SYNTHESIS AND CHARACTERISATION OF CARBON DOTS FROM LEMON JUICE

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ABSTRACT

Luminescent carbon dots have gained intensive interests in recent years due to their unique optical properties and widespread applications. In this work, luminescent carbon dots were synthesised from lemon juice by hydrothermal method. The as prepared carbon dot solution changes from brown colour to yellow after excitation by UV light. The synthesised carbon dots were then conjugated with 0.5M boric acid solution. Upon capping with boric acid, luminescence intensity increased which is attributed to the fact that capping provides surface passivation and minimise electronic trapping capabilities of surface defects resulting in higher photoluminescence intensity. This good photoluminescence intensity of boric acid-conjugated carbon dots was exploited for sensing glucose. Upon glucose addition the luminescence intensity increases due to the formation of complexes by boric acid-conjugated carbon dots with glucose. The optical properties of the carbon dots were characterised using UV-Visible absorption spectroscopy and photoluminescence spectroscopy. pH of synthesised carbon dots were determined using a digital pH meter. Morphological characterisations were done using high resolution transmission electron microscopy and dynamic light scattering. Antibacterial properties of carbon dots were also studied by agar well diffusion method. From the UV-Visible spectrum, an absorption peak was observed at 400nm which originates from n-π* transition of C=O bond. From photoluminescence spectra, highest PL intensity was observed at an excitation wavelength of 240 nm. pH of carbon dot solution was found to 5.94 and pH increased to 6.18 when few drops of NaOH solution was added. Zeta potential of bare carbon dots and boric acid-conjugated carbon dots was found to be 0.3 and 0.7eV respectively which indicates positive surface charge of carbon dots. Hydrodynamic diameter of carbon dots and boric acid-conjugated carbon dots was around 100nm. From high resolution transmission electron microscopy, particle size was found to be 10nm.From the antibacterial studies, it was clear that carbon dots exhibit significant antibacterial activity against gram-positive bacteria Staphylococcus aureus. The low cost, eco-friendly synthesis method and favourable optical properties of the synthesised carbon dots make them promising candidates for various optical and biomedical applications.

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LIST OF ABBREVIATIONS

NNI	National Nanotechnology Initiative
CD	Carbon Dots
PL	Photoluminescence
QY	Quantum Yield
GQD	Graphene Quantum Dot
CQD	Carbon Quantum Dot
CPD	Carbonised Polymer Dot
CEE	Crosslink Enhanced Emission
RTP	Room Temperature Phosphorescence
ISC	Intersystem Crossing
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
HRTEM	High Resolution Transmission Electron Microscopy
DLS	Dynamic Light Scattering
LED	Light Emitting Diode
ORR	Oxygen Reduction Reaction
OER	Oxygen Evolution Reaction
HER	Hydrogen Evolution Reaction
CLED	CDs Based Light Emitting Diodes
SC	Solar Cells
PDT	Photodynamic Therapy
PTT	Photothermal Therapy
PET	Photo-Induced Electron Transfer
FRET	Fluorescence Resonance Energy Transfer
IFE	Inner Filter Effect
EDTA	Ethylenediaminetetraacetic acid
NCQDS	N-Doped Carbon Quantum Dots

CHAPTER 1

INTRODUCTION

1.1 Nanoscience and Nanotechnology

The word 'nano' is referred to a Greek word meaning 'dwarf' or 'something very small' that depicts one thousand millionth of a meter (10^{-9} m). Nanoscience is the study of structures and molecules on the scales of nanometer ranging between 1 and 100 nm, and technology that utilises it in practical applications such as devices etc. is called nanotechnology [1]. Nanotechnology has the ability to convert the nanoscience theory to useful applications by observing, measuring, manipulating, assembling, controlling, and manufacturing matter at the nanometer scale. The National Nanotechnology Initiative (NNI) in the United States define nanotechnology as 'a science, engineering, and technology conducted at the nanoscale (1-100 nm), where unique phenomena enable novel applications in a wide range of fields, from chemistry, physics, and biology, to medicine, engineering, and electronics'. This definition suggests the presence of two conditions for nanotechnology. The first deals with scale that implies nanotechnology is concerned to use structures by controlling their shape and size at nanometer scale. The second issue has to do with novelty which implies nanotechnology deals with small things in a way that takes advantage of some properties because of the nanoscale.

Nanoscience is a convergence of physics, materials science and biology, which deals with manipulation of materials at atomic and molecular scales while nanotechnology is the ability to observe, measure, manipulate, assemble, control, and manufacture matter at the nanometer scale. Important properties of materials such as the electrical, optical, thermal and mechanical properties are determined by the way molecules and atoms assemble on the nanoscale into larger structures. In nanometer size structures these properties often differ from macroscale.

The American physicist and Nobel laureate Richard Feynman introduce the concept of nanotechnology in 1959 in his lecture entitled 'There's Plenty of Room at the Bottom' [2]. He is considered as the father of modern nanotechnology. After fifteen years Norio Taniguchi, a Japanese scientist was the first to use and define the term 'nanotechnology' in

1974 as 'nanotechnology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule'.

1.2 Classification of Nanomaterials

Nanomaterials are defined as materials possessing, at minimum, one external dimension measuring 1-100 nm. Materials reduced to the nanoscale shows very different properties compared to what they exhibit on a macroscale. Nanomaterials are classified based on the number of dimensions of material, which are outside the nanoscale.

1.2.1 Zero Dimensional Nanomaterials

In zero dimensional (0D) nanomaterials, all the dimensions are measured within the nanoscale, that is, no dimensions are larger than 100 nm. Due to inherent structural properties of 0D nanomaterials, such as ultra small sizes and high surface area-to-volume ratios, they have more active edge sites per unit mass. The edge and quantum confinement effects of 0D nanomaterials endow them with more improved or novel properties such as high photoluminescence, quantum efficiency and chemiluminescence. Compared with bulk high dimensional nanomaterials, 0D nanomaterials are mostly spherical or quasi-spherical nanoparticles with a diameter of less than 100 nm. With novel properties such as optical stability, wavelength dependent photoluminescence, chemical inertness, cellular permeability, and biocompatibility, 0D nanomaterials offers great adaptability to biomedical applications such as nanomedicine, cosmetics, bioelectronics, biosensors etc.

1.2.2 One Dimensional Nanomaterials

In one dimensional nanomaterial, one dimension is outside the nanoscale. Two dimensions are at nanoscale and the other is outside the nanoscale. This leads to needle shaped nanomaterials. It includes nanofibres, nanotubes, nanorods and nanowires.These one dimensional nanomaterials are amorphous or crystalline, single or polycrystalline, and ceramic, or polymeric similar to zero dimensional nanomaterials. These materials exist as stand-alone materials or embedded within another medium.

1.2.3 Two Dimensional Nanomaterials

In two dimensional nanomaterials (2D), two dimensions are outside the nanoscale. The 2D nanomaterials exhibit plate like shapes. It includes nanofilms, nanolayers and nano coatings with nanometer thickness. Two dimensional nanomaterials within general sheet like structures are a newly emerging but very important class of materials. Their lateral dimensions are generally one or several orders of magnitude larger than the thickness, with typical morphologies of nanosheets, nanoribbons, nanoplates, and nanowalls [3]. In terms of mechanical, chemical, and optical attributes, the potential biocompatibility and degradability, 2D nanomaterials have been enthusiastically researched in various biomedical fields including biosensing [4], antibacterial agents [5], bioimaging [6], and cancer therapy [7]. 2D nanomaterials possess the highest specific surface areas and hence they have abundant anchoring sites to load and deliver therapeutic agents [8].

1.2.4 Three Dimensional Nanomaterials

Three dimensional nanomaterials (3D) are materials that are not confined to the nanoscale in any dimension. These materials have arbitrary dimensions above 100 nm. The bulk 3D nanomaterials are composed of a multiple arrangement of nanosized crystals in different orientations. It includes dispersions of nanoparticles, bundles of nanowires and nanotubes, as well as multi nano layers in which the 0D, 1D, and 2D structural elements are in close contact with each other and form interfaces.

1.3 Carbon Dots

Carbon based materials play significant roles in the development of material science. From the traditional industrial carbon like activated carbon, carbon black etc. to new industrial carbon such as carbon fibres, graphite and new carbon nanomaterials such as graphene and carbon nanotubes (CNTs), fundamental research and applications of carbon based materials are always popular in the field of chemistry, materials, and other interdisciplines due to their environmental friendliness. But macroscopic carbon material lacks the appropriate band gap making it difficult to act as an effective fluorescent material. Carbon dots (CDs), a new rising star in the carbon family, have attracted considerable attention due to their excellent and tunable photoluminescence(PL), high quantum yield(QY), low toxicity, small size, appreciable biocompatibility, and abundant low-cost sources, providing important

applications in many fields including biomedicine, catalysis, optoelectronic devices, and anticounterfeiting [9].
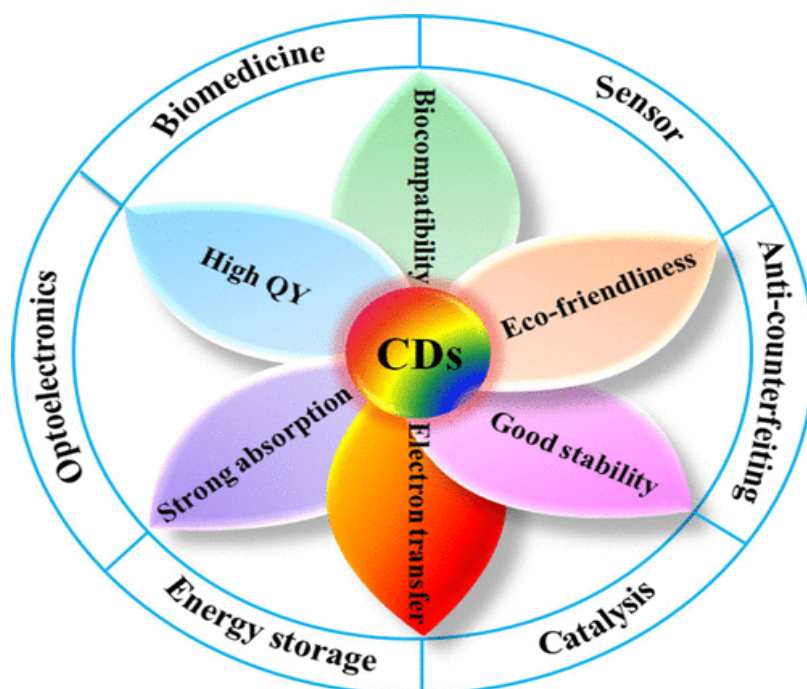
Figure 1.1 : Schematic representation of properties and applications of carbon dots [9]

Carbon dots are typically carbon nanoparticles, with average diameter less than 10 nm [10, 11]. CDs were discovered accidentally during the purification of single walled carbon nanotubes by Xu et al. in 2004 [12]. The structure of CDs is comprised of sp^2 and sp^3 carbon atoms with large number of polymer chains or functional groups attached on their surfaces. CDs have gathered enormous attraction among researchers, owing to their significant and outstanding characteristics such as excellent electron conductivity, photobleaching and photoblinking properties, high photoluminescent quantum yield, fluorescence property, resistance to photodecomposition, alterable excitation and emission attributes, increased electrocatalytic activity, good solubility in aqueous media, excellent biocompatibility, long term chemical stability, cost effectiveness, negligible toxicity and acquaintance of large effective surface areato volume ratio. CDs can be readily functionalised owing to the abundant functional groups present on their surface, and they also exhibit remarkable sensing features such as specific, selective, and multiplex detectability. The presence of abundant functional groups such as amine, carboxyl, hydroxyl, etc., or polymer chains on the surface of CDs result in an excellent solubility in aqueous solutions, as well as making them convenient for being functionalised with other nanomaterials. The fundamental sp^2 or sp^3 carbon skeleton

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of CDs usually displays amorphous carbon form or graphite lattice that are due to the variation in their degree of carbonisation. In addition, the physico-chemical characteristics of CDs which are easily tunable based on their intended usage or application.

1.4 Classification of Carbon Dots

CDs are mainly classified into graphene quantum dots (GQDs), carbon quantum dots (CQDs), and carbonised polymer dots (CPDs) according to their different formation mechanism, micro or nanostructures, and properties, while associations are built among them by changing the graphene layer and carbonisation degree [13].

GQDs possess one or multiple-layer graphite structures connected chemical groups on the surface or edge or within the interlayer defect [14]. They have obvious graphene lattices and are obtained by "top down" prepared methods by oxide cutting larger graphitised carbon materials such as graphene powder, carbon rods, carbon fibers, carbon nanotubes, carbon black, or graphene oxide into small pieces [15-18]. Their optical properties are mainly dominated by the size of π-conjugated domains and the surface or edge structures. GQDs are anisotropic with lateral dimensions larger than their height, but CQDs and CPDs are typically spherical often produced from small molecules, polymers, or biomass by assembling, polymerisation, crosslinking, and carbonisation via 'bottom-up' methods [19,20]. CQDs exhibit multiple layer graphite structures connected surface groups. Intrinsic state luminescence and the quantum confinement effect of size are their PL mechanism.

CPDs showing aggregated or crosslinked and carbonised polymer hybrid nanostructures, were first put forward in 2018 according to the formation process, structures, and PL mechanism [21]. They possess special 'core-shell' nanostructures consisting of carbon cores less than 20 nm with highly dehydrated crosslinking polymer frames or slight graphitization and shells of abundant functional group or polymer chains, which endows CPDs with higher stability better compatibility, easier modification and functionalization, as well as wider application. In particular, different from the PL feature and mechanism of GQDs and CQDs, the optical properties of CPDs mainly originate from the molecular state and crosslink enhanced emission (CEE) effect which makes the relationship between structure and performance of CPDs more controllable.
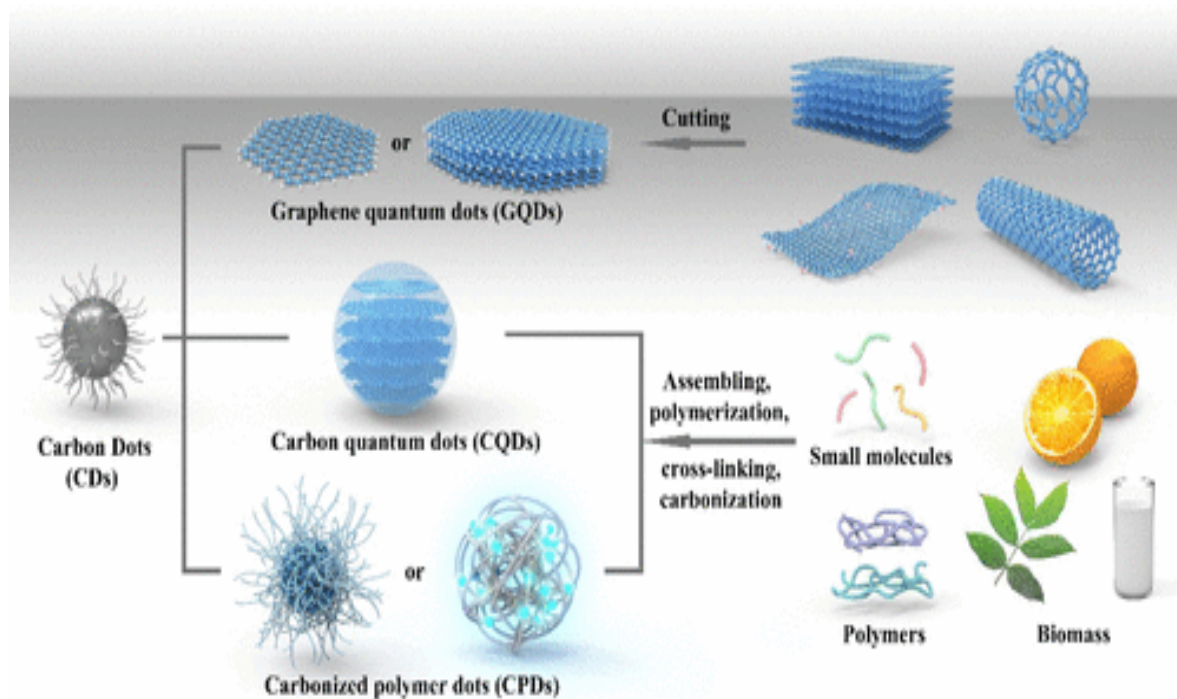
Figure 1.2 : Classification of CDs: including graphene quantum dots (GQDs), carbon quantum dots (CQDs), and carbonized polymer dots (CPDs), and their main preparation approaches [9].

1.5 Properties of Carbon Dots

1.5.1 Absorbance

Carbon dots prepared from different carbon sources or via different synthetic methods always present different absorption behaviours. However, they typically exhibit strong absorption in the ultraviolet (UV) region (200-400 nm), with a tail extending into the visible range, where the absorption bands are assigned to the π-π* transition of the C=C bond or the n-π* transition of the C=O or C=N bond [22-26]. In particular, some CDs with red or NIR emission usually possess π-conjugated electrons in the sp^2 domains or the connected surface groups or polymer chains, which results in their long-wavelength absorption in the range 500-800 nm [27,28]. Therefore, the absorption features of CDs are mainly affected by types and content of surface groups, size of π-conjugated domains, and variation of the oxygen or nitrogen content in carbon cores. Surface engineering modifies the corresponding absorption spectra of CDs that alters their emission spectra also [29]. Doping of heteroatom also regulates absorption spectra for doped CDs by altering % hetero atom because of their

alteration in the π-π* energy level [30]. Surface defects ingrained in CDs are considered to be responsible for broad spectral features in their absorption spectra [31]. Moreover, carbonyl and amino functionalities promotes red shifts of band maxima in UV-visible spectra due to the variations in HOMO-LUMO energy levels of CDs because of fictionalisations [32].

1.5.2 Photoluminescence

Carbon dots show excellent PL behaviours. Appearance of photoluminescent peaks (λem) and their intensities depend on excitation wavelengths (λex). The PL maximum for CDs commonly appears in the blue and green region of the spectra [33]. PL behaviour of CDs is controlled by varying the initial precursors and synthetic methodologies and by surface engineering. Interestingly, PL that generates from carbon dots almost remains unchanged under irradiation for long time [34] compared to organic dyes that are susceptible to photobleaching.

Photoluminescence is one of the most appealing properties of carbon dots from both fundamental and applied perspectives. Compared with other fluorescent materials such as traditional quantum dots (QDs) containing cadmium or lead, rare earth nanomaterials, and organic dyes, carbon dots have advantages of better light stability, higher quantum yield, abundant low cost sources, lower toxicity, and excellent biocompatibility, providing more important applications in diverse fields [9].

The brightness of photoluminescence is quantitatively reflected by the quantum yield value, which is greatly affected by carbon sources, synthetic approaches, and post passivation. In a broad sense, carbon dots prepared by 'top down' routes generally possess a relatively low quantum yield compared with 'bottom up' routes. Thus quantum yield of GQDs is always lower than those of CQDs and CPDs. From the perspective of formation and photoluminescence mechanism of carbon dots, more defects formed during the process of oxide cutting carbon resources result in the lower quantum yield of GQDs [35,36]. However surface modification reduces nonradiative recombination and enhance the integrity of the π-conjugated system of GQDs, which has been widely used to improve the PL behaviours and regulate the band gaps of the GQDs. For CPDs the CEE effect of crosslinked sub-fluorophores, supramolecular interaction, as well as molecular state emission contribute to their higher quantum yield as compared to completely carbonised carbon dots (CQDs and GQDs) [37]. Now, the quantum yield of 99% has been reported for CPDs in solution.

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Abundant carbon sources and diverse synthetic methods promotes the PL behaviour of carbon dots.

There are some differences among the morphology, surface groups, or nanostructures of CPDs, CQDs, or GQDs, while most of them show similar excitation dependent emission, that is the PL emission commonly red-shifts with the increase of excitation wave length. Thus, the PL of carbon dots are easily regulated by controlling the excitation wavelength, without changing their chemical structure or size, which is helpful in multicolour bioimaging [38]. The excitation dependent PL behaviour and broad PL profile of CDs come from the multiple PL centres and wide distribution of different levels. On the other hand, researchers have devoted to carbon dots with narrow bandwidth emission due to their advantages in bioimaging and optoelectronic devices. The purity, uniform size distribution, single PL centre, and simple energy levels play key roles in preparing the narrow emissive carbon dots.

1.5.3 Phosphorescence

Room temperature phosphorescence (RTP), one of the attractive properties of carbon dots, is produced by two critical processes: (1) intersystem crossing (ISC) from the lowest excited singlet state (S_1) to a triplet state (Tn), and (2) radiative transition from the lowest excited triplet state (T_1) to the ground state (S_0) [39].Thus an effective ISC process and suppressed nonradiative decay are critical for generating RTP from organics. It has been proven hat C=O and C=N relevant moieties contribute the production of RTP because of their strong spin-orbit coupling, causing low singlet–triplet splitting energy [40]. Meanwhile, heteroatoms doping like N,P, and halogens can promote the n-π* transition of C=O and C=N, favouring the ISC process. However, in order to produce RTP, matrices such as poly(vinyl alcohol) (PVA) and filter paper are used to fix emissive species, stabilise the produced T_1 species, and prevent the CDs from quenching. For CPDs, RTP are easily achieved through appropriate design without additional matrices, because covalently crosslinked frameworks, polymer chains, supramolecular interactions are beneficial for immobilising their emissive centres, further effectively suppressing the nonradiative transitions [41].Great progress has been made in preparing RTP carbon dots, from embedding CDs in various matrices to matrix free CDs as well as expanding applications in multimodal anticounterfeiting and bioimaging.

1.6 Methods of Synthesis of Carbon Dots

The methods of synthesis of nanomaterials include two approaches, depending on the precursors used: top down and bottom up. In the first category, the 'top down' synthesis, nanoparticles are obtained by reducing the size of macroscopic systems at nanometres. The reduction of particle size is achieved through different physical and chemical methods.

The methods of synthesis of 'top down' are replaced or improved due to imperfections in the structure of the obtained material. This is a major limitation because the surface chemistry and other physical properties of the nanoparticles are strongly dependent on the structure of the material. In addition, they may contain significant amounts of impurities. The particles have relatively wide distribution of different sizes and shapes.

In bottom up approach, the components of atomic or molecular dimensions are assembled together to form nanoparticles. It is much more used for the elaboration of nanoparticles, because it allows to control size. This method is much more versatile, that is why a multitude of nanoparticle synthesis techniques have been developed following the principle of self assembly.

1.6.1 Laser Ablation / Irradiation Method

Laser ablation or irradiation is a single step method that offers various advantages over the traditional chemical synthesis techniques in the production of nanomaterials. The main advantages are the use of fewer chemical precursors and fewer by-products. Rapid production of nanomaterials including carbon dots are achieved by adjusting the parameters of the incident laser beam. Thermal evaporation and explosive spraying are the two processes that are used to obtain nanomaterials by laser ablation. By thermal evaporation, the irradiation of the laser causes the solid target to form a plasma at very high temperatures and pressures of the solid-liquid interface. The plasma expands adiabatically, interacts with the surrounding medium, condenses, and leads to a rapid formation of clusters. The explosive spraying makes the target surface molten, resulting in the formation of nanodroplets upon laser incidence. These nano droplets are then ejected into the liquid to react with the liquid medium on their surface and form nanomaterials [42].

1.6.2 Hydrothermal Method

Hydrothermal methodology is the most promising method in recent years for the synthesis of carbon dots due to their environment friendly, nontoxic, low cost, and easy operational technique. In this method, a solution of organic precursors is sealed in a hydrothermal synthetic reactor where reaction occurs at high temperature and pressure. The hydrothermal method consists of synthesising nanomaterials through hydrolysis reactions at high temperatures of the various compounds directly in an autoclave, that are stainless steel cylinders, which withstands high temperature and pressures for a long time. There is also a group of solvothermal synthesis methods, related to hydrothermal methods, this group of methods are based on the use of organic solvents and supercritical CO_2 [43]. It is possible to obtain the nanoparticles by adjusting the processing conditions, including temperature, pressure, or concentration of precursors. The main parameters of the hydrothermal synthesis, which define both the process kinetics and the properties of the resulting products are the initial pH of the solution, the duration, the temperature of the synthesis and the pressure in the system.

1.6.3 Microwave-Assisted Method

The microwave assisted method is a simple, fast, cost-effective, clean and versatile method with high yields of CDs [44]. During synthesis process, the dipolar moments of polar molecules interacts with the alternate electric and magnetic fields in a solvent to trigger molecular-level heating. Compared with other methods, the microwave assisted method for the synthesis of carbon dots has several advantages. The key advantage is that the material processing involves noncontact heating as energy is generated directly inside the material by molecular interaction with the electromagnetic field. Other advantages are the short reaction time, environmentally friendly nature, energy saving, and uniform selective distribution of the internal energy of the sample to enable a high yield of pure and size regulated carbon dots [45]. Reproducibility, safety, and excellent control of experimental parameters are achieved using this process. Microwave process is critical for reducing the size and increasing the product yield in a short period of time.

1.6.4 Sonochemical Method

The sonochemical method is one of the recently discovered techniques that offers a versatile way to obtain nanostructures for different applications. This represents the use of high intensity ultrasounds, which induce synthesis conditions distinct from other conventional methods. These specific conditions are generated during acoustic cavitation, which produce high temperatures and high pressures in the centre of the bubbles formed in the solution. The bubbles are obtained when the solutions are exposed to high intensity ultrasound. The reaction conditions in the sonochemical method are the speed of noise in the liquid should be in the range 1000-1500 ms^{-1}, the ultrasound wavelength is required between 10 cm and 100 μm, and the frequency range from 20 kHz to 15 MHz.

Two types of materials such as volatile and non-volatile precursors, have been used in the sonochemical method as a raw material for the production of nanomaterials through different reaction mechanisms [46]. If volatile compounds are used as precursors in the sonochemical method, it is called primary sonochemistry, and if non-volatile are used, it is called secondary sonochemistry. In primary sonochemistry, free metal atoms are generated from volatile organometalic compounds, by bond dissociation, facilitated by the high temperature produced during bubble collapse. The obtained atoms are injected into the liquid phase and subsequent nucleation gives rise to nanoparticles of various materials in the presence of a suitable template. In the latter case, the sonochemical reactions occur just outside the balloon, which is subjected to the reaction with the radicals. Radicals diffuse into the liquid, result in reaction and generate nanomaterials.

1.7 Characterisation Techniques of Carbon Dots

1.7.1 UV- Visible Spectroscopy

UV–Visible spectroscopy involves measuring the transmittance or reflectance of a light beam after it passes through or reflected from the sample surface. From transmittance measurements, size, shape information of some nanoparticles etc. are obtained by analysing the absorption peak position and profile in the UV–Vis spectrum. By monitoring absorption peak intensity, one can also determine nanoparticle concentrations [47]. In the case of semiconductor nanomaterials, UV–Vis spectra are directly related to their band gaps. A blue shift in the absorption band edge corresponds to a decrease in particle size whereas a red shift

suggests an increase in particle size. A sample is placed between a light source and a photodetector, and the intensity of a beam of light is measured before and after passing through the sample. The measurements are compared at each wavelength to quantify the sample's wavelength dependent extinction spectrum. The data is typically plotted as extinction as a function of wavelength. Each spectrum is background corrected using a blank to guarantee that spectral features from the solvent are not included in the sample extinction spectrum. Nanoparticles have optical properties that are sensitive to size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface, which makes UV-Vis spectroscopy a valuable tool for identifying characterising, and studying these materials.

1.7.2 Photoluminescence Spectroscopy

Photoluminescence spectroscopy is a contactless, non-destructive method of probing the electronic structure of materials. Light is directed onto a sample, where it is absorbed and imparts excess energy into the material in a process called photoexcitation. This excess energy is dissipated by the sample through the emission of light, or luminescence. In the case of photoexcitation, this luminescence is called photoluminescence. Photoexcitation causes electrons within a material to move into permissible excited states. When these electrons return to their equilibrium states, the excess energy is released and may include the emission of light (a radiative process) or may not (a nonradiative process). The energy of the emitted light relates to the difference in energy levels between the electron states involved in the transition between the excited state and the equilibrium state. The quantity of the emitted light is related to the relative contribution of the radiative process.

1.7.3 Scanning Electron Microscopy

SEM is a surface imaging method in which the incident electron beam scans across the sample surface and interacts with the sample to generate backscattered and secondary electrons that are used to create an image of the sample [48]. From the image, the size distribution, and shape of nanoparticles are obtained. Under an optimum condition, SEM achieve a resolution better than 1 nm, therefore reveals detailed size, shape, and structural nanomaterial information. It provides information about sample's elemental composition, structural variation and morphology. A beam of electrons was formed by the electron source and accelerated toward the specimen using a positive electrical potential. The electron beam

was confined and focused using metal apertures and magnetic lenses into a thin, focused, monochromatic beam. Electrons in the beam interact with the atoms of the specimen, producing signals that contain information about its surface topography, composition and other electrical properties. These interactions and effects are detected and transformed into an image.

1.7.4 Transmission Electron Microscopy

TEM operates in a very similar way to SEM except that it detects the electrons that pass through a given sample. As a result, it runs at much higher energy levels (50-1,000 kV) than SEM (1–30 kV), and the sample must be very thin so that electron beam can pass through the specimen. For an ideal sample, TEM provides a spatial resolution of 0.1 nm, much higher than that for SEM. TEM is the preferred method to directly measure nanoparticle size, grain size, size distribution, and morphology. Imaging, diffraction and microanalytical information are easily produced and then combined to give detailed insights into the properties and behaviour of nanostructured materials. The beam of electrons passes through the specimen and analyses the internal structure of the specimen in the form of images. Under high resolution TEM (HR-TEM), if the sample is oriented along a zone axis, additional information like crystal structures, lattice parameters and growth direction of nanomaterials are also directly obtained [49].

1.7.5 Dynamic Light Scattering

Dynamic Light Scattering (DLS) is a technique that is used to determine the size distribution profile of small particles in suspension or polymers in solution. In the scope of DLS, temporal fluctuations are usually analysed by means of the intensity or photon auto-correlation function (also known as photon correlation spectroscopy or quasi-elastic light scattering). DLS measures the hydrodynamic size of particles, by the mechanism of light scattering from a laser that passes through the colloidal solution and analyses modulation of the intensity of the scattered light as a function of time. Brownian motion of particles corelates with their hydrodynamic diameter. The smaller the particle, the faster it will diffuse than a larger one and the DLS instrument will generate a correlation function that is mathematically linked with particle size and its time-dependent light scattering capacity. DLS has been used to measure the particle size of colloidal samples, to study the stability of formulations and to detect the presence of aggregation or agglomeration.

1.8 Applications of Carbon Dots

1.8.1 Energy Applications

Accelerated industrial development and economic growth caused rapid consumption of fossil fuels, energy shortage, climate change, and environmental degradation, so exploration of renewable, eco friendly, stable and efficient energy conservation and storage technologies is essential and urgent for the global social development. As a newly developing carbon material, CDs promises wide applications in the field of energy such as photo and electrocatalysts, LEDs, supercapacitors, rechargeable batteries, metal-air batteries, and fuel cells, because of their tunable optical properties, low toxicity, low cost, large specific surface area, excellent electron acceptor or donor characteristics, and electronic conductivity.

1.8.1.1 Catalysis

Carbon dots are used as photocatalysts, electrocatalysts, and photo electrocatalysts based on their different structures, properties, or purposes. To make the best of solar energy, a form of renewable and clean energy, photocatalysis has become the noticeable application. Carbon dots are employed as photocatalysts for pollutants degradation, water splitting, CO_2 reduction, and chemical reactions in the process of solar energy conversion, which are benefitted from their broad light absorption range, from UV to NIR, low cost, excellent photostability, charge separation, and transfer capability. Carbon dots with nitrogen atoms located at the edge size of the aromatic domains enable efficient interfacial electron transfer and further lead to an increased photocatalytic hydrogen generation from water [50]. Usually, CDs hybridised with other nanomaterials such as Fe_2O_3, $g-C_3N_4$, serves as the light absorber or electron acceptor in the heterojunction to improve the photocatalytic performance.

The oxygen reduction reaction (ORR), oxygen evolution reaction (OER), and hydrogen evolution reaction (HER) are the important energy conservation processes between chemical energy and electric power. Considering low cost, chemical inertness, high electron mobility, large specific surface area, abundant surface defects, and active sites, CDs have become the strong competitor for the conventional Pt- and Ir or Ru-based electrocatalysts. For pure CDs electrocatalysts, heteroatom doping (B,P,S, or N) is used to modulate electrical structures and electron-coupling interactions, thus enhancing their electrocatalytic performance. In the process of photoelectrochemical (PEC) water splitting and waste water treatment, carbon dots

act both as photosensitizers to capture more solar energy and electron media to promote charge separation in semiconductors.

1.8.1.2 CDs-Based Light-Emitting Diodes (CLEDs)

LEDs, solid state lighting devices for converting electric energy into light, have been a hotspot in academic research for years, aiming at their applications in liquid crystal displays, full colour displays, and illumination devices in our daily life. As a newly developing fluorescent material, carbon dots are expected to replace expensive rare-earth-based phosphors and toxic metalbased semiconductor QDs in LEDs, because of their abundance, adjustable PL colours, low cost, and environmental friendliness. Generally, CDs can act as phosphors in CLEDs or active layers in electroluminescent devices.

In phosphor-based CLEDs, most CDs are homogenously dispersed in matrix materials such as transparent polymers, starch, silica, or even inorganic salts to prevent aggregation-caused quenching of CDs in the solid or powder state resulting from excessive resonance energy transfer or direct π-π interactions. These common methods need multistep procedures, and the introduction of a thick solid matrix layer depress the luminescence from the chip and increase the cost. In recent years, some self-quenching resistant solid state fluorescent CDs were developed from polymers, inorganic salts, or silane coupling agents and directly used as phosphors in CLEDs, which greatly simplified the preparation process and reduced the cost.

1.8.1.3 Solar Cells

CDs are extensively studied in solar cells (SCs), where a small amount added to the bulk heterojunction has been found to improve efficiency in various works due to their unique optical properties, abundant functional groups such as amino, hydroxyl or carboxyl groups and high electron mobility. The roles of carbon dots playing in SCs mainly include (1) facilitating separation of photo generated electron-hole pairs, suppressing carrier recombination, (2) broadening the light absorption range and (3) improving device environmental stability. Like CPDs-based perovskite SCs, CPDs can dramatically enhance long term stability against high humidity and high temperature, convert the harmful UV light into desirable visible light, and promote carrier separation and electron extraction for improving cell efficiency.

1.8.1.4 Supercapacitors

Supercapacitors show fast charge or discharge rates, high power density and long cycle but inferior energy density, which limits their practical application in energy storage. Carbon dots hybridised with other carbon materials, polymers or metal oxides are reported to improve the electrochemical performance of supercapacitors [51]. CPDs possess continuously interconnected core-shell structures, crosslinked networks, abundant functional groups, which facilitate to form a continuous conductive 3D carbon skeleton with large specific surface area and good wettability, and fabricate the stable and multilayer energy storage devices.

1.8.1.5 Rechargeable Batteries

Rechargeable batteries are considered as one of the most effective energy storage technologies to bridge production and consumption of renewable energy. In Li, Na, or K ion batteries, CDs creates an excellent interface for intercalations between electrodes and electrolytes via the surface engineering, provide more active sites for ion insertion and extraction, help to boost the stability, enhance electron or ion transfer and diffusion, and improve electrochemical properties [52].

1.8.2 Biomedical Applications

One of the most promising and frequently reported applications of CDs is in biomedicine. In vitro cytotoxicity studies on a series of cell lines demonstrates the low toxicity or no toxicity and excellent biocompatibilty of CDs even at a high concentration level. In vivo experiments indicate that CDs are rapidly excreted via the kidney or hepatobiliary system. Besides, no remarkable symptoms of inflammation are observed in the brain, heart, lung, liver, spleen, kidney, testicle, and bladder in rats based on blood biochemistry and haematological analysis [53]. Thus CDs are safe for biomedical applications. In addition, low cost, small size, controllable surface functions, high photostability, and high brightness make CDs suitable for biomedical applications. Biomedical applications of CDs include bioimaging, phototherapy, and drug or gene delivery.

1.8.2.1 Bioimaging

Bioimaging is a technique that directly visualize biological events in real-time and non-invasive ways via probes and detectors. Fluorescence imaging, one of the imaging modalities,

has become a powerful approach for clinical diagnosis due to its convenience, low cost, high sensitivity, non invasiveness and long term observation. However conventional fluorophores like QDs and organic dyes suffer from either toxicity concerns or poor fluorescence performance, which provoke the development of CDs. High photostability, excellent biocompatibility, simple synthetic routes, flexible designability, multicolour emission, deep red or NIR emission and two-or multiphoton PL make CDs the next generation fluorescent probes for both in vitro and in vivo bioimaging. Various carbon dots have been used extensively for imaging cells, microorganisms, and plant tissue.

1.8.2.2 Phototherapy

Phototherapy, including photodynamic therapy (PDT) and photothermal therapy (PTT), is a form of non invasive therapeutic treatment that converts the irradiating light into reactive oxygen species and heat with the help of photosensitizers, including local apoptosis of cancer cells. Carbon dots have gained much attention as promising phototherapeutic agents due to their unique optical properties, high water solubility, and high photostability.

1.8.2.3 Drug / Gene Delivery

Aside from anticancer photo therapies, CDs combines with imaging tools of drugs or genes to form imaging-guided nanohybrids for improving the delivery efficiency or offering benefits in the therapeutic strategy. Drug delivery, the safe and efficient treatment, refers to carrying the medicine to a specific location in the body and releasing it in a sustained manner. Thus the controlled drug release and robust selectivity in drug delivery systems are crucial for increasing local therapeutic effects and minimising side effects of non-infectious and non-cancerous tissue. Carbon dots have advantages in visualising drug accumulation and activities at pathological sites via their fluorescent properties, which are vital for estimating therapeutic efficacies of medicines [54].

Different from other therapy technologies, gene therapy is considered as a durable and possibly curative clinical strategy for various diseases such as inherited human diseases and cancers. Effective vectors in gene therapy can deliver genetic materials into cells, and possess high gene transfection efficiency. Viral vectors with the natural ability to invade and deliver their genetic material have served as effective gene carriers. However, severe safety risks based upon their immunogenicity and their oncogenic potential have kept them far from safe

for clinical use. Carbon dots possess low toxicity, abundant functional groups, and excellent biocompatibility. Importantly, the small size of carbon dots contributes to adequate cellular uptake of vectors, enhancing gene transfection efficiency. In addition, their unique fluorescence are used for tracking the internalization of genes [55]. Thus, CDs are attractive as non-viral vectors in gene therapy.

1.8.3 Optical Applications

Based on their low toxicity, good photostability, aqueous dispersibility, strong PL, and phosphorescence properties, CDs are receiving attention in optical applications including sensor and information encryption.

1.8.3.1 Sensors

Carbon dots have been widely used as fluorescent probes for detecting various analytes in the environment or biological systems due to their intrinsic fluorescent properties, high sensitivity, quick response, low cost, and simple preparation methods. The small size, large specific surface area, and abundant surface functional groups make CDs very reactive and sensitive to the surrounding environment such as temperature, ionic strength, and solvent, resulting in changes to their properties, especially optical properties, for instance, the enhancement or activation(turn-on) and quenching (turn-off) of fluorescence. Theoretically, the detection mechanisms mainly include photo-induced electron transfer (PET), fluorescence resonance energy transfer (FRET), and the inner filter effect (IFE). Carbon dots are commonly used to detect cations and anions such as Pt^{2+}, Hg^{2+}, Zn^{2+}, Fe^{3+}, $ONOO^-$, and ClO^- which can bind the surface groups of carbon dots like carboxylate and amino groups through coordination or electrostatic interaction or free radical reaction. Yang's group designed a reversible "off-on" fluorescent nanosensors for selectively detecting ethylenediaminetetraacetic acid (EDTA) and Zn^{2+} [26]. Orange emissive CPDs with pH-sensitive fluorescence immobilized in medical cotton cloth that are used for wound pH monitoring via both fluorescence and visible colorimetric changes [56]. In addition, benefitting from the dual-model response to pH changing as well as the established analytical method, wound pH could be both predicted theoretically and estimated visually in the case of blood contamination and long term observations. Besides supplications in ions and pH sensing, CDs also show selectivity toward a wide range of biomolecules including amino acids (such as cysteine), glutathione, and vitamins such as vitamin B_{12}, vitamin B_7, ascorbic

acid, formaldehyde, glucose, DNA, and proteins which were associated with health issues and diseases. Therefore CDs provides valuable insights into the diagnostics and early precaution of diseases. Because of the low toxicity, excellent biocompatibility, and photostability, CDs also provide promising probes for efficiently targeting and imaging cancer cells, identifying and detecting bacteria by virtue of their nature or modified ligands.

1.8.3.2 Information Encryption

Information encryption is conducive to protecting valuable things from being replicated. Compared with conventional anticounterfeiting technology such as fluorescence printing and plasmonic security labels or materials including organometallic complexes and pure organic compounds with RTP, CDs with RTP provide a smart choice for applications in anticounterfeiting data and encryption due to their environmentally friendly feature, easy handling, facile design , and low cost.

Initially, embedding CDs into various matrices such as PVA, filter paper, crystalline Mn-framework was used to obtain CDs-based RTP materials for anticounterfeiting. However these host matrices only allow specific guest CDs to generate RTP and lack universality. Li et al. reported a universal approach to activate multicolour RTP of CDs in a boric acid matrix for anticounterfeit technology and information encryption [57]. The introduction of B in composites reduces the energy gap between the singlet and triplet state, facilitating the ISC between S_1 and T_1, the formed glassy state which protects the excited triplet state of CDs from nonradiative deactivation.

1.9 Scope of the work

Carbon dots have emerged as most precious gifts in nanotechnology because of their magical properties and applications. Luminescent semiconductor quantum dots have also aroused intensive attention due to their optical properties and promising biomedical applications. However, typical QDs contain heavy metals as necessary elements, which bring severe disease to human beings and damage to the environment. Hence nontoxic and benign quantum dots with similar optical properties are required for practical applications. Carbon dots have emerged and gained much interest as a new class of carbon nanomaterials especially suited for biological applications owing to their characteristic advantages such as non-toxicity, excellent biocompatibility, element abundance, unique optical properties,

outstanding colloidal stability, low cost and easy surface functionalisation. These advantages endow them with great potential applications in different areas like sensing, bioimaging, nano drug, drug delivery, energy, food industry, agriculture, photocatalysis and electrocatalysis. In this work luminescent water soluble carbon dots were synthesised using lemon juice as precursor via low-cost, eco-friendly hydrothermal synthesis method. Synthesised carbon dots showed good luminescence intensity and this property of carbon dots was exploited for sensing applications.

CHAPTER 2

REVIEW OF LITERATURE

Zehui Zhang et al. synthesized highly photoluminescent carbon dots from egg white by one - step hydrothermal strategy. The synthesised CDs with a diameter of 2.1 nm, were well dispersed in aqueous solution, and showed excellent pH stability. CDs have high quantum yield of 61% and were used as a probe for detecting metal ions and in living cell imaging [58]. Shahla Ahmadian et al. synthesised carbon dots from grape fruit, lemon, turmeric extracts by hydrothermal method. Then Fe_3O_4 (magnetite) nanoparticles was achieved using these biocompatible capping agents. Magnetite-carbon dots were synthesised as a novel magnetite and photoluminescent nanocomposite. Carbon dots were used as a non-toxic photoluminescence sensor for detecting of Escherichia coli (E.coli) bacteria[59]. Shoujun Zhu et al. prepared highly photoluminescent carbon dots by hydrothermal method. The reaction was conducted by first condensing citric acid and ethylenediamine, where upon they formed polymer like CDs, which were then carbonised to form the CDs. The chemical structure and PL mechanism were investigated in detail. Synthesised CDs were used for multicolor patterning, bioimaging, and as biosensor reagent capable of detecting Fe^{3+} in biosystems [60].

Zhi Yang et al. synthesised nitrogen-doped, carbon rich, highly photoluminescent carbon dots from ammonium citrate under hydrothermal conditions. The obtained nitrogen doped carbon dots possess bright blue luminescence, short fluorescence life time, pH-sensitivity and excellent stability at a high salt concentration. They have potential to be used for pH sensors, cell imaging, solar cells, and photocatalysis [61]. Gang Huang et al. synthesised photoluminescence CDs from sugarcane molasses derived from industrial waste via simple, environmentally friendly hydrothermal pyrolysis method. The spherical C-dots with a diameter of 1.9 nm emitted blue photoluminescence under UV light at 365 nm and quantum yield was assessed both in vitro and in vivo, demonstrating that the C-dots possessed excellent biocompatibility with MCF-7 cells, red blood cells, BSA and the main organs [62]. Tathagata Pal et al. synthesised multicolour fluorescence carbon dots from curcumin by one-step facile hydrothermal method and was used in vitro and in vivo bioimaging and other applications. The synthesised carbon dots have a size 4-5 nm with high colloidal stability.

The study suggest s that the multifunctional potentiality of CDs serves as high-performance optical nanoprobes and are a suitable alternative for various biolabeling and contrasting agents[63].

Hua Xu et al. synthesised fluorescent carbon dots from aloe for selective detection of tartrazine in food samples. The prepared C-dots were spherical shape with an average diameter of 5nm and emit bright yellow photoluminescence with a quantum yield of approximately 10.37%. The surface of the C-dots was rich in hydroxyl groups and presented various merits including high fluorescent quantum yield, excellent photostability, low toxicity, and satisfactory solubility. One of the widely used synthetic food colorants, tartrazine, could result in a strong fluorescence quenching of the C-dots through a static quenching process. The decrease of fluorescence intensity made it possible to determine tartrazine in the linear range extending from 0.25 to 32.50 μm [64]. Swagatika Sahu et al. synthesised highly photoluminescent carbon dots with a PL quantum yield of 26% from orange juice by one-step hydrothermal treatment. Due to high photostability and low toxicity, these carbon dots are demonstrated as excellent probes in cellular imaging [65]. Jiao Zhou et al. synthesised fluorescent carbon dots using watermelon peel as carbon source. The as prepared carbon dots possess small particle sizes (2.0 nm), strong blue luminescence, acceptable fluorescence life time and good stability in a wide range of pH (2.0 -11.0) and at a high salt concentration. Besides the obtained C-dots have been successfully applied in live cell imaging, indicating these nanoparticles can serve as high performance optical imaging probes [66].

Bui Thi Hoan et al. synthesised carbon dots via hydrothermal method using lemon juices as carbon precursor with different temperatures to control the luminescence of C-dots. The obtained C-dots have strong green light emission and the ability to use its luminescence properties as probes for Mo^{6+} detection application, which is based on Mo^{6+} induced luminescence quenching of C-dots. Thus these CDs have potential application in optoelectronic, labelling and luminescent probing of Mo^{6+} ions [67]. Rajkumar Bandi et al. synthesised highly fluorescent carbon dots with 28% quantum yield by utilising onion waste as a precursor. These CDs exhibited high aqueous dispersibility, excitation dependent fluorescence emission and excellent stability to various effects like pH, high ionic strength and continuous irradiation. Presence of Fe^{3+} ions could result in strong fluorescence quenching, hence these CDs were applied as a fluorescent probe for selective and sensitive

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detection of Fe^{3+} ions. Cytotoxicity studies performed on both normal (HEK-293) and cancerous (HeLa) cells revealed their excellent biocompatibility and they were further employed as fluorescent probes for multicoloured (blue, green and red) imaging of HeLa cells [68]. Hui Ding et al. synthesised red-emitting carbon dots (R-CDs) with high quantum yield of 28% in water by heating ethanol solution of pulp-free lemon juice. The obtained R-CDs were monodispersed with an average diameter of 4.6 nm, exhibited excitation independent emission at 631 nm. Synthesised CDs have low cytotoxicity and good photostability which allow R-CDs to be employed as luminescent probes for in vitro/ in vivo bioimaging. A detailed study on the physical properties and structural compositions of the sodium borohydride ($NaBH_4$) reduced R-CDs with orange emission suggested that surface states on the R-CD surfaces and nitrogen derived structures in the R-CD cores synergistically caused their intense red luminescence [69].

Hui Ding et al. synthesised near-infrared emitting carbon dots with a quantum yield of 31% in water by heating a formamide solution of pulp-free lemon juice. The as-obtained NIR-CDs possess an average size of 5.7 nm and a nitrogen content of approximately 20%, and exhibit excitation-independent photoluminescence emission at 704 nm. Due to good photostability and low cytotoxicity, these NIR-CDs are used as an excellent fluorescent probe for bioimaging[70]. Severino S. Monte-Filho et al. synthesised highly fluorescent carbon dots from lemon and onion juices by one step microwave assisted carbonisation method for determination of riboflavin in multivitamin or mineral supplements. CDs displayed satisfactory optical properties, a high quantum yield of 23.6 % and excellent water solubility, and the particle size was 4.23-8.22 nm with an average diameter of 6.15 nm. An efficient fluorescent resonance energy transfer (FRET) between the CDs and riboflavin was achieved with CDs acting as donor and riboflavin as acceptor [71]. Yue Xu et al. synthesised fluorescent carbon quantum dots (CQDs) by one-step hydrothermal treatment with apple juice as raw material. The result indicated that the fluorescence could be quenched by Hg^{2+} with specificity. Based on this phenomenon, a selective and sensitive sensor was constructed for the detection of Hg^{2+} in phosphate solutions (pH 7.0). The fluorescence intensity showed linear responses with Hg^{2+} concentration ranging from 5.0 to 100.0 nM and 1.0 to 50 μM [72].

Liu Yanan et al. synthesised CDs possessing down and up conversion photoluminescence from carrot juice by hydrothermal treatment. The surface of obtained CDs were highly

covered with hydroxyl groups and nitrogen groups without further modification. The quantum yield of obtained CDs was as high as 5.16%. CD cultured cells exhibited distinguished blue, green, and red colors, respectively, during in vitro imaging when excited by three wavelength lasers under a confocal microscope. It is used for biological applications due to excellent optical properties, biocompatibility, low cytotoxicity, and good cellular imaging capability [73]. Yongli Liu et al. developed selective and sensitive chemo sensor for lead ions using fluorescent carbon dots prepared from chocolate by one-step hydrothermal method. The fluorescent carbon dots exhibited excitation- dependent behaviour with the maximum excitation at 280 nm. This novel chemo sensor demonstrated high sensitivity and selectivity for detection of Pb^{2+} based on the efficient fluorescence quenching effect, which was ascribed to the special chelation between Pb^{2+} and hydroxyl groups on the surface of carbon dots [74]. Chi-Lin Li et al. synthesised photoluminescent carbon dots for the detection of cobalt ions. The C-dots (mean diameter 3.6 ± 0.3 nm) prepared from L-cysteine through a simple hydrothermal process at 300^0C for 2h have a quantum yield of 13.2%. The C dots have strong blue PL with a maximum PL intensity at 395 nm under an excitation wavelength of 325 nm [75].

Meiquin He et al. synthesised fluorescent carbon dots from lemon juice via hydrothermal reaction. The obtained CQDs are with an average size of 3.1 nm. They reveal uniform morphology and well crystalline and generates bright blue-green light emission under UV or blue light irradiation. Fluorescence from these CQDs is mainly induced by the presence of oxygen- containing groups on the surface and edge of the CQDs. CQDs are used for imaging plant cells [76]. Tapas Kumar Mondal et al. synthesised water-soluble, nitrogen- doped, fluorescent carbon quantum dots (NCQDs) from lemon juice and ammonia by hydrothermal treatment. These new NCQDs consist of a ring type moiety (porphyrin/chlorine) in the centre surrounded by the graphitic network and serve as an efficient fluorescent probe for label-free, sensitive, and selective detection of Fe^{3+} [77].

CHAPTER 3

OBJECTIVES OF THE WORK

The important objectives of the present work are,

1. Synthesis of luminescent carbon dots by a wet chemical hydrothermal method from lemon juice.

2. Preparation of boric acid-conjugated carbon dots

3. Optical characterization of the carbon dots using UV-Visible and Photoluminescence spectroscopy.

4. Morphological characterization of the carbon dots using Dynamic Light Scattering and High Resolution Transmission Electron Microscopy.

5. Detection of Glucose using boric acid-conjugated carbon dots

6. Antibacterial study of the synthesized carbon dots.

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

- Lemon juice
- 0.5 M Boric acid
- Glucose
- Distilled water

4.2 Experimental Methods

4.2.1 Synthesis of Luminescent Carbon Dots from Lemon Juice

Carbon dots were synthesised from lemon juice by hydrothermal method using a thermal magnetic stirrer. 50ml of filtered lemon juice was taken in a 250 ml beaker. The beaker was placed on the magnetic stirrer and heated until a yellowish-brown colour was produced. The beaker was cooled to room temperature and the solution was centrifuged at 10000 rpm for ten minutes to discard large molecules. Appearance of carbon dots in UV light is depicted in **Figure 4.2**.

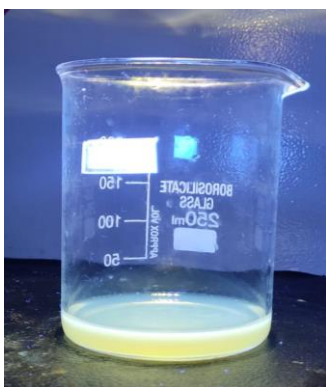**Figure 4.1 : Schematic representation of synthesis of carbon dots from lemon juice.**

Figure 4.2 : Carbon dots under UV light

4.2.2 Preparation of 0.5 M Boric Acid

About 0.30915 g of boric acid was accurately weighed and dissolved in 10 ml distilled water.

4.2.3 Preparation of Boric Acid-Conjugated Carbon Dots

5 ml of Carbon dot solution and 2ml of 0.5M boric acid was mixed in a beaker using a magnetic stirrer for 2 minutes.

4.2.4 Characterization Techniques

Optical properties are characterised by using UV-Visible spectroscopy and Photoluminescence spectroscopy. Charge and size of the carbon dots are characterised by Dynamic Light Scattering (DLS). Particle morphologies were investigated using High Resolution Transmission Electron Microscope. The UV visible spectroscopic studies were done using Perkin Elmer Lambda 365 UV-Visible spectrophotometer. The room temperature luminescence measurements of CDs were studied using JascoFP-750 spectrophotometer at medium sensitivity. Dynamic Light Scattering (DLS) measurements were carried out in HORIBA Scientific nanoparticles analyser SZ-100.

4.2.5 pH Studies

pH value of the Carbon dots solution was determined using a digital pH meter

4.2.6 Detection of Glucose

3 ml boric acid-conjugated carbon dot solution was taken in a cuvette and different concentration glucose was added and photoluminescence intensity was measured.

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4.2.7 Antibacterial Studies

Antibacterial assay of the synthesised carbon dots were assessed using agar well diffusion method. Gram positive organism Staphylococcus aureus was used as the test pathogens for the study of antibacterial properties of carbon dots. Agar well diffusion method is widely used to evaluate the antimicrobial activity of the test sample. Mueller-Hinton agar (15-20 mL) was poured on glass Petri plates of same size and allowed to solidify. Standardized inoculum of the test organism was uniformly spread on the surface of the plates using sterile cotton swab. Four wells with a diameter of 8 mm (20 mm apart from one another) were punched aseptically with a sterile cork borer in each plate. The test sample (50 and 100 μL) was added into the wells T1 and T2 directly from sample. Gentamycin (40μl from 4 mg/ml stock) and the solvent used for sample dilution were added as positive and negative control respectively. The plates were incubated for 24 h at 36ºC ± 1ºC, under aerobic conditions. After incubation, the plates were observed and the zone of bacterial growth inhibition around the wells was measured in mm.

Culture media Details:

Muller Hinton Agar medium (HIMEDIA- M173) is used for determination of susceptibility of microorganisms to antimicrobial agents. Suspended 38 grams in 1000 ml distilled water. Heated until it boils to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cooled to 45-50°C. Mixed well and poured into sterile petri plates.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 UV - Visible Absorption Studies

In understanding the behaviour of carbon dots, optical absorption studies have an important role. UV –Visible absorption studies of the synthesized carbon dots is depicted in **Figure 5.1**. It was found that there was an absorption peak positioned at around 400nm. The absorption peak at 400nm originates from the n-π * transition of C=O bonds.
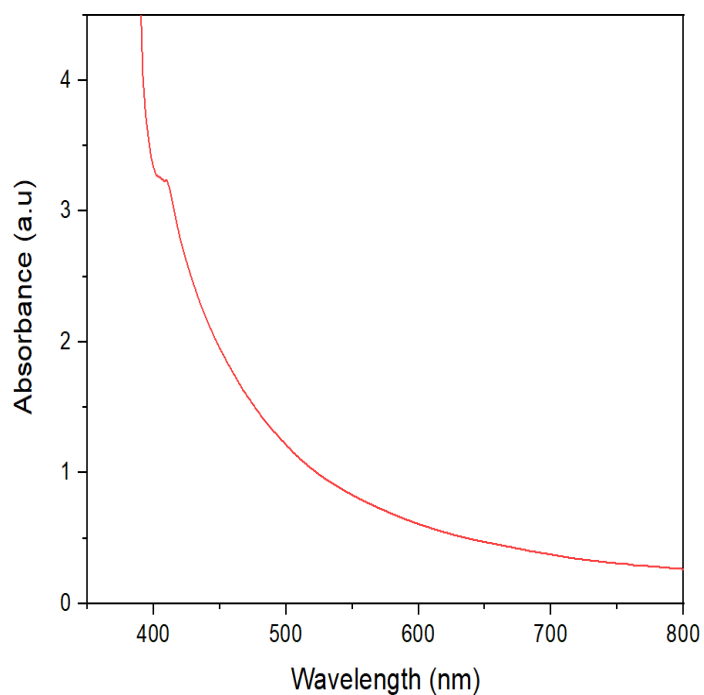

Figure 5.1. UV-Visible absorption spectra of carbon dots from lemon juice

5.2 Photoluminescence Studies

The luminescence property of the synthesized carbon dots and boric acid-conjugated carbon dots were studied by photoluminescence spectroscopy. The intensity of PL depends on the number of particles excited at a particular wavelength. The highest PL intensity was observed at an excitation wavelength of 240nm, because of the fact that the largest number of particles being excited at 240nm. From the data, it was found that the synthesized carbon dots show considerable photoluminescence properties with a band centered at around 620nm which is the characteristic emission band of carbon dots.
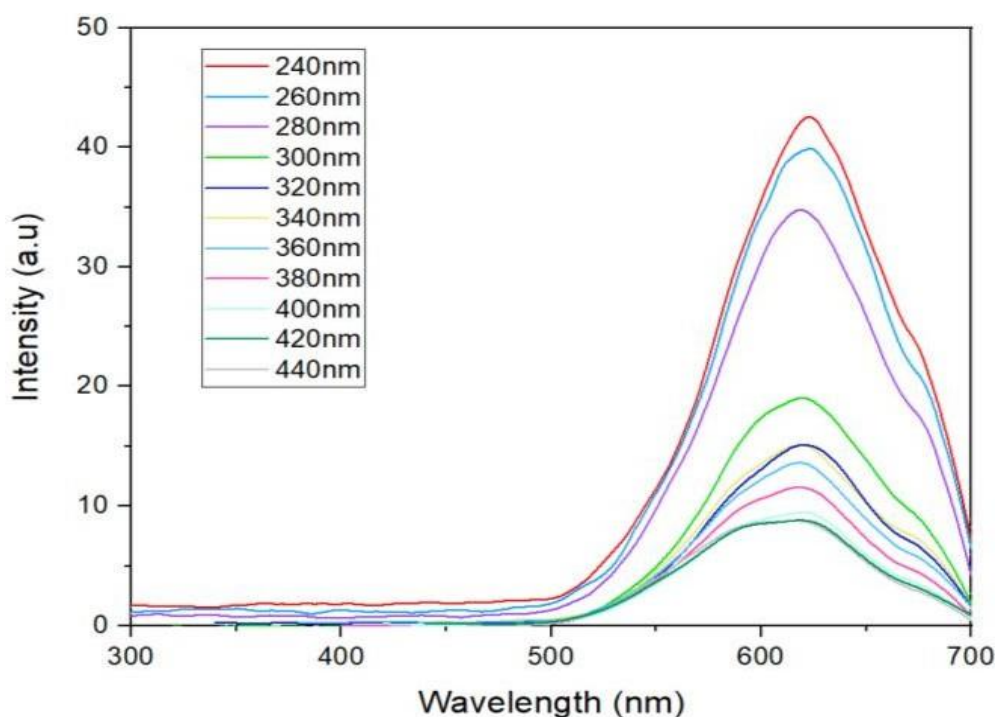

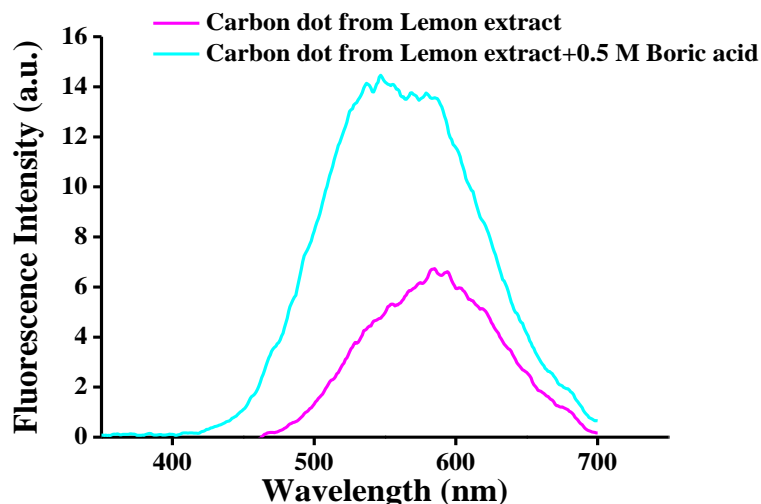**Figure 5.2 :Emission spectra of carbon dots from lemon juice at different excitation wavelengths**

Figure5.3:Emission spectra of carbon dots alone and carbon dots coated with boric acid

The carbon dots synthesised from lemon juice was capped with 0.5 M boric acid and the photoluminescence of two samples were analysed. It was found that upon capping the luminescence intensity increased which is attributed to the fact that capping provides surface passivation and minimize electronic trapping capabilities of surface defects, resulting in higher photoluminescence intensity.

5.3 pH Studies

pH of the synthesised carbon dots was determined. pH of carbon dot solution was found to be 5.94. When few drops of NaOH solution was added, pH increased to 6.18.
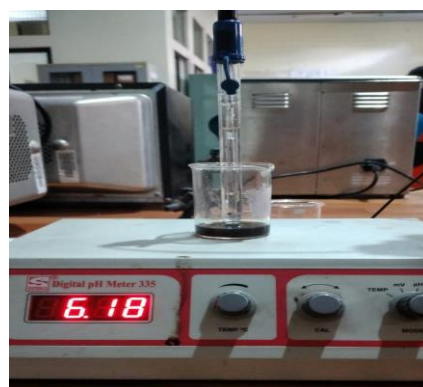

Figure 5.4 :(a) pH of synthesised carbon dot and (b) pH of carbon dots with few drops of NaOH

5.4 Dynamic Light Scattering Studies

Dynamic light scattering (DLS), which is also referred to as photon correlation spectroscopy (PCS) or quasi-elastic light scattering, is used to determine the nanoparticle size and to analyze the zeta potential. It determines particle size distribution, particle zeta potential (related to the magnitude of the electrical charge at the particle surface) and molecular weight of large polymeric substances dispersed in water. From the study of dynamic light Scattering, the size distribution properties of the synthesized carbon dots and boric acid- conjugated carbon dots were analysed. Hydrodynamic diameter of carbon dots alone and boric acid conjugated carbon dots were found to be near 100nm shown in **figure 5.5** and **figure 5.6**.

Zeta potential of bare carbon dots and boric acid-conjugated carbon dots were recorded, both shows slightly positive potential of 0.3 and 0.7 eV respectively which is depicted in **figure 5.7** and **figure 5.8** that indicates the positive surface charge of carbon dots.
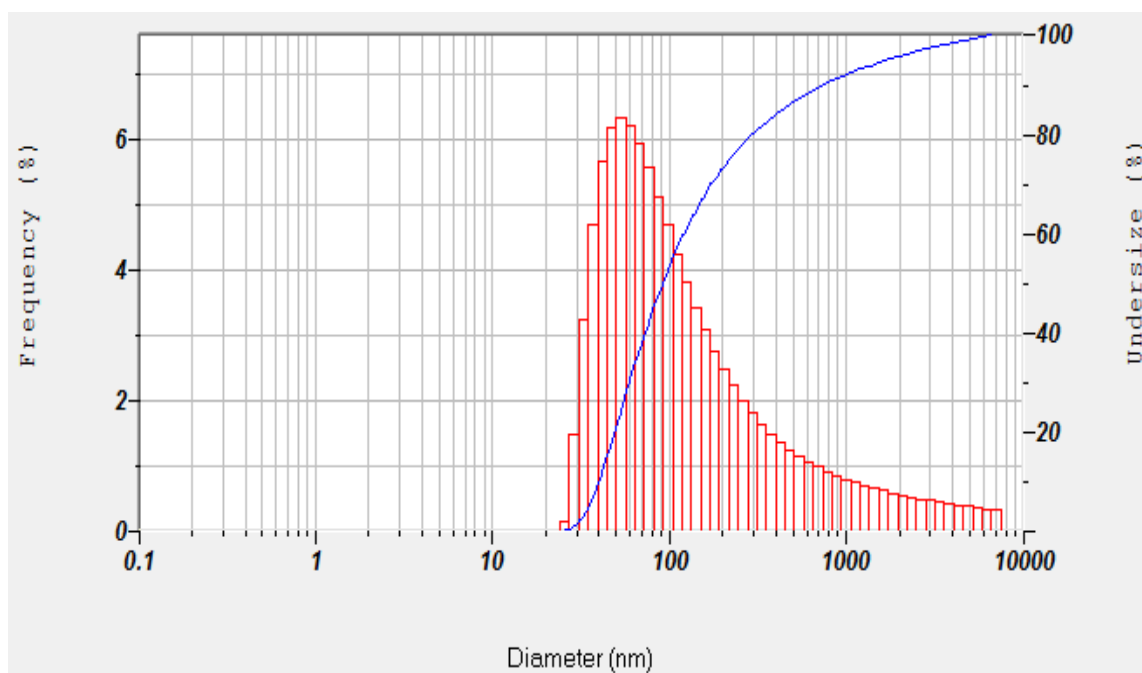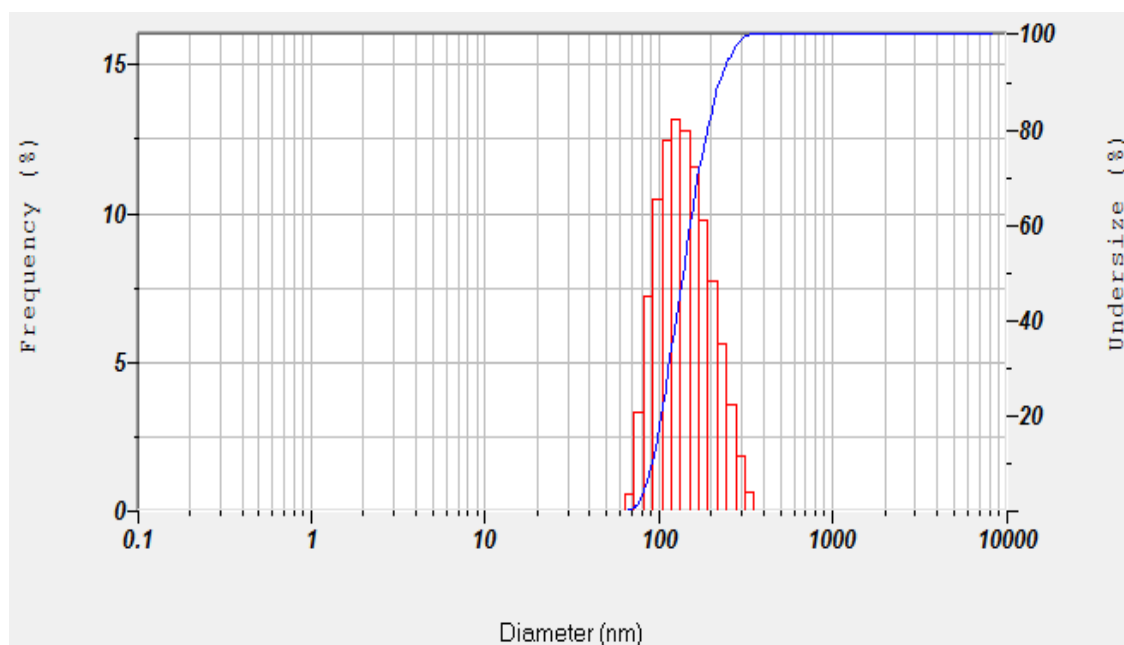

Figure 5.5 : Particle size of Carbon dots alone

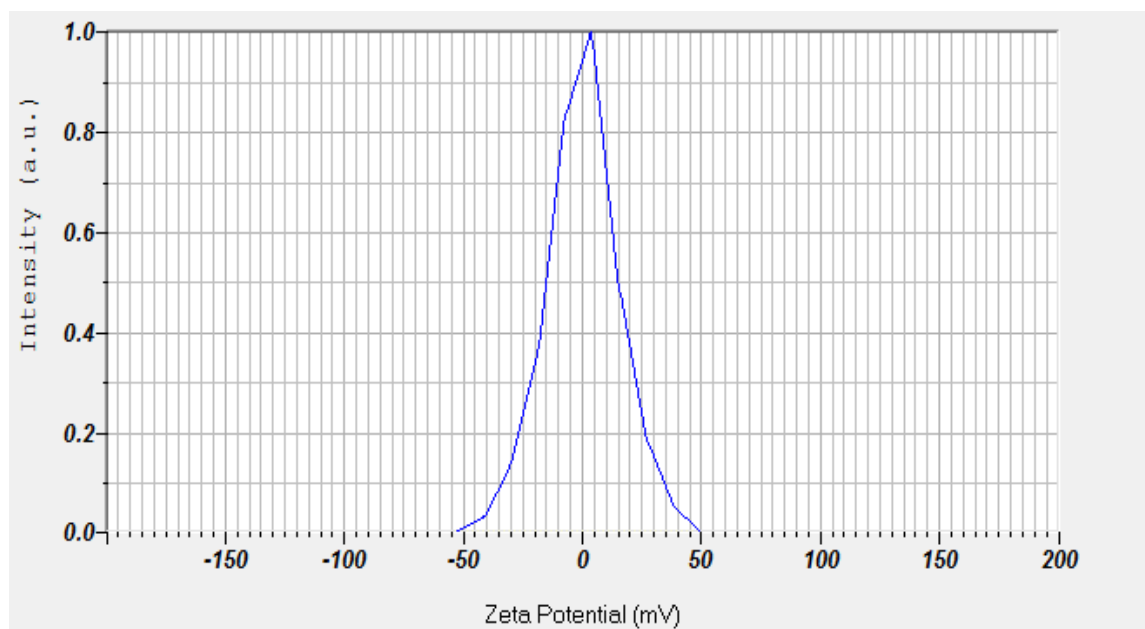**Figure 5.6: Particle size of boric acid-conjugated carbon dots**

Figure 5.7: Zeta potential graph of Carbon dots alone
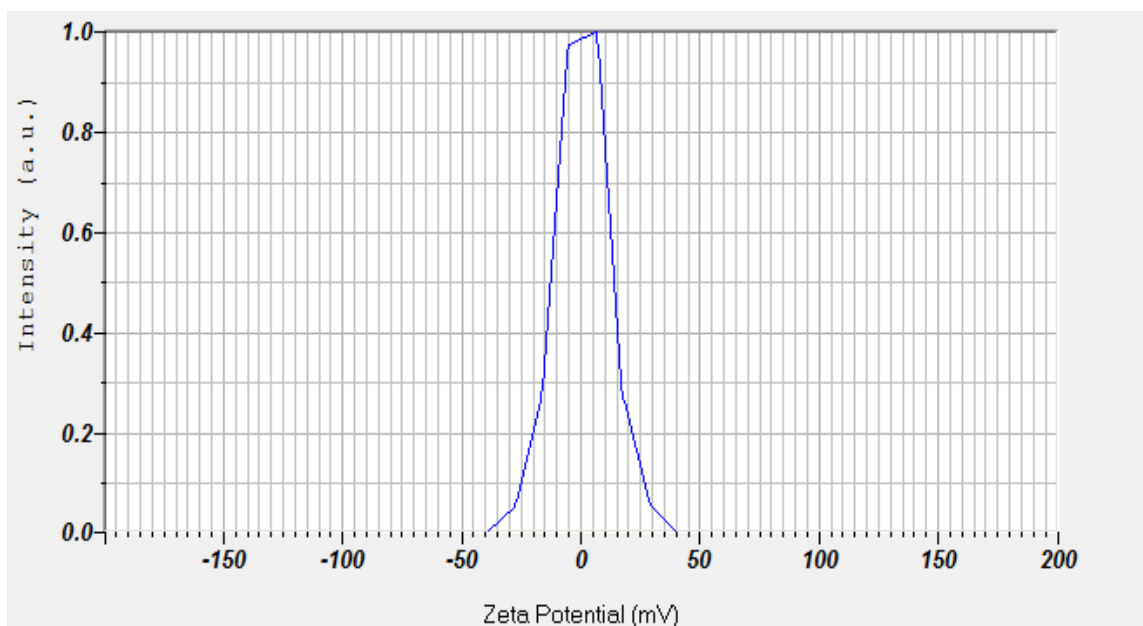
Figure 5.8 : Zeta potential graph of boric acid-conjugated Carbon dots

5.5 High Resolution Transmission Electron Microscopy

The morphology of carbon dots was characterized in terms of shape and particle size using Transmission Electron Microscope. The HR TEM image of carbon dots synthesised from lemon juice is depicted in **Figure 5.9**. Average particle size was found to be 10 nm.
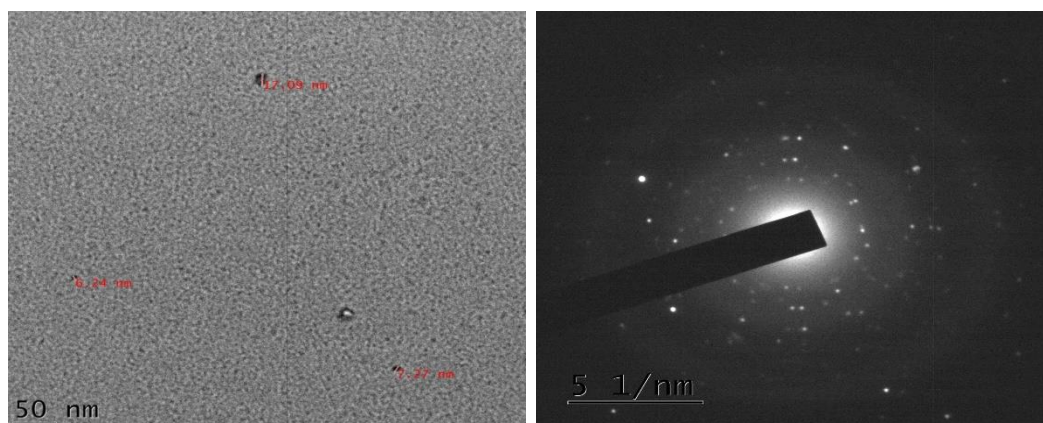
Figure 5.9: HR-TEM image and SAED pattern of carbon dots synthesised from lemon juice.

5.6 Detection of Glucose
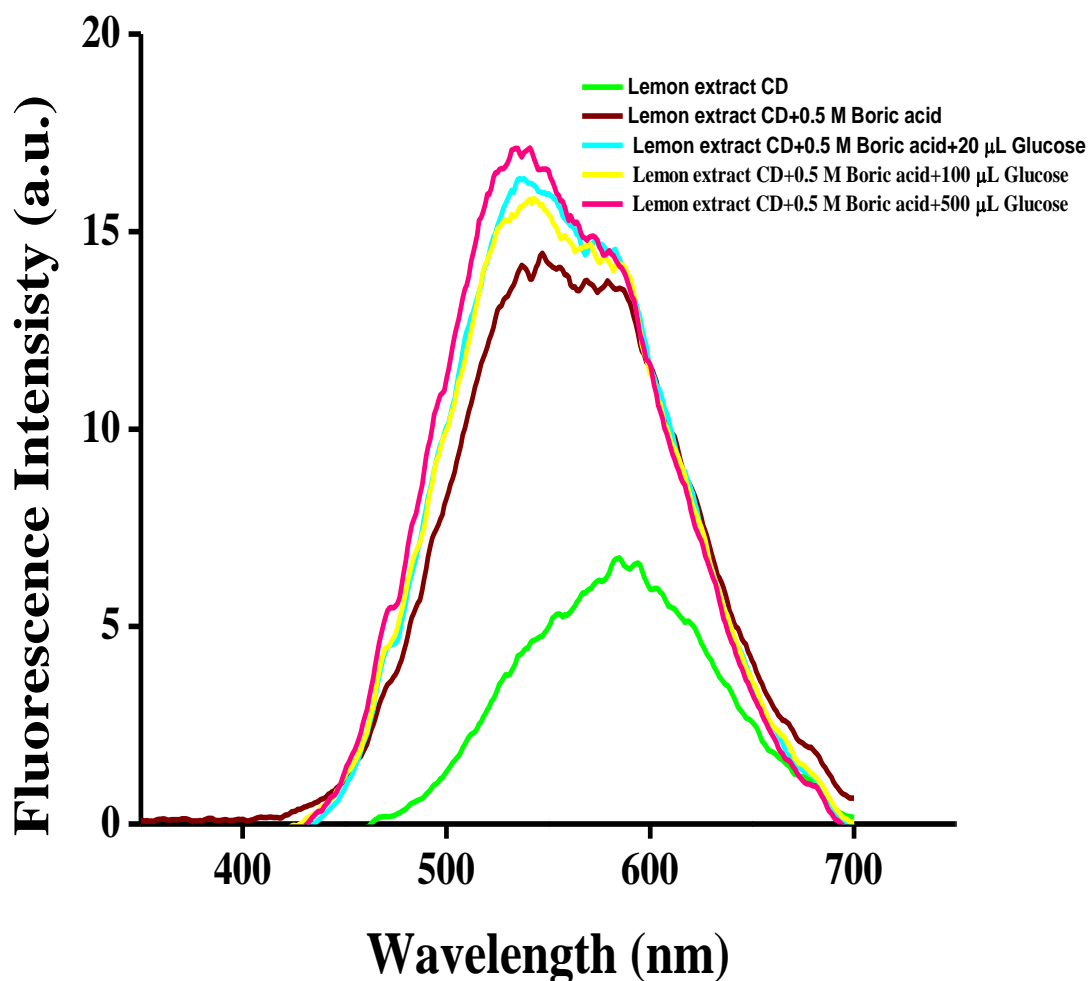
Figure 5.10 : The photoluminescence emission spectra of boric acid conjugated carbon dots in the presence of different concentration of glucose.

Glucose is an important biomarker indicating diabetics and related physiological conditions. Boric acid conjugated-carbon dots synthesized from lemon juice shows good luminescence intensity and was utilized for the sensing of glucose. The stock solution of glucose (0.1 M) was prepared. Solutions of diluted concentration of glucose (0.0001 M) was then prepared by

diluting the stock solution using distilled water. To 3mL of boric acid-conjugated carbon dots taken in a cuvette, varying amount of glucose was added for various experiments. The solutions were mixed and was shaken for 3 seconds and then excited at 240 nm. The corresponding emission values during each addition were recorded. The gradual addition of increasing concentration of glucose solution to boric acid capped carbon dots, the luminescence intensity was found to be increasing. It is due to the aggregation of carbon dots with glucose which is in turn due to the formation of complex between boric acid conjugated carbon dots and glucose [78].

5.7 Antibacterial Studies

The antibacterial activities of carbon dots against gram positive bacteria Staphylococcus aureus was analysed. **Table 5.1** gives the antibacterial activity of synthesised carbon dots. From the data it was clear that the carbon dots exhibit good anti-bacterial activity against gram positive bacteria Staphylococcus aureus. The zone of inhibition of the synthesised carbon dots is depicted in **Figure 5.11.**

Microorganism	Zone of inhibition (mm)		
Staphylococcus aureus	Standard Gentamycin (160mcg)	T1(50μl from10mg/ml)	T2(100μl from 10mg/ml)
	20 mm	+ve (17 mm)	+ve (22 mm)

Table 5.1: Antibacterial activity of carbon dots
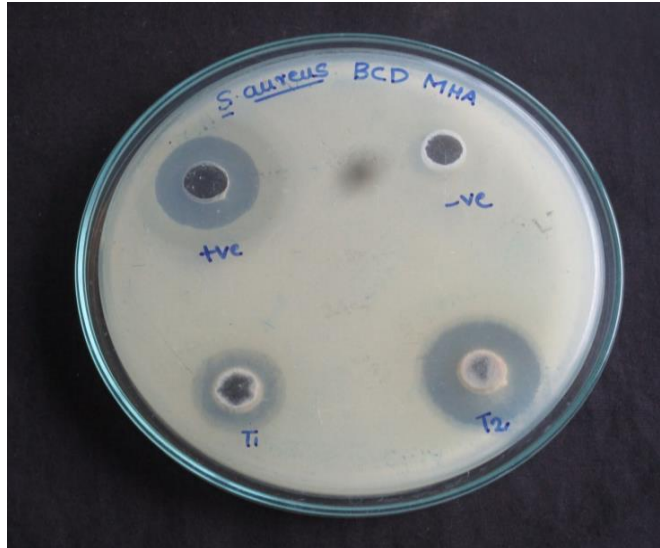
Figure 5.11: The zone of inhibition of the synthesised carbon dots by using Staphylococcus aureus

CHAPTER 6

CONCLUSION

Luminescent carbon dots were successfully synthesised from lemon juice by hydrothermal method. Then, the synthesised carbon dots were conjugated with 0.5M boric acid solution. The carbon dots were characterised by UV-Visible absorption spectroscopy, photoluminescence spectroscopy, dynamic light scattering and high resolution transmission electron microscopy. pH of the synthesised carbon dots were also measured. Antibacterial properties were studied by agar well diffusion method. In the UV-Visible spectra, an absorption peak was observed at 400 nm which originates from n-π^* transition of C=O bond. The carbon dot solution changes from brown colour to yellow after excitation by UV light. From the photoluminescence spectra, highest PL intensity was observed at an excitation wavelength of 240 nm. The emission peak was found to be at 620 nm. Upon capping carbon dots with 0.5 M boric acid, luminescence intensity increased, which is attributed to the fact that capping provides surface passivation and minimise electronic trapping capabilities of surface defects, resulting in high photoluminescence intensity. From the DLS data, Zeta potential of bare carbon dots and boric acid-conjugated carbon dots was found to be 0.3 and 0.7 eV respectively which indicates positive surface charge of carbon dots. The average hydrodynamic diameter of carbon dots and boric acid-conjugated carbon dots were found to be around 100nm.From high resolution transmission electron microscopy, morphology of carbon dots were analysed and particle size was found to be 10nm. Boric acid-conjugated carbon dots exhibits good luminescence intensity and was utilised for sensing of glucose. Upon glucose addition, luminescence intensity increases due to aggregation of carbon dots with glucose which is in turn due to complexation between boric acid-conjugated carbon dots and glucose. From the antibacterial studies, it was clear that the carbon dots exhibit significant antibacterial activity against gram positive bacteria Staphylococcus aureus. These results suggest that the synthesised carbon dots have potential application in sensing, bioimaging and many other fields.

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