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CARBON DOTS: A REVIEW ON RECENT ADVANCEMENT IN PROPERTIES, SYNTHESIS AND BIOMEDICAL APPLICATIONS

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ABSTRACT

Luminescent carbon quantum dots (CQDs) represent a novel form of carbon nanomaterials with a size below 10 nm. It was first discovered in the electrophoretic analysis of fluorescent single-walled carbon nanotube fragments. Carbon Dots (CDs) mainly includes carbon nanodots, carbon quantum dots, graphene quantum dots, and carbonized polymer dots. Carbon dots have tunable emission with a different excitation wavelength and abundant surface functional groups which will act as a platform for the attachment of several drugs and ligands. CQDs are also suitable for surface passivation by various organic and inorganic molecules and by surface passivation physical and fluorescence properties are enhanced. As well as carboxyl moieties on the surface of CDs imparts solubility and biocompatibility. Due to This unique combination of characteristics, along with the ease with which they can be synthesized, carbon dots are drawing significant attention as a novel nanoparticle and a promising approach in Bioimaging, biosensing, Targeted drug delivery, gene delivery, and other applications. Recently a triple conjugated system was also developed with carbon dots (C-dots), which have gained tremendous success in Cancer Therapy. In this review, we present some of the most recent applications of carbon dots in biomedicals and concentrating on their fluorescence properties, types, synthesis, and surface modifications.

KEYWORDS: CQDs, CDs, C-dots.

INTRODUCTION

Carbon Dots (CDs) are novel carbon nanostructures, with unique optical and fluorescent properties.[1] They can be described as small, surface-functionalized carbonaceous nanoparticulate systems.[2] In 1984, Russian physicist Ekimov first discovered the QD in glass crystals.[3] After 1984 C-dots were accidentally discovered in 2004 during the separation of single-walled Carbon nanotubes (CNTs). Based on these findings, in 2006, Sun et al. synthesized fluorocarbon NPs with a diameter of less than 10 nm, naming them C-dots.[2] Different compounds belonging to group II to VI e.g. Ag, Cd, Zn, Hg, etc lead to the formation of QD. These have become an obligatory tool for traceable targeted delivery, biomedical research, and different therapy applications.[4,5] They generally possess an sp2 conjugated core and contain suitable oxygen content in the forms of multiple oxygen-containing species represented by carboxyl, hydroxyl, and aldehyde groups.[1] Depending on their structure, CDs are classified into three categories, graphene quantum dots (GQDs), carbon nanodots (CNDs), and polymer dots (PDs). All types of CDs exhibit fluorescence properties despite their dissimilar structure, size, and surface functional groups.[6,7] Their unique characteristics makes them versatile for several applications.[8] Until now, the applications of C dots have been widely demonstrated in sensing, bioimaging, nanomedicine, catalysis.[9] Photothermal as well as photodynamic therapy and drug/gene delivery carriers. CDs could also be applied for the determination of cellular levels of biomolecules and ions, such as Cu^{2+}, Hg^{2+}, NO^{-}, C H O, etc.[10,11] Great success of carbon dot is dominantly benefited from the easy accessibility of its preparation and synthesis methods with different physical or chemical approaches. These methods are generally divided into two subcategories: top-down and bottom-up approaches.[12,13] Due to easy functionalization and biocompatibility C-dots have gained increasing research attention in the past decade.[14] Functional groups incorporation on the surface of C-dots has resulted in a useful tool for the treatment of various diseases and also applied for the delivery of anticancer drugs. Hence Targeted drug delivery has become one of the prominent properties of c-dots.[15]

Carbon dots

Carbon dots are small nanoparticles (generally < 10 nm) that are water soluble, highly photoluminescent, inexpensive to make, have good biocompatibility, and are believed to be nontoxic.[16] CDs are formed either by a top-down method, which relies on the fragmentation of

carbon allotropes such as nanotubes, graphene and fullerenes, or by a bottom-up method where carbon hydrates are introduced as a carbon source to react with solvents in special synthesis conditions.[17] Carbon dots (CDs), one kind of fluorescent carbon nanomaterials with size less than 10 nm and emissive wavelength from blue to near-infrared (NIR) region, have attracted increasing attention worldwide in biomedical fields.[18,20] The main two characteristics which are truly recurrent in every type of CD are the small size and the surface functionalization layer, which is typically very dense and disordered.[21] High-resolution transmission electron microscopy (HR-TEM) imaging and atomic force microscopy (AFM) imaging reveal the spherical nanocrystal design of C-dots.[22] Nasal, oral, parental, and pulmonary are the various routes for administration of C-dots.C-dots fabricated from natural precursors, such as plants and trees, can act as drugs for different diseases. For example, C-dots fabricated from ginger and tea inhibit HeLa, HepG2, MCF-7, and MDAMB-231 cells.[23]

Types of carbon dots

CDs are classified into three categories based on their structure and these categories are graphene quantum dots (GQDs), carbon nanodots (CNDs), and polymer dots (PDs) as illustrated in Fig. 1.[24,25] According to the research, graphene QDs have been proved to be derivations of the graphene/graphite and other graphitic three-dimensional materials through top down synthetic approaches. Normally G-QDs have layered structures and lateral size up to 100 nm.[26] On the other hand, CNDs possess a spherical shape and can have a crystalline graphite-like lattice in carbon quantum dots (CQDs), or an amorphous structure in carbon nanoparticles (CNPs).[17] PDs are aggregated or cross-linked polymers prepared from linear polymers or monomers. In addition, the carbon core and the connected polymer chains can self-assemble to form PDs.[27,28]

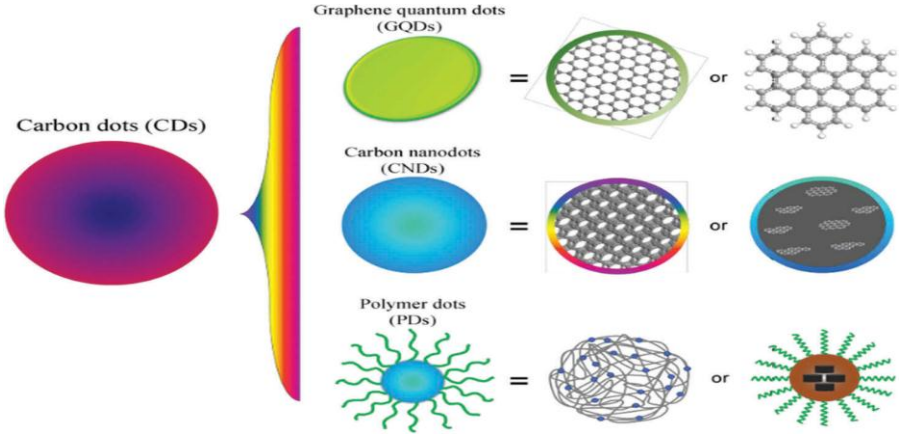

Fig. 1. Representation of three types of carbon dots.

PROPERTIES

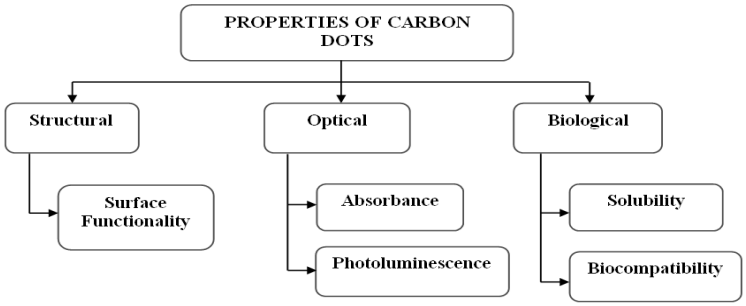

Fig. 2. Flowchart of properties of Carbon dots.

Structural property

CDs are quasi-spherical carbon nanoparticles with usually less than 10 nm diameter.[29] they are fundamentally composed of C, H and O elements, due to the presence of carboxylic acid moieties, render them superior water solubility and functionalization with different species.[30] The surface properties of CDs are characterized using transmission electron microscopy (TEM) and atomic force microscopy (AFM).[31] CQDs are generally referred to as carbogenic compounds having amorphous and crystalline structures with surface functional groups. Many researchers conveyed the existence of crystalline sp2 carbon in CQDs which showed poor crystallinity.[32] They can be doped with N, S, P, and B heteroatoms resulting in a chemically modifiable structure which enhances and render additional functional properties.[33] Also, covalent binding enables them to be consequently functionalized

with organic, polymeric, inorganic, or biologic species.[34]

Optical Properties

- **Absorbance**

CDs usually exhibit strong absorption in the UV region with a tail extending to the visible range.[35] Absorption in the UV- visible region is usually estimated as the π-π∗ transition of sp2 conjugated carbon and n-π∗ transition of hybridization with a heteroatom such as N, S, P, etc. The absorption property can be improved through surface modification.[36] Most of the c dots have an absorption band of around 260–320 nm. However, the absorbance of C-dots was found to increase to longer wavelength after surface passivation with organosilane up to 350–550 nm.[35,37] Besides, the compact structure within the CDs, the interactions among groups make the energy gap change, resulting in variation of the absorption.[38]

- **Photoluminescence**

In general, one uniform feature of the Photoluminescence of CDs depends on the emission wavelength and intensity. The reason for this unique phenomenon may be the optical selection of nanoparticles with different sizes or CDs with different emissive traps on the surface.[39] Studies of the optical properties of small-sized C-dots are controversial due to the exact mechanisms of PL, which remain unclear.[40] The molecular state emission, crosslinking, surface passivation enhances the emission effect.[41] Four universally accepted photoluminescence mechanisms are: carbon core state luminescence from conjugated -domain with quantum confinement effect, surface state luminescence from the interaction of surface groups and carbon core, molecular state luminescence from the specific molecular structure and crosslink enhanced emission effect.[42]

Biological Properties

Magnificent success has been made in engineering CDs bio-probes with good stability. However, the biocompatibility of the functionalized CDs is still a critical issue for the applications in live cells and tissues.[43] Systematic cytotoxicity evaluations were carried out on both raw CDs and Surface functionalized CDs in the last few years.[44] The cytotoxicity of the CDs passivated with functional groups, such as PEG, PPEI-EI, PEI, etc. The PEGylated CDs were non-cytotoxic up to concentrations much higher than that is necessary for cell imaging and related applications.[45] Recently carbon dots have been synthesized from natural sources such as orange juice, jaggery, sugar, etc as they contain carbohydrate, hence C-dots derived from bio-sourced become Exceptionally biocompatible.[47]

Surface modification

Good water solubility, prominent photostability, and biocompatibility render C-dots an effective alternative to Q-dots.[48] Surface engineering involves surface passivation by active functional moieties via different bonds such as covalent, hydrogen, and ionic bonds. Surface-engineered C-dots results in accurate drug delivery, diagnostic imaging, and biosensing.[48] Surface functionalization can be employed via covalent and non-covalent mechanisms such as amide coupling, esterification, sulfonation, polymerization, complexation, and electrostatic interaction as depicted in Fig. 3.[7,49] The majority of CDs are rich in oxygen-containing groups which makes them feasible in covalent bonding. Surface passivation via covalent bonding of amine-containing agents is a common method to improve the Photoluminescence of CDs, which showed an important influence on the properties of CDs.[50] Surface functionalization by chemical approach is one of the attractive since it directly leads to oxygen-containing functionalized carbon dots with precisely engineered surface properties and it suitably selects the carbon source size, shape, and physical properties.[51] Amine-coated C-dots were fabricated by Fan and co-workers by surface engineering, –COOH was converted to a –NH2 group followed by carbonization of glucose to develop amino-coated C-dots with improved surface functionalization.[52] Instead of random functionalization of different chemical groups on the CDs, the advanced effort has been made on grafting specific receptors on the CDs. This has been efficient for CDs to be used as nanoprobes in biosensing applications. Specific molecules of different sizes, structures, and functionality are attached to the CD's surface to create binding affinity. CDs decorated with tyrosinase can be a nanoprobe that is efficient and sensitive in detecting levodopa.[53] boric acid can also be functionalized onto the CDs, which shows great sensitivity in terms of quenching toward the presence of glucose.[54] Sun and co-workers firstly decorated CDs of no detectable PL with PEG, then bright luminescence emission was observed by surface passivation. Since then, various biomacromolecules were linked to CDs to receive satisfactory results or improve the specificity of analytical detection or bioimaging[2,55] The antibody is another candidate that is being used to tag on CDs for the development of nanoprobes. Studies have demonstrated that the CDs tagged with goat IgG antibodies can detect the presence of human IgG.[56]

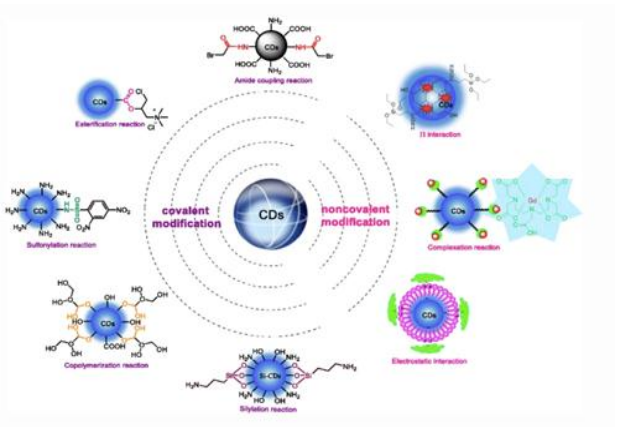

Fig. 3 Representation of various surface modifications.

Synthesis

CDs can be synthesized mainly via two routes as illustrated in Fig. 4. (i) top down approach and (ii) bottom-up approach. Top-down approach refers to breaking down larger carbon structures via chemical oxidation, discharge, electrochemical oxidation, and ultrasonic methods.[57] In the bottom-up approach, CQDs are fabricated from molecular precursors such as citric acid, sucrose and glucose through microwave exposure, thermal decomposition, hydrothermal treatment, template- based routes and plasma treatment.[85]

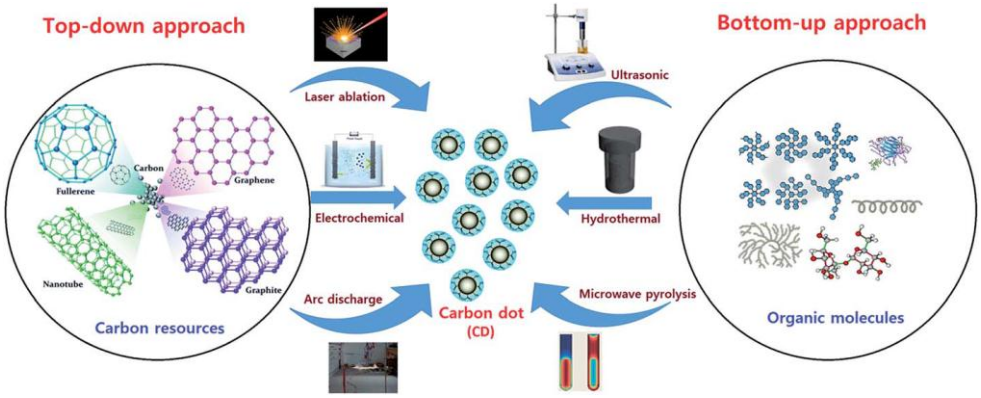

Fig. 4. Representation of the possible synthesis methods to prepare carbon dots.

Top down approach

- **Arc Discharge**

In 2014 Arora and Sharma.[59] indicated that arc discharge[60] is a method to reorganize the carbon atoms decomposed from the bulk carbon precursors in the anodic electrode driven by the gas plasma generated in a sealed reactor. In order to produce a high-energy plasma the temperature in the reactor can reach as high as 4,000K under electric current in the cathode the carbon vapor assembly to form CQDs. The preparation of CQDs by arc discharge method was originated in 2004.[61] During the arc discharge process, electrons are emitted either through heating or with field emission due to the large electric field generated. The plasma formed has a mixture of carbon vapour, inert gas and vapours of the catalyst by triggering the arc between two electrodes, which subsequently evaporate the metal of interest into metal vapour. Later, the metal vapour nucleates and forms nanoparticles in the surrounding medium and the metal vapour can either be quenched by mixing with cold gas or by the surrounding atmosphere. The vaporization is the effect of the energy transfer from the arc to the anode made of graphite doped with the catalyst.[62,63]

- **Laser Ablation**

Laser ablation was the first method used to produce CDs in a controlled way.[64] It is a top-down method which is frequently used for the production of many different types of nanoparticles, through the interaction of a pulsed laser beam with the surface of a target (solid precursor) and the consequent ejection of materials in the form of nanoparticles.[65] For the synthesis of CDs laser ablation is another standard method used by the researchers. CDs were prepared using toluene as the carbon source via laser irradiation technique by Yu et al .They controlled the size of CDs using laser furnace.[66] Carbon dots were fabricated by laser ablation of a carbon target (hot-pressing of graphite powder and cement mixture) in the presence of argon gas and containing water vapour at 900 ºC and 75 kPa , using Nd : YAG laser by Sun et al.19.C-dots obtained with some modification exhibited bright luminescence emission property.[64] Sun et al.19

- **Electrochemical Approach**

A redox reaction is exploited by electrochemical approach which is a type of top-down approach occurring in an electrochemical cell under the influence of an electric current applied between two electrodes (solids) separated by the electrolyte (liquid).[67] Graphene quantum dots (GQDs) and graphene oxide quantum dots (GOQDs) are synthesized by electrochemical exfoliation with the average size of 2-3 nm, showing blue to green fluorescence under 365 nm UV radiation. GQDs and GOQDs are with tunable functional groups which can be easily prepared by varying the concentration of alkali hydroxide in the electrolyte.[68] 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.[69] Peng et al. fabricated TTDDA passivated CDs with an average size of 5 nm from carbohydrates by dehydrating with conc. H2SO4.[70]

Bottom up approach

- **Hydrothermal approach**

Hydrothermal synthesis method is being used by most of the researchers as a cheap, eco-friendly and low cost route to synthesize CDs from saccharides, amines, organic acids and their derivatives.[71,72] In a typical approach, small organic molecules and/or polymers are dissolved in water or organic solvent to form the reaction precursor, which was then transferred to a Teflon-lined stainless steel autoclave. At relatively high temperature the organic molecules and/or polymers are merged together to form carbon seeding cores and then grow into

CQDs with a particle size of less 10 nm[73] Highly photoluminescent CQDs with a QY of 26% in one step by hydrothermal treatment of orange juice followed by centrifugation was prepared by Mohapatra et al. These CQDs with sizes of 1.5–4.5 nm were applied in bioimaging due to their high photostability and low toxicity.[74]

- **Ultrasonic Approach**

There are limited numbers of studies, where researchers used ultrasonic treatment method to synthesize CDs. For example, Li et al. synthesized water soluble fluorescent CDs through the acid assisted ultrasonic treatment of glucose having a size in the range of 5–10 nm. In the same year, Li et al. synthesized water soluble fluorescent CDs using one-step H2O2 assisted ultrasonic treatment method from activated carbon. According to the TEM results, the average size of CDs was ranging from 5 to 10 nm, and the surface of CDs was rich in hydroxyl groups.[75,76]

- **Microwave pyrolysis**

To synthesize CQDs microwave irradiation of organic compounds is a rapid and low-cost method.[77-80] Green luminescent CQDs were obtained within one minute under microwave irradiation by using sucrose as the carbon source and diethylene glycol (DEG) as the reaction media.[79] Energy efficient internal heating resulted in microwave irradiation by direct coupling of microwave energy with dipoles and /or ions that are present in the reaction mixture. Microwaves heat the reaction mixture on a molecular basis by direct interaction with the molecules of solvents, reagents and catalysts.[81] A facile microwave pyrolysis approach to synthesize CQDs was reported by Zhu et al 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 CQDs exhibited an excitation-dependent PL properties.[77]

Characterization

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, X-Ray diffraction (XRD), transmission electron microscope (TEM), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), U.V spectroscopy.[82]

Microscopic evaluation

Electron microscopy is an essential tool for characterizing any nanomaterial because it allows direct observation of size, shape, structure. TEM and SEM are useful tools to check the exfoliation of bundles and the purity of the material.[83]

- **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. Transmission electron microscopy is broadly utilized as a part of the characterization of carbon dots (C-dots).[84] Zhang et al. synthesized C-dots by acid oxidation of graphite, their lattice spacing was mostly less than 0.3 nm, indicating most of the dots were actually separate grapheme.[85]

- **Scanning electron microscopy**

By scanning the surface of a C-dots sample with a focused electron beam SEM produces images which interacts with the atoms of C-dots during which the charges are accumulated to form an image. Fig. 5. shows an example of SEM and TEM image of C-dots.[86]

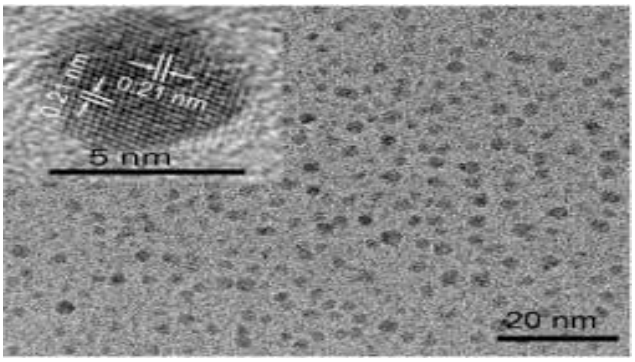

Fig. 5 Representation of SEM and TEM.

- **X-ray diffraction**

XRD is one of the characterization techniques of the C-dots, mainly used to provide the information on crystal structure and particle size.[87] X-ray diffraction also used to determine the crystalline phases of CQD.[88] Local measurements of the sizes and diameters of CDs can be obtained by the microscopy techniques such as SEM and TEM, but if the information is required on the larger scale to obtain a statistical picture of the sample rather than local information, diffraction techniques are usually used to provide this important information.[89] Fig. 6 depicts a representative XRD pattern, showing a diffraction peak at around 20° reflecting the crystalline structure. A lattice spacing of 0.45 nm which is greater than bulk graphite (*0.35 nm) indicating the certain amorphous character of the Carbon-Dots.[90] Bourlinos et al. calcined octadecyl ammonium citrate salt at 300 °C to prepare C-dots, the XRD pattern displayed the existence of highly disordered carbon and surface-modified carbon alkyl groups.[91]

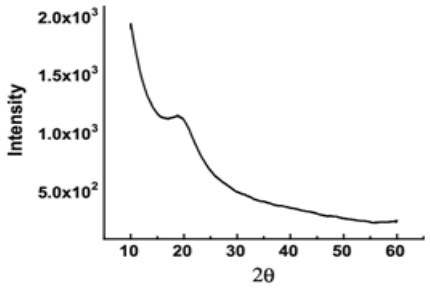

Fig. 6. Graphical representation of X-ray diffraction.

Spectroscopic evaluation

- **Ultra violet absorption**

The carbon dots prepared using various techniques, usually shows Strong (UV) absorption but still the positions of absorption peaks of UV are entirely different for different techniques used for the preparation.[18] UV e Visible measurements were also carried out to study the absorption characteristics of the CDOT and that of a mixture of the CDOT and N719 - a popular dye commonly used as a sensitizing dye in the fabrication of dye sensitized solar cells.[77,80,92]

- **Fourier transform infrared**

Fourier transform infrared (FTIR) spectroscopy usually complements X-ray photoelectron spectroscopy (XPS), illuminating distinct functional units through recording of typical vibration bands.[93] Functionalization by binding functional groups onto the CDs through chemical or physical interactions is a common practice to enhance the solubility and increase the selectivity of CDs and the FT-IR has been aimed at characterizing such surface modifications.[94,95] For the determination of the functional groups that are present on the surface of carbon dots, FTIR has been used. Functionalization of carbon dots by partial oxidation, carboxyl or carboxylic acid groups, hydroxyl groups are abundant on the surface of C-dots and so for the investigation of these groups containing oxygen FTIR is utilized.[82]

Applications

Many carboxylic functional groups on the C-dots surface and large surface area of C-dots allow for synthesis of multimodal probes and therapeutic conjugates, so they facilitated the explorations in the field of nanomedicine, diagnosis and cancer therapy and have various applications as shown in Fig. 7.[96] They not only showed low toxicity, but also can be rapidly excreted from the body,[97] which meet the critical criteria for clinical use.[98]

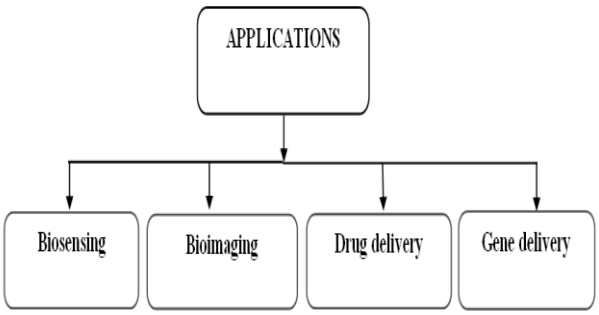

Fig. 7: Flow chart of applications of carbon dots.

- **Biosensing**

An interesting application of CDs is in the field of sensing. A wide variety of biological/chemical sensors were developed based on the fluorescence properties and surface functional groups of C dots, such as the detection of Hg2+ and biological thiols.[99] The changes in their fluorescence property take place via different mechanisms, such as resonance energy transfer, inner filter effect, photo-induced electron and charge transfer.[100] Liu et al. (2016) proposed a switch on fluorescence method for sensing ascorbic acid (AA) in food samples. CQDs-MnO2 probe was used to sense of AA in fresh fruits, vegetables and fruit juices. The limit of detection for AA is 42 nm, with a wide concentration of a linear range of 0.18-90 Mμ.[101]

- **Bioimaging**

CDs are considered as a potential candidate for bioimaging application due to its unique fluorescent nature, high photobleaching resistivity, less cytotoxicity, and better aqueous solubility.[102,103] In-vitro imaging: Bhunia et al synthesized a series of C dots with different fluorescence, and realized target recognition by surface modification of C dots with folic acid.[104] In vivo: Yang et al. obtained C-dots and ZnS-doped C-dots from chemical processing of raw nanomaterial by laser ablation.[105] After PEG1500N modifications, they were used for in vivo optical imaging of mice. C-dots or ZnS-doped C-dots solutions were injected to the back of mice (subcutaneous injection), front extremity (intradermal injection) and whole body (intravenous injection), both C-dots and ZnS-doped C dots emitted strong fluorescence in vivo.[42]

- **Drug delivery**

Yang et al. 1, who grafted the common drug carrier β-cyclodextrin to CDs, to induce and also enhance controlled targeting. An advantage of this method is that the drug is not bound directly on the CD's surface but to β-cyclodextrin, thus inducing low cytotoxicity in normal cells and preventing the uncontrolled leakage.[106] Recently Kong et al. fabricated a citric acid and ethylenediamine based CDs for drug delivery systems. According to their results they observed that DOX could be quickly loaded on CDs through electrostatic interaction and the CDs/DOX complexes showed better cellular uptake and antitumor efficiency on the breast cancer MCF-7 cells compared with free DOX.[107]

- **Gene therapy**

Wu et al. used folate-conjugated reduction-sensitive polyethyleneimine passivated CDs for targeted EGFR and cyclinB1 siRNA delivery and targeting in H460 lung cancer cells. The combined siRNAs were released in reducing intracellular conditions and increased the anti-cancer activity in H460 cells.[108] Liu et al evaluated the transport capacity of C dots, and found that C dots not only had similar DNA transfer ability to positively charged PEI-25K, but also had fluorescence property to exhibit the distribution of plasmid DNA during the transfer process and provide detailed information for the research of plasmid DNA physiological function.[109]

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