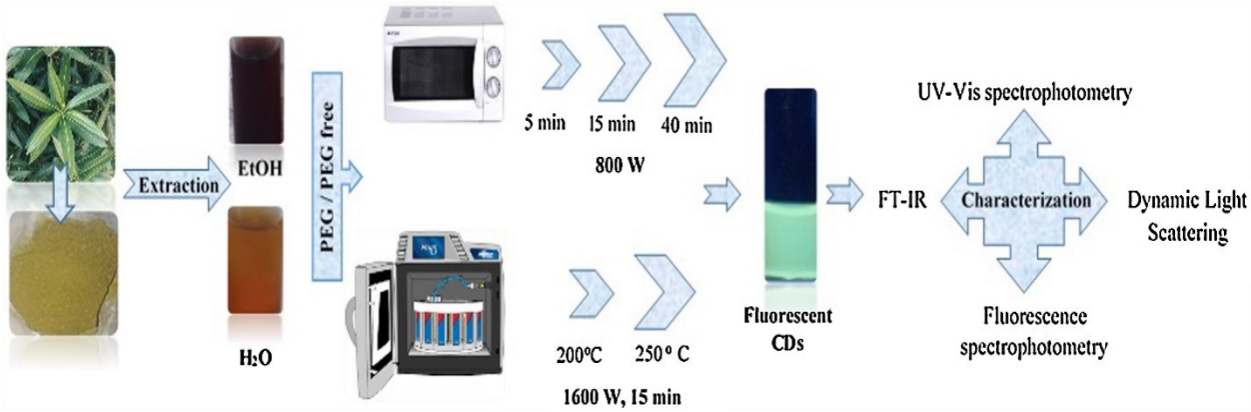


Figure 6. Schematic illustration of the fabrication process. Reproduced with permission from Ref. [149]. 2019, Elsevier.

Table 1. Advantages and disadvantages of different synthetic methods of carbon nanodots.

Synthetic Method	Advantages	Disadvantages	References
Sonochemical/Ultra-sonic fabrication	Easy-operation	Expensive cost of energy	[108–116]
Hydrothermal synthesis	Cost-effective, environmentally benign, non-toxic	No uniformity in size	[124–131]
Carbonization/Pyrolysis	No solvent required, cost-effective, bulk-production	No uniformity in distribution of size	[132–136]
Electrochemical synthesis	Easy, cost-effective, environmentally benign	Uniformity in size distribution	[137–142]
Microwave-assisted synthesis	Fast, cost-effective, environmentally benign	No uniformity in distribution of size	[143–151]

Table 2. Methods for the conversion of biobased and chemical feedstock into functionalized carbon nanodots.

Sources	Synthetic Methods	References
Carbon, Silver nitrate liquid solution	Sonochemical synthesis	[112]
Saccharum officinarum (Sugarcane)	Hydrothermal synthesis	[124]
Coconut water	Ultrasonication synthesis	[113]
o-phenylenediamine	Hydrothermal synthesis	[129]
Waste biomass	Carbonization synthesis	[136]
Ethanol	Electrochemical carbonization synthesis	[139]
Graphene	Sonochemical synthesis	[108]
p-phenylenediamine and urea	Hydrothermal synthesis	[130]
Mangifera indica (Mango)	Microwave-assisted hydrothermal synthesis	[147]
Chitosan	Hydrothermal carbonization synthesis	[140]

3. Applications of Carbon Dots (CDs)

There are various applications which are associated with carbon dots. CDs also show a number of biomedical applications. The application of CDs is shown in Figure 7.

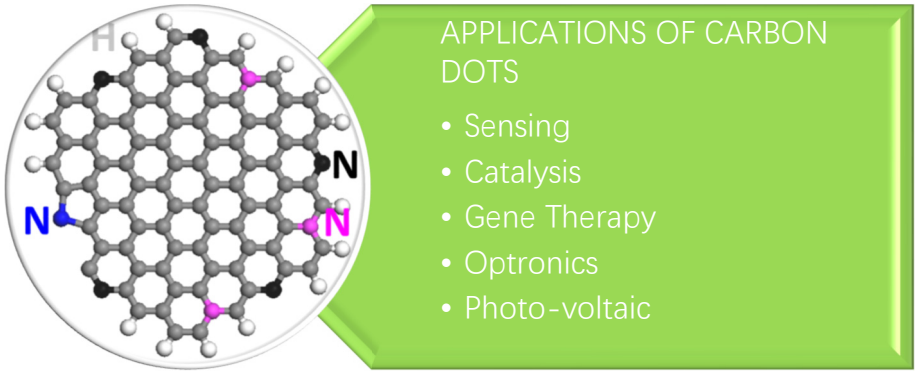


Figure 7. Application of Carbon dots.

3.1. Sensing

One of the most common and potentially significant uses of CDs is sensing [152–154]. Due to their superior optical qualities, high fluorescence sensitivity to the surrounding environment [155,156], and ability to function as effective electron donors [157–159], CDs are frequently suggested as detectors for a variety of harmful substances, including heavy metals such as mercury [160–162], copper, and iron [163–166]. To make CDs more sensitive to one or more of these analytes, persistent work is being conducted in this direction. Only a handful of studies, however, have attempted to examine the interactions of CDs with metal ions at a more fundamental level; for example, Goncalves and colleagues demonstrated that the fluorescence emissions of both CQD solution and CQDs immobilized in sol–gel are sensitive to the presence of Hg^{2+} [167]. In their study, laser-ablated and NH_2-PEG_{200} and *N*-acetyl-L-cysteine-passivated CQDs were used as fluorescent probes. It was observed that the fluorescence intensity of the CQDs is efficiently quenched by micro molar amounts of Hg^{2+} with a Stern–Volmer constant of 1.3×10^5 M^{-1}. Therefore, judging from the relatively large magnitude of the Stern–Volmer constant [168], the quenching provoked by Hg^{2+} is probably due to the static quenching arising from the formation of a stable non-fluorescent complex between CQD and Hg^{2+}. A substantial improvement in the sensitivity down to nanomolars was later realized by replacing the laser-ablated CQDs with N-CQDs. Again, static quenching is thought to be responsible for the quenching of fluorescence, but with a much larger Stern–Volmer constant of 1.4×10^7 M^{-1}, two orders of magnitude higher than that of the previous system [169]. It was suggested that the presence of the nitrogen element in the N-CQDs, most probably -CN groups on the N-CQD surface, is responsible for the much-improved performance of Hg^{2+} sensing.

3.2. Bio Imaging Probes

An intriguing application of C dots is their use as a potential agent for in vivo and in vitro bioimaging of cells and species due to their photoluminescence, which is an important property of C dots [170–172]. The bioimaging of cells and tissues is an important part of the diagnosis of many diseases, particularly cancer. Various fluorescent systems for diagnostic purposes have been reported, ranging from organic and inorganic dyes to the most recent nanoparticle-based systems.

To be considered suitable for use as an imaging probe, a bioimaging agent must have excellent biocompatibility, a tunable emission spectrum, and be free of cytotoxicity. Rapid progress in implementing a new class of nanoparticles has resulted in a material that meets these criteria and can be used for both diagnostic and therapeutic purposes. Chemical functionalization is used to successfully conjugate the required drug molecule to the fluorescent nanoprobes for these theranostic applications. Sahu et al. [173] reported the synthesis of C dots from orange juice hydrothermal treatment. This was one of the first examples of making fluorescent C dots from readily available natural resources [174,175]. The C dots were non-cytotoxic and efficiently taken up by MG-63 human osteosarcoma cells for cellular imaging.

The "central dogma" states that genetic information flows from DNA to RNA to proteins. Researchers investigated the physiological activity of RNA during cancer research by using RNA dynamics in cellular functions and the real-time monitoring of their temporo-spatial distribution. The experiments were carried out using fluorescent carbon dots created by the one-pot hydrothermal treatment of o-, m-, or p-phenylenediamines with triethylenetetramine by Chen et al. [176]. Because carbon has excellent biocompatibility and negligible cytotoxicity, there has been a lot of interest in using carbon nanodots as bioimaging probes instead of other types of nanoparticles. C dots are ideal candidates for theranostic applications due to their ease of synthesis, acceptable emission spectra, high photostability, and lack of cytotoxicity.

Tao et al. [177] used a mixed acid treatment to create C dots from carbon nanotubes (CNTs) and graphite. Under UV light, the C dots emit a strong yellow fluorescence with no cellular toxicity. They also demonstrated in vivo bioimaging in the near-infrared region using a rat model, and this experiment exemplified the possibilities for the development of fluorescent imaging probes in both the ultraviolet (UV) and infrared (IR) range spectra.

3.3. Photodynamic Therapy

Photodynamic therapy is a relatively new advancement in biomedical nanotechnology that uses energy transfer to destroy damaged cells and tissues. This method is useful in dealing with cancer cells because it effectively targets and destroys malignant tissue while leaving normal, healthy tissue alone. This targeted destruction in photodynamic therapy can be accomplished with fluorescent C dots that have adequate photostability [178].

Shi et al. [179] used the hydrothermal method to create N-doped C dots from rapeseed flowers and bee pollen. The authors demonstrated that C dots had no cytotoxic effect up to a limiting concentration of 0.5 mg/mL after this successful large-scale synthesis. Human colon carcinoma cells were imaged successfully in this study, and the C dots were found to have good photostability and biocompatibility.

Wang et al. [180] reported C dot synthesis from the condensation carbonization of linear polyethylenic amine (PEA) analogues and citric acid (CA) of different ratios. The authors successfully demonstrated that the extent of conjugated π-domains with CN in the carbon backbone was correlated with their photoluminescence quantum yield. The main conclusion from this study is that the emission arises not only from the sp^2/sp^3 carbon core and surface passivation of C nanodots, but also from the molecular fluorophores integrated into the C dot framework. This work provided an insight into the excellent biocompatibility, low cytotoxicity, and enhanced bioimaging properties of N-doped C dots, which opens the possibilities for new bioimaging applications.

Bankoti et al. [181] fabricated C dots from onion peel powder waste using the microwave method and studied cell imaging and wound healing aspects. The C dots exhibited stable fluorescence at an excitation wavelength of 450 nm and an emission wavelength of 520 nm at variable pH, along with the ability to scavenge free radicals, which can be further explored for antioxidant activity. The radical scavenging ability leads to an enhanced wound healing ability in a full-thickness wound in a rat model.

3.4. Photocatalysis

There has been significant research interest in photocatalysts over the past decade due to the scenario of environmental safety and sustainable energy. The applications of nanomaterials for the efficient fabrication of photocatalysts made the journey fast and effective.

Ming et al. [182] successfully developed C dots using a one-pot electrochemical method that only used water as the main reagent. This is an extremely promising synthetic methodology because it is a green protocol that is also cost-effective, with good photocatalytic activity of C dots for methyl orange degradation.

Song et al. [183] devised a two-step hydrothermal method for the creation of a C dot–WO_2 photocatalyst. The authors used this system to photocatalytically degrade rhodamine

B. It is worth noting that the reaction rate constant reported in this study is 0.01942 min^{-1}, which is approximately 7.7 times higher than the catalytic rate using WO_2 alone.

For photocatalytic hydrogen generation, a C-dot/g-C_3N_4 system was used. The authors created C dots from rapeseed flower pollen and hydrothermally incorporated them into g-C_3N_4. Under visible light irradiation, this system was able to photocatalytically generate hydrogen via sound with an output greater than that of bulk g-C_3N_4.

3.5. Biological Sensors and Chemical Sensors

There is great interest in using nanoparticles as biochemical sensors because C dots have been found to be useful in sensing chemical compounds or elements. Based on the properties of C dots, particularly their fluorescence properties and surface-functionalized chemical groups, various sensors for biological and chemical applications have been developed.

Qu et al. [184] developed ratiometric fluorescent nano-sensors using C dots in a single step of microwave-assisted synthesis. This research is significant in C-dot sensor research because the developed nanosensors are multi-sensory and can detect temperature, pH, and metal ions such as Fe (III). Because it can detect and estimate multiple metabolic parameters at the same time, this exciting feature is proving to be widely applicable in the biological environment. The sensory mechanism is non-cytotoxic and based on ratiometric fluorescence, which is a promising feature for future research.

Vedamalai et al. [185] developed C dots that are highly sensitive to copper (II) ions in cancer cells. They used a relatively simple hydrothermal synthesis method based on ortho-phenylenediamine (OPD). The orange color was caused by the formation of the $Cu(OPD)_2$ complex on the surface of the C dots. Further investigation revealed that the C dots were highly water dispersible, photostable, chemically stable, and biocompatible.

Shi et al. [186] used C dots to detect Cu(II) ions in living cells as well. The hydrothermal pyrolysis of leeks resulted in blue and green fluorescent C dots. In a single step of hydrothermal carbonization, the C dots were modified with boronic acid using phenylboronic acid as the precursor. This C-dot-based sensor successfully detected blood sugar levels and demonstrated good selectivity with minimal chemical interference from other species [187].

Nie et al. [188] used a novel bottom-up method to develop a pH sensor out of C dots. This method yielded C dots with high crystallinity and stability. The procedure involved a one-pot synthesis with high reproducibility using chloroform and diethylamine. The authors were able to use the technique for cancer diagnosis after successfully implementing the pH detection of two C dots with different emission wavelengths.

Wang et al. [189] described an intriguing C-dot sensor for hemoglobin detection (Hb). The C dots were developed from glycine using an electrochemical method that included multiple steps, such as electro-oxidation, electro-polymerization, carbonization, and passivation. The authors successfully validated the sensitivity of Hb detection and discovered that the luminescence intensity varied inversely with Hb concentration in the 0.05–250 nM range.

3.6. Drug Delivery

Carbon dots' excellent biocompatibility and clearance from the body meet the requirements for in vivo applications. Carbon dots with rich and tunable function groups, such as amino, carboxyl, or hydroxyl, can carry therapeutic agents, resulting in theranosticnanomedicines [190–195]. The bright emission of carbon dots allows for the dynamic and real-time monitoring of drug distribution and response. Zheng et al. [196] used carbon dots synthesized through the thermal pyrolysis of citric acid and polyene polyamine to transport oxaliplatin, a platinum-based drug, because platinum-based drugs are the most effective anticancer drugs and are used in more than 50% of clinical cancer patients' chemotherapeutic treatments.

3.7. Micro-Fluidic Marker

The study of fluidic physics at the micro-scale is now best conducted using microfluidic systems. Because of their considerably higher surface-to-volume ratio, surface tension and viscosity dominate those of inertia, making the fluid easier to control. Static laminar flows and dynamic droplet formation are typical microfluidic situations. Both exhibit many advantages, including minimal reagent use, high sensitivity, and high output, which leads to a wide range of applications in bioassays, chemical reactions, drug delivery, etc. The majority of applications rely on the microfluidic circuit's ability to visualize fluid flow. However, the biocompatibility and cheap cost of the fluorescent materials currently in use cannot be balanced, which is a critical issue for microfluidic applications, particularly for bio-applications. Sun's colleagues used carbon dots, synthesized by heating glucose and urea in a microwave, to visualize microfluid flows for the first time to address this problem [197–199]. The scientists used carbon dots dissolved in the deionized water as a fluorescent marker to investigate the dynamics of the mixture of glycerol and deionized water. When the interface is ruptured by an electric field above a threshold, fast mixing occurs at the microscale. In addition to laminar flow, the authors also synthesized monodispersed droplets in a flow focusing system, where the continuous phase was mineral oil while the aqueous solution of carbon dots appeared as the dispersed phase. The diameter of the droplets will shrink because a higher capillary number results in a greater interfacial shear force. Additionally, the authors successfully demonstrated the multiple component droplet, merged droplet, and double emulsion, each of which has a distinct core-shell structure. To more accurately determine the speed of the flow field, luminescent seeding carbon dots were made via a mixture of carbon dots (liquid state) and polystyrene microparticles [200–202].

3.8. Bioimaging

Carbon dots have significant advantages over fluorescent organic dyes and genetically engineered fluorescent proteins, such as high PL quantum yield, photostability, and resistance to metabolic degradation, which endows them with enormous potential for use in bioapplications. While the toxicity testing of carbon dots is required before exploring their bioapplications, Yang et al. [203] used human breast cancer MCF-7 cells and human colorectal adenocarcinoma HT-29 cells (previously reported by other scientists, Yang modified and used it) to assess the in vitro toxicity of carbon dots synthesized by the laser ablation of graphite powder and cement with PEG1500N [204–206] as a surface passivation agent. All the observations of cell proliferation, mortality, and viability from both cell lines indicated that the carbon dots exhibited superior biocompatibility, even at concentrations as high as 50 mg/mL, which is much higher than the practical application demand, for example, in living cell imaging.

3.9. Carbon Dots Chiral Photonics

Chirality is essential in a number of practical application fields, such as chiral drug recognition, chiral molecular biology, and chiral chemistry [207–209]. As a result, as previously proposed by M. Va'zquez-Nakagawa et al. [210], chirality and carbon dots can be combined to form intriguing chiral optics based on carbon dots. The carbon dots used in their groundbreaking research were created by chemically exfoliating graphite with strong sulfuric and nitric acids. The carbon dots' surface carboxylic acid groups were subsequently converted to acid chlorides using thionyl chloride. When the acid chlorides and the (R) or (S)-2-phenyl-1-propanol reacted simultaneously, enantiomerically pure esters and chiral carbon dots were created (chiral molecular). Enantiomerically esters and chiral carbon dots were formed, and their formation was verified using ^{13}C-NMR and FTIR spectroscopy. The presence of phenyl substituents was suggested by the appearance of peaks in the ^{13}C–NMR. The recent work in this field is the most notable development in the chiral regulation of bioreactions for chiral carbon dots. Xin et al. [211] described the destruction of the cell walls of gram-positive and gram-negative bacteria via carbon dots in the presence of D-glutamic

acid, which resulted in the fatality of bacteria. In contrast, the carbon dots formed in the presence of L-glutamic acid demonstrated an insignificant effect on bacterial cells. This implied that antimicrobial nanoagents with chirality can be synthesized from carbon dots. The D-form and L-form of cysteine-based carbon dots were used to regulate the chirality of the enzyme. For instance, L-form cysteine carbon dots reduce the enzymatic activity while D-form cysteine carbon dots enhance the enzymatic activity of the enzyme. According to Li et al. [212], these cysteine-based carbon nanodots have the capacity to affect cellular energy metabolism. We anticipate that other chiral carbon dots-based applications will be investigated in the future [213], and that carbon dots with chirality will emerge as a novel but exciting topic because of their wide applications.

4. Conclusions

Carbon dots have drawn rigorous attention since they possess outstanding photoluminescence, fluorescence, biocompatibility, sensing and imaging, photostability, excellent colloidal stability, eco-friendly synthesis, low toxicity, and are cost-effective. In this review, widespread synthesis procedures have been discussed in detail, including bottom-up and top-down methods, along with biological and eco-friendly synthetic ways. This concludes numerous synthesizing routes that could be helpful to many scientific and research areas, since carbon nanodots can be easily synthesized for various applications. Earlier, the synthetic methods were limited because of unreliable quantum yields. However, in recent years, the synthesizing methods have seen a remarkable lift in yield, hence enhancing their use in different fields for varied applications. Despite many advancements in the field of carbon nanodots, there is still room for improvement in its synthetic methods. Several bio-related fields are left undiscovered and need special attention.

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