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Review Article

# MULTI-FUNCTIONAL CARBON DOTS: A SYSTEMATIC OVERVIEW

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**ABSTRACT**

Carbon dots (CDs) have emerged as a potential material in the multifarious fields of biomedical applications due to their numerous advantageous properties including tunable fluorescence, water solubility, biocompatibility, low toxicity, small size and ease of modification, inexpensive scale-up production, and versatile conjugation with other targeted nanoparticles. Thus, CDs became a preferable choice in various biomedical applications such as nanocarriers for drugs, therapeutic genes, photo sensitizers, unique electronic, fluorescent, photo luminescent, chemiluminescent, and electro chemiluminescent, drug/gene delivery and optoelectronics properties are what gives them potential in sensing and antibacterial molecules. Further, their potentials have also been verified in multifunctional diagnostic platforms, cellular and bacterial bio-imaging, development of nanomedicine, etc. This present review provides a concise insight into the progress and evolution in the field of carbon dots research with respect to synthesis methods and materials available in bio-imaging, theranostic, cancer, gene therapy, diagnostics, etc. Further, our discussion is extended to explore the role of CDs in nanomedicine and nano theranostic, biotherapy which is the future of biomedicine and also serves to discuss the various properties of carbon dots which allow chemotherapy and gene therapy to be safer and more target-specific, resulting in the reduction of side effects experienced by patients and also the overall increase in patient compliance and quality of life and representative studies on their activities against bacteria, fungi, and viruses reviewed and discussed. This study will thus help biomedical researchers in percuss the potential of CDs to overcome various existing technological challenges.

**Keywords:** Carbon dots, Sensing, Bio-imaging probes, Biotherapy, Drug delivery, Multi-drug resistance, Gene therapy


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## INTRODUCTION

Carbon nanodots are nano-crystals of nano materials having zero dimensions are smaller than 10 nm [1]. They show various size reliant optical properties such as photoluminescence, chemiluminescence, electrochemical luminescence and photoinduced electron transfer [2]. Besides, the high remarkable aqueous dispersibility, biocompatibility, good elasticity in modification, high resistance to photobleaching and chemical inertness make it well applicable in bio-imaging, bio-sensing, chemical-sensing, biomedical applications [3-8]. Being a new kind of fluorescent nanomaterial and having excellent biocompatibility, CDs are widely used in the area of bio-imaging both *in vitro* and *in vivo* and in diagnosis purposes, eco-friendliness, conductivity, desirable optical properties and low toxicity, carbon dots have revolutionized the biomedical field, in Photothermal as well as photodynamic therapy and drug/gene delivery carriers [9-11]. CDs could also be applied for the determination of cellular levels of biomolecules and ions (bio-sensor), such as $Cu^{2+}$, $Hg^{2+}$, $NO^{3-}$, $C_6H_{12}O_6$, $H_2O_2$, etc. CDs could also act as a promising photocatalyst after co-doping with heteroatoms, such as nitrogen, phosphorus, sulphur, and certain metal ions, such as Cu, Zn, Ti, etc. Incorporation of these elements improves the electron-donation and acceptance ability of the CDs and promotes redox reaction on the surface of CDs [12-18]. These properties of CDs are being employed for wastewater treatment and hydrogen generation [19, 20]. CDs are as well appropriate for surface passivation and chemical modification with several polymeric, inorganic, organic, or biological materials. The physical and fluorescence characteristics are improved by surface passivation.

In 2004, during electrophoretic purification of single-walled carbon nanotubes fluorescent carbon nanoparticles were accidentally discovered by Xu *et al.* Fluorescent-based quantum dots are of two types namely graphene quantum dots and carbon quantum dot. They make up a new class of semiconductor nano-crystals with a size range between 2 and 10 nm called quantum dots, and have received extensive attention due to their great potential like intrinsic photoluminescence [21]. Currently, these types of carbon dots emerged as efficient, superior and universal fluorophores, based on their characteristics, CDs have been combined with semiconductor nanoparticles such as $Ag_3PO_4$, $TiO_2$, and $Fe_2O_3$ to improve their photocatalytic property [22]. These polymeric dots are cross-linked or aggregated polymer, prepared from linear monomer or polymers this type of dots is an aggregation of carbon core and connected polymer chains [23]. The three main executive parameters-the quantum confinement effects, the surface state, and the molecule state-are very important in the design of fluorescent CDs. CDs can be designed to have various functional groups including hydroxyl, carboxyl, carbonyl, ether, and epoxy in addition to their easy functionalization with amine, phosphorous, sulphur, and boron-containing heteroatoms containing functional groups with the different organic, polymeric, and biological materials during the preparation process. Various chemical precursors have been identified as the source of CDs, such as citric acid, glycerol, l-ascorbic acid, glucose, citric acid-urea and thiourea. To convert these precursors into fluorescent CDs various synthetic processes are used, such as ultrasonication, simple heating, arc discharge, solvothermal, hydrothermal, chemical oxidation, and laser ablation [24-40]. Plentiful efforts have been made to expand the usability of CDs to fulfil the growing demand for high-performance techniques, such as bio-imaging, drug-gene delivery, chemical sensing, as well as photocatalysis and kills microorganisms.

CDs are with their effective light-harvesting over a very broad spectral range from UV to near-IR, the photoexcited CDs are capable of producing reactive oxygen species, which are known to kill/inhibit microorganisms. According to existing research results, the major processes responsible for the antimicrobial effects of CDs are likely associated with the generation of reactive oxygen species. The mechanism of action includes the adhesion of CDs to the bacterial surface, the photoinduced production of reactive oxygen species, the disruption and penetration of the bacterial cell wall/membrane, the induction of oxidative stress with damages to DNA and RNA, leading to the inhibitions of gene expressions, and the induction of oxidative damages to proteins and other intracellular biomolecules. Under visible/natural light illumination, CDs in contact with the bacteria cell can efficiently generate ROS by activating the oxygen in air or water, leading to the production of hydroxyl free radicals and singlet oxygen

($1O_2$), which can destroy some of the critical biomolecules in cell and lead to cell death [41]. However, it is also important to monitor the dimensions of CDs during its synthesis to attain uniform properties for a particular application. A large number of reports entrenched the methods of purifying the as-prepared CDs via post-treatment, for example, centrifugation, filtration, gel-electrophoresis and column chromatography. Besides, monitoring the dimensions of CDs during its formation is also preferred [44]. In this current review article, we have elucidated the novel progress of small molecule-derived CDs in the field of biomedical as well as chemical applications to date and their future perspective.

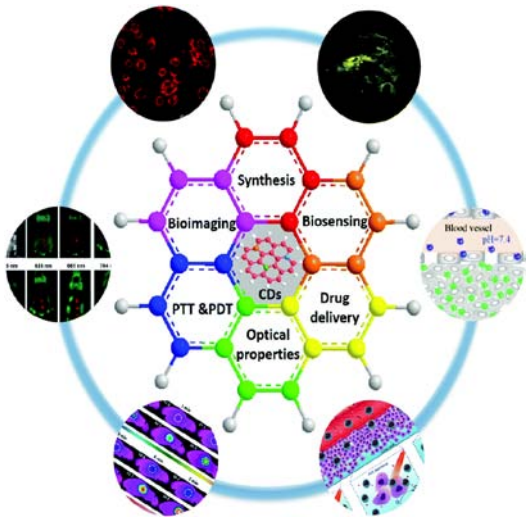


**Fig. 1: Schematic illustration of the topic of this review, showing the recent trends in applications of CDs in biomedicine, including bioimaging, biosensing, and cancer therapy, [Reprinted with permission from 20, 53, 54, 74, 82 and 143. Copyright 2017 American Chemical Society, Copyright 2012 and 2018 John Wiley and Sons, Copyright 2012 and 2017 royal society of chemistry Copyright 2016 Springer]**

## Methods of synthesis of CDs

CDs can be synthesized mainly via two routes: (i) top-down approach and (ii) bottom-up approach. In top-down techniques, the substances mainly consisting of carbon atoms such as graphite, oxidized graphite, carbon soot, activated carbon, carbon nanotubes, etc. are subjected to relatively vigorous heating conditions, such as electrochemical exfoliation, oxidative acid treatment, laser ablation, and arc discharge, to exfoliate the bulk carbon materials into quantum dots of sizes below 10 nm. In recent years, large-scale synthesis of high-quality CDs with controlled size distribution was achieved via an electrochemical approach using ultrapure water as a solvent, and thus electrochemical synthesis is the method of the choice for synthesizing the homogeneous morphological CDs. The homogeneousness in morphology is usually achieved by varying the applied potential at the electrode. Top-down approach refers to breaking down larger carbon structures via chemical oxidation, discharge, electrochemical oxidation, and ultrasonic methods. However, drawbacks of this approach include the requirement of expensive materials, harsh reaction conditions, and long reaction time. On the other hand, the bottom-up approach refers to the conversion of smaller carbon structures into CDs of the desired size. This bottom-up approach is consisting of hydrothermal treatment, ultrasonic treatment, thermal decomposition, pyrolysis, carbonization, microwave synthesis and solvothermal method to synthesize CDs. The syntheses of CDs through "top-down" approaches usually require a separate step for surface functionalization/passivation, but in the case of "bottom-up" approaches, no separate step is necessary, and the surface passivation can be accomplished in a one-pot synthesis. In "bottom-up" approaches, small organic precursors can be polymerized and carbonized into CDs by means of hydrothermal/solvothermal synthesis, pyrolysis, microwave-assisted polymerization, and carbonization. Here this review discussed on both the top-down and bottom-up approach for the synthesis of CDs obtained from small organic molecules and with special significance on various applications and bacterial detection, the antibacterial effect of CDs. Tables 1,2,3, and 4 summarized the different synthetic methods for CDs preparation from different molecules and fig. 2 shows different synthetic methods for the preparation of CDs

### Top-down approach

In the "top-down" methodology, CDs are synthesized by electrochemical oxidation, laser ablation and arc discharge method.

### Electrochemical method

Electrochemical/chemical oxidation is the top-down synthetic route for the synthesis of CDs, because of several remarkable advantages, such as high yield, high purity, low cost, and easy control over size. However, tedious purification process of synthesized particles can be considered as a main disadvantage of this method. The electrochemical method is one of the most prominent methods used to synthesize ultrapure CDs from larger molecular matter like carbon nanotube, graphene, graphite, and carbon fiber by an electrolytic process where larger organic molecules are used as an electrode in the presence of proper electrolytes. Zhou *et al.* first reported synthesis of CDs from multiwalled carbon nanotubes in the presence of tetrabutylammonium perchlorate as electrolyte [42]. Zheng *et al.* synthesized water-soluble pure CDs by an electrochemical method using graphite as electrode in the presence of phosphate buffer at neutral pH. The as-prepared CDs were successfully applied as potential biosensor [43]. Li *et al.* prepared crystalline CDs by an electrochemical method from graphite. The as-prepared CDs exhibited size-dependent upconversion photoluminescence (PL) properties and are used in photocatalysis [44]. Later, Ray *et al.* used carbon soots as the carbon source for the synthesis of CDs, and this approach can be used for the mg scale synthesis of CDs [45]. Recently, CD with polyaniline hybrid was synthesized by an electrochemical technique with high QY and purity. The as-synthesized CD-polyaniline composite reported to exhibit high capacitance and used in energy-related devices. Electrochemical soaking is a powerful method to prepare CDs using various bulk carbon materials as precursors [46-50]. In another investigation, Nakamura *et al.* reported the fabrication of nanocrystalline CDs based on an electrochemical synthesis method [31]. They applied 1-propanol as carbon source and similar to previous works, they used two Pt electrodes along with an Ag/AgCl electrode as a reference. The reaction was performed in a basic medium by adding of KOH to solution. A constant potential of 6.5 V (100 mA) was applied to the working electrode. The obtained CDs were collected after 4.5 h and 7.5 h. According to their report, both CDs produced after 4.5 and 8.5 h showed a similar pattern of spherical geometry with an average diameter of 3 and 4 nm, respectively [51]. Furthermore, it was revealed that the CD properties significantly depended on the electrolysis time spent in the process.

### Chemical ablation

Strong oxidizing acids carbonize small organic molecules to carbonaceous materials, which can be further cut into small sheets by controlled oxidation. This method may suffer from harsh conditions and drastic processes. Peng and Travas-Sejdic reported a facile aqueous solution-based procedure to produce luminescent CDs using carbohydrates as precursor materials [52]. First, they produced carbonaceous materials via dehydrating carbohydrates using concentrated sulphuric acid. Then, the obtained carbonaceous materials were treated with nitric acid and cleaved into tiny CDs. Finally, as the passivation step, a number of amino-terminated surface passivation reagents including ethylenediamine, oleylamine, bis (3-aminopropyl) terminated poly (ethylene glycol) (PEG1500N) and 4,7,10-trioxa-1,13-tridecanediamine (TTDDA) were investigated. Compared to all passivized CDs, TTDDA passivized CDs showed the highest QY when excited at 360 nm. Surface passivation was the critical step for the photoluminescence of these CDs. It was also found that prolonged nitric acid treatment resulted in a blue-shift in the

maximum emission wavelength, possibly because of a decrement in the particle size. Nontoxic nature and multicolour emission capabilities of these CDs make them good candidates in biomedical research.

**Laser ablation**

The laser ablation method uses a high-energy laser pulse to irradiate the surface of the target to a thermodynamic state in which high temperature and high pressure are generated, rapidly heats up and evaporates into a plasma state, and then the vapor crystallizes to form a nanoparticle. Laser ablation is an effective method to prepare CDs with narrow size distribution, good water solubility, and fluorescence characteristics. However, its complicated operation and high cost limit its application. In laser ablation route, complex organic macromolecules are exposed under laser radiation operated in pulsed mode and nanosized carbon particles are detached from the larger molecular structures. The laser ablation technique can involve three steps: (1) the carbon materials absorb the high energy by the laser pulse; (2) electrons are stripped from the atoms through photoelectric and thermionic emission; and (3) a high electric field produces a strong repulsive force between positive ions and solid material, breaking down CDs [53]. Synthesis of CDs by a laser ablation technique was first reported by Sun *et al.* in 2006 from graphite powder [54]. They synthesized CDs upon laser excitation from a Nd: YAG (1064 nm, 10 Hz) source in an atmosphere of argon at 900 °C and 75 kPa. Thongpool *et al.* synthesized CDs from bulk graphite in the presence of ethanol using a Nd: YAG laser of wavelength 1064 nm. The synthesized CDs showed a broad absorption spectrum peaked at 325 nm [55]. Presently, photoluminescent CDs of ~3 nm size have been synthesized by a laser irradiation technique from carbon glassy particles in the presence of polyethylene glycol 200. CDs so prepared are applied in bioimaging for cancer epithelial human cells [56]. Recently, Li and colleagues prepared CDs by laser ablation of a carbon target in a water vapour company with a carrier gas (argon) at 75 kPa and 900 °C. CDs with bright luminescence emission were obtained after refluxing in HNO3 for up to 12 h and passivation of the surface by organic polymers such as PEG1500N or poly propionyl ethyleneimine-co-ethyleneimine (PPEI-EI) [57].

**Ultrasonic treatment**

Ultrasonic treatment is also a very convenient method as the large carbon materials can be broken down by the action of very high energy of ultrasonic sound wave. Wang *et al.* synthesized N-doped CDs from ascorbic acid and ammonia via ultrasonic treatment [58, 59]. Dang *et al.* fabricated CDs using oligomer-polyamide resin as the carbon source by ultrasonic treatment. The as-prepared CDs were well dispersed, had low crystallinity, and functional groups at the surface. Lu *et al.* reported the use of an ultrasonic-assisted, liquid-phase exfoliation technique to prepare graphene carbon dots. Briefly, graphite can be well dispersed in organic solvent and the graphite layers cleave apart and are exfoliated by the surface energy for van der Waals forces of graphite layers under the ultrasonication process. This study supported that sonication can enhance the exfoliation effects and dispersion in the organic solvent [60, 61].

**Arc discharge method**

CDs by an arc discharge method had been an accidental event. This method was first reported by Xu *et al.* during the synthesis of single-walled carbon nanotubes SWCNTs [62]. Electrical discharge across two graphite electrodes results in the formation of small carbon fragment or CDs. Bottini *et al.* reported CDs derived from pristine and single-walled carbon nanotube by means of an arc discharge method with bright PL in the violet-blue and blue-green region, respectively [63]. Recently, Boron-and nitrogen-doped CDs were synthesized by the arc discharge method from graphite. They used $B_2H_6$ for doping boron and $NH_3$ for nitrogen [64].

**Acid oxidizing exfoliation method**

In acid oxidizing exfoliation methods, strong acids such as $HNO_3$, $H_2SO_4$, and even $KMnO_4$ have been widely used to exfoliate CDs by the oxidation of carbon materials [65]. Hu *et al.* reported the oxidizing of coal with $H_2O_2$ to prepare CDs to escape the side effects of the strong acids; damage the original structure of graphitic precursors as costly purification and extreme preparation conditions with toxic chemicals.


**Fig. 2: Representation of the possible synthesis methods to prepare carbon dots, [Reproduced with permission from [52]. Copyright Royal Society of chemistry, 2017]**

**Bottom-up approach**

In the bottom-up approach, CDs as bulk carbon materials are formed as the precursors change to particle forms via chemical and physical techniques, including hydrothermal, solvothermal, microwave-assisted, and thermal pyrolysis. Presently, there has been much interest in the development of bottom-up approaches for the preparation of CDs due to the precise control of precursor molecules, ease of techniques, low cost, and practicality and convenience of the procedure with generally nontoxic precursors. The features of bottom-up methods for the preparation of CDs are summarized in tables.

**Hydrothermal synthesis/Solvothermal treatment**

Hydrothermal synthesis method is being used by most of researchers as a cheap, eco-friendly, easy to handle and low-cost route to synthesize CDs from saccharides, amines, organic acids and their derivatives and from diverse carbon-based precursors. In this methodology, a solution of organic precursors is sealed in a hydrothermal synthetic reactor where the reaction occurs at high temperature and pressure. In a typical procedure, the precursor's usually small organic molecules are dissolved in a suitable solvent and heated to high temperatures (100–200 °C) in the absence of air in a Teflon-lined autoclave. The small organic moieties join together to form carbogenic cores and then grow into CDs ranging from 2 to 10 nm in size. Zhang *et al.* first reported a one-pot hydrothermal method to make CD from ascorbic acid in the presence of ethanol as solvent. QY and average particle sizes of their synthesized CDs were 6.79% and ~2 nm, respectively [67]. Pang *et al.* reported the synthesis of carbon doped nitrogen and sulphur in CDs (NS-CDs) derived from methionine by a hydrothermal method. Zhu *et al.* reported that the highest quantum as high as about 80% of CDs that is almost equal to fluorescent dyes. They used citric acid and ethylenediamine as carbon and nitrogen sources to be utilized in

ionization to the condensation, polymerization, and carbonization steps by hydrothermal treatment at 150–300 °C for 5h to prepare polymer-like and carbonaceous CDs. Even the utilization of amino acids such as serine and cystine is reported in the preparation of CDs [68].

Solvothermal carbonization followed by organic solvent extraction is a common technique to synthesize CDs. Ideally, carbon-yielding compounds were heated in a high boiling point organic solvents, this is then followed by extraction and concentration procedure. Bhunia *et al.* fabricated two types of CDs, hydrophilic and hydrophobic with a diameters less than 10 nm from carbohydrates carbonization [69]. The hydrophobic CD was produced by mixing different amounts of carbohydrate with octadecylamine and octadecene before heating to 70–300 °C for 10–30 min. The hydrophilic ones can be produced by heating an aqueous solution of carbohydrate within wide range of pH [70]. The hydrophilic CDs with red and yellow emissions can also be fabricated by mixing an aqueous solution of carbohydrate with concentrated phosphoric acid, then heating at 80–90 °C for 60 min. Problems arising from CDs synthesis include;

(i) Carbonaceous aggregation during carbonization, which can be bypassed using electrochemical synthesis, solution chemist-try, or confined pyrolysis methods.

(ii) Uniformity and size control, which is crucial for uniform characteristics and mechanistic study, can be optimized through post-treatment, such as centrifugation, gel electrophoresis, and dialysis.

(iii) Surface characteristics that are crucial for solubility and selected applications, can be tuned during synthesis or posttreatment.

**Pyrolysis method**

Pyrolysis is a simplistic method to synthesize CDs from organic compounds by simple chemical reactions carried out at very high temperatures in the presence of strong acid or alkali. Pyrolysis is an irreversible thermal decomposition reaction in which decomposition of organic materials take place in an inert atmosphere. It involves physical as well as chemical changes in organic materials resulting in solid residue containing carbon. Generally, pyrolysis takes place at very high temperatures and under controlled pressure. Bourlinos *et al.* synthesized Gd (III)-doped CDs having diameter ~ 3.2 nm with dual fluorescence via pyrolysis method. They prepared a mixture of tris(hydroxymethyl) aminomethane (Tris base), gadopentetic acid, and betaine hydrochloride to fabricate Gd (III)-CDs followed by the pyrolysis at 250 °C temperature [71]. Martindale *et al.* synthesized CDs of average diameter ~6 nm by pyrolysis of citric acid at 180 °C for generation of hydrogen fuel-utilizing solar energy [72]. Guo *et al.* synthesized stable CDs from hair (keratin) by a one-step pyrolysis method at 200 °C for 24 h of reaction time. They successfully recovered CDs and used their CDs in the detection of $Hg^{2+}$with higher sensitivity and selectivity [73]. Recently, Rong *et al.* synthesized highly photoluminescent nitrogen-doped CDs (N-CDs) derived from guanidinium chloride and citric acid by a pyrolysis method and fluorescence quenching observed in the presence of $Fe^{3+}$. N-CDs obtained by their synthesis were profoundly used in metal ion detections and in bioimaging [74]. Zhu *et al.* reported a facile microwave pyrolysis approach to synthesize CDs by combining poly (ethylene glycol) (PEG200) and a saccharide (glucose, fructose, etc.) in water to form a transparent solution, followed by heating in a microwave oven. The obtained CDs exhibited excitation-dependent photoluminescence properties. This is a simple, fast and environment-friendly preparation method for CDs rich in oxygen-containing groups [75], which would become the coordination sites of metal ions for the design of carbon-based electrocatalysts. It is of great importance to control the size during the preparation of discrete CDs with tunable and uniform sizes can be prepared via canned pyrolysis of an organic precursor in nanoreactors (fig. 3). Three steps were used as follows: (i) absorbing the organic precursor into porous nanoreactors via capillary force, (ii) pyrolysis of the organic precursor coned in the nanoreactors into carbonaceous matter, (iii) release of the as-synthesized CDs by removing the nanoreactors. The size and size distribution of the CDs produced from this method are dictated by the texture parameters of the nanoreactors.

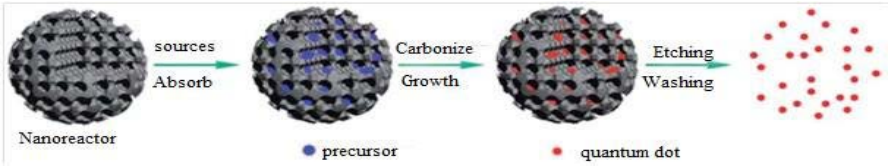


**Fig. 3: Schematic illustration of the preparation of CDs via confined pyrolysis of an organic precursor in nanoreactors. [Adapted with permission.62 copyright 2012, Royal society of chemistry]**

**Carbonization synthesis**

Carbonization of the precursor molecules is one of the best, inexpensive, simple, and ultrafast one-step methods to fabricate CDs. Carbonization is a chemical process in which solid residues with higher content of carbon are formed from organic materials by prolonged pyrolysis in an inert atmosphere. Wei *et al.* synthesized N-doped CDs using this ultrafast carbonization method within two min from glucose as a carbon source, and ethylenediamine as the nitrogen source [76, 77]. The observed size of the CDs was in the range of 1 to 7 nm with 49% of QY.

**Microwave irradiation method**

Microwave-assisted synthesis is a fast, low-cost, scalable and non-toxic, energy-efficient method to synthesize CDs via the irradiation of electromagnetic radiations having a wavelength ranging from 1 mm to 1 m through the reaction mixture containing the precursor molecules. In this methodology, carbonization of the small organic molecule occurs by microwave heating within a very short period of time [78, 79]. Zhu *et al.* synthesized fluorescent CDs having size ~ 3.7 nm using microwave irradiation for the first time [80]. They heated the aqueous solution of saccharides and polyethylene glycol in a domestic microwave oven (500 W) for nearly 3 min. Kiran *et al.* used citric acid as a carbon source and 3-aminophenyl boronic acidas the passivation agent to fabricate CDs. They heated the aqueous solution of citric acid, and 3-aminophenyl boronic acid in a microwave oven (1200 W) for 4 min and the average diameter of the obtained CDs was ranging from 2 to 5 nm [81]. Recently Cao *et al.* synthesized CDs from the aqueous solution of glucose and arginine using microwave-assisted pyrolysis in a microwave oven (700 W) for near about 10 min. The average diameter of the as-obtained CDs was between 1 and 7 nm [82]. Using sucrose as the carbon source and diethylene glycol as the reaction media, green luminescent CDs were obtained within one minute under microwave irradiation. These DEG-stabilized CDs could be well-dispersed in water with a transparent appearance. With an increase in the excitation wavelength, the intensity of the PL first increased to a maximum (360 nm) excitation) and then decreased. However, no perceptible shift of the PL peak over an excitation range from 320 to 380 nm could be observed. Moreover, these DEG-CDs could be efficiently ingested by C6 glioma cells and exhibited low cytotoxicity, suggesting their potential in bioimaging. Liu *et al.* promoted microwave-mediated pyrolysis of citric acid with various amine molecules to synthesize highly luminescent CDs. Several other researchers have also reported the microwave-assisted synthesis of CDs.

**Thermal decomposition**

This method offers various advantages, such as easy to operate, less time consuming, low cost, and large-scale production. In thermal

decomposition, a substance or compound decomposes chemically by the action of heat. Thermal decomposition reactions are generally endothermic. This type of decomposition reactions are either irreversible (decomposition of starch, proteins) or reversible (decomposition of ammonium chloride, limestone) [83]. Wang *et al.* synthesized CDs by this method from citric acid. They heated citric acid on a hot plate at 200 °C for 30 min; neutralized with sodium hydroxide solution, and finally dialyzed for purification. The size of CDs was observed within the range from 0.7 to 1 nm [84]. These CDs showed both excitation-dependent as well as independent photoluminescent properties, and different QY depending on different synthesis conditions. Wan *et al.* used the thermal decomposition of 1-butyl 3-methyl imidazolium bromide and l-cysteine for the synthesis of CDs at 240 °C [85]. Some other researchers also reported the synthesis of CDs from small organic molecules via this method.

**Table 1: Synthesis of CDs from small organic molecule via top-down approach**

| S. No. | Source | Method of preparation | Doping (d)/surface passivating (p) agents | Color | Size (nm) | Ref. |
|---|---|---|---|---|---|---|
| 1. | Carbon soot | Chemical oxidation | - | Green | 2-5 | [123] |
| 2. | Oligomer polyamide resin | Ultrasonic treatment | Silane Coupling agent (p) | Bright white | 2-4 | [126] |
| 3. | Graphite powder | Laser ablation | - | Red, black, and blue | 1.5, 1.6 and 1.8 | [128] |
| 4. | Carbohydrates | Chemical oxidation | (TTDDA) 4,7,10-trioxa-1,13-tridecanediamine (P) | Red, blue, green, and yellow | 5 | [130] |
| 5. | Carbon nanotube | Electrochemical synthesis | - | Blue | 2.8±0.5 | [132] |
| 6. | Tolune | Laser ablation | - | Red, black and blue | 2-3.9, 3-10.0,10-17.2 and 13-20.5 | [125] |
| 7. | Graphite electrode | Electrochemical synthesis | - | Bright yellow | 4±0.2 | [131] |
| 8. | Sodium citrate and urea | Electrochemical synthesis | - | Blue | 1.0-3.5 | [127] |
| 9. | Low molecular-weight alcohols | Electrochemical synthesis | - | Red and Blue | 2.1, 2.9,3.5 and 4.3 | [129] |
| 10. | Ascorbic acid and ammonia | Ultrasonic treatment | Silane Coupling agent (p) | Bright blue | 2-4 | [124] |

**Table 2: Synthesis of CDs from small organic molecule via hydrothermal treatment**

| S. No. | Source | Method of preparation | Doping (d) surface passivating (p) agent | Color | Size (nm) | Ref. |
|---|---|---|---|---|---|---|
| 1. | Dopamine | Hydrothermal Treatment | - | Blue, yellow, green | 3.8 | [133] |
| 2. | Streptomycin | Hydrothermal Treatment | - | Violet | 2.97 | [138] |
| 3. | Sodium citrate | Hydrothermal Treatment | - | Blue | 1.59 | [141] |
| 4. | Glucosamine HCL | Hydrothermal Treatment | Glucosamine HCL (d) | Green | 15-70 | [146] |
| 5. | Glucose, monopotassium phosphate | Hydrothermal Treatment | - | Violet | 1.83-3.83 | [146] |
| 6. | Citric acid and ethylene diamine | Hydrothermal Treatment | - | Blue | 2-6 | [135] |
| 7. | Histidine, NAOH | Hydrothermal Treatment | - | Blue | 3-5 | [151] |
| 8. | bPEL, ammonium persulfate | Hydrothermal Treatment | bPEL | Blue | 3-4 | [144] |
| 9. | Ammoinum citrate, ethylenediamine | Hydrothermal Treatment | N (d) | Blue | 4.8 | [153] |
| 10. | Citric acid | Hydrothermal Treatment | Isoleucine (d) | Violet | 6-15 | [152] |
| 11. | L-Serine, L-Cystine | Hydrothermal Treatment | N,S (d) | Orange | 2.6 | [148] |
| 12. | Ammonium citrate | Hydrothermal Treatment | Ethylene diamine (d) | Indigo | 4.8 | [142] |
| 13. | 1-Octadecane 1-hexadecylamine | Hydrothermal Treatment | Dihydrolipoic acid (P) | Yellow | 6-8 | [136] |
| 14. | Citric acid, ethanediamine | Hydrothermal Treatment | - | Violet | <5 | [137] |
| 15. | Citric acid, GSH | Hydrothermal Treatment | - | Blue | 2.5-3 | [140] |
| 16. | Citric acid, NAOH | Hydrothermal Treatment | - | Green | 11.3 | [147] |
| 17. | Folic acid, Phosphoric acid | Hydrothermal Treatment | Folic acid, Phosphoric acid (d) | Indigo | 13.2±1.6 | [140] |
| 18. | Citric acid, $NH_3$. $H_20$ | Hydrothermal Treatment | N (d) | Blue | 2 | [145] |
| 19. | Glucose | Hydrothermal Treatment | - | Blue | 1.65 | [149] |
| 20. | Sodium nitrate, histidine | Hydrothermal Treatment | - | Indigo | 1.5 | [143] |
| 21. | L-Phenylalaninol | Hydrothermal Treatment | - | Violet | 2.8 | [139] |
| 22. | APTS (3-Aminopropyl) (Triethoxysilane), Glycerol | Hydrothermal Treatment | - | Violet | 9±0.5 | [150] |

**Applications of CDs**

**Gene and biomedicine/drug delivery**

CDs have been micro-sized, they are readily available for cell uptake and more biocompatible to reduce cytotoxic effects, thus, they are likely to be safe, potent, and good delivery vectors and nanostructured materials in conjugate with the drug(s) can improve the drug delivery systems with respect to the drugs absorption, distribution and elimination. Currently, CDs have received increasing attention for drug delivery due to their superior properties such as fluorescence emission, and resultant cell membrane permeability, low toxicity, chemical inertness, water-solubility, easy synthesis, potential functionalization, and drug loading. Several researchers have applied CDs in drug delivery systems. For example, Wang *et al.* synthesized doxorubicin (DOX)-loaded CDs, which showed potential for application in both cell imaging and cancer therapy [86]. Initially, they prepared hollow CDs from bovine serum albumin by solvothermal reaction (6.8 nm in diameter, pore size of 2 nm and QY=7.5%) and then the produced particles were loaded with DOX. The sonicated solution of BSA (10 mg), ultrapure water (5 ml) and ethanol (10 ml) was heated at 180 °C for 12 h and then cooled to room temperature. Hollow CDs were centrifuged (10,000 rpm) and then added to DOX (0.1 mg $ml^{-1}$) and stirred for a couple of hours for loading of DOX into Hollow CDs. Fluorescence images of A549 cells confirmed that hollow CDs could be internalized by A549 cells and were mainly localized in the cytoplasm but could not enter the nucleus. Cell viability and cellular uptake results suggest that the Hollow CDs show low toxicity and act as a potential platform in drug delivery field. pH-triggered drug release, rapid cellular uptake, excitation-dependent and excellent biocompatibility were reported as the prominent advantages of designed HCDs-based drug delivery system [87]. Thakur *et al.* reported designing of antibiotic-conjugated CDs via a microwave-

assisted method using gum arabic as the precursor, which used a theranostic agent for controlled drug release, bioimaging and enhanced antimicrobial activity. In this work, CDs served as a carrier for ciprofloxacin hydrochloride, a broad-spectrum antibiotic, which was attached to the surface of synthesized CDs. The Cipro carbon dots showed good biocompatibility on Vero cells as compared to free ciprofloxacin (1.2 mmol) and ciprofloxacin release from CDs depended extremely on physiological conditions. These CDs exhibited improved antimicrobial effect against both gram-negative (*E. coli*) and gram-positive (*S. aureus*) microorganisms and also showed bright green fluorescent when live imaging was applied to view yeast cells under the fluorescent microscope and also give an effective new nanocarrier for controlled drug release with a high antimicrobial activity under physiological conditions [88].

**Table 3: Synthesis of CDs from small organic molecules via decomposition, carbonization, pyrolysis, solvothermal, and Ultrasonic treatment**

| S. No. | Source | Method of preparation | Doping (d) surface passivating (p) agent | Color | Size (nm) | Ref. |
|---|---|---|---|---|---|---|
| 1. | $CCl_4$, $NANH_2$ | Solvothermal treatment | N (d) | Blue, Cyan, Kelly, and yellow | 3.3 | [154] |
| 2. | Hydroquinone | Solvothermal method | $BBR_3$ (d) | Blue | 16 | [144] |
| 3. | $Sicl_4$, Hydroquinone | Solvothermal method | Si (d) | Blue | 7±2 | [125] |
| 4. | Glucose, HCl/NaOH | Ultrasonic treatment | - | Blue | <5 | [160] |
| 5. | Active carbon, $H_2O_2$ | Ultrasonic treatment | - | Blue, green, yellow, red | 5-10 | [157] |
| 6. | Glucose | Carbonization | Ethylene diamine (d), phosphoric acid (p) | Green | 1-7 | [162] |
| 7. | Citric acid | Carbonization | - | Blue | 4.8-9 | [164] |
| 8. | 6-O-(O-O-dilauroyl-tartaryl)-D-glucose | Carbonization | Green | Green | 2.4±0.5 | [147] |
| 9. | Tris base, betaine Hcl | Pyrolysis | Gadopetetic acid (d) | Purple, Green | 3.2 | [161] |
| 10. | GDs | Pyrolysis | L-glutamic acid | Blue, green and red | 4.66-1.24 | [165] |
| 11. | D-Glucose | Pyrolysis | L-Aspartic acid (d) | Yellow | 2.28±0.42 | [159] |
| 12. | Sodium alginate | Pyrolysis | - | Blue | <10 | [144] |
| 13. | Citric acid | Pyrolysis | Diethylenetriamine (p) | Indigo | 5-8 | [155] |
| 14. | Citric acid, N-(β-Aminoethyl)-γ-aminopropyl methyl dimethoxy silane | Thermal decomposition | AEAPMS (p) | Blue | 0.9 | [124] |
| 15. | Citric acid | Thermal decomposition | DETA (p) | Blue | 3-5.5 | [138] |
| 16. | Citric acid | Thermal decomposition | Ruthenium (III) | Blue | 6.8±2.3 | [156] |
| 17. | Citric acid | Thermal decomposition | - | Blue | 0.7-1.0 | [163] |
| 18. | Citric acid | Thermal treatment | Dicyanamide (d) | Green | 8-16 | [158] |
| 19. | L-Cystein | Thermal Decomposition | 1-butyl 3-methyl imidazolium bromide | Blue, yellow, red, green | 1.0-3.5 | [150] |

**Table 4: Synthesis of CDs from small organic molecule via microwave treatment**

| S. No. | Source | Method of preparation | Doping (d) surface passivating (p) agent | Color | Size (nm) | Refs. No. |
|---|---|---|---|---|---|---|
| 1. | Glycerol | Microwave synthesis | PEI (d, p) | Blue | 9±1.1 | [155] |
| 2. | Citric acid Urea | Microwave-assisted synthesis | - | Green | 2-6 | [166] |
| 3. | Arginine and glucose | Microwave synthesis | - | Blue | 1-7 | [169] |
| 4. | Triammonium Citrate | Microwave synthesis | - | Indigo | 6.6 | [171] |
| 5. | Citric acid | Microwave synthesis | Tryptophan (d) | Indigo | 2.6 | [167] |
| 6. | Glycerol | Microwave synthesis | TTDA (p) | Blue, turquoise, green, jacinth, and red | 5 | [134] |
| 7. | Saccharides and polyethylene glycol | Microwave synthesis | - | Blue | 3.7 | [164] |
| 8. | Carbohydrates and inorganic salts | Microwave synthesis | - | Blue, green, yellow | 2.1 | [152] |
| 9. | Citric acid | Microwave synthesis | RNase A (d) | Blue | 25-45 | [168] |
| 10. | Citric acid | Microwave synthesis | Boric acid (d) | Indigo | 2-6 | [170] |
| 11. | Citric acid | Microwae synthesis | 3-Aminophenyl boronic acid (d) | Indigo | 2-5 | [164] |

Cationic CDs have shown great potential as gene carriers and delivery applications because of their ability of electrostatic interaction with positively charged functionalized CDs and negatively charged nucleic acids. Cao *et al.* prepared positively charged CDs from porphyra polysaccharide and ethylenediamine precursors with a high QY of 57.3% to induce the neuronal differentiation of adult stem cells through nonviral gene deliver [89]. Gene transfection is faster and more efficient in neuronal induction from the adult stem cells by using these plasmid DNA-loaded CDs that can be used in bioimaging, gene delivery, and tissue engineering. Yang *et al.* reported turn on-off theranostic fluorescent CDs against hyaluronidase (HAase) in cancer cells for self-targeted imaging and drug delivery. Negatively charged CDs were modified with cationic polyethyleneimine (PEI) through electrostatic interaction to prepare P-CDs and functionalized with hyaluronic acid-Doxorubicin conjugate (P-CDs/HA-Dox) and these nanoprobe can pass into the cells readily with targeting specify to the CD44 receptor on the cancer cell. HA can be degraded to tetra saccharide units in the presence of the HAase enzyme [90]. Therefore, Dox can be released from a P-CDs/HA-Dox nanoprobe into cancer cells because of the enzyme-triggered drug delivery and induce apoptosis in Hela cancer cells. Therefore, this study clearly showed that CDs can be successfully used in the targeted bioimaging and delivery vehicles for image-guided chemotherapy. Tables 5 and 6 summarized some of the methods for the delivery of QDs.

CDs is a carbon material attracting tremendous interest in distinct fields of biomedicine. A facile and green synthesis of DNA-CDs using

genomic DNA isolated from Escherichia coli has been reported. The DNA-CDs were purified by column centrifugation-based technique. During the course of the formation of DNA-CDs, it was assumed that nitrogen is released by the thermal degradation of ribose which resulted in the formation of several new bonds (C−OH, N−O, and N−P) where many covalent bonds of the DNA were retained. The presence of ample amino and hydroxyl groups enables further functionalization. The remarkable biocompatibility warranted the DNA-CDs to be used in the design of novel type of fluorescent probes for bioimaging and drug delivery and CDs synthesized from carbon nano powder have high binding affinity to calcified bones *in vivo* with specificity [91, 92], and the bone-binding ability of the CDs was not significantly altered by surface passivation, which demonstrated the promising applications of CDs as highly bone-specific bioimaging agents and drug carriers.

**Table 5: Methods used for the delivery of carbon dots**

| S. No. | Strategy | Mode of action | Examples | Targeted cells | Refs |
|---|---|---|---|---|---|
| 1. | Facilitated delivery | Peptide-mediated | Histidine-Arginine-rich peptide gH625 (Herps simplex virus derived-peptide) | A549(lung adenocarcinomal cytosol) HeLa (cervical adenocarcinoma; cytosol | [172] |
| | | | JB577 peptide (palmitoylated) | HEK, COS-1, A549, primary fibroblast, chick embryo, rat hippocampal neurons(cytosol) | [176] |
| | | | LAH, sweet arrow peptide | COS-1 (African green monkey kidney) | [181] |
| | | | Chemoseletive Peptides | A549 | [179] |
| | | | Chitosan | L929 (murine fibrosarcoma) | [174] |
| | | | Liposomes | B16F10 (mouse melanoma) | [178] |
| | | Polymers | Triblock copolymers | Panc-1 | [183] |
| | | Small molecule | Lactose | Hela, Araki Sasaki (human corneal epithelium) | [182] |
| | | | Galactose | HepG2 (Hepatocyte), MCF-7 | [175] |
| | | | Gambogic acid | HepG2 | [173] |
| **2.** | **Active Delivery** | Nanoneedle Injection | | HeLa | [177] |
| | | Reversible membrane Permeabilization | | Rat cardiomyocyte (H9C2) | [180] |
| | | Nanochannel electroporation | | A549 | [173] |
| | | Nanoblade | | HeLa | [177] |
| | | Microfluidic cell 'squeezing' | | HeLa | [173] |
| **3.** | **Passive uptake** | QD surface character/chaarge | | Human primary epithelial | [180] |

**Table 6: A summary on CD use in drug delivery**

| S. No. | Source of CD | Drug/Method | Disease/Model system | Efficiency | Reference number |
|---|---|---|---|---|---|
| 1. | FA-Gd CD green CDs synthesized from crab shell doped with $Gd^{+}$ and conjugated with folic acid | Targeted drug delivery of doxorubicin | HeLa cell line | Significantly higher to toxicity towards HeLa cells and less toxicity in vivo (zebrafish embryos and other cell lines) | [185] |
| 2. | MSN-SS-$CD_{PAA}$-DOX CQD synthesized by hydrothermal polymerization method using poly-acrylic acid | Multifunctional nanosystem (targeted and controlled delivery of drug doxorubicin along with bioimaging | *In vitro* (human prostrate cancer cell line) | High therapeutic effect against cancers and good biocompatibility and stability silica particles containing the drug doxorubicin | [185] |
| 3. | CQD (Nitric acid oxidation of candle soot) | Phototherapy | Cell line | Higley cytotoxic to cancer cells | [188] |
| 4. | CQD hydrothermal treatment of citric acid, hyaluronic and ethylenediamine | Bio-nanoplatform (CQD-HA-$SiO_4$-DOX | Cancer cell line | Low cytotoxicity | [191] |
| 5. | MSN-SS-CDHA-DOX CQD synthesized by decomposition of citric acid and conjugated with HA, which were further mesoporous silica nanoparticles enclosing the anti-tumor drug, doxorubicin | Multifunctional nanosystem (targeted and controlled drug delivery of doxorubicin along with bioimaging) | *In vivo* mouse model | High therapeutic efficiency towards cancer cells | [187] |
| 6. | CQD-Asp (Thermolysis of d-glucose and 1-aspartic acid | - | *In vivo* mouse model of brain tumor | High biocompatibility and less toxicity | [193] |
| 7. | CQD (hydrothermal treatment of citric acid monohydrate, with diethylene glycol bis ether) | - | Both *in vitro*and*in vivo* model of glioma (brain cancer) | Successful targeting of glioma | [190] |
| 8. | CQDs Pt(IV)@PEG-(PAH/DMMA) CQD, prepared by thermal pyrolysis of citric acid, conjugated with PEG-(PAH/DMMA) | Cisplatin | Both *in vitro*and*in vivo* model | High tumor inhibition efficiency and low side effects | [184] |
| 9. | CQD-PEG-Ag acid oxidation of carbon nanotube and graphite | Radiotherapy | Cell lines | Cytotoxic to cancer cells | [189] |
| 10. | mPEG-OAL-DOX/CQD (CQD, prepared by pyrolysis of citric acid, cross-linked with PEGylated oxidized alginate (mPEG-OAL) | Doxorubicin | *In vitro* cell model | Cytotoxic specifically to cancer cells | [186] |
| 11. | CQDs Microwave synthesis method using acrylic acid and ethylene diamine followed by functionalization with glycidyl methacrylate | Targeted cancer drug delivery | Nanogel (copoymerized with zwitterionic amini acid ornithine methacrylamide) | Low cytotoxicity | [192] |

**Table 7: Role of CDs in gene delivery system**

| S. No. | Source molecule | Ligand attached | Drug/gene delivery | Cell type | Refs. No. |
|---|---|---|---|---|---|
| 1. | Sorbitol and sodium hydroxide | Folic acid | DOX | HeLa | [196] |
| 2. | EDTA | Mesoporous silica nanoparticles (MSPs) | DOX | HeLa | [199] |
| 3. | β-Cyclodextrin(βCD), oligoethylenimine (OEI) and Phosphoric acid | OEI/CD | DOX | H1299 | [194] |
| 4. | Polyethyleneimine and fluorinated diglycidyl ethers | Flourine doped | siRNA/DNA | HeLa cells | [202] |
| 5. | Citric acid and tryptophan | PEI | siRNA | MGC-803 | [197] |
| 6. | Citric acid and Polyene polyamine | - | Oxaliplatin | Hepatic cancer cells | [204] |
| 7. | Glycerol and polyethyleneimine | Fc-rPEI(folate conjugated reducible PEI rPEI) | siRNA | H460 | [194] |
| 8. | Arginine and glucose | - | pSOX-9 | Chondrogenic differentiation of mouse embryogenic Fibroblasts | [200] |
| 9. | ATP (Adenosine Triphosphate) moreover, polyethyleneimine (PEI) | Hyaluronic acid (HA) | DOX | HeLa cells | [198] |
| 10. | Urea and citric acid | Carboxyl groups on CDs | DOX | HepG2 and HL-7702 | [203] |
| 11. | D-Glucose (2.5 mmol) and L-glutamic acid | Polydopamine coated | DOX | HeLa cells | [195] |
| 12. | Carbon nanopowder | Transferrin | DOX | Glioblastoma cells; CHLA-266, DAOY,CHLA-200 and SJGBM2 cells | [205] |
| 13. | Branched polyethyleneimine, Hyaluronic acid | Hyaluronate (HA) and polyethyleneimine (PEI) | DNA/RNA | HeLa Cells | [201] |
| 14. | Citric acid and o-phenylenediamine | - | DOX | HeLa, mouse fibroblast cells (L929) | [198] |

**Bioimaging**

CDs have similar remarkable fluorescent properties but extremely low cytotoxicity, which makes them strong candidates to be used to design novel bioimaging probes. The researcher also selected the blue luminescent N-CDs to incubate with a human cervical cancer cell line for 2 h under different channels, clearly visualized the fluorescent imaging of HeLa cells. As a control, the HeLa cells untreated with N-CDs did not show any fluorescence. To confirm the potential application of S, N-CDs as a bioimaging probe, and also conducted *in vitro* cellular uptake experiments in MCF-7 cells, which was recorded by laser scanning confocal microscopy [93-95]. Polythiophene phenyl propionic acid-derived red-emissive CDs were synthesized by Ge *et al.* and used for both *in vitro* and *in vivo* imaging [96]. For *in vitro* bioimaging, HeLa cells were treated with the CDs which showed red fluorescence localized in the cytoplasm when excited at 542 nm. They also intravenously injected CDs in the HeLa-tumor-bearing mice and observed that the CDs were mostly accumulated inside the tumor due to enhanced permeation and retention effect. The various bioimaging applications are summarised in table 8.

**Table 8: Role of CDs in bioimaging applications**

| S. No. | Source molecule | Color | Application (bio-imaging) | Refs. No. |
|---|---|---|---|---|
| 1. | Glycine | Green | MCF-7 cell | [208] |
| 2. | Glycerol solvent | Blue | HeLa Cell | [215] |
| 3. | Carbon soot | Blue-yellow | HepG2 cell | [215] |
| 4. | Activated carbon | Blue/yellow/green | COS-7cells | [211] |
| 5. | Graphene oxide and ammonia | Green | HeLa Cells | [217] |
| 6. | Glucose TTDDA | Green | HeLa, MCF-7, NH-3T3 cells | [207] |
| 7. | Citric acid and ethylenediamine | Blue | MC3T3 cell | [219] |
| 8. | Sucrose and oil acid | Green | 16HBE CELL | [212] |
| 9. | Graphene oxide and Dimethylformamide | Green | HeLa Cells | [221] |
| 10. | Graphite Powder | Green/Blue | A549 cell | [209] |
| 11. | Polycyclic aromatic hydrocarbon | Green | MCF-7 cell | [219] |
| 12. | Folic acid | Blue/Green | U87glioma cell | [213] |
| 13. | Citric acid, PEG diamine, and Glycerin | Blue | Cholesterol imaging | [216] |
| 14. | Urea, polyethylene glycol | Blue | L929 cells | [220] |
| 15. | Citric acid, urea, and sodium fluoride | Red | Glioma C6 cells | [207] |
| 16. | Citric acid, phosphoric acid, and ethylene diamine | Red, green | Raw 2647 cells, PA and FL imaging of mice tumors | [223] |
| 17. | Glycerol polyethyleneimine | Blue/green/red | COS-7 cell | [212] |
| 18. | CX-72 carbon black | Green | MCF-7 cell | [225] |
| 19. | Graphite rods and hydrazine | Yellow | Neutrospheres cells, pancreas progenitor cells, and cardiac progenitor cells were performed | [206] |
| 20. | Carbon nanotubes and graphite | Yellow | *In vivo* NIR fluorescence imaging in mice | [222] |
| 21. | Carbon fibers | Green | 147D Cell | [218] |
| 22. | Glucose, monopotassium phosphate | Green | HepG2 cell | [227] |
| 23. | Graphene oxide and DMF | Green | MG-63 cell | [210] |
| 24. | Carbon soot | Blue-yellow | HepG2cell | [224] |
| 25. | Activated carbon | Blue/yellow/green | COS-7 | [214] |
| 26. | Citric acid, AEAPMS and silica | Blue | BGC823 cell | [226] |

**Sensor and biosensors**

Fluorescence CDs can be used as sensors for the detection and identification of a wide range of analytes, that is, cations, anions, drugs, small molecules, and macromolecules, depending on high sensitivity and selectivity, and the easy operation as benign biocompatible, and low-cost device applications. There are three main strategies to design CDs as a sensor material: As the prepared CDs interact with the analyte, the fluorescence signals could be changed; Specific receptors or special functional groups can be conjugated via post-modification on CDs to generate sensing ability; and Quenchers, fluorophores, and substrates integrations of CDs could be used as sensory materials [97-99]. The functional groups on the surface can be interacted with several metal ions such as $Ag^{+}$, $Au^{3+}$, $Fe^{3+}$, $Cr^{3+}$, $Cu^{2}$+, $Eu^{2+}$, $As^{3+}$, $Hg^{2+}$, $Pb^{2+}$, $Sn^{2+}$, $Co^{2+}$, and their binary and ternary mixtures with nonspecific sensing [101, 102]. The types of precursors and their surface state can be designated the quenching responsive of CDs to specific analytes.

Atashi *et al.* also demonstrated the same "on-off-on" fluorescent using $Cu^{2+}$and D-penicillamine. It is obvious that these studies clearly reveal that the fluorescent effects of CDs from chitin nanofibers was quenched or "turned off" after the addition of $Cu^{2+}$ions, whereas the fluorescent was "turned on" again in the presence of D-penicillamine as the $Cu^{2+}$ions were bound to D-penicillamine instead of CDs with high affinity [103-105]. Therefore, using a specific ligand and competitive binding interaction can be used in the design of very specific sensors for biomedical and environmental applications. The utilization of CDs as biosensing devices to recognize specific biological molecules such as glucose, amino acids, peptides, nucleotides, proteins, DNA, vitamins, cells, and bacteria has attracted great attention, especially for clinical sample analysis, early diagnosis of sickness, and so on. For example, the glucose level in the human body is of vital importance for the treatment of diabetes and/or cancerous diseases [97]. Moreover, Li *et al.* showed the biosensing effects of mannose-modified CDs against bacteria labelling by high selectivity of the CDs that bind to a specific lectin unit of the filegalle of the wild type E. coli K12 strain. In addition, these CDs can be successfully used in the labelling of bacteria by the fluorescence detection method in the real samples, including tap water, apple juice, and human urine [107]. Residue corresponding to antibiotics was determined by a CD-based composite sensor where either PL quenching (turn off) or enhancement (turn on) was observed. Antibiotics or their residues like tetracycline, cephalexin, ciprofloxacin, norfloxacin, oxytetracycline, and chlortetracycline have been detected from raw milk, egg, meat, and human urine sample. Estrogen drugs those were used in animals, birds, for fast growth can also be traced out by CD-based sensor very effectively [108-113]. Consequently, the utilization of different CDs in the recognition of different biomolecules is a viable procedure and offers a great advantage over the common diagnostic procedures in many aspects in biomedical applications summarised in table 9, 10 and also discussed in this review.

**Table 9: Role of CDs in bio-sensing application**

| S. No. | Precursor molecule | Color | Application (bio-sensing) | Ref. |
|---|---|---|---|---|
| 1. | Oxalic acid (OA) and urea | Blue | $Fe^{3+}$and $Ag^{+}$ | [331] |
| 2. | Fullerenes (C60) | Blue | $Fe^{3+}$ | [229] |
| 3. | $SiCl_4$ hydroquinone | Blue | $Fe^{3+}$, $H_2O_2$ and melamine | [331] |
| 4. | Lactose and NAOH | Blue | Folic acid | [333] |
| 5. | Citric acid, aminoguanidine | Blue | Nitric oxide (NO) | [333] |
| 6. | Galactose and *m*-aminophenyl boronic acid | Blue | Galactose | [335] |
| 7. | L-Glutamic acid | Blue, green, red | $H_2O_2$ | [228] |
| 8. | Citric acid and melamine | Blue | Glutathione | [332] |
| 9. | $BBr_3$, hydroquinone | Blue | $H_2O_2$, and glucose | [334] |
| 10. | Dopamine and (3-aminopropyl) triethoxysilane, glycerol | Blue | $Ag^{+}$ | [330] |

**Table 10: Role of CDs in chemical-sensing application**

| S. No. | Precursor molecule | Application (chemical-sensing | Ref. |
|---|---|---|---|
| 1. | Sodium citrate and citric acid | $Hg^{+}$ | [341] |
| 2. | Citric acid, $NH_3$. $H_2O$ | $Hg^{+}$ | [346] |
| 3. | Ammonium citrate and ethylenediamine | $Hg^{+}$ | [338] |
| 4. | Sodium citrate and citric acid | $Hg^{+}$ | [344] |
| 5. | Ethylenediaminetetra acetic acid (EDTA) | $Hg^{+}$ | [351] |
| 6. | Folic acid and 3-aminopropyl trimethoxy silane | $Fe^{+}$ | [340] |
| 7. | Cetylpyridinium bromide (CPB) | $Fe^{+}$ | [356] |
| 8. | Citric acid | $Fe^{+}$ | [358] |
| 9. | Citric, thiourea | $Fe^{+}$ | [343] |
| 10. | Ethylene glycol | $Fe^{+}$ | [352] |
| 11. | Polycyclic aromatic hydrocarbon (PAH) | $Fe^{+}$ | [347] |
| 12. | Graphite rods | $Fe^{+}$ | [353] |
| 13. | Phenolphthalein and ethylenediamine | $Hg^{+}$, lemon yellow dye, $Fe^{2+}$and $H_2O_2$ | [359] |
| 14. | Phenylenediamine | $Fe^{+}$ | [337] |
| 15. | D-sorbitol | $Fe^{+}$ | [348] |
| 16. | Citric acid | $Fe^{+}$, and $I^{+}$ | [357] |
| 17. | Uric acid | $Ag^{+}$and $Hg^{2+}$ | [336] |
| 18. | $CCl_4$ as a carbon and diamines as nitrogen precursors | $Ag^{+}$ | [350] |
| 19. | Citric acid and amino acid | $Ag^{+}$ | [338] |
| 20. | 1,2 diaminobenzene | $Ag^{+}$ | [345] |
| 21. | Uric acids | $Ag^{+}$ | [340] |
| 22. | Urea, polyethylene glycol | $Ag^{+}$ | [345] |
| 23. | Citric acid and guanidine thiocyanate | $Ag^{+}$ | [351] |
| 24. | Citric acid, polyethyleneimine for BPEI-CQDs | $Cu^{2+}$ | [341] |
| 25. | Citric acid | Selenite ($SeO_3^{2-}$) | [331] |
| 26. | Ammonium citrate and ethylenediamine | $I^{-}$ | [342] |
| 27. | Citric acid, and 1,6,-diaminohexane hydrochloride | $Cr6^{+}$ | [349] |
| 28. | Sodium alginate | Ascorbic acid | [345] |

**Electrocatalytic/energy**

CDs have been used in energy conversion and storage as well as electrocatalytic and photocatalytic devices, owing to their outstanding features such as low cost, broad optical absorbance, high photo and chemical stability, environmental friendless and nontoxicity, and scalable synthesis methods. Hu *et al.* reported ZnO nanorode-functionalized CDs (ZnO@CDs) as an energy conversion and storage material in photoelectrochemical (PEC) water splitting from solar to hydrogen energy conversion. ZnO@CDs as a photoanode enhanced the Photo-electrochemical activity compared with the bare ZnO nanorodes for solar water splitting, due to the extended-spectrum response range improving the photo conversion efficiency. This study cornered out that functionalization of the CD surfaces with photosensitive materials can improve the photo-electrochemical activity for solar conversion [114]. The various applications are summarised in table 11.

**Table 11: Role of CDs in electrocatalytic application**

| S. No. | Nanomaterial | Source molecule | Photocatalysis applicaaation/role of support | Ref. No. |
|---|---|---|---|---|
| 1. | N doped GDs-$ZnNb_2O_6$/g-$C_3N_4$ hetero structures | Urea for g-$C_3N_4$and $C_6H_5O_7(NH_4)_3$, NAOH for NGDs | $H_2$ generation | [345] |
| 2. | CDs | Citric acid | $H_2$ generation | [368] |
| 3. | CDs/$TiO_2$ | Vitamin C | $H_2$ generation | [346] |
| 4. | CDs/$TiO_2$ | Graphite | $H_2$ generation | [367] |
| 5. | PEG 1500N-functionalized CDs with Au/Pt doping | Carbon-based | $H_2$ generation and $CO_2$ Photoreduction | [346] |
| 6. | Au-doped CDs | Carbon-based | $CO_2$ Photoreduction | [348] |
| 7. | g-$C_3N_4$ | Urea or melamine | Conversion of $CO_2$into methanol | [351] |
| 8. | Reduced graphene oxide/ZnO | Graphene Oxide | $CO_2$ Photoreduction | [354] |
| 9. | CDs/$la_2Ti_2O_7$ | Vitamine C and ethanol | Rhodamine B (RhB) | [364] |
| 10. | Ultrafine amorphous iron oxyhydroxide/ultrathin g-$C_3N_4$ | Urea | Degradation of Rhodamine B, methylene blue, and methyl orange | [361] |
| 11. | CDs/$Bi_2O3$ | L-Ascorbic acid | Degradation of Rhodamine b | [360] |
| 12. | S, N doped GDs/g-$C_3N_4$ | Citric acid and thio urea | Rhodamine B (RhB) degradation | [356] |
| 13. | CDs/g-$C_3N_4$ | Citric acid, ethylenediamine | Degradation of Rhodamine B and tetracycline hydrochloride (TC-HCl) | [349] |
| 14. | CDs/Ag/$Ag_2O$ | Glucose | Rhodamine b | [353] |
| 15. | S, N doped GDs/$TiO_2$ | Citric acid for c-dots and urea/thiourea for N, S | Degradation of Rhodamine B | [358] |
| 16. | CDs/g-$C_3N_4$/$MoO_3$ | Citric acid, urea and dicyandiamide | Degradation of tetracycline (TC) | [365] |
| 17. | Ag-CDs/g-$C_3N_4$ | Citric acid, ethylenediamine | Naproxcen | [362] |
| 18. | Pb-CDs-$TiO_2$ | Ascorbic acid and kollicoat | Degradation of RBX,CRB, and CNB dye | [366] |
| 19. | CDs/$ZnFe_2O_4$ | L-Ascorbic acid, glycol and deionized water | NO removal | [347] |
| 20. | CDs/$Bi_2WO_6$ | Citric acid, ethylenediamine | Degradation of methyl orange and bisphenol A | [352] |
| 21. | Ultrafine amorphous iron oxyhydroxide/ultrathin g-C3N4 nanosheets | Urea | Methyl Orange | [361] |
| 22. | N doped CDs | Glucose and ammonia | Photodegradation of methyl orange | [350] |
| 23. | La/Cu/Zr/CDs | D-Fructose, NaOH | Degradation of ampicillin antibiotic, malachite green | [355] |
| 24. | CDs/nitrogen-doped ZnO | Carbon black pigment | Degradation malachite green | [357] |
| 25. | Fe (III)/CDs | Oxidative coupling of Xylene by anhydrous $FeCl_3$ | $H_2O_2$ reduction | [363] |
| 26. | N doped CDs/$TiO_2$ | Glycerol and TTDDA | Degradation of methylene blue | [359] |

**Biomedicine delivery system**

It is an attractive prospect to combine medical therapy and bioimaging diagnostics for visual drug distribution and monitoring of their effects. A multifunctional theranostic agent (CD-Oxa) was prepared by the conjugation of an anticancer agent (oxidized oxaliplatin, oxa(IV)–COOH) onto the surface of CDs containing amine groups. CD-Oxa successfully integrates the optical properties of the CDs and the therapeutic performance of Oxa. The *in vitro* results indicated that CD-Oxa possesses good biocompatibility, bioimaging function, and anticancer effects. The *in vivo* results demonstrate that it is possible to follow the track or distribution of the drug by monitoring the fluorescence signal of CD-Oxa, which helps customize the injection time and dosage of the medicine (fig. 5)

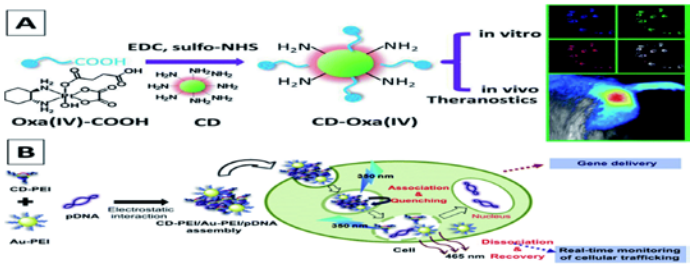


**Fig. 5: Synthetic scheme for CD-Oxa and its applications in bioimaging and theranostics, [Adapted with permission.53 Copyright 2014, Wiley-VCH. (B) A schematic illustration for the gene delivery and real-time monitoring of cellular trafficking utilizing CD-PEI/Au-PEI/pDNA assembled nanohybrids. Adapted with permission.112 Copyright 2013, Elsevier]**

**Anti-fungi/Anti-viral effects**

The fortuitous discovery of CDs concerted efforts have been devoted to discover novel nanomaterial-based strategy for combating the infectious disease with high selectivity/specificity to overcome multidrug-resistant bacterial infection. The recent studies have proved that the doping of commonly used antibiotics, e. g., ciprofloxacin on CDs' surface remarkably increases the selectivity and specificity of the antibiotics which makes the CDs an efficient platform to construct a novel drug delivery system and enhance the efficacy/selectivity of the existing antibacterial agents [115]. Yang *et al.* synthesized of a novel kind of CDs using glycerol as a carbon source and 3-[2-(2 aminoethylamino) ethylamino]propyl-trimethoxysilane as a surface passivating agent [116, 117]. The as-synthesized CDs showed the capabilities of selective recognition of Gram-positive bacteria and remarkable antibacterial activity. Recently, Huang, *et al.* found that CDs synthesized from benzoxazine monomer could block the infection of life-threatening flaviviruses (Japanese encephalitis, Zika, and dengue viruses) and non-enveloped viruses (porcine parvovirus and adenovirus-associated virus) *in vitro*, probably via directly binding to the surface of the virion and eventually impeding the first step of virus-cell interaction [118]. There have been a few reported on the anti-fungi activities of CDs are shown in table 12 is a brief summary of CDs samples and their various antimicrobial uses.

**Table 12: Summary of carbon dots samples and their antimicrobial actions/results**

| CDs configuration | Light activation | Microorganism | Highlight of antimicrobial action | Refs. |
|---|---|---|---|---|
| Dot sample from Carbonization synthesis coupled with ampicillin | Visible light | *E. Coli* | The MIC value decreased to 14 µg/ml from free ampicillin of 25 µg/ml | [360] |
| Dot sample carrying penicillin | Visible light | *S. aureus, E. coli* (DH5α), MDR *E. coli*, MRSA | The treatment at 100 µg/ml, inhibited more than 50% 0f MDR E. coli and MRSA | [368] |
| Dot sample from carbonization in polymer films | Blue Light | *S. aureus, E. coli, K. pneumoniae* | Light irradiation for 60 min caused up to 5 logs of inhibition effects | [366] |
| Dot sample with $Na_2W_4O_{13}/WO_3$ | Visible light | *E. coli* | The treatment for 100 min inactivated about $2x10^7$ CFU/ml, of *E. Coli, cells* | [370] |
| Dot sample from Carbonization synthesis coupled with ZnO in hydrogel | 660 nm and 808 nm light | *S. aureus, E. coli* | With the dual-light irradiation, inactivated 99.9% of the bacteria | [362] |
| Dot sample from electrochemical processing of carbon rod and then coupled with $TiO_2$ | Visible light | *S. aureus, E. coli* | The treatment at 1 mg/ml for 1 h reduced>7 logs and 1.82 logs viable cells, respectively | [364] |
| Dot sample from carbon nano powders combined with $H_2O_2$ | White light | *E. coli* | A mixture of 10 µg/ml dots and 8.82 mmol $H_2O_2$ reduced 2.46 logs of viable cells | [371] |
| EDA-CDs, EPA-CDs, $PEI_{600}$-CDs and $PEI_{1200}$-CDs (all from functionalization of CNP) | Visible light | *B. Subtilis* | EDA-CDs treatment at 0.1 mg/ml for 1 h reduced 3.26 logs of viable cells, while EPA-CDs treatment barely showed any reduction. $PEI_{600}$-CDs and $PEI_{1200}$-CDs treatment at 0.1 mg/ml for 1h reduced>7 logs and 1.82 logs viable cells, respectively. | [374] |
| EDA-CDs (from chemical functionalization of CNPs) | Visible light | *S. aureus, E. coli* | EDA-CDs treatment for 30 min reduced ~4 logs *E. coli viable cell numbers* | [363] |
| EDA-CDs, EPA-CDs (both from chemical functionalization of CNPs) | - | Human noroviruses virus-like particles (VLPs) | EDA-CDs and EPA-CDs at 5 µg/ml inhibited 100 % and 85-99%, of the binding of VLP to histo-blood group antigens receptors on human cells. | [375] |
| Dot sample made from benzoxazine monomer | - | Japanese encephalitis, Zika and dengue viruses, and porcine parvovirus and adenovirus-associated viruses | The dots could directly bind to the surface of the virion, and 95 eventually impede the first step of virus-cell interaction | [377] |
| Dot sample made from vitamin C | - | *R. Solani and P. grisea fungi* | The treatment at 300 µg/ml significantly inhabited the growth of the fungi | [373] |
| Dot sample carrying ciprofloxacin hydrochloride | | *S. aureus, E. coli* | The MIC value lower for, *E. coli* than that for *E. coli* | [376] |
| Dot sample from carbonization synthesis doped with Au | - | *C. albicans* fungus | Antifungal activity with $MIC_{80}$ ~ 250 µg/ml | [361] |
| Dot sample carrying metronidazole | - | *P. gingivalis* | Only selectively inhibiting obligate anaerobes | [372] |
| Dot sample from PEG-diamine and ascorbic acid as a precursor | - | Pseudorabies virus, porcine reproductive and respiratory syndrome virus | Significantly inhibited the multiplication of the viruses | [365] |
| Dot sample from carbonization synthesis carrying quaternary ammonium moieties | - | *S. aureus* | Killing the Gram-positive bacteria and also staining the dead cells for fluorescent analysis | [366] |
| Dot sample from carbonization of ammonium citric coupled with spermidine | - | *P. aeruginosa,* MRSA | Antibacterial activities against all of the tested bacteria. | [369] |

**Photothermal therapy (PTT)**

CDs with idiosyncratic optical properties, robust stability, and remarkable biocompatibility are of significant importance manifesting potential applications in bioimaging and PTT of various kinds of carcinomas [119]. Yang *et al.* used dopamine as a carbon source to synthesize CDs via a facile hydrothermal process. The as-synthesized CDs was subjected to *in vitro* PTT study after irradiation with an 808 nm laser (1.5W cm−2); 100% tumor cell eradication was reported with no serious side effects to the normal tissues [120]. Moreover, Wang *et al.* reported novel self-assembled red-emissive CDs@Au nanoflowers were fabricated and demonstrated efficient photothermal properties under 750 nm laser irradiation, and fluorescence imaging abilities [121, 122].

**Photodynamic therapy (PDT)**

Carbon dots in photodynamic therapy (PDT) Photodynamic therapy (PDT) offers low toxicity, minimal invasiveness, and targeted therapy towards cancer. It comprises three main factors, a light source, a photosensitizer, and a radical. Here, a laser excites the photosensitizer to generate reactive oxygen that eventually destroys the cancer cells shown in fig. 6. CDs are shown in table 13 is a brief summary of CDs samples and their various photo-dynamic and photothermal therapy.

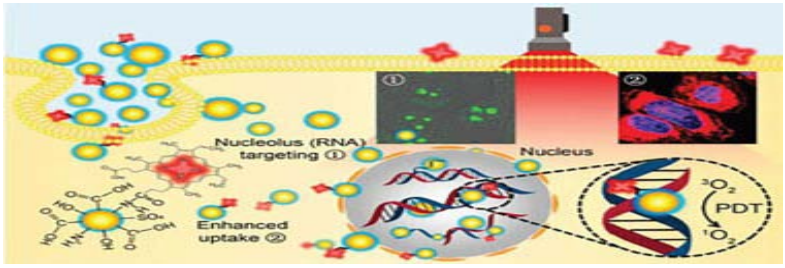


**Fig. 6: Graphical representation showing the preparation carbon dots, and its improved nucleus-targeted photodynamic therapy application, [Reproduced with permission (DeRosa, 2002), Copyright 2018, American Chemical Society]**

**Table 13: Role of CDs in photo-dynamic therapy (PDT) and photo-thermal (PTT) therapy**

| S. No. | Source molecule | Ligand attached | Targeted cell type | Refs. |
|---|---|---|---|---|
| 1. | Dopamine | - | HeLa cells | [419] |
| 2. | Citric acid and urea | - | HeLa | [423] |
| 3. | Urea | - | HeLa cells | [425] |
| 4. | Polythiophene phenyl propionic acid | - | HeLa cells | [421] |
| 5. | Citric acid and 5, 10, 15, 20-tetrakis(4-aminophenyl) porphyrin | Cetuximab(C225) | HCC827 and MDA-MB-231 cells | [427] |
| 6. | *m*-Phenylenediamine and L-Cysteine | Protoporphyrnix | HeLa | [420] |
| 7. | Diaminohexane and carboxylic group of Ce6 | Ce6-HA (hyaluronate) | B16F10 melanoma | [426] |
| 8. | EDTA-2Na and $CuCl_2$ | - | Murine melanoma (B 16) cells | [424] |
| 9. | Acrylic acid, 1,2-ethylenediamine (EDA) and $Mg(OH)_2$ | Mg/N | HePG2 | [418] |
| 10. | Hydrophobic cyanin dye and poly (ethylene glycol) | - | HePG2, CT26 | [422] |

**CONCLUSION**

In this article, recent developments in the field of CDs, concentrating on their synthetic approaches, surface modification methods, various optical properties and their applications in bioimaging, photocatalysis, biosensing and drug delivery and anti-fungal effects and antiviral effects have been discussed. Furthermore, the superior recognition capabilities of CDs in biosensors and theranostic applications also make them the favourable choice for the development of new diagnostic and treatment devices in many biomedical and environmental applications as well as the early determination of different kinds of sicknesses and environmental contaminations. In the inquisition for novel alternative antimicrobial approaches that are not only effective in mitigating the threat of resistant microorganisms but also benign and nontoxic, CDs have emerged to represent a promising new platform for visible/natural light-activated microbicidal agents. The excellent potential of the CDs platform in the killing/inhibition of bacteria, fungi, and viruses, including some multi-drug resistant species, has been demonstrated in many reported studies, so has been the path towards theragnostic uses, as highlighted in this review article.

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**AUTHORS CONTRIBUTIONS**

All the author have contributed equally.

**CONFLICTS OF INTERESTS**

There are no conflicts of interest

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