Chapter 1:

Introduction

Carbon is an interesting element because of its unusual electronic configuration which gives it a tendency to form chains and different bonds. It is very versatile in utilising not only covalent interactions but also physical weaker interactions like van der Waals forces, hydrophobic bonds, π-π and cation-π bonds giving rise to a range of allotropes, organic and nano- materials.

1.1 Nanocarbon Family Allotropes

Nano-carbon family has fascinated researchers in the past years due to exceptional intrinsic characteristics conferred by the type of carbon arrangement. Unique structures result from quantum-confined carbon core like fullerenes, graphene, single and multi-walled carbon nanotubes and carbon quantum dots. Each of these structures results in having distinctive properties which have been harnessed in advancing technologies by researchers for multi-field industrial applications [1,2].

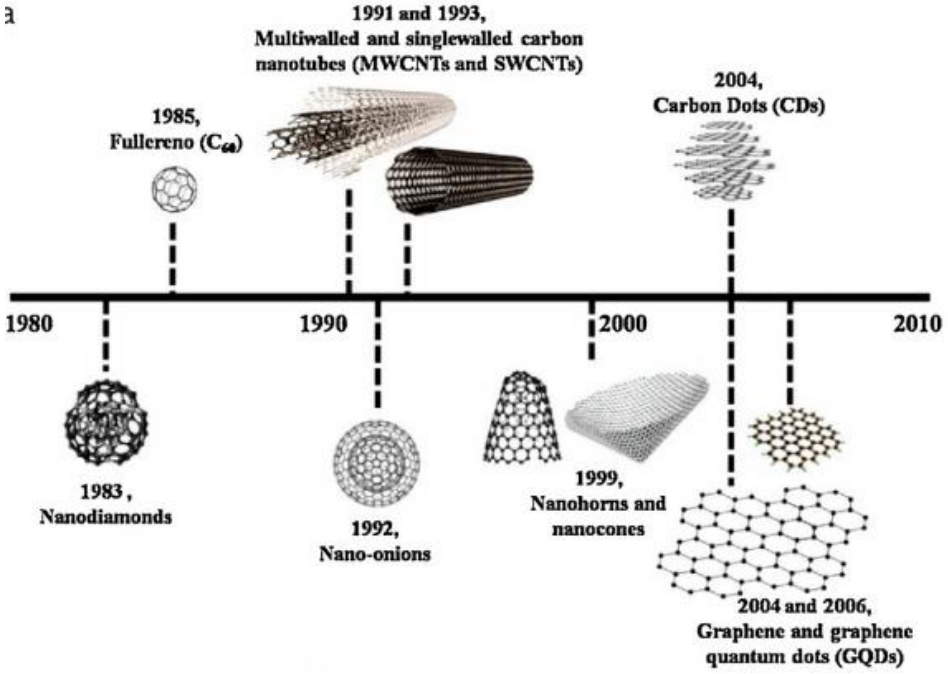

Figure 1.1: The chronological order of the discovery of new carbon nanomaterials (*Adapted from S.N. Baker, G.A. Baker. Angew. Chem 49 (2010) 6726–6744*)

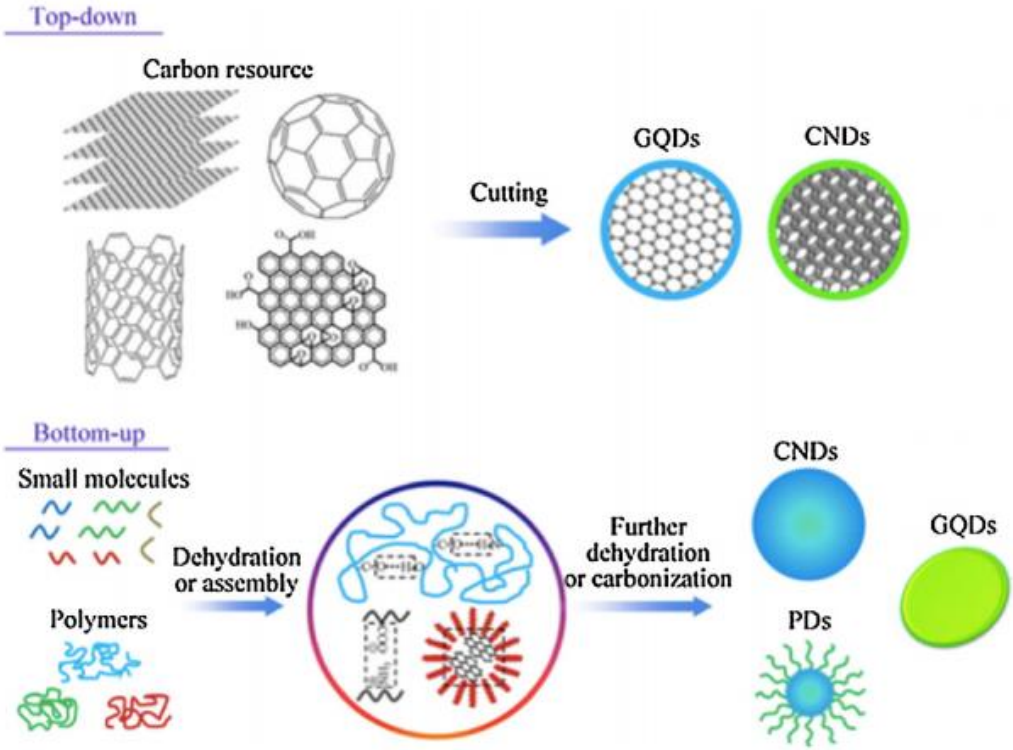

Figure 1.2: Schematic Illustrating the Chemical Structure after Synthesis Process of CQDs (*Adapted from Y. Xu, M. Wu, Y. Liu, X.Z. Feng, X.B. Yin, X.W. He, Y.K. Zhang, Chem.- Eur. J. 19 (2013) 2276–2283*)

Fullerenes or “buckyballs” are closed-meshlike carbon allotropes that were accidently discovered by Kroto, Curl and Smalley in 1985 for which they later received the Nobel Prize in Chemistry in 1996 [3]. Before their accidental discovery, only graphite, diamond and amorphous carbon were known. Intense research followed to develop new technologies based on the structural properties of these nano-cages especially in electronics and biomedical engineering. Soon after single- and multi-walled carbon nanotubes having diameters in few nanometres came into limelight. Although much credit of their discovery in 1991 is often given to Sumio Iijima, many earlier reports describe their observation of these hollow carbon tubes found through chemical vapour growth technique similar to fullerenes [4]. They have been exploited for many nanotechnology and electronic applications because of their high conductivity and tensile strength. Nanotubes can be laterally joined end-to-end to produce a carbon tube of infinite length because of their high propensity to form van der Waals bonds between two tubes leading to very high length-to-diameter ratios. They have also been used as drug carriers using the hollow cavity [5]. They form perfect symmetry along their translational axis as they can be imagined to be joined at perfect Bravais lattice points when a 2D sheet is folded. Multiple tubes folded about the same longitudinal axis form onion layers called multi-walled carbon nanotubes [6].

1.2 Graphene

Finally in 2004, graphene was re-discovered by Andre Geim and Kotsya Novoselov revolutionalising research in material science. Repeated segregation of graphite layers yielded single-layer graphene using a simple scotch tape for which few years later they were awarded the Nobel Prize in Physics in 2010 [7]. Graphene is the backbone for most graphitic forms of carbon like fullerenes (0D), carbon nanotube

(1D), single-layer graphene sheets (2D) and graphite (3D). During the past several years, various methods for producing graphene have been developed by researchers such as micromechanical exfoliation, chemical vapour deposition (CVD) for epitaxial growth and chemical synthesis by separating individual layers from graphite [8]. Although the latter method is most commonly used to produce bulk graphene, it often is accompanied by several defects. The purest graphene is still produced by using scotch tape method. Interestingly, optical microscopy can differentiate between the numbers of graphitic layers constituting inhomogenous graphene sample. Now these layers are routinely characterized using atomic force microscopy (AFM), transmission electron microscopy (TEM), scanning tunneling microscopy (STM), X-ray diffraction (XRD) and Raman spectroscopy. Raman spectroscopy is often used in graphene studies to draw inferences based on signature D and G bands.

Graphene has a range of unusual properties like the 'quantum Hall effect' at room temperature, an ambipolar electric field effect along with ballistic conduction of charge carriers, high elasticity and tunable band gap [9]. It has an optical transmission rate of more than 98% in the visible bandwidth region, an improvement over its closest competitor, indium tin oxide (ITO). Graphene has also been reported to have a mechanical strength similar to that of the so-called "theoretical strength of defect-free solids" making it both the thinnest and strongest material. It has an exceptionally high Young's modulus of 1.0 TPa and high tensile strength [10]. Latest industrial applications often make use of its high thermal conductivity, electron mobility and specific surface.

Graphene materials vary in number of layers, surface chemistry, lateral dimension, defect density and quality of the individual graphene sheet with respect to the composition or purity. Few-layer graphene (FLG) is defined as thin graphitic

flake-like stacks of 2-10 graphene layers [11]. Through thermal exfoliation by intercalation of sulfate, nitrate or other ions between the layers of crystalline graphite followed by rapid thermal heating, massive expansion of natural graphite follows because of internal pressure between the layers. These FLG samples are often used as reinforcing agents in composite materials or further processed into graphene/ graphene oxide [12]. Ultrathin graphite has been defined as a material with thickness greater than 10 sheets (3-5 nm) but less than 100 nm and thus falls within the scope of nanomaterials having at least one dimension within the range 1-100 nm [13].

Recently, biomedical applications of 'Graphene Family Nanomaterials (GFNs) are continuously emerging. The properties which make GFNs amenable for interest in biological applications include their non-covalent interactions, purity, number of individual layers, lateral dimension and surface area, surface chemistry and homogeneity. Miniscule nanoparticles (<10 nm diameter) have a high surface area:volume ratio, i.e. significant fraction of their atoms exposed on their surfaces. Sheet of monolayer single-atom thick graphene represents the theoretical maximum surface area of a sp^2-hybridized carbon (about 2600 m^2/g) where every atom lies on the surface, exposed to the surrounding medium on two sides [14]. This makes graphene a suitable substrate for physical adsorption of molecules or catalytic chemical reaction, making them of interest to understand the biological response to these materials. The number of graphene layers in a GFN is an important factor as it determines the specific surface area and bending stiffness.

Various GFNs have been utilized for the fabrication of functionalized biosystems integrated with nucleic acids (NAs), aptamers, enzymes, peptides, antibodies, other functional proteins and even bacterial/mammalian cells [15]. The ability of graphene to quench electron donors and to protect biomolecules from

enzymatic cleavage has been widely used for constructing biosensors and drug delivery vehicles [16]. In addition, the transportation capability of these materials in living cells and *in vivo* systems, have revealed the potential of graphene in biological studies and nanomedicine [17]. These new-found nanomaterials are providing fascinating opportunities for biotechnological applications because of their unique structural characteristics and properties. In-depth knowledge of fundamentals as well as its applications are very necessary for harnessing the true potential of these materials.

1.3 Graphene Oxide

Graphene oxide (GO) is a highly oxidized form of modified graphene identified by oxygen-containing functional groups at interstitial and boundary sites which create defects in the purely symmetrical honeycomb lattice of graphene. It is most commonly produced by the harsh oxidation of crystalline graphite to separate its layers inter-connected by van der Waals forces followed by sonication or other dispersion methods to produce an oxidised monolayer in aqueous suspension [18]. These single-atom-thick carbon-skeletal sheets contain carboxylate groups on the periphery, where they provide pH dependent negative zeta potential contributing to its high colloidal stability. The basal lattice is usually interspersed with uncharged but polar hydroxyl (-OH) and epoxide (-O-) functional groups. The planar lattice also contains graphenic patches capable of forming hydrophobic and π-π interactions relevant to adsorption of a multitude of hydrophobic molecules. Another modification of graphene is reduced graphene oxide (rGO) which is synthesized by treating GO under reducing conditions but only partial reduction can be achieved by high-temperature thermal treatment or chemical treatments with hydrazine or other reducing agents. Reduced GO is often preferred to restore graphene-like physical

parameters like electrical conductivity. Other changes during reduction of GO include increased hydrophobicity, reduced oxygen content, new holes or defects in the carbon lattice due to carbon monoxide/dioxide liberation, reduced surface charge and water dispersibility [19]. GO having lateral dimension less than 100 nm, is often referred to as nano-GO. The small size of these materials makes them suitable for being used for biological applications which facilitates their cell entry and dispersion stability [13, 20].

Graphene oxide has been employed as a universal substrate for attachment to various biomolecules or cells. These molecules form biologically-modified outer corona which makes the composition more biocompatible, selective and soluble. Therefore, several studies have focused on graphene modification and functionalization [21]. GO can easily be functionalised using proteins through physical adsorption or strong covalent bonding as they interact through their varied surface functional groups and secondary structures. These interactions can be tuned by changing physical conditions and the type of protein. GO has been tethered with various enzymes and proteins, to make powerful biosystems with unique properties. Various proteinaceous molecules have been immobilised to form a matrix on GO like horseradish peroxidase (HRP) and lysozyme [22]. They spontaneously self-assemble onto GO through the oxygen-containing groups enriching individual GO sheets. Such strategies make it possible to immobilize biomolecules on GO surface without any surface modifications or additional coupling agents. Such biosystems have been used for many applications like the development of FRET biosensors [23]. Graphenic surfaces have been found to have strong affinity towards nucleic acids via π-π stacking interactions between the ringed structures. GO has found propensity for

being used as a powerful building element for nanoscale bioelectronic interfaces with biomolecules, cells and tissues, owing to its coupling ability with cell membranes.

Many promising results about graphene chemistry have been reported in the past several years, which offer a new avenue for the surface modification and functionalization of graphene and its oxidised form with biomolecules. Further exploration will provide better ideas for the applications of graphene in biotechnology *in vitro* and *in vivo*, such as graphene-based cellular probing, diagnostics, drug delivery and therapy.

1.4 Carbon Quantum Dots

Another fascinating member of the nanocarbon lineage are carbon quantum dots (CQDs). They were also accidently discovered during the purification of single-walled carbon nanotubes through preparative electrophoresis in 2004 by Xu et al [24]. Since then, they have captivated many researchers at both fundamental and application-oriented levels by their unusual tunable luminescence for which they are also referred to as carbon nanolights. Photoluminescence (PL) λ_{ex} was found to have dependence in many reports. It remains elusive whether this was caused because of emissive traps on their surfaces, different sizes of CQDs or some other mechanism [25]. These are miniscule sp^2/sp^3 carbonaceous cores typically having oxygen and nitrogen-containing functional groups or modified attached groups on their surface creating defects. Chemical groups can be modulated on the surface of CQDs [26]. They mostly have diameters within 1-10 nm and good aqueous solubility, dispersibility and stability for months. They may be crystalline or amorphous in their organisation depending on the starting material and synthesis route. Some CQDs have been found to possess diamond-like arrangement while most of them are graphitic in

their organisation. Most CQDs absorb in the UV region (230-320 nm) while the tail of the spectrum extends to the visible region. Small shoulders represent π - π* and n- π* transitions of carbon-carbon or carbon-heteroatom double bonds present in functional groups [27]. The slight changes in the absorbance peak positions give slight indication of the composition differences in the synthesis of CQDs. They have been found in some daily eatables that we consume like bread, jaggery, cornflakes and biscuit. Hence, it can be concluded that CQDs have been consumed for a long time [28]. They are predicted to replace conventional semiconductor quantum dots in future as they align with the core principle of sustainable development. Some methods like reduction, base catalysis and self-exothermic synthesis ensure efficient CQD synthesis at room temperature without external energy input. Many solution-based and solid phase optical probes have been designed using them [29].

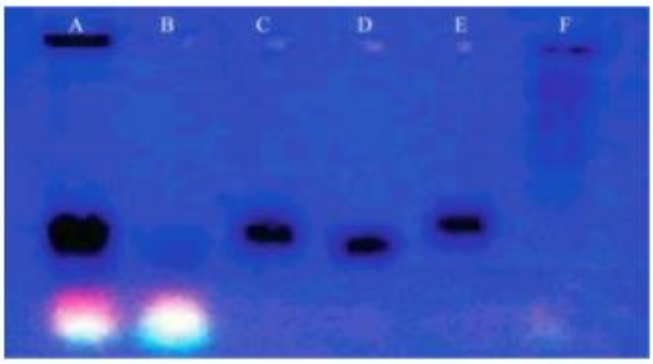

Figure 1.3: Electrophoretic profile that led to the discovery of CQDs in 1% agarose gel under 365 nm UV light (*Adapted from X. Xu, R. Ray, Y. Gu, H. J. Ploehn, L. Gearheart, K. Raker, W. A. Scrivens, J. Am. Chem. Soc. 2004, 126, 12736–12737*)

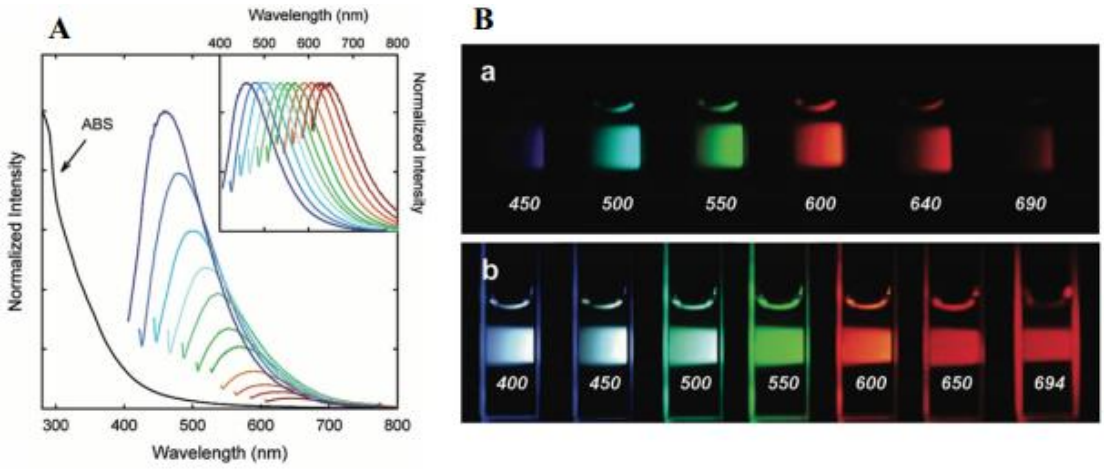

Figure 1.4: The absorption and PL emission spectra of CQDs after different incident excitation wavelength. (*Adapted from Y.P. Sun et al J. Am. Chem. Soc. 128 (2006) 7756–7757*)

The direct competitors of CQDs are traditional metal-semiconductor quantum dots which are typically chalcogenides (selenides, sulphides or tellurides) of metals like zinc, cadmium or lead like CdTe, ZnSe and PbS. Compared to these and other organic dyes, CQDs are superior in having high aqueous solubility, facile modification, high resistance to photobleaching and non-toxicity [30]. Their non-blinking nature and high photostability make them advantageous for various long-term imaging uses. CQDs are benign to the environment, very biocompatible and chemically quite inert. This makes them highly suitable for use in biomedical, biosensing, drug delivery and tissue engineering applications. CQDs usually show emission in the blue-green region which largely depends on their surface chemistry and synthesis route [31,32].

1.4.1 Type of Luminescence

Since CQDs are very good electron donors and acceptors, they have been popularly harnessed for their **chemiluminescence** (CL) or **electrochemical luminescence** (ECL) towards applications in catalysis, sensing and optoelectronics

[33]. By introducing oxidants, holes can be injected on the CQD surface which in turn greatly enhances energy release in the form of CL emission by electron-hole annihilation [34]. The dual ability of CQDs to act as both electron donor and acceptor offers much potential for their use in optoelectronics, catalysis and sensing. ECL has been linked to the oxidised state of the CQD surface [35]. Recently, **phosphorescence** was observed in CQDs synthesized by confining their rotational and vibrational movement in a rigid matrix [36]. **Up-conversion photoluminescence** (UCPL) has been an uncertain occurrence in CQDs backed by few reports claiming to observe the phenomenon and others not. Most CQDs and GQDs do not exhibit UCPL and the observed peak may signify a normal fluorescence peak excited by some leaking component from the excitation of second-order diffraction light in the monochromator of the fluorescence spectrophotometer. This may be eliminated by adding a suitable long-pass filter in the excitation pathway before deducing UCPL [37, 38]. Another fascinating aspect of CQDs that is being explored is the **photoinduced electron transfer** property (PET). CQDs have been checked for photoresponse, electron transfer and photoinduced charge separation processes as they have an active electron accepting/ donating surface [39]. Such efforts are bound to advance the current mechanistic understanding and many applications in catalysis and related light-energy conversion reactions.

1.4.2 Synthesis Methods

Typically, the order of reactions that follow CQD formation is- dehydration, condensation, polymerization, carbonization and passivation including sp^3- to sp^2-carbon transformation [40]. Depending on the route and conditions of synthesis and the precursor for reaction, carbonization maybe imperfect which leads to amorphous structures like polymer chains and functional groups within the formed quantum dots.

They also inherit some characteristics from their original precursors [40, 41]. CQDs can be synthesized through top-down as well as bottom-up approaches. Most often, top-down approaches include nano-chiselling GO, CNT, carbon fibre or pristine graphite while bottom-up methods involve carbonization of complex organic molecules [42]. Modifications can be introduced during the process or post-treatment. Generally, the points to be considered while producing CQDs are to get rid of the carbonaceous aggregates that often accompany the treatment, maintain a uniform size and tune the optimum surface property according to the required application. There are many existing methods to synthesize carbon quantum dots like microwave irradiation, chemical acid oxidation, hydrothermal method, carbonisation and thermal pyrolysis, arc discharge, laser ablation, electrooxidation and ultrasonic synthesis [25, 43]. Each of these methods has its own set of advantages and disadvantages. The most advantageous point in the synthesis of CQDs is that any organic carbon-containing source can be used as a precursor. This renders them as very inexpensive and abundant material for use in latest fluorescence-based applications [44]. It is imperative to gain control over the size of CQDs to maintain uniformity within the samples. Most samples contain a mixture of molecules, oligomers, polymer clusters and carbon core leading to high polydispersity [40, 45]. Generally, many approaches are adopted to achieve this either during the treatment or post-process like centrifugation, filtration, dialysis, column chromatography and gel-electrophoresis. Through surface passivation and doping, CQDs can be tuned to have specific functional groups and chemical moieties on their surface which largely affect their photoluminescence. Sulphur and nitrogen doping has often been found to enhance the optical properties of CQDs during synthesis [26, 46]. The solvent also offers weak but

important surface passivation which allows for an effect on the photoluminescence [47].

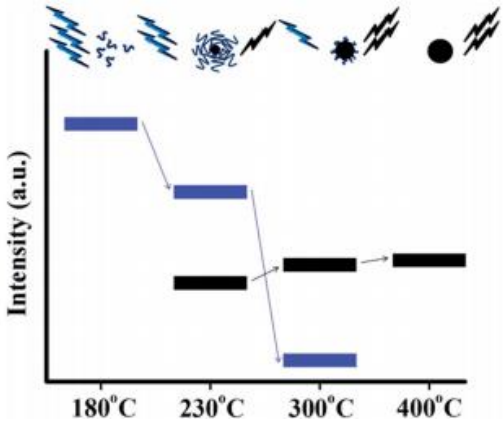

Figure 1.5: Schematic Representation of the PL emission characteristics of different species produced at different temperatures for organic fluorophores (blue) and carbonaceous cores (black) (*Adapted from M. J. Krysmann, A. Kelarakis, P. Dallas and E. P. Giannelis, J. Am. Chem. Soc., 2011, 134, 747*)

Strong oxidising acids like sulphuric and nitric acids are used in **chemical ablation** method which is a harsh treatment to carbonize small organic molecules [48]. **Electrochemical carbonization** has also successfully been used by introducing platinum electrodes for converting alcohols into CQDs. It was reported that the degree of graphitization and size is directly correlated to the applied potential. Graphitic rods in water as electrolytes have also been used to yield a light brown solution which upon purification leads to a good yield of CQDs [49, 50]. Another successfully used technique that has been used often for CQD synthesis is **laser ablation**. Typically, a suspension of carbon material is mixed in a suitable solvent and a pulsed laser maybe used for a short irradiation period after which the supernatant is procured from the centrifuged sample [51]. Another frequently used low-cost method to synthesize CQDs very rapidly, is through **microwave irradiation**. Highly luminescent CQDs have been prepared through this procedure [52]. **Arc discharge** is an often used

technique for synthesizing CQDs by top-down approach followed by oxidation of hanging carbon moieties [53]. CQDs have also been created through **reverse micelle process** by manipulating the surfactant, water and reactant ratio [54].

Hydrothermal method is one of the most commonly used CQD synthesis methods as it utilizes some carbon source treated under high temperature and pressure in a one-pot synthesis step without the need of any additional step or chemical reagents. This makes it a low-cost, non-toxic, environmental-friendly route to produce novel CQDs from any possible organic carbon precursor source [55]. Proteins have been used as a raw material for CQD synthesis as it allows the incorporation of nitrogen rich moieties naturally. Few such examples include silk, wool and milk [29, 56]. This process usually has four steps-dehydration, polymerization, carbonization and passivation [57]. CQDs synthesized using hydrothermal method, usually absorb in the UV region and emit in the blue-green region. Through this method, most CQDs synthesized have been found to possess negative zeta potential because of the electronegative surface charge contributed by the functional groups. **Solvothermal carbonization** is another similar technique which employs carbonization of compounds followed by extraction with an organic solvent. Organic compounds are subjected to heat treatment in organic solvents having high boiling point, followed by extraction and concentration of CQDs [58].

Table 1.1: Different Biomass Used as Carbon Precursor Sources for CQD Synthesis

Biomass	Q.Y.	PL	Application	Reference
Sweet Pepper	19.3	Blue	ClO^- detection	*Analyst*, 2013, 138, 6551–6557
Saffron	23.6	Green-Blue	Prilocaine detection	*Sens. Actuators*, B, 2017, 253, 451–460
Lignin	21	Blue	Multi-colour imaging	*Green Chem.*, 2018, 20, 1383–1390
Grass	-	Blue	-	*Green Chem.*, 2012, 14, 3141–3145
Chitosan	13	Blue	Nitroaromatics detection	*ACS Appl. Mater. Interfaces*, 2016, 8, 17478–17488
Coriander leaves	6.48	Green	Fe^{3+} detection	*Analyst*, 2015, 140, 4260–4269
Silkworm	46	Blue	Cell imaging	*J. Mater. Chem. B*, 2016, 4, 387–393
Pumpkin	9.42	Yellow	pH sensing	*J. Mater. Chem. B*, 2015, 3, 6813–6819
Potato	6.14	Blue	Multicolour imaging	*New J. Chem.*, 2014, 38, 6152–6160
Lentils	10	Blue	Thioridazine HCl detection	*RSC Adv.*, 2016, 6, 104467–104473
Fingernails	42.8	Blue	Sunset yellow detection	*Sens. Actuators, B*, 2018, 267, 494–501
Garlic	13	Blue	Fe^{3+} detection	*Sens. Actuators, B*, 2016, 223, 689–696
Prawn	9	Blue	Cu^{2+} detection	*Sens. Actuators, B*, 2016, 224, 396–403
Banana	8.95	Green	-	*RSC Adv.*, 2013, 3, 8286–8290

New sustainable methods are being developed to cause least effect on the environment. Hydrogen peroxide has often been used to make CQDs at room temperature as the exothermic reaction allows for the heat requirement of the reaction without supplementing external heat, metal impurities or acids [29, 59].

1.4.3 CQD Modification

The high surface-charge ratio makes the highly unstable surface states of CQDs temporary traps for charge carriers leading to radiative recombination and reduced quantum yield. Engineering the surface of CQDs through **surface functionalization** or **passivation** can be a powerful strategy to tune their surface properties according to specific applications. Many methods can be adopted for adding functional groups and molecules mainly by using the oxygen-rich functional groups on the surface of CQDs. Covalent bonding, coordination bonding, π–π interactions and sol-gel processes have been employed for attaching various

molecules [25, 60]. Amine-functionalised molecules have often been used to enhance the fluorescence of CQDs linked by covalent bonding. Förster Resonance Energy Transfer (FRET) strategies have also been attempted by using a FRET pair molecule complementing CQDs. Coordination chemistry involves bonding strategies between carboxylate edges and positively-charged ions. Sol-gel approach can be adopted for surface functionalisation of CQDs within a short duration by using a coordinating solvent for the polymerization process [61]. Biomolecules like proteins and amino acids can help in enhancing emission, biocompatibility and targeted delivery [62].

Doping of CQDs is a prevalent practice which has been found to immensely enhance the photoluminescence of CQDs by most commonly using boron, nitrogen, sulphur, phosphorus and sometimes metal elements [25, 63]. Nitrogen is most frequently used as a dopant as it is said to enhance the photoluminescence by causing an upward shift of Fermi level and electrons in the conduction band. It may introduce new surface states that enhance radiative recombination. These elements have shown to cause changes when used in different combinations and ratios. Metals like Na and Mg have also been used to preserve the functional groups [64]. Sulphur often changes the lattice spacing and oxygen content of CQDs leading to a red-shift in photoluminescence [65].

In many recent efforts, CQDs have been been integrated with inorganic nanoparticle cores such as titania, iron and zinc oxide to exploit the combined properties of both to form **nanohybrids** [49,66]. In addition to the photoluminescent property of CQDs, magnetic, mechanical or other optical properties can be combined to form a robust system. These systems hold great potential for biolabelling, segregation strategies or photocatalysis.

1.4.4 Photoluminescence mechanism

Many efforts have been made towards understanding the origin of photophysicochemical processes that lead to the brilliant fluorescence phenomena in CQDs but till date it remains an elusive subject. Many reasons have been associated with the origin of the optoelectronic behaviour of CQDs like quantum effect governed by size, surface states arising because of defects and various functional groups, surface passivation, degree of p-conjugation and electron-hole pair recombination [30, 67]. At lower temperatures, the photoluminescence is contributed by molecular fluorophores whereas at higher temperature effects caused by the carbonaceous core predominate [68]. Often, multiple fluorescence intensity levels have been observed with single CQD suggesting the presence of multiple chromophoric units within the core and surface emissive states. Reduced CQDs seem to have multiple levels as compared to oxidized CQDs which show single emission level. Hence, the surface chemistry is largely linked with these processes with size effect playing a role as well as it has been hypothesized to decrease the HOMO-LUMO gap with decreasing size of CQDs [49, 69]. These tiny particles often show excitation-dependent fluorescence. CQDs also show a broad emission peak with a large Stokes' shift as compared to organic dyes which could be because of multiple narrow peaks caused by inhomogenously prepared solutions containing CQDs with different sizes, diverse PL centers and surface chemistry [70].

Surface oxidation and functional groups largely determine the emission peak of CQDs by having defects that cause a red-shift and affecting the band-gap in addition to quantum confinement [71]. Quantum yield refers to the ratio of photons emitted to the photons absorbed by CQDs. Many surface modification and synthesis steps have been reported to help in gaining higher quantum yield. Most naturally-

derived CQDs emit blue or green fluorescence. The blue fluorescence might arise from n–π* transition of the carbon-oxygen groups or the π–π* transitions of the carbon core states. On the other hand, the green fluorescence may arise from the n–π* transitions of the edge states [29, 72].

1.4.5 Biological Applications

Most commercially available organic fluorophores like fluorescein, Alexa Fluor and BODIPYs have been commonly used in different areas of life sciences. These organic molecules can easily enter cells but suffer from few disadvantages like narrow absorption spectrum, small Stokes' shift, photobleaching, bad solubility and cellular toxicity. Core-shell QDs with heavy metals are better bioimaging agents and show quantum effect. They are inert, photochemically stable and brighter with a large Stokes' shift. However, their potential cytotoxicity is a concern because of toxic surface ligands/coatings, leakage of toxic metal core and aggregation [73]. The discovery of CQDs as bioprobes and bioimaging agents is a boon in current scientific research. This green alternative has been reported to possess good biocompatibility *in vitro* and *in vivo*. Several reports show that CQDs do not show any cytotoxicity upto a concentration of 0.4 mg/ml [74]. They are non-biotoxic to cells at concentrations necessary for **fluorescence bioimaging**. They have been used for multimodal bioimaging of cells and tissues. Since the pioneering work by Sun and co-workers on using CQDs for cell imaging, many groups have followed suit changing the excitation wavelength. Mostly, CQDs are seen to distribute evenly within the cytoplasm and seldom enter the nucleus [75]. Targeted staining using CQDs for specific imaging has been utilised by attaching special ligands to them. Tranferrin, folic acid and hyaluronic acid have been used as ligands to specifically identify cancer cells [76]. These steps often require multiple purification steps which lead to low yield. As such,

CQDs may directly be fabricated using these molecules as precursor. As contrast agents, they can be used to differentiate between specific tissues in mammals through *in vivo* imaging. Efforts have been made to combine the optical resolution capabilities of CQDs with magnetic resonance imaging (MRI) scanning for better anatomical and physiological interpretations [77]. Fluorescence of CQDs in the Near Infra-red (NIR) region has been used for *in vivo* imaging. The multiphoton fluorescence of CQDs in the visible and NIR region surpasses the usable wavelength range offered by organic dyes by many orders of magnitude [78]. Some CQDs have also been seen to cross the blood-brain barrier (BBB) like L-aspartic acid derived and Angiopep-2 functionalized CQDs [76, 79]. Even though mouse is the most commonly used model for imaging applications, many other animals have been experimented on, like zebrafish, mosquito and fruit fly. The optical transparency and ease of genetic manipulation make zebrafish very good candidates for understanding the imbibition, localization and metabolism of CQDs. It has been observed that CQDs show biocompatibility and long-term stability entering through the digestive system and even through skin. They have high affinity for melanin. The lack of tissue specificity still limits the *in vivo* applications of CQDs [80]. Using confocal microscopy, within the optical window, one can image using CQDs up to hundreds of micrometers deep.

Another area where CQDs have been widely explored is **biosensing**. They apply principles like inner filter effect (IFE), fluorescence resonance energy transfer (FRET), electron transfer (ET), aggregation induced emission enhancement (AIEE) effect, aggregation induced emission quenching (AIEQ) effect, static quenching effect (SQE) or dynamic quenching effect (DQE) [29, 81]. CQDs offer a range of advantages over other dyes and sensors. They are highly photostable and provide excitation-dependent multi-colour emission over a wide wavelength range. They are

hydrophilic with good aqueous dispersibility, very biocompatible, non-cytotoxic and cell permeable. They can easily be functionalised with a range of molecules for easy monitoring of health parameters like key metabolic molecules, metal ions, local cell pH, DNA and protein changes, even cells and bacteria [29, 82]. Single-stranded DNA may be attached to the CQD surface which after binding to its complementary strand, leads to the recovery of fluorescence earlier quenched by the DNA-CQD π-π interaction. Intracellular probes have also been designed to detect toxic metabolites through FRET strategy [83]. CQDs are very versatile nanoprobes having a huge potential for utilisation in biosensing. Now, solid phase sensing is also gaining popularity in the form of fingerprint tests, paper-based sensors, fibres and hydrogels [29, 84].

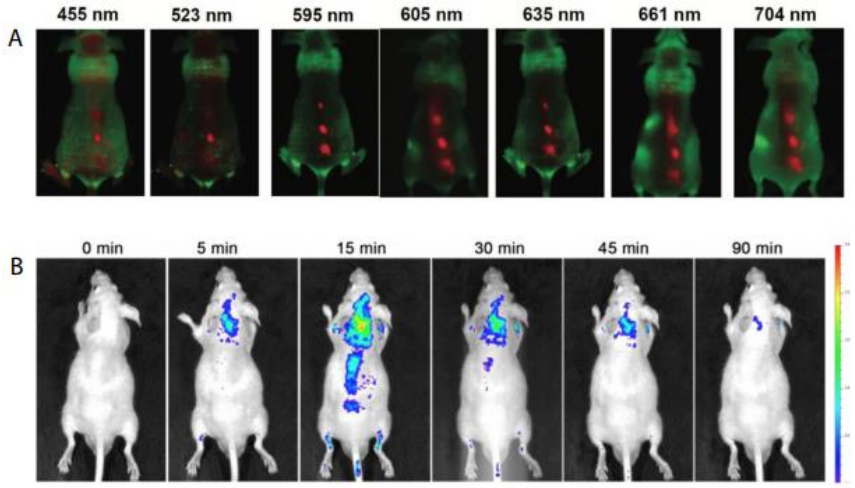

Figure 1.6: *In vivo* fluorescence image of a mouse injected with red-emitting CQDs (*Adapted from H. Tao, K. Yang, Z. Ma, J. Wan, Y. Zhang, Z. Kang and Z. Liu Small, 8 (2012) 281*)

CQDs can efficiently be used in **biomedical drug delivery** systems by incorporating them into drug-loaded vehicles. They can lend their excellent fluorescence bioimaging capability for tracking drug response, delivery and activity combining 'therapy' as well as 'diagnostics' known as theranostic approaches.

Attempts to bind cancer drugs, genes, antibacterial drugs and neuro disease drugs for controlled release have been done [85]. The release of attached drug moiety leads to fluorescence recovery of the CQDs which can be monitored over time. These molecules are attached via covalent and non-covalent bonds, the former providing more control for targeted release through pH changes or light irradiation. Recent efforts have been made to synthesise CQDs using molecular precursors having the required properties which is remnant in final CQDs to simplify the tedious multi-step processes. Even dual colour-CQD systems with FRET capabilities have been devised for efficient tracking of drug delivery [86].

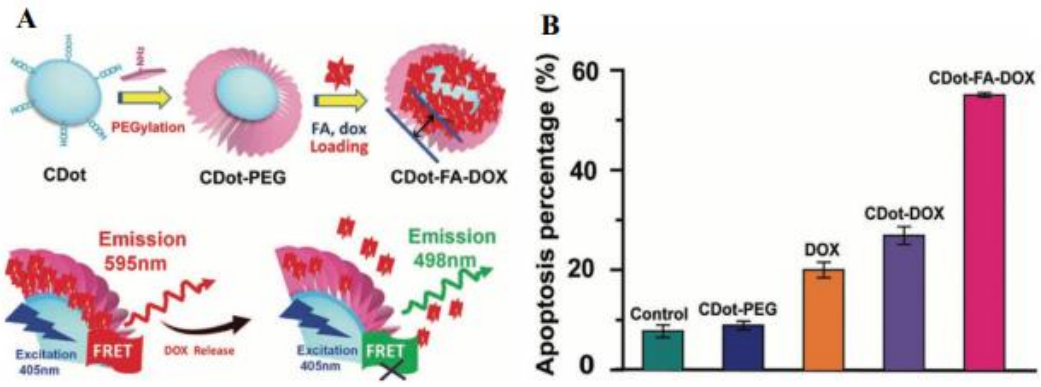

Figure 1.7: A Schematic Illustrating the functionalisation of PEG-coated CQDs with FA and Dox delivery for cancer therapy (*Adapted from J. Tang, B. Kong, H. Wu, M. Xu, Y.C. Wang, Y.L. Wang, D.Y. Zhao, G.F. Zheng, Adv. Mater. 25 (2013) 6569–6574*)

1.4.6 Optoelectronic Applications

CQDs have widely been used in **solar cell** applications. For **dye-sensitized solar cells**, they possess certain advantages over their organic dye counterparts like resistance to photobleaching, long-term stability and low toxicity [87]. In combination, CQDs may help in the suppression of charge recombination resulting in better photoelectric conversion efficiency. CQDs have also been used in composite

organic solar cells by incorporating them in a polymer matrix or dispersing them in a thin layer. They offer enhanced absorption in the UV-violet region and also emission in the lower visible wavelength range compared to most polymer composite capabilities. Therefore, more wavelength range is covered leading to higher power conversion efficiency [88].

Another application of CQDs that has generated interest is for making **supercapacitors**. CQDs are embedded in a composite-based electrode through uniform dispersion. This causes the electrode to have a good specific capacitance and an ultra-high current density. The hybrid composite shows excellent cycling stability and electrochemical performance [89].

CQDs have been widely used in **light-emitting diode (LED)**-based applications due to their ease of synthesis, cheap production, eco-friendly and non-toxic nature and stable bright long-range fluorescence. Many CQDs have been reported to show a broad visible light fluorescence on excitation in the UV region. CQDs embedded in a polymer matrix compose flexible fluorescent materials that utilise the fluorescence of evenly dispersed CQDs which do not show solid-state quenching in such composites [90]. These films are cheap, have robust mechanical, thermal strength and stability and can be used for making flexible solid lighting systems. In many LED devices, an emissive layer of CQD is often sandwiched between electron transport and hole transport layers. By varying the voltage/current, fluorescence emission to white, blue, cyan or magenta can be tuned using the same CQDs [91].

1.4.7 Catalysis Applications

Renewable energy in the form of hydrogen evolution through photoelectrochemical reactions is greatly needed to be harnessed and is one of the most invested areas of research. CQDs act as an electron reservoir and help in separating electron-hole pairs for photoinduced charge separation [92]. They also help in sensitizing materials for better **photocatalytic** evolution of hydrogen. Up-converting CQDs have also been used for utilising the NIR portion of the sun's energy for harvesting. After absorption in the IR region, they emit in the visible region for activating various chemical cycles [93].

In many photocatalytic applications like fuel cells and water splitting, a very common reaction that accompanies these processes is **oxygen reduction reaction**. CQDs have been utilized in electrocatalyzing this activity via a reduction pathway. Compared to commercial catalysts, CQDs show greater stability and tolerance to methanol and carbon oxide making their use more favourable for such reactions [94].

1.4.8 Chemical Sensing

By observing the changes in fluorescence intensity of CQDs, a range of molecules can be detected including ions, pH, radicals, organic compounds and biomolecules. The most often and easily detected ions by CQDs are iron, mercury, chromium and iodine radicals [25]. Many rapid and efficient protocols have been described for single or dual detection of molecules and ions based on FRET, on-off signalling, competitive interaction with CQDs and selective detection from a mixture. Such strategies provide an eco-friendly approach without the need for additional steps, toxic reagents or additional steps. Ratiometric FRET or double-probe strategies have also been successfully exercised inside cell environment [95].

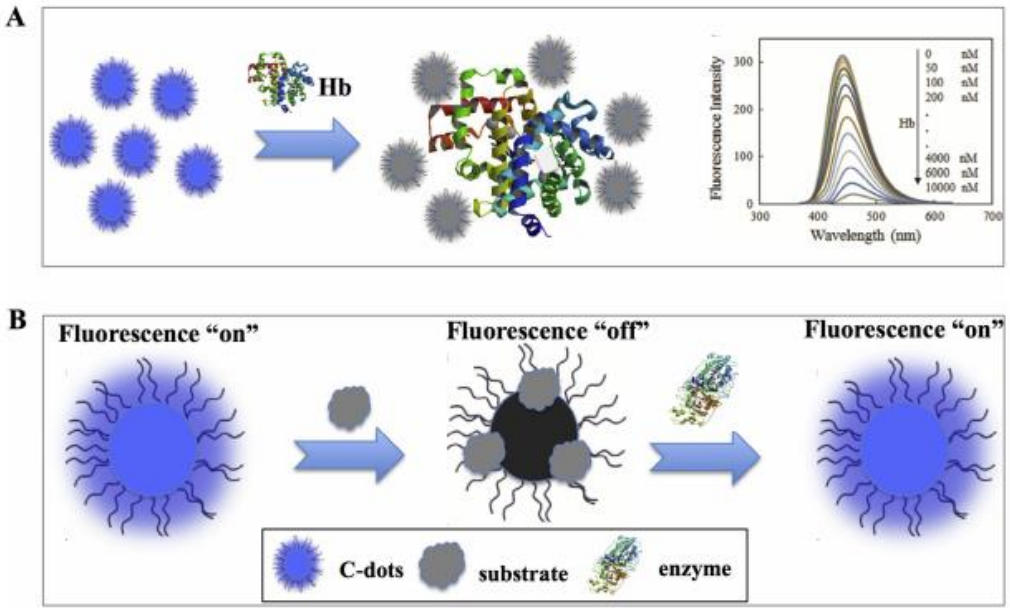

Figure 1.8: A schematic of a Typical On-Off-On Fluorescence Quenching Mechanism for the detection of an analyte (*Adapted from A. Barati, M. Shamsipur, H. Abdollahi, Biosens. Bioelectron. 71 (2015) 470– 475*)

1.4.9 Challenges with CQDs

Most CQDs have quantum yields less than 10% due to oxygen-containing defect states which lend to non-radiative decay and purification steps. Proper doping/surface passivation steps can largely increase the quantum yield. Most CQDs do not emit in the red/near infra-red (NIR) region, making them not very useful for *in vivo* imaging applications as light of larger wavelength does not scatter or get absorbed by tissues. Since most tissues autofluoresce at green wavelengths, blue-green emitting CQDs may not be ideal for such purposes [96].

1.5 Literature Review

Fluorescent nano-carbonaceous material has quickly made a place in recent advanced materials. Due to a huge potential in many applications, their capability has been harnessed in biomedicine, catalysis, solar cell use, sensing and optoelectronics.

Generally, materials are graphene quantum dots (GQDs), carbon quantum dots/carbon nanodots (CQDs/CNDs) or polymeric dots (PDs) [42, 97]. Methods for both top-down and bottom-up approaches have evolved for the synthesis of these carbon dots. Top-down methods that have been often used involve etching of bulk graphitic materials like graphene oxide, candle soot, carbon nanotubes and carbon rods. These materials, in their innate form, have very good sp^2 hybridised organisation which makes their band gap inefficient to deliver fluorescence. Hence the surface chemistry is modified by using strong oxidising acids which cut them into tiny fragments and decorate the nano-sized carbon with oxygen-containing functional groups. Other methods include electrochemistry, laser ablation, arc discharge and hydrothermal/solvothermal steps to break larger graphitic chunks [25, 42, 98].

On the other hand, bottoms-up approach usually involves dehydration and carbonization of organic molecular precursors like polymers and biological compounds. Mostly the methods that exist for bottom-up synthesis of fluorescent nano-carbon include hydrothermal, solvothermal, microwave, pyrolysis, laser ablation and arc discharge techniques. Largely, all such samples contain non-uniform size distribution of carbon dots and high polydispersity index which are dispersed among larger aggregates. This happens due to the uncontrollable chain of reactions in these processes. Some methods have been found to be helpful in maintaining a control over uniformity of CQDs like introducing intramolecular oxidative polycyclic aromatic hydrocarbons (PAHs) and later processing steps like dialysis, electrophoresis and filtration can help in getting rid of larger undesirable particulate moieties [99].

Surface modification or "passivation" is used to enhance the quantum yield of CQDs by methods like chemical crosslinking with PEG-NH_2 or surface reduction [100]. GQDs differ from CQDs by having mostly an anisotropic carbon core with

functional groups on the edges with some crystallinity and average lattice size of 0.24 nm corresponding to the (001) plane of carbon. On the other hand, CQDs possess an amorphous nature but some crystalline short-range order usually showing an interplanar spacing of 0.34 nm corresponding to the (002) plane of graphitic lattice. Moreover, GQDs consist of very few layers of graphene having a high aspect ratio as compared to the quasispherical morphology of CQDs. The hybridisation coefficient between the outer functionalised surface and carbon core impacts the PL intensity [101].

GO has often been used as a raw material for CQDs as it can be an ideal model for understanding the complex PL mechanism of carbon dots. They have single-layered sp^2 bonded carbon and oxygen-rich functional groups at the edges via sp^3 bonds whose electronic transitions help in determining the fluorescence. Through these systems, it is speculated that the carbene groups at the zig-zag edges of CQDs contribute more towards PL emission as compared to size effect due to quantum confinement [98, 102]. In addition to the ongoing debate on the exact PL mechanism of CQDs, it has been observed that even after differing the layers or size of CQDs, the emission stays mostly in the blue wavelength region and the same spectral line shapes and peak positions are observed. This suggests the involvement of surface states. The amine content also plays a role in modulating the PL intensity. It has been reported to make the band gap narrower and cause redshift in the emission spectra having higher HOMO orbital than that with hydrogen-terminated CQDs [103].

No matter what mode of synthesis is adopted for CQDs, they always seem to have similar excited-state behaviour. The common hypothesis is that the sp^2 arranged carbon is the origin for photoinduced carriers where they are initially stored, slowly leaking into surface emissive traps on CQDs. The proportion and combination of each

of these traps helps in determining the quantum yield, radiative recombination of carriers and photostability. Therefore, both the intrinsic bandgap by the carbon core and extrinsic fluorescence by the surface functional groups both collectively contribute to the fluorescence of CQDs [29, 71, 104]. It has also been shown that the carbonization temperature is important in bottoms-up synthesis approach of CQDs. Near hydrothermal temperature of 150°C, initially molecular state of fluorophore formation occurs, which embeds into the core or is attached on the surface giving rise to PL. As the temperature is increased, the surface state starts dominating consisting of a carbon core with functional groups on the surface. QY is higher in the molecular state but photostability is higher in the surface state. Interestingly, another mechanism of PL has been observed when polymer-aggregated fluorescent dots were synthesized from non-conjugated polymers. It was observed that the reduced vibrations and rotations in the polymer in non-radiative decay caused by increased vibrations and rotations [105].

CQDs have been explored for their interactions with biomolecules like proteins, DNA and lipids. Many reports show the role of CQDs in the inhibition of protein fibrillation and aggregation. These include experiments with insulin, human islet amyloid polypeptide (hIAPP), transferrin, amyloid beta 33–42 (a key fragment of Aβ 42), human serum albumin, chicken ovalbumin and haemoglobin [76, 106]. It was observed that CQDs derived from organic precursors were successful in inhibiting protein fibrillation but those derived from GO were not. This was probably because of abundant nitrogen-containing functional groups on the surface of CQDs that form hydrogen bonds with proteins which helps to keep them separated [107]. The effect of CQDs on enzymatic activity has often been investigated. CQDs have been reported to boost the activity of hemeprotein equine cytochrome c through electrostatic

interactions. They increased the catalytic activity of laccase, which further increased on irradiating with visible light, but the activity of porcine pancreatic lipase was diminished. This can be used for inhibiting fat absorption to treat obesity. The exact location of an enzyme has also been elucidated using CQDs through reverse engineering [108].

Many studies have started to focus on the protein detection capability of important protein biomarkers through either fluorescence enhancement or quenching of CQDs. This enables the estimation of protein concentration in real samples. "On-off" fluorescence systems and inner filter effect have been used for strategic detection using small molecules and ions. The resulting reaction products may absorb light in the excitation wavelength range of CQDs. These protein-CQD interactions are usually physical rather than chemical [109].

Another important biomolecule to interact with CQDs is nuceic acid (DNA/RNA). CQDs interact differently with single-stranded DNA as compared to double-stranded DNA, which helps in understanding their conformations. Positively-charged CQDs bind to negatively charged phosphate groups in DNA via electrostatic interactions. By binding to the major groove of DNA, CQDs may change their conformation to the transient Z form of DNA. Research has also focussed on delivery of NA sequences like siRNA into cells and subsequent monitoring through fluorescence signals [110].

CQDs have been used as an important tool for understanding their interaction with phospholipids and their entry into cells through biomembranes. They have been shown to cause chemical alterations to membranes and FRET transfers with dyes embedded in them. They help in understanding cell environment, intracellular

trafficking and processes [111]. Fluorescent signals from bacterial membranes using CQDs have helped in identifying and differentiating bacterial species. Depending on their surface charge, they can also be antibacterial. The mode of entry of CQDs through membranes depends on time, dose and is partially energy-dependent in addition to normal passive diffusion. They may enter through caveolae- and clathrin-mediated endocytosis [112]. These revelations form important basis of designing biolabels, drug carriers and fluorescent probes.

Often, the photocatalytic activity, photostability, use of the whole spectrum for light are enhanced when CQDs are coupled with complexes like ZnO, silver orthophosphate, monoclinic bismuth vanadate (BiVO4) and (RhB)/TiO2 complex [25, 113]. CQDs are amazing tools that offer flexibility in manipulation techniques and at the same time the ease of use. This field is definitely worth exploring to harness more information and application-based inventions.

1.6 References

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