Carbon Quantum Dots: Synthesis, Characterization and Biomedical Applications

Inderbir SINGH[1]*, Riya ARORA[1], Hardik DHIMAN[1] and Rakesh PAHWA[2]

[1]Department of Pharmaceutics, Chitkara College of Pharmacy, Chitkara University, Rajpura-140401, Patiala, Punjab, India

[2]Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra – 136119, Haryana, India

ABSTRACT

Carbon Quantum Dots (CQD) are small carbon nanoparticles having size less than 10nm comprising distinctive properties and have become an obligatory tool for traceable targeted delivery, biomedical research and different therapy applications. The objective of the present work was to consolidate the up to date literature on synthesis, characterization techniques and biomedical applications of CQD. Two types of synthetic methods viz. top-down approach and bottom-up approach were utilized for the synthesis of CQD. Top-down approach includes the arc-discharge method, laser ablation method and electrochemical method. On the other hand bottom-up approach includes the thermal method, microwave assisted method, hydrothermal and aqueous method and template method. In this review, we have explained the recent progress of CQD in the biomedical field, focusing on their synthetic methods, characterization followed by the different applications. Carbon dots have extensive adequacy for in-vivo and in-vitro bioimaging and drug delivery studies. Although more cytotoxicity studies of carbon dots are needed to be carried out still the data above suggest the bright future of carbon dots in drug delivery and bioimaging studies.

Key words: Carbon Quantum Dots (CQD), Nanoparticles, Quantum Yield, Carbon dots, Photoluminescence, Nanocomposites.

INTRODUCTION

Luminescent semiconductor nano crystals of size 1-10 nanometres with rich surface chemistry and unique optical properties are called as Quantm Dots (QD). Different compounds belonging to group II to VI and III to V e.g. Ag, Cd, Zn, Hg, Se, Ln, Pb, P, and Te etc leads to the formation of QD. These have become an obligatory tool for traceable targeted delivery, biomedical research and different therapy applications. Long-term fluorescence imaging and detecting the property of these nanoparticles have made them imperative in biomedical research. Different properties of QD such as resistance to photobleaching, superior signal brightness, larger absorption coefficients, light emission, and contemporaneous excitation of different fluorescence colors make them unique as well as indispensable. Advancement in quantum surface chemistry study has led to the development of polymer-encapsulated probes have high fluorescent properties and are stable under the complexed biological conditions. To use QD in biological studies, it is extremely important to cap or passivate ZnS or CdS layer around the QD (CdSe). This layering of ZnS or CdS leads to the improvement of the fluorescence quantum yield of QD and provide protection against photo-oxidation. QD have a major impact in molecular diagnostics and in tissue molecular biology. The basic purpose for opting QD emerged from their incomparable and engrossing optical properties that are not generally feasible for an individual molecule or bulk semiconductor, in addition to resistance against photobleaching. They have the ability to elucidate the pharmacokinetics and pharmacodynamics of drug applicant and serve as a 'traceable drug delivery system.[1-5]

In 1984, Russian physicist Ekimov first discovered the QD in glass crystals. After 1984, the systemic advancement in the pharmaceutical sciences was driven, and a relationship was established between the size and band gap for semiconductor nano particles (by applying a particle in a sphere model) approximation to the wave function for bulk semiconductors.

In the beginning, the studies were limited to CdSe/CdS and CdSe/ZnS QD but later on other "core–shell" QD were developed and studied. eg: ZnSe/CdSe.

Cadmium was the chief component in the composition of traditional QD but the use of cadmium was limited as the leakage of cadmium ions leads to cytotoxicity.[5]

In the traditional composition of QD, cadmium was frequently used as the chief component, but cytotoxicity caused by leaked cadmium ions leads to the discovery of more biocompatible QD. With the increasing demand for more biocompatible QD, the emphasis shifted toward the

development of cadmium-free QD (CFQDs) having high chemical stability, low toxicity, and different pharmaceutical applications. This leads to the formation of different QD such as Carbon QD (CQDs), graphene QD (GQDs), silicon QD (SQDs) etc.[5, 6]

CQD were first obtained through the purification process of single-walled carbon nanotubes through preparative electrophoresis in the year 2004. They were first indicated as "carbon nanoparticles" but later adopted the term "Carbon dots" eliciting similar properties to inorganic QD. Carbon dots have gathered wide attention and considerable potential in biological applications. Also, biocompatibility has been touted as main lead of the carbon dots in the branch of nanoparticles applications. Carbon dots mainly comprise of carbon which is an abundant and nontoxic element and they endows distinguished structural and electronic properties that are different from other nanoparticle families.[5]

Advanced device applications were achieved for QD when their intrinsic properties were successfully tuned by doping with the heteroatoms. Because of their biocompatibility, low toxicity, strong photoluminescence, synthetic and photograph steadiness carbon dots have become a fascinating material for bioimaging, detection of different analytes. C-dots ordinarily contain discrete, quasispherical nanoparticles (NPs) with sizes beneath 10 nm. Sp_2 characterized CQD consist of different functional groups such as carbonyl, ether, epoxy, amino, carboxylic acid and the hydroxyl group on their surface. The presence of such groups on carbon dots leads to their high hydrophilicity. Captivating photoluminescence (PL) properties of carbon dots are subject to their edge shape, size, deformities and surface passivation.[7] Highly bright CQD which are soluble in oil can also fabricate by hot injection method with B and N co-doping by taking 1,2-Hexadecanediol as a carbon precursor and surface passivation material.[8]

Advantages of CQD

- Inexpensive - CQD are inexpensive and abundant thus making them rising star as a nanocarbon member.
- Photostability**:** Stability and composition of CQD lead to their greater photostability when compared with organic dyes and traditional QD.
- Broader excitation and narrow emission: CQD have a more sharply defined emission peak and broader excitation spectra than organic dyes and other cadmium-based QD.

- Biological properties**:** superior biological properties of carbon dots, such as hydrophilicity, low toxicity, chemical stability and good biocompatibility, ensure them with promised applications in the biosensor, drug delivery, and bioimaging.
- Luminescence: They have high luminescence as compared to other QD.
- Aqueous Stability: CQD have high aqueous stability as compared to other cadmium-based QD and organic dyes.
- Electronic properties- outstanding electronic properties of carbon-based QD as electron donors and acceptors cause the electrochemical luminescence and chemiluminescence, empowers them with wide potentials in optronics, catalysis, and sensors.
- Chemical inertness- Chemical stability of carbon dots is very high as compared to other QD (traditional or metallic).[9]

SYNTHESIS

Synthetic techniques for CQDs are categorized into two classes, "bottom-up" and "top-down" courses. These can be accomplished by means of chemical, electrochemical or physical systems.[10] Top–down strategies involve the fragmentation of carbon matter into carbon nanoparticles, and the strategies comprised of arc discharge, laser ablation, electrochemical approach and etc. On the other hand, Bottom up strategies incorporate template strategy, thermal routes, pyrolytic process, hydrothermal and aqueous method, supported synthetic technique, reverse micelle technique, microwave assisted strategy, substance oxidation.[11]

The yield of CQDs could be enhanced during arrangement or post-treatment. Alteration of CQDs is additionally vital to get favorable surface properties which are key for solvency and applications.[12]

Moreover, carbon precursors, for example, ground coffee, utilized tea, grass and light sediment are also used to develop Carbon dots. Development of carbon dots by these precursors is plentiful and economic.[10]

In addition, a green approach for easy and one step synthesis of fluorescent carbon dots can be achieved from wool (natural and nontoxic substance) for sensing the glycophosate detection.[13]

Different synthetic approaches are mentioned in fig.1.

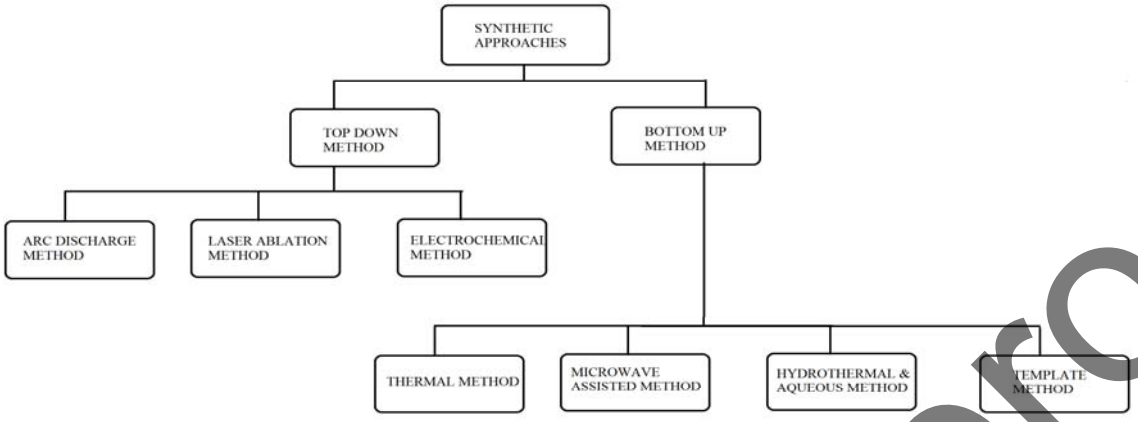

Figure 1. Different methods for the synthesis of CQD.

Top-Down Approaches

Arc-discharge Method

This strategy can be employed to develop Carbon dots (C-dots) from crude carbon nanotube soot (sediment). The Crude material (sediment) was oxidized with 3.3 M HNO_3 to introduce carboxyl groups, the resulted matter was then extracted with NaOH/ basic solution of pH 8.4, a stable dark colored suspension was obtained. Gel electrophoresis was conducted to purify the extracted matter.[10] Separation of a quick moving band of highly fluorescent carbon-dot was found to be 18 nm. Pristine and nitric acid oxidized carbon nanotubes were employed to outline the photoluminescent nanoparticles; latter being developed by electric arc technique. Fluorescent nanoparticles derived by the pristine carbon nanotube were hydrophobic and possess limited distribution. On the other hand, the fluorescent nanoparticles derived by the oxidized carbon nanotube had the capacity to accumulate when scattered in water, as they were externally combined with oxygen and covered by a thin layer of carbon, and demonstrated a more extensive division. Carbon nanoparticles obtained by the arc discharge method have low yield, in addition, arc discharge soot method comprised a number of composite segments. However, the purification of these segments was hard.[11]

Laser Ablation

This is a type of method in which synthesis of fluorescent carbon dots accomplished by laser irradiation of C- target (carbon target). Sun et al.[14] initially heated a blend of cement and graphite

powder in order to prepare carbon target, then fabricated carbon nanoparticles by means of laser ablation/removal of the carbon target in a stream of argon gas conveying water vapor at 900 °C and 75 kPa. The nanoscale carbon particles were obtained in aggregated form possessing different sizes with no distinguishable photoluminescence (PL). An aqueous solution of HNO_3 (nitric acid) was treated with sample followed by refluxing for 12 h, later Polyethylene glycol (PEG1500N) or poly(propionyl ethyleneimine-co ethyleneimine) (PPEI-EI) was reacted with the sample, and the passivated Carbon dots (C-dots) were highly photoluminescent having size about 5nm. Activation of these carbon dots at 400 nm, the fluorescence quantum yield was around 4 % to more than 10 %.[11]

In addition, doping of carbon dots with inorganic salts (like zinc acetate and Na_2S or NaOH) further resulted into enhanced quantum yield, in which the dopants (e.g. ZnO and ZnS) possibly served as a helper passivating mediator for the Carbon dots. When activated at 450 nm, doped carbon dots showed strong Photoluminescence (quantum yield 45%). PL C-dots of different colors were prepared in different solvents and aqueous medium, provided that the organic particles performed as passivation ligands.[10]

Laser ablation has numerous points of interest, for example, effortlessness, Different types of nanostructures can be prepared by this technique, but this method requires a high amount of carbon matter for the development of carbon targets. Laser irradiation develops the different sized carbon nanoparticles, large particles are effortlessly disposed of during the centrifugation process, so the resulted carbon nanoparticles posses low yield, and the use proficiency of carbon matter was also less.[11] Carbon particles can also fabricate in ethanol via laser ablation technique.[15]

Electrochemical Method

Lu et al.[16] utilized high purity rods of graphite and profoundly situated pyrolytic graphite was used as an anode, with a partition of 2 cm platinum wire as a counter electrode, followed by their installation into ionic fluid/water solution. The exfoliation of carbon matter was initiated by the application of static potentials. The process of exfoliation was conducted due to the complex exchange of anionic intercalation from the ionic fluid and anodic oxidative cleavage of water. Until the pH of exfoliation products was neutral, they were washed with ethanol and water. After separation by filter and ultracentrifugation at 15000 rpm at 20°C, carbon dots having 6-8 nm size were obtained with the quantum yield of 2.8-5.2%. Yao et al.[17] settled an anode of spectrum pure

graphite ring and cathode of a titanium tube at the middle of the electrolyzer. An insulated O-ring was used to separate the cathode and anode. Sterilized water was used for an electrolyte medium. Ultrasonic power and electrolytic voltage were applied at one time, and pure blue fluorescent carbon dots of 2-3 nm size were quickly produced without any complex purification. Quantum yield of obtained carbon dots was found to be 8.9%. Resulted carbon dots possessed magnificent fluorescence effect and thermodynamic stability in aqueous solution.[11] The extent of carbon dots can be managed by changing the current density. Larger carbon dots with longer emission wavelength can be formed by lowering the current density.[10] Photoluminescent carbon dots having 3 nm in size is specifically manufactured by electrochemical stunning of multi-walled carbon nanotubes.[2] Water-soluble carbon dots can also be prepared by the chemical oxidation treatment of the flour.[18]

Bottom-up Approaches

Thermal Routes

For the preparation of carbon dots (C-dots), burning sediment of candles was utilized as a starting material. Treatment of sediment with an oxidant, for example, HNO_3 and H_2O_2/AcOH resulted in the formation of carbon dots. Polyacrylamide gel electrophoresis was conducted to isolate the obtained carbon dots and demonstrated that carbon dots with higher versatility possessed photoluminescence at shorter emission wavelengths. The quantum yield estimations of carbon dots extended up to 0.8% to 1.9%. Sediment from natural gas was treated with HNO_3, followed by the neutralization with $NaHCO_3$. Finally, purification was done by dialysis which prompted the development of photoluminescent carbon dots. Metal nanostructures were formed on the surface of carbon dots by separately adding metal salts, including $AgNO_3$, Cu $(NO_3)_2$, and PdCl2, to the carbon dots solution in the presence of reducing agent (ascorbic acid).[10]

Soot based method is easy and clear.[19] However, QY (quantum yield) of the fluorescent carbon nanoparticle is very less (<0.1%) to any valuable purpose. An enhanced soot based technique is developed for the production of fluorescent carbon nanoparticles (CNP) of 2-6 nm size and QY around 3%.

There are 3 discrete improvements in the accompanying adjusted method.

Initially, a basic isolation method for a small-sized fluorescent molecule from heterogeneous particle mixture is developed. The technique is appropriate for the synthesis of these particles (on milligram scale).

The second method demonstrates, large particles are less fluorescent than smaller one and hence isolation of small molecule enhances the QY (<0.1% to ~3%) as they are more fluorescent.

The third technique state that particles which are small in size go into the cell with no further functionalization and fluorescence property of molecule may be utilized for cell imaging application based on fluorescence.[19]

Soot based method produce particle mixture of various colors and isolation of these various colored particles is difficult by gel electrophoresis.[19]

Microwave Assisted Method

Guan et al.[20] investigated this method for development of luminescent carbon dots with the folic acid molecules as both nitrogen and carbon sources. Initially, a blend was formulated by dissolving folic acid (15mg) in 3 mL diethylene glycol, and this blend was placed in the domestic microwave oven of 750W and it was heated for the 40s. A red brown colored suspension was obtained and its dialysis was carried for 3 days against pure water. Luminescent nanoparticles of carbon nitride were developed after post treatment, having a size range around 4.51 nm. Under activation at 360nm, QY of carbon nitride nanoparticles was found to be 18.9 %. Even when the emission peak excited at various wavelengths (from 320 nm to 420 nm) its position remained about constant (at 460 nm). Wang et al.[21] introduced an easy one-stage microwave assisted way to formulate water-solvent phosphorus-containing Carbon dots. In this method, a mixture of 2 mL phytic acid (70%) and 1 mL ethylenediamine was prepared with 25 mL ultrapure water, and the resulted turbid mixture was heated for around 8 min in a microwave stove of 700W. Furthermore purification of crude substance resulted into development of phosphorus-containing carbon dots, also the aromatic structures of these developed C-dots were covalently attached to phosphorus groups. The phosphorus-containing Carbon dots, when activated at low wavelengths indicated two peak emissions, while single peak was indicated at 525 nm (green fluorescence) by the nanoparticles when activated at high wavelengths (360-460nm). Quantum yield of the subsequent phosphorus–containing Carbon dots was 21.65 %.[11]

In comparison with other approaches, microwave assisted method is more convenient and rapidly heat the carbon precursors. This method also simplifies the synthesis process, so the carbon dots are readily obtained within a few minutes with improved QY.[11]

Another approach to develop CQD under this method involves heating of transparent aqueous solution of PEG_{200} and saccharides in a microwave oven working at 500 W for 2–10 min.

Resulted Carbon dots when activated at the wavelengths extending from 330 to 460 nm demonstrated interesting λex-subordinate photoluminescence (PL) properties. The quantum yield of Carbon dots extended from 3.1% to 6.3%. Likewise, carbon dots were developed by treating glucose with alkaline (or acidic solution) under ultrasonication for 4 hours. PL emission of the Carbon dots covered the whole visible to near infrared (NIR) spectral region. The C-dots had up-change PL properties when activated at 700–1000 nm, demonstrating outflow in the wavelength range of 450–750 nm.[10]

Hydrothermal and Aqueous Based Method

In order to develop PL carbon dots, used coffee was exposed to the hydrothermal treatment. Before grinding into fine powder, utilized coffee beans were kept in an oven for drying. Later was then autoclaved and calcined in air at 300 °C for 2 h. The Carbon dots were prepared by four successive stages:

- Dehydration,
- Polymerization,
- Carbonization, and
- Passivation.

Likewise, a green method was conducted to develop carbon dots from utilized green tea at 300 °C for 2 h. Resuspend the subsequent dark carbonized powder in the sterile water and later on dialysis was conducted to purify the Carbon dots. In the development and passivation of carbon dots, the rich catechins present in the green tea likely played a key role. Four unique molecules (cadaverine, glycine, ethylene diamine-tetra acetic acid (EDTA) 2-amino-2-hydroxymethyl-propane-1,3-diol (TRIS)) comprising either a carboxyl group or an ether group or both in aqueous solvent were independently calcined hydrothermally at 300 °C for 2 h. The outcome revealed that agents having both carboxyl and amino groups were beneficial for the development of highly water-diffusible and photo luminescent carbon dots.

Furthermore, in a nitrogen atmosphere ethylene diamine-tetra acetic acid (EDTA) was utilized to form carbon dots at 400 °C for 2 h. Few EDTA precursors were not entirely degraded, which were then employed to prepare carbon dots , prompting to enhanced hydrophilicity. EDTA (i.e., ethylene diamine-tetra acetic acid) containing either a carboxylic group or an amino group or may contains both in the aqueous solution were individually calcined hydrothermally for 2 h at

300 ^{0}C. Results showed that precursors which contain both the groups i.e. carboxyl group and an amino group were beneficial for the photoluminescent and highly water dispersible carbon dots.

To develop organosilane-functionalized carbon dots, (3-Aminopropyl) trimethoxysilane (APTMS) was used as a precursor at 300 ^{0}C for 2 h, in the absence of the additional passivating agent. Likewise, 4-aminoantipyrine and ammonium citrate was taken as another carbon precursor to develop the carbon dots in the air at 300 ^{0}C for 2 h.Various organic ammonium species were attached through a covalent bond to the surface which served as a surface modifiers and altered the hydrophilic nature.[10]

An another approach under this method for the development of carbon dots, various carbohydrates like Sucrose, glucose, starch was used in the presence of strong acid like H_2SO_4, as starting material. These solutions were further treated with nitric acid and carboxyl group was introduced on their surfaces to develop a class of carbon nanomaterials which include carbon dots. For further enhancement of their photoluminescence intensity, surface passivation with organic molecules and polymers was required.[10]

Development of CQD with glucose as a precursor is easy and simple through a hydrothermal process. To increase the fluorescence emission, ethylene diamine can be used as a passivated agent.[22]

Template Method

This approach has also been utilized for the preparation of nanosized carbon dots. This approach contains two stages:

(i) developing carbon dots through calcination in the appropriate mesoporous silicon spheres or template, and

(ii) Etching to erase supports and create the nanosized carbon dots.

Zong et al.[23] concluded a technique for the utilization of mesoporous spheres of silica as hard templates, these spheres of silica were saturated with a blended solution of citric acid and complex salts. After mesoporous supports were calcinated and expelled the photostability of succeeding carbon dots along with mono-dispersion demonstrated fabulous luminescence properties. Whereas Yang et al.[24] reported a method for developing uniformly morphologic PL carbon dots by utilizing soft hard template approach. Copolymer Pluronic P123 was utilized in this approach as a soft template while a hard template requested was mesoporous silica, various organic molecules like diamine benzene (DAB), 1,3,5- trimethylbenzene (TMB) etc. were

sources of carbon. After template removal, passivation, and carbonization, carbon dots so obtained with compositions, tunable sizes and crystalline degrees were having additional high stability properties, up-conversion PL and PL effectiveness as high as 3.3~4.7 %.

The difficulty of aggregate formation was successfully eliminated through this soft hard template approach and development of carbon dots was permitted with a narrow distribution of size due to confinement of size.

Lai et al.[25] concluded the development of carbon dots in the nanoparticles of mesoporous silica which served as a nanoreactor to control the size distribution. Initially they developed mesoporous silica nanoparticles ($mSiO_2$); furthermore, the $mSiO_2$ nanoparticles were blended with PEG-NH_2 and glycerol, then heated to 30 min at 230 °C; at last, extraction of crude items was done by centrifugation to acquire the carbon dots nano-composites without scratching. Simultaneously capping PEG onto the surface of $mSiO_2$, the subsequent carbon dots indicated additional improvement in the QY, biocompatibility, and colloidal stability. During the synthetic process of carbon dots, corrosive acid or base was expected to etch the template because the formation of mesoporous silica was difficult. This strategy was time consuming and expensive. Moreover, the template was hard to be etched off completely due to high-temperature pyrolysis of the template, also the process of separation and purification was difficult, and the QY was limited.[11]Properties of different synthetic methods are summarized in the table no. 1.

Table 1. Properties of different synthetic methods

S No.	Synthetic methods	Size range	Quantum yield (QY)	Advantages	Disadvantages
1	Arc discharge method	---	-	Most attainable method	Harsh conditions, posses Low quantum yield and composite method
2	Laser ablation method	5 nm	QY ranges between 4%-10%	Effortlessness, effective technique, different sized nanoparticles can be prepared.	Large amount of carbon matter is required, poor control over sizes, low quantum yield.
3	Electrochemical method	6-8 nm	QY ranges between 2.8%-8.9%	Stable method, extent of carbon dots can be managed by changing current density, water-soluble carbon dot can also be prepared.	Complex method
4	Thermal route	2-6 nm	QY ranges between 0.1%-3%	Easy and straightforward method, have fluorescence property, appropriate method for particles (on milligram scale).	Low quantum yield
5	Microwave assisted method	4.51 nm	The PL QY and phosphorus containg QY ranges between 3.1%-6.3% and 21.65% respectively	Simple and convenient method, inexpensive and eco-friendly method.	Poor control over sizes.
6	Hydrothermal and aqueous-based method	-	-	Highly water dispersible carbon dots can be prepared, inexpensive, non-toxic	Poor control over sizes
7	Template method	-	-	Carbon dots have biocompatibility and colloidal stability	Time consuming and expensive method, have limited quantum yield

CHARACTERIZATION OF CQD

Keeping in mind the goal to attain the information about synthetic properties of carbon dots, numerous techniques may be utilized in order to characterize the carbon dots, for example, Nuclear magnetic resonance (NMR), X-Ray diffraction (XRD), transmission electron microscope (TEM), Fourier transform infrared spectroscopy (FTIR), U.V spectroscopy, photoluminescence.[11]

Transmission Electron Microscopy

TEM can be used to identify the ultrastructure of the samples as it possessed a high resolution of 0.1-0.2 nm. TEM has a wide demand in science, pharmaceutical, material science and other research and development departments. Morphology study of the nanoparticles can be studied by this technique, in order to understand their information regarding shape, size, and dispersion etc.

Transmission electron microscopy is broadly utilized as a part of the characterization of carbon dots (C-dots). To determine the fine structure of carbon dots, High- resolution transmission electron microscopy can also be utilized. Crystalline nature of carbon dots can be classified into two types of lattice fringes, named as interlayer spacing and in-plane lattice spacing, respectively. Interlayer spacing typically focused at around 0.34 nm, while in-plane lattice spacing focused at 0.24 nm.[11]

Zhang et al.[26] carried out acid oxidation of graphite in order to synthesize carbon dots and their lattice spacing was generally below than 0.3 nm, demonstrating that large portion of the dots were actually separate graphenes.

Shinde et al.[27] synthesized carbon dots from multi-walled carbon nanotubes dots by means of electrochemical technique, and at the same time, two kinds of lattice fringes were observed in the HRTEM image.[11]

X-Ray Diffraction Method

X-ray diffraction method is efficiently used for the characterization of the carbon dots and to obtain the information of particle size, phase purity and crystal structure.[11, 28] X- ray diffraction method also determines the crystalline phases of CQD.[29] Liu et al.[30] synthesized the carbon dots by utilizing hexa-perihexabenzocoronene as the precursor. Carbon dots having a size of ~60 nm in breadth and 2~3 nm thickness were produced, after pyrolysis at high temperature, surface functionalization, reduction treatment and oxidative peeling. So obtained carbon dots possessed fluorescence quantum yield of 3.8%. Mao et al.[31] developed photoluminescent carbon dots with

the glycerol through a one-stage pyrolysis of poly(acrylic acid). Various structure and optical features of the carbon dots were altogether examined. X-ray diffraction design demonstrated a wide peak near 2θ=24°, further affirming the white fluorescent carbon dots graphite structure. Bourlinos et al.[2] synthesized carbon dots through calcination of ammonium citrate salt at 300 ^{0}C, the relating X-ray diffraction design showed two reflections which were superimposed, which confirm the presence of exceptionally carbon alkyl groups which are surface modified.[11]

Fourier Transform Infrared Spectroscopy (FTIR)

For determination of the functional groups that are present on the surface of carbon dots, FTIR or Fourier transform infrared spectroscopy has also been used.[10] Carbon dots are mostly comprised of oxygen, carbon, and hydrogen. Due to the development of carbon dots by partial oxidation of carbon precursor, carboxyl or carboxylic acid groups, hydroxyl groups, and ether/epoxy are abundant on the surface of C-dots and so for the investigation of these groups containing oxygen FTIR or Fourier transform infrared spectroscopy is a useful device.

Before applying, changes were required to be made with C-dots for balancing out potential wells on the energy surface, lesser cytotoxicity, and higher fluorescence quantum yield. Altered C-dots can be characterized using Infrared spectroscopy so as to decide if they were passivated adequately. Peng et. al.[32] developed carbon dots of size 1-4 nm by the compound oxidation of carbon strands of the size of a micron, 1~4 nm carbon dots, the particles so formed broke up in a polar solvent and were soluble in water; dimethyl sulfoxide and dimethyl formamide being the examples. The Infrared range of these was recorded. Peaks of characteristic absorption at 1724 cm^{-1} and 3307 cm^{-1} proposed carboxyl group's appearance on their surface; the presence of double bond was shown by the peak of absorption at 1579 cm^{-1}, and the presence of ether linkage was implied by absorption peak at 1097 cm^{-1}.[11]

Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance strategy is often utilized to obtain the structural information of carbon dots. Hybrid types of C-atoms in the crystalline network and binding mode between carbon atoms is determined by the NMR. Tian et al.[33] utilized natural gas burning sediment as a carbon source and conducted the refluxing with nitric acid which resulted in the development of carbon dots. Aromatic (sp^2) carbons show resonance in the region extending from 90-180 ppm whereas aliphatic (sp^3) carbons show resonance in the region extending from 8-80 ppm, structural insights of carbon dots is determined with the help of nuclear magnetic resonance

measurements by distinguishing sp^3 carbons from sp^2 ones. The Absence of aliphatic carbons was indicated by 13C NMR range, which depicted the absence of a single peak, below 120 ppm. Within the region extending from 120-150 ppm, a sequence of peaks appeared and most of these peaks were emerged from aromatic carbons. 13C nuclear magnetic resonance spectroscopic estimations affirmed that the carbon dots were developed from sp^2 carbons.[11]

U.V. Spectroscopy

Strong (UV) absorption is usually shown by the carbon dots prepared using various techniques, but still the positions of absorption peaks of UV are entirely different for different techniques used for the preparation of C-dots. [12] C-dots having size 3.8, 1.5-3, 1.2 nm transmit at the NIR, Visible (400-700 nm) and UV (350 nm) regions, respectively.[10] Li et al.[34] added active carbon (4.0g) into 70mL of hydrogen peroxide for making a suspension and sonicated it for 2 hours at room temperature. After filtration, fluorescent water-soluble C-dots were obtained and had a diameter of range 5-10 nm, and typical absorption of an aromatic pi framework was represented by the common UV-visible absorption band peak at 250~300 nm. Wang et. al.[35] immediately added 0.5 g citrus extract anhydrous into AEAPMS (N-(β-aminoethyl)-γ-aminopropyl methyl dimethoxy silane) solution with vigorous stirring at 240 °C and maintaining same for 1 minute. Amorphous carbon nanoparticles having a diameter of ~0.9 nm were incorporated after natural cooling and purification, they were also very luminescent (quantum yield=47%). C-dots so manufactured were having a strong UV-visible absorption peak at 360 nm. Dong et. al.[36] used carbonation of citrus acid for forming photoluminescent C-dots at 200°C. Carbon dots so formed were nanosheets of 0.5-2.0 nm thickness and ~15 nm in width, demonstrating UV absorption at 362 nm in the absorption range, the nanoparticles were consistent in size and this was evident by the narrow peak width. The maximum emission wavelength kept unaltered at a point when activated at various excitation wavelengths. Tang et. al.[37] conducted pyrolysis of glucose solution assisted by microwave for the preparation of C-dots, the diameter of so obtained C-dots was 1.65nm with fluorescence quantum yield of 7~10%. Two evident UV absorption peaks at 228 and 282 nm were indicated by the aqueous solution of these C-dots. The intensity of both UV absorption peaks increased by extending the microwave heating time, whereas the peak positions remained unaltered, and showed no connection with nanoparticles size.[11]

Photoluminescence

As another class of nanomaterials, C-dots have tempted remarkable consideration in the past decade. From an essential perspective to property and application, photoluminescence is the most intriguing characterizations of carbon dots. Carbon dots possessed certain optical properties which may reflect not just impacts from particles of various sizes in the sample. In addition to this, various emissive sites are distributed on each carbon dot. However, investigations on the optical properties of carbon dots which are small in size are dubious; because the accurate mechanism of photoluminescence is unclear. One exceptional feature of the photoluminescence of the carbon dots is the clear λex-dependence of the emission wavelength and intensity. By utilizing surfactant modified silica sphere as carriers and resols as carbon precursors, carbon dots having size 1.5~2.5 nm were synthesized followed by the surface passivation with PEG1500N. Resulted quantum yield of passivated carbon dots was characterized to be 14.7 %. Suspension of passivated carbon dots showed strong blue luminescence, when excited at 365 nm. These carbon dots have broad emission spectra, extending from 430 to 580 nm, and they exhibit λexdependent photoluminescence (PL) emission. Brilliant and vibrant photoluminescence of carbon dots can be ascribed to the presence of surface energy trap settled by surface passivation.[11]

Biodistribution and Pharmacokinetics

S.C and I.V injections are the basic routes of administration of CQD. As these QD reach the systematic circulation they identify the target and bound to it. After getting attached to the target, light is emitted by each quantum dot. The color of the fluorescence depends on the size of the QD and can be easily detected and identified by various techniques.

CQD or C-dots, are small semiconductor nanocrystals of size 1-10 nanometres obtained with different surface passivation processes either by modification or functionalization. These have very low toxicity and high fluorescent, and thus have numerous applications in bioanalysis, bioimaging, drug delivery and other related areas. So it is necessary to consider the biosafety studies of carbon dots which includes biodistribution, pharmacokinetics etc.

Yang et al.[38] in 2009 were the first to investigate the biodistribution pattern of carbon dots by combining 13C labeling and whole body imaging. The biodistribution and translocation of C-dots in mice were concluded. It was found that carbon dots cannot cross the blood brain barrier (BBB), but can easily distribute into the whole body. In certain organs like spleen, liver and kidney moderate accumulation was observed.[39, 40]

Likewise, Yang et al.[38], Tao et al.[39] conducted the toxicity and biodistribution study by labeling the carbon dots by ^{125}I. Pharmacokinetic analysis of carbon dots was done by a two-compartment model. Distribution $t_{1/2}$ of C-dots was 0.1 h and clearance half-life was 2.1 h. The distribution pattern of carbon dots was similar to that of Yang et al. as moderate accumulation was observed in spleen, liver, kidney except for brain.

Biodistribution studies of carbon dots were also carried out by Li et al.[34] in mice using i.v. injection. Imaging of dissected and sliced organs was done under 405nm excitation and 500nm emission. Blue fluorescence was detected in different body organs including spleen, liver, heart, kidney, lungs, small intestine and brain. High concentration of carbon dots was noticed in the spleen.

In other exposure pathways, due to high hydrophilicity and small size carbon dots exhibited free translocation in the body. Biodistribution of Gd-carbon dots was investigated by Xu et al.[41] after intra-tracheal instillation. Gd^{3+} quantification was done so as to measure the biodistribution of QD. Accumulation of QD in liver, kidney, lungs, heart and spleen was reported. Tumor-bearing mice had almost same distribution with an accumulation of 10% injected dose per gram of tissue (ID g^{-1}) in the tumor. The report recommended that carbon dots can freely translocate as well as distribute in different organs of the body.[42]

CYTOTOXICITY

The ability of certain chemicals or mediator cells to destroy living cells is called as cytotoxicity. These mediators or chemicals can induce necrosis (accidental cell death) or apoptosis (programmed cell death) in healthy living cells of humans and animals. Carbon dots have been considered as possible replacements for organic dyes and metallic quantum in bioimaging, due to their chemical stability, broad excitation ranges, and excellent fluorescent properties. Biocompatibility is considered important for their application in cell labeling and imaging thus making them the most important property of QD. Toxicity of carbon dots is a basic concern. Studies related to cytotoxicity of carbon dots have been conducted by different scientists, organizations, and institutes but only a few results and reports are present right now.

Zhang et al investigated the cytotoxicity of carbon quantum dots on rat mesangial cells. No apparent cytotoxicity and much better biosafety property of carbon dots were reported for biological fluorescent probe application.[18]

Fluorescent carbon dots were synthesized and evaluated for cytotoxicity.[19] Various indicators like cell viability, malondialdehyde, total reactive oxygen species, glutathione and lactate dehydrogenase were evaluated using human bronchial epithelial (16HBE) cell line. The results showed that carbon dots significantly increased the membrane permeability of 16HBE cells. Carbon dots induces oxidative stress which exhausts the antioxidant defenses of cells leading to decreased cell viability. Therefore, surface modification of carbon dots could minimize its cytotoxicity.

Sun and co-workers[14] conducted in vitro and in vivo cytotoxicity studies of carbon dots. Viability, proliferation and cell mortality of MCF-7 cells (human breast cells) and HT-29 cells (human colorectal adenocarcinoma) were determined by conducting Trypan Blue and MTT assays after exposing them to carbon dots. Carbon dots thus used were synthesized using PEG1500N laser ablation and surface passivation techniques.

Carbon dots as agents have been employed for in vivo testing in mice. They give bright fluorescent appearance in solution form. No acute toxicological response was reported when carbon dots solution were injected intravenously in mice. These carbon dots were excreted primarily via urine within ~3 h of injection,[38] thus signifying the nontoxic nature of carbon dots. Fluorescence of carbon dots in liver and kidney can be observed after 4 h of intravenous injection. High accumulation of carbon dots leads to higher fluorescence in the kidney as compared to that in liver. Urine being the chief excretion pathway of carbon dots also leads to higher fluorescence.

No toxicity was reported when carbon dots were administered intravenously in male CD-1 mice. Even exposure of carbon dots for 28 days did not show any toxic effects.[38]

All the above experiments lead to the conclusion that carbon dots have extensive adequacy for in-vivo and in-vitro bioimaging and drug delivery studies. Different research has suggested that carbon dots will have biocompatibility almost equivalent to that of FDA-approved organic dyes used in optical imaging eg. Indocynine green. Although more cytotoxicity studies of carbon dots are needed to be carried out still the data above suggest the bright future of carbon dots in drug delivery and bioimaging studies.

BIOMEDICAL APPLICATIONS

Bioimaging

CQD play an important role in the biomedical applications; Bioimaging is one of the essential applications which can be defined as the process in which images of living organisms are produced with the help of magnetic resonance imaging, x-rays, ultrasound, etc. It is also use to determine the 3-D structure information.[43]

CQDs have numerous advantages over semiconductor QD because of their biocompatibility, low toxicity, strong photoluminescence, etc. These properties make the CQD very advantageous to visualize the biological systems both in vivo and in vitro. This is important to know that CQD themselves are nontoxic but it is the surface passivating agent on the surface of CQD which is mainly responsible for the cytotoxicity. The surface passivating agent of low toxicity can be safely use for in vivo imaging at higher concentrations. For example: In toxicity evaluation, when PEGylated CQD were introduced intravenously 8-40 mg kg^{-1} (CQD/body weight) into mice for up to 28 days showed no significant toxic effects in vivo. When mice were exposed to various dosages of CQD and NaCl control, all the physiological indicators were at same levels. Therefore, at various exposure levels, CQD indicated non-toxicity and the duration beyond those can be used for in vivo imaging studies.[44]

CQD also possess the fluorescence property with biocompatibility and low biotoxicity, this fluorescence property of CQD made them potential candidates for fluorescence bioimaging and multimodal bioimaging both in vivo and in vitro.

For example- PEGylated CQD was labeled on E.coli ATCC25922 and confocal microscopy images were produced at different excitation wavelengths. It was demonstrated that CQD can be used as a fluorescence contrast agent in mice. An aqueous solution of PEGylated CQD was injected subcutaneously into mice and at various excitation wavelengths, the fluorescence images were obtained. A noticeable contrast was observed for imaging in both green and red emissions. Similar results were obtained when similar experiment was conducted on nude mice. More precisely, fluorescence imaging was done with excitation at various wavelengths ranging from 455 nm to 704 nm along with a subcutaneous introduction of an aqueous solution of CQD in to mice. Excitation at 595 nm showed the best fluorescence contrast.

Another property is Multimodal bioimaging which can be defined as the combination of optical imaging and magnetic resonance imaging (MRI) modalities. MRI demonstrates high spatial

resolution and the potential to obtain the anatomical and physiological information. On the other hand, rapid screening was determined by the optical imaging. For example: For multi-modality bioimaging, iron oxide doped CQD (IO-CQDS) were fabricated. Organic precursors with small Fe_3O_4 nanoparticles (approximate size of 6 nm) were thermally decomposed and lead to the formation of IO-CQDS. Intravenous injection of IO-CQDS was injected into rats for in vivo bioimaging and fluorescence signals appeared in the spleen slide samples. The combination of various imaging technology with the fluorescent imaging of CQD is also beneficial due to the biocompatibility of CQD.[12]

Loading of CQD with enzyme responsive mesoporous silica nanocarriers having pH switchable zwitterionic surface can be utilize for targeted imaging and drug delivery to tumour.[45]

Targeted Drug Delivery

CQD are one of the most effective carbon-based materials which can be used for various biological applications due to their biocompatibility. Insignificant cytotoxicity of CQDs makes them potential candidates for safe, effective and targeted delivery. CQD are the attractive candidates for theranostic agents (can be defined as the agent having both therapeutic and diagnostic capabilities). For example- A multifunctional theranostic agent (CD-Oxa) was developed when Surface of carbon dots containing amine groups was conjugated with an anticancer agent (oxidized oxaliplatin, oxa(IV)-COOH). CD-Oxa profitably combines the therapeutic properties of Oxa and optical properties of the carbon dots. They possess better biocompatibility, bioimaging feature, and anticancer effects for in vitro study. The in vivo study reveals that distribution of drug can be followed by monitoring the fluorescence signal of CD-Oxa, which assists to customize the dose of medicament along with the injection time. To deliver the DNA to cells, an assembly was prepared by coupling of CQDs with gold nanoparticles followed by the conjugation with PEI-pDNA. The experimental study revealed that there is a possible delivery of cells with the aid of CQDs.

Delivery of doxorubicinorubicin (anti-cancer) in a multimodality fashion can be possible with the use of CQD functionalized gold nanorods. Under physiological and Hixson-Crowell standard conditions, haloperidol (anti-psychotic) grafted CQD with cysteamine hydrochloride can be used for controlled release for up to 40 h. In addition of bioimaging, Conjugation of ciprofloxacin (broad spectrum antibiotic) with CQD under physiological conditions also gave an efficient new nanocarrier for controlled drug release.[12,44,45]

Nanomedicine

CQD being the small fluorescent nanoparticles serve as a better alternative to other fluorescent nanomaterials. CQDs have the appreciable application in nanomedicine as they do not possess any kind of toxicity in animals. In an experiment, CQDs were injected intravenously in mice and evaluation was conducted after 4 weeks which concluded that there was no significant effect on organs and their internal functions. Insignificant effect and low cytotoxicity level allow them to be used for in vivo studies. In plasma samples, highly biocompatible CQDs was supported by prothrombin time assays concluded that CQD do not affect the thrombin activity. Also, they do not lead to coagulation of blood.

CQDs have an attractive application in photodynamic therapy (therapy that uses special drugs which activate by light for the treatment of superficial tumors). Cancer cells MCF-7 and MDA-MB-231 are effectively inhibited by the CQDs. In addition, CQDs are promising photosensitizers as they are able to produce reactive oxygen species and the selective localization of CQDs into tumors makes them suitable candidates for photosensitization.

Route of administration and surface coating, both factors influence the circulation and uptake of CQDs. There is rapid and effective excretion of CQDs from the body when they are administered through the parental route (intravenous, intramuscular and subcutaneous injection). In the photodynamic therapy, the up-conversion property of CQDs plays an important role in the treatment of deep-seated tumors. Conjugation of CQDs with protoporphyrin IX (a conventional photosensitizer) followed by the indirect excitation (800 nm) of photosensitizer via FRET. Excitation at 800 nm wavelength comes under the phototherapeutic window and is able to penetrate four times deeper in human tissue as compared to excitation at 630 nm light which has been used in clinical photodynamic therapy.

CQDs also play a significant role in radiotherapy, coating of a silver shell (C-Ag-PEG CQDs) on PEG-CQDs make them accessible to be used as radiosensitizers in Du145 cells. Availability of CQDs as nanocarriers make them efficient for tracking and delivery of genes or drugs, bPEI (Branched Polyethylenimine) CQDs possess considerable potential for gene delivery. CQDs also have an appreciable application in control drug release, control on a release of Doxorubicin in HeLa cells can be achieved by loading of CQDs with Doxorubicin. However, it is not clear whether the CQDs can specifically target a disease state or not, thus limiting their efficacy in therapeutic applications.[12,44,45]

Biosensing

CQD have wide application in biosensing, certain properties of CQDs like high water solubility, surface modification flexibility, better cell permeation, low toxicity, high biocompatibility make them potential biosensors. Cellulose copper, glucose, nucleic acid, iron, potassium, phosphate can be monitored visually with the aid of CQD based biosensors. CQD can be utilized as a successful fluorescent sensing agent for the detection of nucleic acid with a selective single-base mismatch. The idea involves the adsorption of single stranded DNA (ssDNA) which was fluorescently labeled via CQD by means of pi-pi association, which was accustomed by extensive fluorescence quenching, further hybridized with its target to consequence for the formation of double stranded DNA. As a result, it was observed that the ssDNA was desorbed from the surface of CQDs which was accompanied with a successive revival of fluorescence, prying with the target DNA.

Detection and imaging of mitochondrial H_2O_2 were verified by means of CQD based FRET (fluorescence resonance energy transfer). CQDs fill in as a contributor of energy transfer and transporter for the sensing framework. Covalently linked CQDs with an H_2O_2 recognition element, boronate secured fluorescin can be utilized for the imaging of H_2O_2 which was endogenously produced in RAW 264.7 macrophages cells.[12]

The principle of FRET (Forster resonance energy transfer) and homogenous assay was employed to develop an immunosensor for 4,4-dibrominated biphenyl (PBB15) detection- an organic pollutant that causes the endocrine system disturbance. The immunosensor consist of antibodies of PBB15, functionalized with gold nanoparticles (AuNP) that behave as the fluorescence acceptor and PBB15 antigens labeled with carbon quantum dot behave as a fluorescence donor. FRET resulted that CQDs fluorescence was efficiently quenched by means of AuNP. On adding PBB15 to the solution, competitive immunoreactions took place and antigens labeled with CQDs were unconfined from the surface of AuNP and resulted in fluorescence recovery. This particular immunosensor utilized as a fine example for the immunoassay development to identify the analytes with preferable antigens and antibodies.[12,44,45]

Vitamin B_{12} coated CQD serve as a ratiometric nanosensor that exhibit an excellent selectivity for phenolic carbofuran. [46] In addition to this, CQD use as a fluorescent probe for recognizing the small bioanalytes (anti-bacterial drugs). An experimental study was conducted on such example, which involves the production of fluorescent N-CQDs from glutamic acid. These N-CQDs were

produced by one-step pyrolysis technique. The resulted N-CQDs were further utilized for the amoxicillin (anti-bacterial) detection. Other small bioanalytes like ascorbic acid, dopamine, and glucose were also detected by CQD.[12,44,45]

Photocatalysis

Lately, the process of photocatalysis has increased colossal force as greener options in natural synthesis. Awareness in the photocatalytic process has been roused to some degree by the acknowledgment that sunlight is adequately an unlimited source of energy. Nonetheless, the elevated vitality of ultraviolet and short wavelength visible light can unfavorably harm natural compounds. The exhibited capacity of outfitting the extended wavelength light and vitality conversion with CQDs solution groups offers a fantastic open door for their utilization as photocatalysts in the natural synthetic process. A current review has shown that CQDs possessing size range 1-4 nm are powerful NIR light-determined photocatalysts for specifically oxidizing the alcohols into benzaldehydes with great conversion proficiency (92%) and specificity (100%), because of their incredible catalytic action for H_2O_2 deterioration and NIR light determined electron transfer activity. Doping of 171 CQDs and fitting the surface groups can adequately modulate the photocatalytic activity of CQDs. In contrast, CQDs possessing size range of 5-10nm combined via electrochemical removal of graphite indicated light-instigated proton features in solution form, which can serve as an acid catalyst for catalyzing a sequence of natural changes in watery medium under visible light. As a standout amongst the most well-known photocatalysts, TiO_2 has been utilized as a part of the expulsion of natural contaminations and for generating H_2 via water splitting. However, a noteworthy downside in its photocatalytic proficiency dwells in its insufficient usage of visible light as the illumination source. Since a bandgap of bulk TiO_2 comes under the UV area (3.0–3.2 eV), just under 5% of sunlight is used by TiO_2. So, bandgap engineering through a conceivable change of TiO_2-based medium is one of the conceivable ways to deal with upgrade the execution of TiO_2 photocatalysts. In perspective of their appealing optical properties and up-change specifically, a nanocomposite of TiO_2 and CQDs is required to understand the productive utilization of the full sunlight spectrum. By utilizing methylene blue (MB) as the model agent, it is demonstrated that nanocomposites of CQDs and TiO_2 can totally deteriorate MB (50 mg/ mL) under visible light illumination in 25 min, where just 0.5% of MB is debased when immaculate TiO_2 is utilized as a photocatalyst. Apart from gathering visible light and changing over it to short wavelength light by up-

transformation, which thusly energizes TiO_2 to frame pairs of electron–hole, it is trusted that the nanocomposites of CQDs encourage the relocation of electrons from TiO_2 and the electrons can carry unreservedly along the directing ways of the CQDs, permitting charge partition, adjustment and obstructing recombination, accordingly creating extensive openings (holes) at the TiO_2 surface. Long-lived openings then record for the greatly improved photocatalytic action of the CQD-TiO_2 nanocomposites. In like manner, comparative conduct was seen with TiO_2-CQD nanotube composites TiO_2-CQD nanosheet nanocomposites and TiO_2-CQD nanotube composites in the photocatalytic deterioration of rhodamine (Rh) and MB respectively.[12,44,46] Different applications of various delivery systems are summarized in Table 2.

Table 2. Different applications of delivery systems.

S No.	Delivery system	Applications	References
1	Carboxylic functionalized CQD	For DNA detection and fabrication of DNA biosensor	47
2	Photoluminscent CQD	For Fe^{3+} detection in biosystems as a biosensor reagent	48
3	CQD	For multiphoton bioimaging	49
4		For optical bioimaging in vivo and in vitro	50
5		For photocatalytic energy conversion as photocatalyst	51
6	CQD loaded with mesoporous silica nanocarriers	For targeted drug delivery and imaging to tumor	45
7	Carbon nanotubes	For treatment of tumors	52
8		For chemotherapeutic drug delivery	52
9		For in vivo cancer therapy as drug delivery agent	52

CONCLUSION

1. In this review, we have elaborated the recent advancement in CQD, emphasizing on their synthetic methods, characterization followed by their biomedical applications.
2. The unique properties of CQD are beneficial for potentiable applications in biomedical science and research.

3. Carbon dots have extensive adequacy for in-vivo and in-vitro bioimaging and drug delivery studies.
4. Because of their biocompatibility, low toxicity, strong photoluminescence, synthetic and photograph steadiness carbon dots have become a fascinating material for bioimaging, detection of different analytes.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr Madhu Chitkara (Vice Chancellor, Chitkara University, Rajpura, Punjab, India) and Dr Sandeep Arora (Dean, Chitkara University, Rajpura, Punjab, India) for support and institutional facilities.

Conflict of interest: No conflict of interest was declared by the authors.

REFERENCES

1. Bradburne CE, Delehanty JB, Gemmill KB, Mei BC, Mattoussi H, Susumu K, Blanco-Canosa JB, Dawson PE, Medintz IL. Cytotoxicity of Quantum Dots used for in vitro cellular labeling: Role of Quantum Dots surface ligand, delivery modality, cell type, and direct comparison to organic fluorophores. Bioconjug Chem. 2013;24(9):1570-1583.
2. Bourlinos AB, Stassinopoulos A, Anglos D, Zboril R, Karakassides M, Giannelis EP. Surface functionalized carbogenic Quantum Dots. Small 2008,4(4):455-458.
3. Klostranec JM, Chan WCW. Quantum Dots in Biological and Biomedical Research: Recent Progress and Present Challenges. Adv Mater. 2006;18(15):1953-1964.
4. Modani S, Kharwade M, Nijhawan M. Quantum Dots: A novelty of medical field with multiple applications. Int J Curr Pharm Res. 2013;5(4):55-59.
5. Zhu JJ, Li JJ, Huang HP, Cheng FF. Quantum dots for DNA biosensing. Springer Briefs in Molecular Science 2013;1-91.
6. Chen N, He Y, Su Y, Li X, Huang Q, Wang H, Zhang X, Tai R, Fan C. The cytotoxicity of cadmium-based quantum dots. Biomater 2012;33(5):1238-1244.
7. Qi L, Gao X. Emerging application of Quantum Dots for drug delivery and therapy. Drug Deliv. 2008;5(3):263-267.
8. Tan L, Huang G, Liu T, Fu C, Zhou Y, Zhu Z, Meng X. Synthesis of highly bright oil-soluble Carbon Quantum Dots by hot-injection method with N and B co-doping. J Nanosci Nanotechnol. 2016;16(3):2652–2657.
9. Tope S, Saudagar S, Kale N, Khambayat S, Bhise K. Therapeutic application of Quantum Dot (QD). The Pharma Innovation 2014;2(12):86-105.
10. Roy P, Chen PC, Periasamy AP, Chen YN, Chang HT. Photoluminescent carbon nanodots: Synthesis, physicochemical properties and analytical applications. Mater Today 2015;18:447-458.
11. Zuo P, Lu X, Sun Z, Guo Y, He H. A review on syntheses, properties, characterization and bioanalytical applications of fluorescent carbon dots. Microchim Acta 2016;183(2):519-542.
12. Wang YF, Hu A. Carbon Quantum Dots: synthesis, properties and applications. J Mater Chem C. 2014;2:6921-6939.
13. Wang L, Bi Y, Hou, J, Li H, Xu Y, Wang B, Ding H, Ding L. Facile, green and clean one-

step synthesis of carbon dots from wool: Application as a sensor for glyphosate detection based on the inner filter effect. Talanta 2016;160:268-275.

14. Sun YP, Zhou B, Lin Y, Wang W, Fernando KA, Pathak P, Meziani MJ, Harruff BA, Wang X, Wang H, Luo PG, Yang H, Kose ME, Chen B, Veca LM, Xie SY. Quantum-Sized Carbon Dots for Bright and Colorful Photoluminescence. J Am Chem Soc. 2006;128(24):7756–7757.
15. Thongpool V, Asanithi P, Limsuwan P. Synthesis of Carbon Particles using Laser Ablation in Ethanol. Procedia Engg. 2012;32:1054-1060.
16. Lu J, Yang J, Wang J, Lim A, Wang S, Loh KP. One-pot synthesis of fluorescent carbon nanoribbons, nanoparticles, and graphene by the exfoliation of graphite in ionic liquids. ACS Nano. 2009;3:2367–2375.
17. Yao S, Hu YF, Li GK. A one-step sonoelectrochemical preparation method of pure blue fluorescent carbon nanoparticles under a high intensity electric field. Carbon 2014;66:77–83.
18. Zhang Z, Duan Y, Yu Y, Yan Z, Chen J. Carbon Quantum Dots: synthesis, characterization, and assessment of cytocompatibility. J Mater Sci Mater Med. 2015;26(213):1-7.
19. Zhang X, He X, Li Y, Zhang Z, Ma Y, Li F, Liu J. A cytotoxicity study of fluorescent carbon nanodots using human bronchial epithelial cells. J Nanosci Nanotechnol. 2013;13(8):5254-5259.
20. Guan W, Gu W, Ye L, Guo C, Su S, Xu P and Xue M. Microwave-assisted polyol synthesis of carbon nitride dots from folic acid for cell imaging. Int J Nanomed. 2014;9:5071–5078.
21. Wang W, Li YM, Cheng L, Cao Z, Liu W . Water-soluble and phosphorus-containing carbon dots with strong green fluorescence for cell labeling. J Mater Chem B. 2014;2:46–48.
22. Kang YF, Li YH, Fang YW, Xu Y, Wei XM, Yin XB. Carbon Quantum Dots for zebrafish fluorescence imaging. Scientific Reports 2015;5:11835.
23. Zong J, Zhu YH, Yang XL, Shen J, Li C. Synthesis of photoluminescent carbogenic dots using mesoporous silica spheres as nanoreactors. Chem Comm. 2011;47:764-766.

24. Yang YX, Wu DQ, Han S, Hu P, Liu R. Bottom-up fabrication of photoluminescent carbon dots with uniform morphology via a soft-hard template approach. Chem Comm. 2013;49:4920–4922.
25. Lai CW, Hsiao YH, Peng YK, Chou PT. Facile synthesis of highly emissive carbon dots from pyrolysis of glycerol; gram scale production of carbon dots/mSiO2for cell imaging and drug release. J Mater Chem. 2012;22:14403–14409.
26. Zhang M, Bai LL, Shang WH, Xie W, Ma H, Fu Y, Fang D, Sun H, Fan L, Han M, Liu C, Yang S . Facile synthesis of water-soluble, highly fluorescent graphene Quantum Dots as a robust biological label for stem cells. J Mater Chem. 2012;22:7461–7467.
27. Shinde DB, Pillai VK. Electrochemical preparation of luminescent graphene Quantum Dots from multiwalled carbon nanotubes. Chem-A Eur J. 2012;18:12522–12528.
28. Thambiraj S and Shankaran DR. Green synthesis of highly fluorescent Carbon Quantum Dots from sugarcane bagasse pulp. Applied Surface Sci. 2016;30:435-443.
29. Zhao C, Li W, Liang Y, Tian Y, Zhang Q. Synthesis of BiOBr/CQD microspheres with enhanced photoactivity and photostability under visible light irradiation. Applied Catalysis A: General. 2016;527:127–136.
30. Liu RL, Wu DQ, Feng XL, Mullen K. Bottom-up fabrication of photoluminescent graphene Quantum Dots with uniform morphology. J Am Chem Soc. 2011;133(39):15221–15223.
31. Mao LH, Tang WQ, Deng ZY, Liu SS, Wang CF, Chen S. Facile access to white fluorescent carbon dots toward light-emitting devices. Ind Eng Chem Res. 2014;53:6417–6425.
32. Peng J, Gao W, Gupta BK, Liu Z, Romero-Aburto R, Ge L, Song L, Alemany LB, Zhan X, Gao G, Vithayathil SA, Kaipparettu BA, Marti AA, Hayashi T, Zhu JJ, Ajayan PM. Graphene Quantum Dots derived from carbon fibers. Nano Lett. 2012;12:844–849.
33. Tian L, Ghosh D, Chen W, Pradhan S, Chang X, Chen S. Nanosized carbon particles from natural gas soot. Chem Mater. 2009;21(13):2803–2809.
34. Li HT, He XD, Liu Y, Yu H, Kang Z, Lee ST. Synthesis of fluorescent carbon nanoparticles directly from active carbon via a one- step ultrasonic treatment. Mater Res Bull. 2011;46(1):147–151.

35. Wang F, Xie Z, Zhang H, Liu C, Zhang Y. Highly luminescent organosilane-functionalized carbon dots. Adv Funct Mater. 2011;21(6):1027-1031.
36. Dong YQ, Shao JW, Chen C, Li H, Wang R, Chi Y, Lin X, Chen G. Blue luminescent graphene QD and graphene oxide prepared by tuning the carbonization degree of citric acid. Carbon 2012; 50:4738–4743.
37. Tang LB, Ji RB, Cao XK, Lin J, Jiang H, Li X, Teng KS, Luk CM, Zeng S, Hao J, Lau SP. Deep ultraviolet photoluminescence of water-soluble self-passivated graphene Quantum Dots. ACS Nano 2012;6:5102–5110.
38. Yang ST, Wang X, Wang H, Lu F, Luo PG, Cao L, Meziani MJ, Liu JH, Liu Y, Chen M, Huang Y, Sun YP. Carbon dots as nontoxic and high performance fluorescence imaging agents. J Phys Chem C. 2009;113(42):18110–18114.
39. Tao H, Yang K, Ma Z, Wan J, Zhang Y, Kang Z, Liu Z. In vivo NIR fluorescence imaging, biodistribution, and toxicology of photoluminescent carbon dots produced from carbon nanotubes and graphite. Small 2012;8:281-290.
40. Li N, Liang X, Wang L, Li ZH, Li P, Zhu Y, Song J. Biodistribution study of carbogenic dots in cells and in vivo for optical imaging. J Nanopart Res. 2012;14:1177.
41. Xu Y, Jia XH, Yin XB, He XW, Zhang YK. Carbon quantum dot stabilized gadolinium nanoprobe prepared via a one-pot hydrothermal approach for magnetic resonance and fluorescence dual modality bioimaging. Anal Chem. 2014;86:12122–12129.
42. Chen C, Wang H. Biomedical Applications and Toxicology of Carbon Nanomaterials. ISBN: 978-3-527-33871-9, 2016, pp. 60-62.
43. Shen LM, Liu J. New Development in Carbon Quantum Dots technical applications. Talanta 2016;156-157:245-256.
44. Lim SY, Shen W, Gao Z. Carbon Quantum Dots and their applications. Chem Soc Rev. 2015;44(1):362-381.
45. Liu Z, Chen X, Zhang X, Gooding JJ, Zhou Y. Carbon-Quantum-Dots loaded mesoporous silica nanocarriers with pH-switchable zwitterionic surface and enzyme responsive pore-cap for targeted imaging and drug delivery to tumor. Adv Healthcare Mater. 2016;5(12):1401-1407.

46. Campos BB, Caceres RC, Bandosz TJ, Jimenez JJ, Castellon ER, Esteves da Silva JCG, Algarra M. Carbon dots coated with vitamin B12 as selective ratiometric nanosensor for phenolic carbofuran. Sensors Actuators B Chem. 2017;239:553-561.
47. Loo AH, Sofer Z, Bousa D, Ulbrich P, Bonanni A and Pumera M. Carboxylic CQD as a Fluorescent Sensing Platform for DNA Detection**.** ACS Appl. Mater. Interfaces 2016; 8(3): 1951-1957.
48. Zhu S, Meng Q, Wang L, Zhang J, Song Y, Jin H, Zhang K, Sun H, Wang H, Yang B. Highly photoluminescent carbon dots for multicolor patterning, sensors, and bioimaging. Angewandte Chemie. 2013;52(14):3953-3957.
49. Cao L, Wang X, Meziani MJ, Lu F, Wang H, Luo PG, Lin Y, Harruff BA, Veca LM, Murray D, Xie SY and Sun YP. Carbon dots for multiphoton bioimaging. J Am Chem Soc. 2007;129(37):11318-11319.
50. Luo PG, Sahu S, Yang ST, Sonkar SK, Wang J, Wang H, LeCroy GE, Cao L, Sun YP. Carbon "quantum" dots for optical bioimaging. J Mater Chem B. 2013;1(16):2116-2127.
51. Fernando KAS, Sahu S, Liu Y, Lewis WK, Guliants EA, Jafariyan A, Wang P, Bunker CE, Sun YP . CQD and applications in photocatalytic energy conversion. ACS Appl Mater Interface 2015;7(16):8363–8376.
52. Elhissi AMA, Ahmed W, Hassan IU, Dhanak VR, Emanuele AD**.** Carbon nanotubes in cancer therapy and drug delivery. J Drug Del. 2012; 2012:1-10.