

# **SYNTHESIS AND CHARACTERISATION OF CARBON DOTS FROM ORANGE JUICE**

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## **ABSTRACT**

Carbon dots find a wide variety of applications in bioimaging, biosensing, drug delivery, explosive detection, food safety, energy conversion, photocatalysis etc, because of their outstanding chemical and physical properties together with biocompatibilities. In this work, carbon dots were synthesized from orange juice extract by a wet chemical method method. The optical and morphological properties of synthesized carbon dots were characterized by UV-visible spectroscopy, Photoluminescence spectroscopy, Dynamic Light Scattering and Transmission Electron Microscopy. Antimicrobial properties of carbon dots were studied by Diffusion Assay Method. The synthesised carbon dots was successfully conjugated with boric acid and was utilized for the detection of glucose. The luminescence intensity of carbon dots was found to be increasing upon glucose addition which was due to the formation of complex between the boric acid conjugated carbon dots and glucose. In the present work, photoluminescent, cost effective and biocompatible carbon dots were synthesised which are promising candidates for various optical and biological applications.

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# CHAPTER 1

## INTRODUCTION

### 1.1 NANOSCIENCE AND NANOTECHNOLOGY

**Nanoscience** is the study, manipulation and engineering of matter, particles and structures on the nanometer scale (one millionth of a millimeter, the scale of atoms and molecules). Important properties of materials, such as the electrical, optical, thermal and mechanical properties, are determined by the way molecules and atoms assemble on nanoscale into larger structures. Moreover, in nanometer size structures these properties are often different than on macroscale, because quantum mechanical effects become most important. Nanoscience is an emerging area of science which concerns itself with the study of materials that have very small dimensions.

**Nanotechnology** is the application of nanoscience leading to the use of nanomaterials and nano size components in useful products[1]. Nanotechnology provides the ability to design custom-made materials and products with new enhanced properties, new nano electronics components, new types of “smart” medicines and sensors, and even interfaces between electronics and biological systems. Nanotechnology is now identified as an area related to research and technology development at the atomic, molecular and macromolecular levels. It is an important field of modern research dealing with synthesis, strategy and manipulation of particle’s structure ranging from approximately 1 to 100 nm in size [2]. The concept of nanotechnology was first described by renowned physicist Richard Feynman in his talk ‘There's Plenty of Room at the Bottom.’[3]

Nanoscience and nanotechnology are being envisaged as the science and technology of the future. They are being expected to transform understanding of most of the natural phenomenon. Nanotechnology was predicted to revolutionize every aspect of human life. Nanotechnology as defined by size is naturally broad, including fields of science as diverse as surface science, molecular biology, organic chemistry, molecular engineering etc. It is a field of research and innovation concerned with building 'things' generally, materials and devices - on the scale of atoms and molecules. A nanometer is one billionth of a meter ie, ten times the diameter of a hydrogen atom. The diameter of a human hair is on average, 80,000 nanometers. At such scales, the ordinary rules of physics and chemistry no longer apply. The material characteristics, such

as their color, strength, conductivity and reactivity, differs substantially between the nanoscale and the macro. Carbon 'nanotubes' are 100 times stronger than steel but six times lighter. Nanotechnology is hailed as having the potential to increase the efficiency of energy consumption, help to clean the environment, and solve major health problems. It is able to massively increase manufacturing production at significantly reduced costs. Products of nanotechnology are smaller, cheaper, lighter yet more functional and requires less energy and fewer raw materials to manufacture.

## **1.2 CLASSIFICATION OF NANOMATERIAL**

Nanomaterials are classified into different types on the basis of morphology, size and physical and chemical properties. On the basis of dimensions nanomaterials are classified as zero dimensional, one dimensional, two dimensional and three dimensional nanomaterials.

### **1.2.1 ZERO DIMENSIONAL NANOMATERIAL**

Zero dimensional materials in all the dimensions are measured within the nanoscale. The most common representation of nanomaterials are nanoparticles. Nanoparticles are amorphous or crystalline. It is single crystalline or polycrystalline. It composed of single or multichemical elements. It exhibits various shapes and forms[4]. They exist individually or incorporated into a matrix. They are metallic, ceramic or polymeric which includes graphene quantum dots, carbon quantum dots, inorganic quantum dots, fullerenes etc. A major feature that discriminates various types of nanostructures is their dimensionality. The word “nano” stems from the Greek word “nanos”, which means “dwarf”. This word “nano” has been assigned to indicate the number  $10^{-9}$ , i.e., one billionth of any unit. A rich variety of physical and chemical methods have been developed for fabricating zero dimensional nanomaterials with well-controlled dimensions. Recently, zero dimensional nanomaterials such as uniform particles arrays (quantum dots), heterogeneous particles arrays, core shell quantum dots are synthesized by various methods.

### **1.2.2 ONE DIMENSIONAL NANOMATERIALS.**

In this type of nanomaterials, one dimension is outside the nanoscale. This leads to needle like-shaped nanomaterials. They include nanotubes, nano rods, and nanowires. They are amorphous or crystalline, single crystalline or polycrystalline [5]. They are metallic, ceramic, or polymeric. The smallest possible crystalline wires with cross-section as small as a single atom engineered



in cylindrical confinement. Carbon nanotubes, a natural semi-one dimensional nanostructure, are used as a template for synthesis. Confinement provides mechanical stabilization and prevents linear atomic chains from disintegration; other structures of one dimensional nanowires are predicted to be mechanically stable even upon isolation from the templates.

### **1.2.3 TWO DIMENSIONAL NANOMATERIALS**

Here two of the dimensions are not confined to the nanoscale. Two dimensional nanomaterials exhibit plate like shapes. They include nanofilms, nanolayers, and nanocoatings. They are amorphous or crystalline, various chemical compositions and are used as a single layer or multilayer structures [6]. Metallic, ceramic, or polymeric. Two dimensional materials are crystalline consisting of a two-dimensional single layer of atoms. The most important representative graphene was discovered in 2004. Thin films with nanoscale thicknesses are considered nanostructures, but are sometimes not considered nanomaterials because they do not exist separately from the substrate.

### **1.2.4 THREE DIMENSIONAL NANOMATERIALS**

Bulk nanomaterials are materials that are not confined to nanoscale in any dimension. These materials are thus characterized by having three arbitrary dimensions above 100 nm. In terms of nanoscale structures, bulk nanomaterials are composed of a multiple arrangement of nanosized crystals, most typically in different orientation[7]. With respect to the presence of features at the nanoscale, three dimensional nanomaterials contains dispersion of nanoparticles, bundles of nanowire and nanotube as well as multinanolayers. Box-shaped graphene (BSG) nanostructure is an example of three dimensional nanomaterial. This nanostructure is a multilayer system of parallel hollow nanochannels located along the surface and having quadrangular cross-section. The thickness of the channel walls is approximately equal to 1 nm. The typical width of channel facets makes about 25 nm.

## **1.3 PROPERTIES OF NANOMATERIALS.**

Nanomaterials have various properties such as optical, magnetic and electronic properties.

### **1.3.1 OPTICAL PROPERTIES.**

The colour of a material is determined by the wavelength of light absorbed and emitted by it. Nanomaterials of different sizes have different electronic structures and different energy level

separation[8]. Light induced transition between these levels determines the colour of the materials. Smaller nanoparticles are blue in colour and larger are red in colour. Thus nanoparticles are made to emit or absorb specific wavelength of light merely by controlling their size. Nanomaterials having small particle sizes exhibit enhanced optical emission as well as nonlinear optical properties due to the quantum confinement effect. Synthesis, characterization, and measurement of optical properties of nanomaterials with different anisotropic shapes have also drawn significant attention.

### **1.3.2 ELECTRONIC PROPERTIES**

Nanomaterials of different sizes have different electronic structures and different energy level separation. The electrical conductivity of nanotubes and nanorods are very high due to electron confinement to particular direction. Nanotechnology in electronics provides faster, smaller and enhanced handheld devices. It provides advanced display technologies with conductive nanomaterials, data storage, quantum computing, printable and flexible electronics, and magnetic nanoparticles for data storage.

### **1.3.3 MECHANICAL PROPERTIES**

Mechanical strength, toughness and Young's modulus of the nanomaterials are very high. Carbon nanotubes (CNTs) have attracted significant research attention in the field of nanomaterials ever since it was discovered. CNTs possess excellent mechanical qualities specifically high mechanical strength. This has resulted in its diverse applications in real world such as space elevator, better solar cells, faster computer chips, thinner electronic devices, faster flywheels, etc. The excellent mechanical properties of CNTs are one of the major incentives for studying the mechanical properties of nanoscale materials[9]. Numerous studies have been undertaken to characterize the mechanical properties of CNTs. Laboratory experiments suggest that CNTs have high strengthened elastic modulus. Young's modulus of CNTs are determined by directly measuring the thermal vibrations. It was found that the CNTs possess very high Young's modulus and excellent mechanical properties which make them ideal for fabricating nanoscale fibers for lightweight composites.

### **1.3.4 MAGNETIC PROPERTIES**

Nanostructures exhibit excellent magnetic properties. In nanostructures the magnetic moment of each atoms will interact with the magnetic moments of the other atoms. Hence all the

magnetic moments align in one direction with respect to some symmetry axis of the cluster[10]. The magnetic nanoparticles are used in a range of applications like imaging and bio processing. The large surface area to volume ratio results in developing a proportion of atoms having different magnetic coupling with neighboring atoms leading to differing magnetic properties. The properties of magnetic nanoparticles depends on the synthesis method and chemical structure. In most cases, the magnetic nanoparticles range from 1 to 100 nm in size and exhibits superparamagnetism [11]. Superparamagnetism is caused by thermal effects , the thermal fluctuations are strong enough to spontaneously demagnetize a previously saturated assembly; therefore, these particles have zero coercivity and have no hysteresis. In this state, an external magnetic field is able to magnetize the nanoparticles with much larger magnetic susceptibility. When the field is removed, magnetic nanoparticles exhibit no magnetization. These properties are utilized for controlled therapy and targeted drug delivery.

#### **1.4 METHODS OF SYNTHESIS OF NANOMATERIALS**

Two prevalent strategies adopted for the formation of nanoparticles are bottom-up and top-down. Nanomaterials are made by either cutting down macro structures to the nano scale, top-down approach or by amassing structures from particles and atoms, bottom-up approach [12]. The bottom-up approach is more advantageous than the top-down approach since it has better chance of creating nanostructures with less defects.

##### **1.4.1 BOTTOM-UP APPROACH**

The bottom-up approach is an investment strategy that depends on the individual stocks. In this approach, materials and devices are built from molecular components which assemble themselves chemically by principles of molecular recognition [10]. Bottom up approach alludes to the buildup of a material from the bottom ie molecule by atom, atom by particle or cluster by cluster.

##### **1.4.2 TOP-DOWN APPROACH**

In the top-down approach, nano objects are constructed from larger entities . In this method, breaking up larger particles by the utilize of physical forms like pulverizing, processing or grinding. This method creates smaller devices by using larger ones to direct their assembly. Many technologies that descended from conventional solid state silicon methods for fabricating microprocessors are now capable of creating features smaller than 100 nm.

### **1.4.3 MICROWAVE METHOD**

Microwave Irradiation (MWI) method provides simple and fast routes to the synthesis of nanomaterials since no high temperature and high pressure are needed. By using metal precursors that have large microwave absorption cross-sections relative to the solvent, very high effective reaction temperatures are achieved [13]. The material properties of nanomaterials are dependent on their size. Depending on the field of application, it was therefore essential to have the narrowest and most exact size distribution possible in order to obtain nanoparticles with defined properties. In recent years, microwave-assisted synthesis have established itself as a modern way of producing different nanomaterials in order to effectively influence both size distribution and material properties. Using microwave irradiation it was possible to synthesize nanoparticles with exact parameter control in a short time and also change the particle properties and particle size as required. The fields of application for these produced nanoparticles range from medical uses (drug delivery systems, formulations) to use in many industries, i.e. vehicle manufacturing (coatings, windshields, energy storage), cosmetics (sun protection, shampoo, toothpaste), textile production (outdoor clothing, shoes) and electronics (circuit boards, solar cells, LEDs, touchscreens).

### **1.4.4 HYDROTHERMAL METHOD**

This method is regularly carried out in an autoclave with the reaction in aqueous solution. The temperature within the autoclave are raised over the boiling point of water, reaching the pressure of vapour saturation. Hydrothermal synthesis are defined as a method of synthesis single crystals that depends on the solubility of minerals in hot water under high pressure. The crystal growth is performed in an apparatus consisting of a steel pressure vessel called an autoclave, in which the nutrient is supplied along with water[14]. A temperature gradient is maintained between the opposite ends of the growth chamber. At the hotter end the nutrient solute dissolves, while at the cooler end it is deposited on a seed crystal, growing the desired crystals.

### **1.4.5 MECHANICAL GRINDING**

Mechanical grinding is a top down synthesis, where the materials are prepared not by cluster assembly but by the structural decomposition of coarser- grained structures as a result of severe plastic deformation[15].This has become an important method to make nanocrystalline materials because of its simplicity, the relatively inexpensive equipment needed, and the

applicability to essentially the synthesis of all classes of materials. Mechanical milling is typically achieved using high energy shaker, planetary ball or tumbler mills. The energy transferred to the powder from refractory or steel balls depends on the rotational speed, size and number of balls, ratio of ball to powder mass, the time of milling and the milling atmosphere. Nanoparticles are produced by the shear action during grinding.

#### **1.4.6 SOL-GEL METHOD**

It involves the evolution of inorganic networks through the formation of a colloidal suspension (sol) and gelation of the sol to form a network in a continuous liquid phase (gel). The process involves conversion of monomers into a colloidal solution (*sol*) that acts as the precursors for an integrated network (or *gel*) of either discrete particles or network polymers. Typical precursors are metal alkoxides. [16]. Sol-gel are used for the synthesis of various inorganic and organic hybrid materials. Typical precursors are metal alkoxides and metal chlorides, which undergoes hydrolysis and polycondensation reactions to form a colloid. The basic structure or morphology of the solid phase ranges from discrete colloidal particles to continuous chain like polymer networks.

#### **1.4.7 CO-PRECIPIATION METHOD**

Co-precipitation method is a classical and perhaps the simplest approach for synthesizing iron oxide nanoparticles. Basically, this process involves the precipitation of  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  salt such as chlorides, sulfates, and nitrates aqueous solutions by addition of a base such as NaOH. Co-precipitation method refers to obtain the uniform composition in two or more cations homogeneous solution through precipitation reaction, which is one of important methods for the synthesis of composites containing two or more kinds of metal elements [17]. There are four types of co-precipitation: (1) surface adsorption, (2) mixed-crystal formation, (3) occlusion and (4) mechanical entrapment. (1) and (2) are equilibrium processes, while (3) and (4) arises from kinetics of crystal growth. To improve the size distribution of the as-prepared nanoparticles, various surface ligands, such as surfactants, inorganic molecules, and polymers have been employed as the stabilizing agents during the precipitation process. [18]. The precipitate obtained after drying is heated to the required temperature in appropriate atmosphere to produce the final product. Decomposition temperature of precipitate are generally lower than the temperatures employed in the ceramic method.

## **1.5 CHARACTERIZATION METHODS OF NANOMATERIALS**

Nanomaterials are characterized by various methods such as,

### **1.5.1 ULTRAVIOLET SPECTROSCOPY**

Ultraviolet/Visible/Infrared (UV/Vis/IR) spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample (a quantity known as the extinction, which is defined as the sum of absorbed and scattered light). Absorption of light in the UV/Visible part of the spectrum is 210 – 900 nm. In its simplest form, a sample was placed between a light source and a photodetector, and the intensity of a beam of light was measured before and after passing through the sample. These measurements are compared at each wavelength to quantify the sample's wavelength dependent extinction spectrum[19]. The data was typically plotted as extinction as a function of wavelength. Each spectrum background is corrected using a blank a cuvette filled with only the dispersing medium, to guarantee the spectral features from the solvent that are not included in the sample extinction spectrum.

### **1.5.2 PHOTOLUMINESCENCE SPECTROSCOPY**

Photoluminescence spectra was recorded by measuring the intensity of emitted radiation as a function of either the excitation wavelength or the emission wavelength . In an emission spectrum a fixed wavelength is used to excite the sample and the intensity of emitted radiation is monitored as function of wavelength. When corrected for variations in the source's intensity and the detector's response, a sample's excitation spectrum was nearly identical to its absorbance spectrum. The excitation spectrum provides a convenient means for selecting the best excitation wavelength for a quantitative or qualitative analysis. In an emission spectrum a fixed wavelength was used to excite the sample and the intensity of emitted radiation was monitored as function of wavelength[20].

### **1.5.3 SCANNING ELECTRON MICROSCOPY**

SEM is similar to the optical stereo-binocular microscope to observe the morphology and shape of the specimen. 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 interacts with the atoms of the specimen, producing signals that contains information about its surface topography, composition and other electrical properties[21]. These interactions and effects are detected and transformed into an image.

#### **1.5.4 TRANSMISSION ELECTRON MICROSCOPY**

TEM is the original form of electron microscopy and analogues to the optical microscope. Since the wavelength of electrons are much smaller than that of light, the optimal resolution attainable for TEM images was many orders of magnitude better than that from a light microscope (~0.1 nm).The beam of electrons passes through the specimen and analyzes the internal structure of the specimen in the form of images. The electrons have the poor penetrating capability and gets absorbed in the thick specimen[22]. Therefore, the thickness of the specimen should not be more than few hundred Angstroms (one angstroms = 10<sup>-10</sup> m). However sometimes, slightly thickens samples were used in High Voltage Electron Microscope.

#### **1.5.5 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[23].In the scope of DLS, temporal fluctuations are usually analyzed by means of the intensity or photon auto correlation function also known as photon correlation spectroscopy or quasi elastic light scattering. In the time domain analysis, the autocorrelation function (ACF) usually decays starting from zero delay time and faster dynamics due to smaller particles leads to faster decorrelation of scattered intensity trace. It has been shown that the intensity ACF was the Fourier transformation of the power spectrum and therefore the DLS measurements were equally well performed in the spectral domain.[24] DLS was also used to probe the behavior of complex fluids such as concentrated polymer solutions.

### **1.6 APPLICATION OF NANOMATERIALS**

#### **1.6.1 NEXT GENERATION COMPUTER CHIPS**

The microelectronics sector has paid special attention to miniaturization, which involves reducing the size of circuits like transistors, capacitors, and resistors. A considerable reduction

in their size enables microprocessors developed using these parts, to operate much faster, thus allowing computations at much greater speeds [25]. However, there were a number of technical obstacles to achieving these advancements such as the lack of ultrafine precursors to make these parts, inadequate dissipation of huge amounts of heat generated by these microprocessors because of the faster speeds, poor mean time to failures etc. Nanomaterials helps the industry to overcome these obstacles by offering manufacturers materials with better thermal conductivity, nanocrystalline starting materials, ultra high purity materials and longer lasting, durable interconnections like connections between different parts in the microprocessors.

### **1.6.2 KINETIC ENERGY PENETRATORS WITH IMPROVED LETHALITY**

The Department of Defense (DoD) has been using depleted uranium (DU) projectiles for its battle against hardened targets and armored vehicles of enemies. But DU has residual radioactivity; therefore, it is explosive, harmful carcinogenic, and lethal to personnel who uses them. However, some of the key reasons for the sustained use of DU penetrators are that they have an exclusive self sharpening mechanism on impact with a target, as well as the lack of an appropriate non-explosive, non toxic alternative for DU [26]. Nano crystalline tungsten heavy alloys are used for such self sharpening mechanisms due to their exclusive deformation characteristics, for example, grain boundary sliding. Therefore, nano crystalline tungsten heavy alloys and composites are being assessed for use as alternative DU penetrators.

### **1.6.3 BETTER INSULATION MATERIALS**

Nano crystalline materials manufactured by the sol-gel method gives rise to foam like structures known as “aerogels.” In spite of being extremely lightweight and porous, these aerogels loads equal to 100 times their weight. Aerogels are made up of continuous 3D networks of particles with air or any other fluid, such as a gas trapped at their interstices. Since aerogels are porous and includes air trapped at the interstices, they are used for insulation in homes, offices, etc. This considerably reduces the cooling and heating bills, thus saving power and decreasing the associated environmental pollution. [27] They are also being employed as materials for “smart” windows, which darken when the sun is very bright same as in changeable lenses in sunglasses and prescription spectacles, and lighten when the sun is not shining very brightly.



#### **1.6.4 PHOSPHORS FOR HIGH- DEFINITION TV**

The resolution of a monitor or television is subjected to the size of the pixel. These pixels are fundamentally composed of materials known as “phosphors,” which glow when struck by a stream of electrons within the cathode ray tube (CRT). The resolution enhances with a reduction in the pixel size or the Phosphors. Nano crystalline zinc selenide, cadmium sulfide, zinc sulfide, and lead telluride prepared through the sol-gel methods are potential materials for enhancing the resolution of monitors.[28] The use of nanophosphors is intended to lower the cost of these displays to make personal computers and high definition televisions (HDTVs) affordable for an average household in the United States.

#### **1.6.5 LOW COST FLAT PANEL DISPLAYS**

In the portable laptops computer industry, the demand for flat panel displays are high. Japan is leading in this area, mainly due to its R&D efforts on the materials for these displays. The resolution of these display devices are significantly improved by synthesizing nanocrystalline phosphors, while considerably bringing down the manufacturing costs. Furthermore, the flatpanel displays manufactured using nanomaterials have far higher contrast and brightness compared to the traditional ones due to their improved magnetic and electrical properties.[29]

#### **1.6.6 TOUGHER AND HARDER CUTTING TOOLS**

Cutting tools made of nano crystalline materials like carbides of tantalum, tungsten, and titanium, are a lot harder, much more erosion resistant and wear resistant, and last longer than their traditional (large-grained) equivalents. They also allow the manufacturer to machine several materials much faster, thereby boosting productivity and largely minimizing manufacturing costs. Moreover, miniaturizing microelectronic circuits necessitates micro drills (drill bits having diameters lesser than the thickness of an average human hair [100  $\mu\text{m}$ ]) with improved edge retention and much better wear resistance. Nano crystalline carbides are being used in these micro drills since they are much harder, stronger, and wear-resistant.

#### **1.6.7 ELIMINATION OF POLLUTANTS**

Nano crystalline materials have very large grain boundaries corresponding to their grain size. Therefore, they are very active with regards to their physical, chemical, and mechanical properties. Owing to their improved chemical activity, nanomaterials are employed as catalysts

to react with toxic and noxious gases such as nitrogen oxide and carbon monoxide, in power generation equipment and automobile catalytic converters, to avoid environmental pollution caused when gasoline and coal are burnt.

### **1.6.8 HIGH ENERGY DENSITY BATTERIES**

Traditional and rechargeable batteries are used in nearly all applications that necessitate electric power. These applications include laptop computers, automobiles, toys, electric vehicles, personal stereos, cordless phones, cellular phones, watches, and next generation electric vehicles NGEV that reduces environmental pollution. The energy density storage capacity of these batteries are very low, necessitating frequent recharging. Nano crystalline materials produced using sol-gel methods have a foam like aerogel structure that stores significantly more energy than their traditional equivalents. Hence, they are highly suitable for separator plates in batteries[30]. Moreover, nickel metal hydride Ni-MH batteries made of nanocrystalline nickel and metal hydrides have been predicted to necessitate much lesser recharging and to last considerably longer because of their large grain boundary surface area and improved chemical, physical, and mechanical properties.

### **1.6.9 HIGH POWER MAGNETS**

A magnet's strength is measured in terms of saturation magnetization and coercivity values. These values increase when there is a decrease in the grain size and an increase in the specific surface area per unit volume of the grains of the grains. It has been demonstrated that magnets made of nanocrystalline Yttrium-Samarium Cobalt grains have highly uncommon magnetic properties because of their extremely large surface area. Common applications for these high power rare earth magnets include ultra sensitive analytical instruments, quieter submarines, land-based power generators, automobile alternators, motors for ships, and magnetic resonance imaging (MRI) in medical diagnostics.

### **1.6.10 HIGH-SENSITIVITY SENSORS**

Sensors use their sensitivity to detect the variations in different parameters they are programmed to measure. The parameters include chemical activity, thermal conductivity, electrical resistivity, magnetic permeability, and capacitance. All of these parameters depend a lot on the microstructure (grain size) of the materials used in the sensors. A variation in the sensor's environment was revealed by the sensor material's physical, chemical or mechanical

characteristics, which was leveraged for detection. For example, a carbon monoxide sensor made of zirconium oxide (zirconia) applies its chemical stability to identify whether carbon monoxide is present. [31]When carbon monoxide was present, the oxygen atoms in zirconium oxide reacts with the carbon in carbon monoxide to reduce zirconium oxide partially. This reaction activates a modification in the sensor's characteristics, such as capacitance and conductivity (or resistivity).The rate and the degree of this reaction was significantly increased by a decrease in the grain size. Therefore, sensors made of nanocrystalline materials are highly sensitive to variations in their environment. Common applications for sensors made using nanocrystalline materials are ice detectors on aircraft wings, smoke detectors, automobile engine performance sensors, etc.

#### **1.6.11 AUTOMOBILES WITH GREATER FUEL EFFICIENCY**

Existing automobile engines waste substantial amounts of gasoline, thus adding to environmental pollution by burning the fuel incompletely. A traditional spark plug is not made to burn the gasoline totally and efficiently. This problem was amplified by faulty, or worn-out, spark plug electrodes. Since nanomaterials are harder, stronger and considerably more erosion-resistant and wear-resistant, they are currently being proposed for use as spark plugs. These electrodes extend the service life of the spark plugs and helps to burn fuel far more efficiently and fully. A totally new spark plug design known as the rail plug was also in the prototype stage.

#### **1.6.12 BIO IMAGING**

Quantum dots enters in to the cytosomes and was further detected in small vesicles and finally in large compartments. An interesting advantage of quantum dots at biological point of view was that these particles are nanocrystals with high atomic number atoms (Cadmium, Tellurium etc). It means that they are electron dense and are visualized by transmission electron microscopy. This feature is extremely useful considering that besides provide high quality.

#### **1.6.13 MEDICAL IMAGING AND DISEASE DETECTION**

Many nanomaterials are used as a tool for treating cancerous cells. Different nanomaterials are also be armed with tumor fighting toxic therapeutics to provide the diagnosis and treatment of cancer. They are also utilized for imaging of sentinel node in cancer patients for tumor staging and planning of therapy. This application assists in detecting suitable therapy and stage

for various malignancies like melanoma, breast, lung and gastrointestinal tumors [32]. Nanoparticle sensor arrays were developed as an early detection test for cancer and other diseases.

## **1.7 CARBON DOTS**

Carbon dots (CDs) are zero dimensional carbon-based materials within the size range of a couple of tens of nanometers and be doped with N, S, P, and B heteroatoms. They are chemically modifiable to upgrade and render a few extra useful properties. CDs have numerous characteristic resources such as tunable optoelectronic properties with high photoluminescence and or fluorescence properties, great biocompatibility, and tunable and post modifiable functional groups with simple arrangement strategies. As the precursor materials and synthesis methods significantly decides the applicability of CD materials in numerous areas. The approaches for CD synthesis, counting the impacts of source and doping as well as the response conditions such as temperature and time. This was taken after by the application of CDs in bio imaging, cancer treatment, quality and drug delivery, sensors and biosensors, catalysts, and vitality. CDs are generally defined as a quasi zero dimensional carbon-based material with a size below 20 nm, and fluorescence was their intrinsic property. In 2004, carbon nanoparticles with fluorescence were first reported, which were accidentally obtained from the purification of single walled CNTs. In 2006, Sun and co workers named nanoscale carbon particles synthesized by laser ablation of carbon target as CDs for the first time, but the QY of these surface passivated CDs was only about 10%. [33]

### **1.7.1 TYPES OF CARBON DOTS**

Based on their classification, amorphous quasi-spherical nanodots that lack quantum confinement are considered to be carbon nanodots (CNDs), while spherical quantum dots with quantum confinement and crystalline structures are referred to as carbon quantum dots (CQDs) Fluorescent carbon dots (CDs) are a novel type of fluorescent nanomaterials, which not only possess the specific quantum confinement effects of nanomaterials due to the small size of nanomaterials, but also have good biocompatibility and high fluorescence.

#### **1.7.1.1 LUMINESCENT CARBON DOTS**

Luminescent carbon dots (CDs) have become attractive materials because of their superior photo physical properties and various potential applications. However, most of the formerly developed CDs only have strong blue emission, which limits their further applications,

particularly in bio imaging. The luminescent CDs emit strong green fluorescence with high quantum yield as well as excellent biocompatibility and bio labeling potentials at first, the luminescent CDs exhibited high selectivity for phosgene with a turn-off fluorescence detection [34]. The limit of detection was 81 nm, which was sensitive for the determination of phosgene over other competing toxic pollutants. In addition, the luminescent CDs have shown a three-state "on-off-on" emission with the stepwise addition of  $\text{Ag}^+$  and cysteine (Cys). Luminescent CDs show fluorescence quenching by Ag and fluorescence regaining with further addition of Cys, with lower detection limits of 3.9  $\mu\text{M}$  (Ag) and 3.4  $\mu\text{M}$  (Cys), respectively. The luminescent CDs were utilized to obtain a clear fingerprint. During the drying process, the coffee ring effect and electrostatic interaction between the positive surface charge of amine functionalized CDs and negatively charged fingerprint residues facilitate the formation of clear fingerprints on different platforms

#### **1.7.1.2 DOPED CARBON DOTS**

Doping with heteroatoms (such as nitrogen doping) was an attractive and efficient strategy to enhance the quantum yield of carbon dots. However, the influence of the nitrogen doping on the carbon dots was relatively scarcely reported. The N CDots exhibited high photoluminescence with a quantum yield of 22 % and showed potential applications in multi-photon single molecule imaging [35]. Hetero-atom doping in CDots represents a robust and versatile technique to further improve the chemical composition and structure properties of CDots Sulfur-doped carbon dots (S doped C dots) were synthesized using a simple and straightforward hydrothermal method. The spherical S doped C dots have an average diameter of 4.6 nm and the fluorescence of S-doped C-dots are effectively and selectively quenched by  $\text{Fe}^{++}$  ions.

#### **1.7.1.3 CARBON DOTS BASED ON MAGNETIC PROPERTY**

Several studies have been report combining C dots with magnetic nanomaterials and developing multifunctional systems for biomedical application For example, the combination of magnetite  $\text{Fe}_3\text{O}_4$  nanocrystals and fluorescent C dots in porous carbon was synthesized by the solvothermal method

## **1.8 PROPERTIES OF CARBON DOTS**

### **1.8.1 PHOTOLUMINESCENCE**

Photoluminescence is one of the most fascinating features of CQDs, both from the view of fundamental research and practical application [36]. In general, one uniform feature of the PL for CQDs was the distinct dependence of the emission wavelength and intensity. The reason for this unique phenomenon was the optical selection of nanoparticles with different sizes or CQDs with different emissive traps on the surfaces [37]. The variation of particle size and PL emission are reflected from the broad and excitation-dependent PL emission spectrum. The emission behaviors of CQDs under an irradiation at 470 nm wavelength with various concentration was observed and it was found that the PL strength of the CQDS solution first increased and then decreased as the concentration increased.

### **1.8.2 ELECTROLUMINESCENCE**

Since semiconductor nanocrystals are well-known to display electroluminescence (ECL) and CQDs have inspired various interests for ECL studies which can favorably be used in electrochemical fields [38]. Zhang et al. (2013) reported a CQDs based light-emitting diodes (LED) device, in which the emission color was controlled by the driving current. Color-switchable ECL from the same CQDs ranging from blue to white was observed under different working voltages. In order to understand the luminescence mechanism of CQDs more clearly, the researchers proposed two models based on the band gap emission of the conjugated p domain and the edge effect caused by another surface defect. The PL characteristics of the fluorescence emission of CQDs from the conjugated p domains are derived from the quantum confinement effect (QCE) of p-conjugated electrons in the  $sp^2$  atomic framework and was adjusted by their size, edge configuration, and shape. Fluorescence emission of CQDs associated with surface defects results from  $sp^2$  and  $sp^3$  hybridized carbon and other surface defects of CQDs and even fluorescence intensity and peak position are related to this defect.

### **1.8.3 ABSORBANCE**

Generally, the optical absorption peaks of CQDs within the UV-visible region was as a rule assessed as  $\pi$ -\* transition of  $sp^2$  conjugated carbon and  $n$ \* transition of hybridization with heteroatom such as N, S, P, etc. The absorption property is controlled through surface passivation or modification process. Jiang et al. created a effortless aqueous strategy to synthesize ruddy, green and blue luminescent CQDS by utilizing three isomers of

phenylenediamines. The UV-visible absorption spectra of the as obtained CQDs shown practically equivalent to design. Interestingly, the absorption transitions of these three CODs were red shifted. Demonstrating the electronic bandgaps of the CQDs were smaller than their comparing precursors. Photoluminescence is one of the foremost intriguing highlights of carbon dots.

## **1.9 APPLICATION OF CARBON DOTS**

### **1.9.1 CHEMICAL SENSING**

Fluorescent carbon dots, due to their excellent optical properties, chemical stability, and good solubility in water, in the field of chemical sensing under great attention are widely used in metal ion detection, anion detection, small organic molecules, and biomolecules detection. Like the semiconductor quantum dots, CDs by the interaction with the analyte changes the efficiency of recombination between the surfaces of the electron-hole pairs, which occurred in the fluorescence enhancement and quenching treatment to achieve quantitative or qualitative analysis of the measured object.

### **1.9.2 BIO IMAGING**

Carbon dots have awesome potential for fluorescent bio imaging, and multimodal bio imaging. The images were taken at various excitation wavelengths. Red and green represents the fluorescent signals of the CDs and the tissue auto fluorescence respectively. It was attractive to integrate the multi imaging technology for one agent for comprehensive understanding of the state of the illness. Most popular nanostructured multimodal imaging probes are combinations of magnetic resonance imaging (MRI) and optical imaging modalities. MRI offers high spatial resolution and the capacity to simultaneously obtain physiological and anatomical information, whereas optical imaging allows the rapid screening. The C dots were water-dispersible, displaying bright fluorescence in the visible range upon light excitation. Carbon dots own distinctive advantages marked by multicolor emission profile, small sizes, low cytotoxicity, prominent biocompatibility, and excellent photo stability, enabling them as an ideal candidate for fluorescence imaging.

### **1.9.3 BIOSENSOR**

CDs have been used as biosensor carriers for their high solubility in water, flexibility in surface modification, nontoxicity, excitation dependent multicolor emission, excellent biocompatibility, good cell permeability, and high photo stability. The CDs based biosensors

are used for visual monitoring of glucose, cellular copper, phosphate, iron potassium and nucleic acid CDs are used as an effective fluorescent sensing platform for nucleic acid detection with selectivity single-base mismatch. The general concept was based on the adsorption of the fluorescently labeled single stranded DNA (ssDNA) probe by CD via  $\pi$  interactions, which was accompanied by substantial fluorescence quenching, followed by specific hybridization with its target to form double stranded DNA (dsDNA). This results in desorption of the hybridized dsDNA from the CD surface accompanied with subsequent recovery of fluorescence, probing the target DNA The CDs serve as the donor of energy transfer and the carrier for the sensing system

#### **1.9.4. DRUG DELIVERY**

Drug delivery refers to the approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body some time based on nanoparticles as needed to safely achieve its desired therapeutic effect. It involves scientific site-targeting within the body, or it might involve facilitating systemic pharmacokinetics; in any case, it was typically concerned with both quantity and duration of drug presence. Drug delivery was often approached via a drug's chemical formulation, but it also involves medical devices or drug device combination products the doxorubicin loaded carbon dots also exhibited a good therapeutic effect by eliminating cancer stem cells. This work provides a potential strategy for developing carb

#### **1.9.5. GENE DELIVERY**

The semiconducting nature and the size dependent fluorescence of these nanocrystals have been successfully applied for in vitro, in vivo transfection and for the diagnosis of various diseases One of the most important emerging applications of CDs appears to be traceable drug delivery, because it has the potential to elucidate the pharmacokinetics and pharmacodynamics of drug candidates and to provide the design principles for drug carrier engineering In gene technology, the carbon dots conjugated with oligonucleotide sequences (attached via surface carboxylic acid groups) are targeted to bind with DNA or mRNA. Gene associated drugs are loaded within a CD core or attached to the surface of these nanoparticles through direct conjugation or electrostatic complexation by which CDs protects the gene from degradation by nucleases. This property has been utilized for an assay of single nucleotide polymorphism (SNP). Due to concerns about long term in vivo toxicity and degradation, Recently



nanotechnology based gene delivery is one of the most attractive therapeutic methods for the treatment of various diseases. In drug delivery, size and distribution of particles are critical parameters to target specific organs and tissues[39]. Proteins derived from their secondary structure are suitable materials for drug/gene carriers due to their precise molecular sizes. An ideal nanoparticle formulation for a drug or gene carrier system are achieved long circulation time, low immunogenicity, good biocompatibility, and selective targeting. Gene delivery involves viral and non-viral vectors. Viral vectors are having low loading capacity, large scale manufacturing, quality control cost, and safety factor such as immunogenicity and potential oncogenic. From the stability and safety concern, non viral vectors have more efficiently passing the gene transfection through the biological barriers compared to viral vectors. Organic inorganic, and various hybrid materials are used for nanoparticles. Among these, polymeric nanoparticles have great therapeutic applications due to its wide range of sizes and varieties and is used in sustained and targeted gene delivery for long periods. Biopolymers used for the preparation of nonviral vectors possess several favorable characteristics such as high biocompatibility, low toxicity, good biodegradability, and abundant renewable sources which are used for efficiency delivery of drug/gene to the target site. Choosing a suitable design of nanoparticle structure increases gene transfection efficiency to overcome extracellular and intracellular transfection barriers, the blood stream, the cellular membrane, endosomes, and the nuclear membrane. Nanoparticle in gene delivery depends upon the nature of the polymer charge and its chain length. Furthermore, modifications in the nanoparticles by introducing ligands onto the surface enhances localization and retention in specific target tissue, local delivery of agents to a large volume of tissues for better clinical application. However, biopolymer-based nanoparticle becomes a tool in near future for the precisely targeted delivery of drugs and genes in many therapeutic fields, but toxicological issues and degradation products of nanoparticles are need to be considered before being applied into humans.

#### **1.9.6 ANTIMICROBIAL ACTIVITY**

Carbon dots (CDs) are one of the most recently developed, carbon-based nanomaterials (NMs). Owing to their multiple properties they are used in various applications. Recently, they were also examined for their antibacterial properties. In a few cases, CDs have inherent antibacterial properties, while in many cases, specific synthesis conditions or functionalization routes are followed, either in order to bestow antibacterial properties to CNDs or to enhance their performance[40]. Although much work has been done following this approach, an alternative option is to increase the antibacterial activity of CDs after irradiation at various wavelengths.

For all the above cases, various mechanisms of action have been proposed to explain the observed antibacterial activity. C dots conjugate showed enhanced antimicrobial activity against selective gram strain bacteria.

### **1.9.7 SCOPE OF THE WORK**

CDs as the potent imaging agents play a pivotal role in the real-time imaging of either certain cells, tissues, organs, or in combination, that help in the accurate diagnosis of various forms of cancer and several diseases. Moreover, they have the tendency to efficiently deliver genes or drugs by serving as nanocarriers. Recently carbon dots have attracted considerable attention in biosensing and imaging applications. The synthesized carbon dots appears as promising luminescence materials which can be utilized for various biomedical applications

## CHAPTER 2

### REVIEW OF LITERATURE

Mhetear Tuerhon et al. studied found that carbon dots have become a potential material for biosensing, drug delivery and bioimaging because of their excellent optical properties, high biocompatibility and low toxicity [40]. Bui Thi Ho et al. studied carbon dots emitting green luminescence with quantum yield in the range of 14.86 to 24.89% as a function of hydrothermal temperature. Firstly highly luminescent carbon dots were synthesized by the open pot method directly from lemon juices using different temperatures, time, aging of precursors and diluted solvents to control the luminescence of C dots [41]. Hui Ding and Shang-Bo Yu et al. studied that carbon dots with tunable photoluminescence and a quantum yield of up to 35% in water were hydrothermally synthesized in one pot and separated via column chromatography. These bright and stable luminescence in gradient colours from blue to red under a single wavelength UV light. They exhibited high optical uniformity, that is every sample showed only one peak in the PL excitation spectrum only one peak in the excitation-independent PL emission spectrum, and similar mono exponential fluorescence lifetimes. Although these samples had similar distributions of particle size and graphite structure in their carbon cores, the surface state gradually varied among the samples, especially the degree of oxidation. Therefore, the observed red shift in their emission peaks from 440 to 625 nm was ascribed to a gradual reduction in their band gaps with the increasing incorporation of oxygen species into their surface structures. These energy bands were found to depend on the surface groups and structures but not on the particle size, not as in traditional semiconductor quantum dots. In addition, because of their excellent PL properties and low cytotoxicity, these CDs could be used to image cells in different colors under a single-wavelength light source, and the red-emitting CDs could be used to image live mice because of the strong penetration capability of their fluorescence [42]. Xiaofang Jia et al. studied One-pot green synthesis of optically pH-sensitive carbon dots with upconversion luminescence One-pot fabrication of fluorescent carbon dots was easily developed by directly heating ascorbic acid aqueous solution at 90 °C. These carbon dots possess excitation, pH and polarity-dependent luminescence and upconversion fluorescence properties [43]. Xiaohi wang et al a facile, economic and green one-step microwave synthesis route towards photoluminescent carbon dots is proposed. They are biologically compatible and show favorable optical properties and have potential applications

in biolabeling and bioimaging [44]. Ashni Mewada et al . reported highly economical plant based method for the production of luminescent water soluble carbon dots (C-dot) using Indian water plant *Trapa bispinosa* peel extract without adding any external oxidizing agent at 90 °C. C-dots ranging from 5 to 10 nm were found in the solution with a prominent green fluorescence under UV-light ( $\lambda_{ex} = 365$  nm). UV-vis spectra recorded at different time intervals (30–120 min) displayed signature absorption of C-dots between 400 and 600 nm. Fluorescence spectra of the dispersion after 120 min of synthesis exhibited characteristic emission peaks of C-dots when excited at 350, 400, 450 and 500 nm. C-dots were further analyzed using X-ray diffraction (XRD), Raman Spectroscopy and Thermo-Gravimetric Analysis (TGA). Structure of the C-dots was found to be turbostratic when studied using XRD. C-dots synthesized by our method were found to be exceptionally biocompatible against MDCK cells [45]. Yan Zhuo et al . studied An innovative and green strategy to synthesize carbon dots (CDs) with a quantum yield (QY) of nearly 19.8% has been successfully established for the first time. Subsequently, the possible fluorescence (FL) mechanism was elucidated by fluorescence, UV-vis, high resolution transmission electron microscope (HR-TEM), Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) analyses. Significantly, not only the precursor of CDs and whole synthesis procedure was green, but also the CDs obtained here exhibited various advantages including high fluorescent QY, excellent photostability, non-toxicity and satisfactory stability. Additionally, the CDs were employed for assaying  $Fe^{3+}$  based on direct interactions between  $Fe^{3+}$  and single bond COOH, single bond OH and single bond  $NH_2$  of CDs, resulting in aggregations that facilitate to quench their fluorescence. Eventually, the CDs were applied for cell imaging and coding, demonstrating their potential towards diverse applications [46]. Shaojing et al .studied Nitrogen and sulfur codoped carbon dots (CDs) were prepared from garlic by a hydrothermal method. The as prepared CDs possess good water dispersibility, strong blue fluorescence emission with a fluorescent quantum yield of 17.5%, and excellent photo and pH stabilities. It is also demonstrated that the fluorescence of CDs are resistant to the interference of metal ions, biomolecules, and high ionic strength environments. Combining with low cytotoxicity properties, CDs could be used as an excellent fluorescent probe for cellular multicolor imaging. Moreover, the CDs were also demonstrated to exhibit favorable radical scavenging activity [47]. Wen Liu et al. studied a simple, green, and low-cost way was developed in the synthesis of fluorescent carbon dots (CDs) with well distributed size, using one-pot hydrothermal treatment of rose heart radish. The as-prepared carbon dots exhibit exceptional advantages including high fluorescent quantum yield (13.6%), excellent biocompatibility, low toxicity, and satisfactory chemical stability. More strikingly,

as-synthesized N-CDs generate strong response to  $\text{Fe}^{3+}$  ions and gives rise to the fluorescence quenching. This phenomenon was used to develop a fluorescent method for facile detection of  $\text{Fe}^{3+}$  with a linear range from 0.02 to 40  $\mu\text{M}$  and a detection limit of 0.13  $\mu\text{M}$  ( $\text{S/N} = 3$ ), and further extended to measure environmental water samples with satisfactory recoveries [48]. Hua Xu et al. A simple, economical, and green method for the preparation of water-soluble, high-fluorescent carbon quantum dots (C-dots) has been developed via hydrothermal process using aloe as a carbon source. The synthesized C-dots were characterized by atomic force microscope (AFM), transmission electron microscopy (TEM), fluorescence spectrophotometer, UV–vis absorption spectra as well as Fourier transform infrared spectroscopy (FTIR). The results reveal that the as-prepared C-dots were spherical shape with an average diameter of 5 nm and emit bright yellow photoluminescence (PL) 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. Additionally, we found that 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  $\mu\text{M}$ . This observation was further successfully applied for the determination of tartrazine in food samples collected from local markets, suggesting its great potential toward food routine analysis [49]. Weng jing Wang et al . prepared carbon dots using a green hydrothermal approach with dehydrated shiitake mushroom as the sole carbon source without any additives (these carbon dots are shortly termed as MCDs). Carbonization, surface functionalization and nitrogen doping are involved in the hydrothermal treatment and no further modification or surface passivation is necessary. The derived MCDs are nitrogen doped, oxygen rich with hydroxyl, carboxyl and amine groups, with a diameter of ca. 4.2 nm. MCDs exhibit cell permeable properties and a distinct pH-sensitive/excitation-dependent photoluminescence emission feature within pH 4.0–8.0, providing an optical probe for intracellular pH sensing and multicolor imaging of live HeLa cells. MCDs also show a strong fluorescence response to hemin, which facilitates sensitive fluorescent sensing of hemin with a detection limit of 120  $\text{nmol L}^{-1}$  [50]. Mohammed J Meziani et al. studied and recently reported that small carbon nanoparticles be surface-passivated by organic or biomolecules to become strongly fluorescent. These fluorescent carbon nanoparticles, dubbed “carbon dots”, are successfully used for in vitro cell imaging with both one and two photon excitations, as already demonstrated in the literature. Here we report the first study using carbon dots for optical imaging in live mice. The results suggest that the

carbon dots remain strongly fluorescent in vivo, which, coupled with their biocompatibility and nontoxic characteristics, might offer great potential for imaging and related biomedical applications [51].

**CHAPTER 3**  
**OBJECTIVES OF THE WORK**

**The important objectives of the present work are:**

1. Synthesis of luminescent carbon dots from orange juice by wet chemical method
2. Optical characteriation of carbon dots
3. Morphological characterisation of carbon dots
4. Antimicrobial analysis of carbon dots
5. Sensing of Glucose using the synthesized Boric acid capped Carbon dots

## CHAPTER 4

### MATERIALS AND METHODS

#### 4.1 MATERIALS

- Orange fruit extract
- 0.5 M Boric acid
- Glucose
- Distilled water

#### 4.2 EXPERIMENTAL METHODS

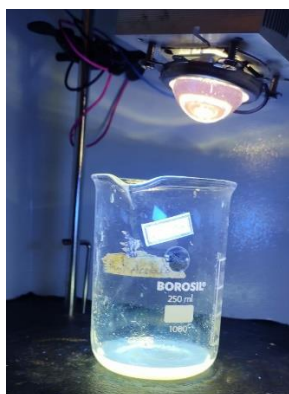
##### 4.2.1 SYNTHESIS OF FLUORESCENT CARBON DOTS BY WET CHEMICAL METHOD

The main precursor used for the synthesis of luminescent carbon dots are orange fruit juice. Carbon dots were synthesised from orange juice by hydrothermal method using a thermal magnetic stirrer. Schematic representation is depicted in **figure 4.2**. 50ml of filtered orange fruit extract was taken in a 250 ml beaker. The essence is then concentrated by providing temperature and constant stirring with the help of magnetic stirrer. The elicit is heated until solution content is reduced. Then the beaker was cooled at room temperature and the resulting solution was centrifuged at 10000 RPM for ten minutes to discard large molecules. Appearance of carbon dots in UV light is depicted in the **figure 4.3**.



**Figure 4.2: Schematic representation of synthesis of Carbon Dots from Orange juice**





**Figure 4.3: Carbon dots under UV light**

#### **4.2.2 PREPARATION OF 0.5M BORIC ACID**

About 0.30915g of Boric Acid was accurately weighed and dissolved in 10ml distilled water.

#### **4.2.3 PREPARATION OF BORIC ACID –CONJUGATED CARBON DOTS**

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

#### **4.2.4 CHARACTERISATION 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 Lambda365 UV-Visible spectrofluorimeter. 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 STUDY**

pH value of the carbon dot solution was determined using a digital pH meter

#### **4.2.6 ANTIBACTERIAL STUDY**

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 petriplates 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  $\mu$ L) was added into the wells T1 and T2 directly from sample. Gentamycin (40 $\mu$ l 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 36 $^{\circ}$ C  $\pm$  1 $^{\circ}$ C, under aerobic conditions. After incubation, the plates were observed and the zone of bacterial growth inhibition around the wells was measured in mm.

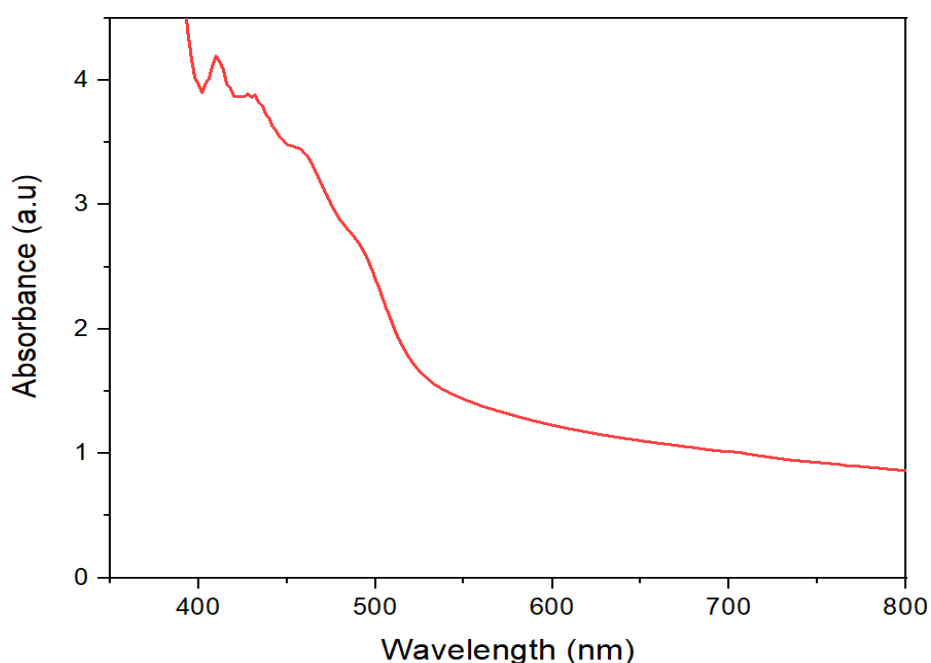
Culture media Details: Muller Hinton Agar medium (HIMEDIA- M173) is used for determination of susceptibility of microorganisms to antimicrobial agents. Suspended 38 grams in 1000 ml distilled water. Heated until it boils to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure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

## CHAPTER 5

### RESULTS AND DISCUSSIONS

#### 5.1 UV-VISIBLE ABSORPTION STUDIES

In understanding the behaviour of QDs, optical absorption studies have an important role. The optical absorption property of carbon dots was recorded using UV-Visible spectrometer as represented in **Figure 5.1**. It was found that there was an absorption peak obtained at 400nm. The absorption peak at 400nm originates from the  $n-\pi^*$  transition of C=O bond of the formed carbon dots from orange juice.

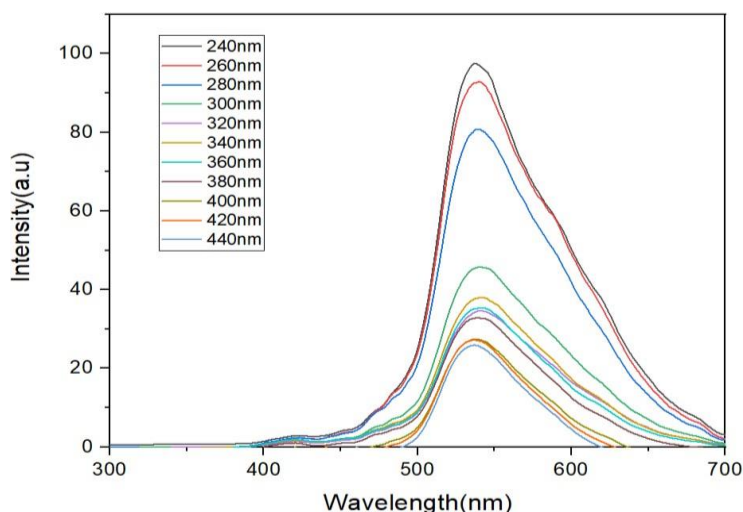


**Figure. 5.1 : The UV-Visible absorption spectra of carbon dots from orange extract**

#### 5.2 PHOTOLUMINESCENCE SPECTROSCOPY

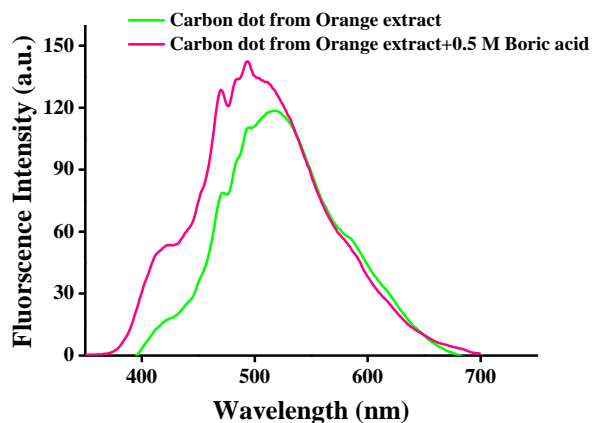
The luminescence property of the synthesized carbon dots was examined by photoluminescence spectroscopy. The intensity of the PL depends on the number of particles excited at a particular wavelength. Maximum PL intensity was observed at an

excitation wavelength of 240 nm for sample from orange juice since largest number of particles are being excited at 240 nm. From the data, it was found that the synthesized carbon dots shows considerable photoluminescence properties. A strong excitation band positioned at around 550 nm was obtained for carbon dots from orange extract. The emission band corresponds to the  $n-\pi^*$  transition of the carbon dots



**Figure.5.2. Emission spectra of carbon dots from juice at different excitationwavelengths.**

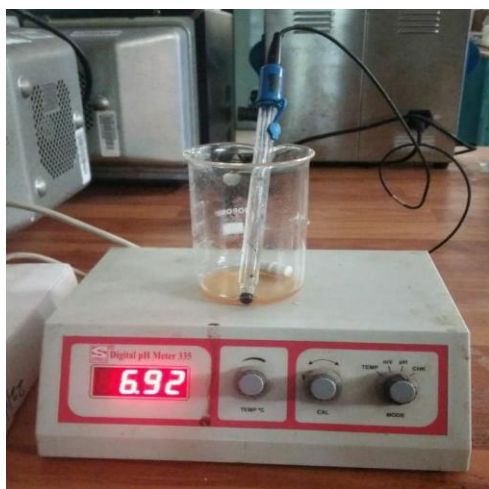
The carbon dots synthesised from orange juice was capped with 0.5 M boric acid and the photoluminescence of two samples were examined. On capping the luminescence intensity increased which is ascribed due to the reason that capping provides surface passivation and reduces electronic trapping abilities of surface defects, resulting in higher photoluminescence intensity.



**Figure 5.3: Emission spectra of carbon dots from orange juice and carbon dots coated with boric acid**

### 5.3 pH STUDIES

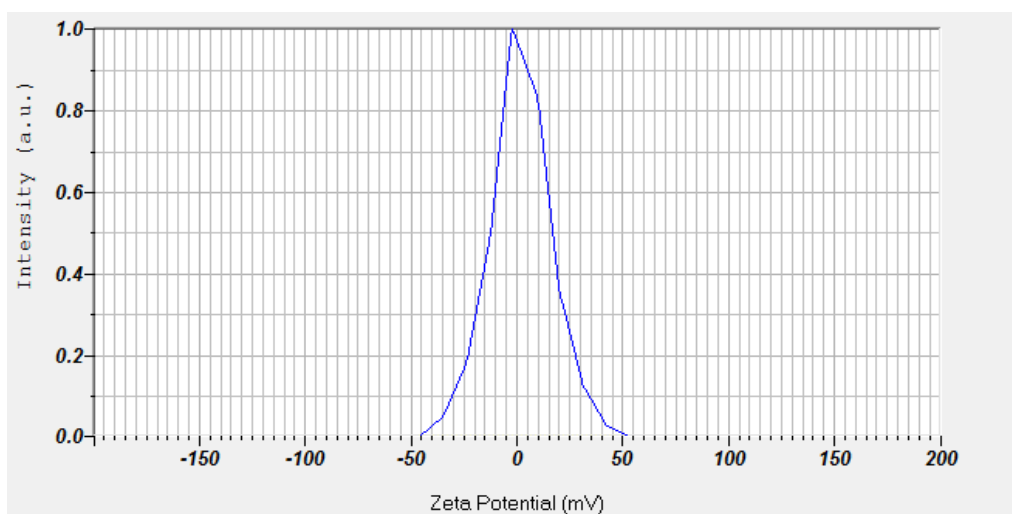
pH value of carbon dots solution was determined using a digital pH meter. pH of synthesised carbon dots was 6.92. When few drops of NaOH was added pH increased to 7.41.



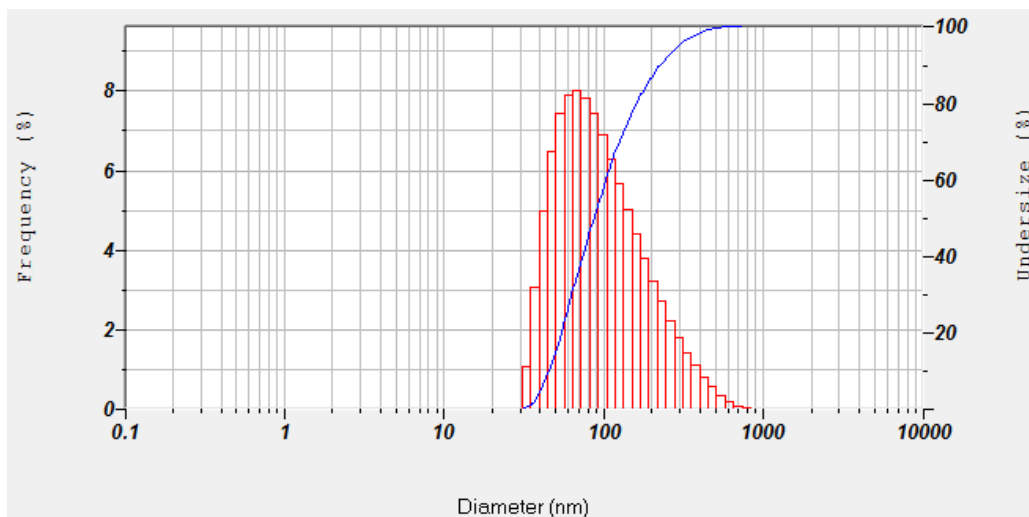
**Figure 5.4 : (a) pH of synthesised carbon dot and (b) pH of carbon dots with few drops of NaOH**

#### 5.4 DYNAMIC LIGHT SCATTERING

From the study of dynamic light scattering, the size distribution properties and zeta potential of the carbon dots were analysed. The carbon dots have hydrodynamic diameter of around 90 nm.( **Figure 5.6**) The zeta potential value of the carbon dots was found to be 1.7 eV which suggest the surface positive charge of the synthesized carbon dots and is depicted in **Figure 5.5**



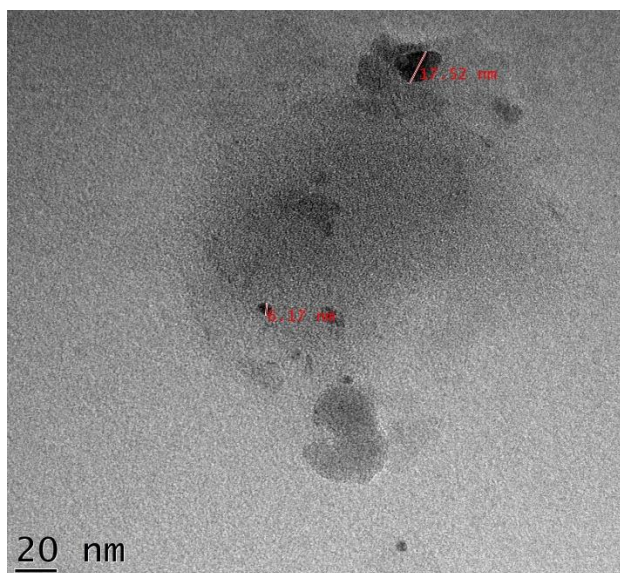
**Figure 5.5 : Zeta potential graph of Carbon dots**



**Figure 5.6 : Particle size of Carbon dots**

### **5.5 TRANSMISSION ELECTRON MICROSCOPY**

The morphology of carbon dots was characterized in terms of shape and particle size using Transmission Electron Microscopy. The HRTEM image of carbon dots are depicted in **Figure 5.7**. From the TEM images, it was observed that assembled spherical nanoparticles with an average size of 12.5 nm were formed.



**Figure 5.7: HR-TEM image of carbon dots synthesised from orange juice.**

## 5.6 ANTIMICROBIAL STUDY.

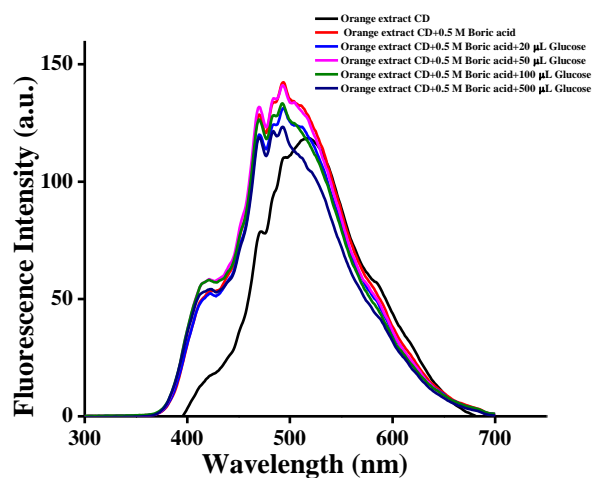
The antibacterial activities of the synthesized carbon dots was studied. The antibacterial property is evaluated using sterile distilled water. The antibacterial activity was assessed using the agar well diffusion assay. Gram positive organism *Staphylococcus aureus* was used as the test pathogens for the study of antibacterial properties of carbon dots. From the results obtained it was found that the carbon dots does not show anti microbial activity (**Figure 5.8**).



**Figure 5.8:** The zone of inhibition of the synthesised carbon dots from orange juice by using *Staphylococcus aureus*



## 5.7 SENSING OF GLUCOSE



**Figure 5.9 : The photoluminescence emission spectra of boric acid conjugated carbon dots in the presence of varying concentration of glucose.**

The carbon dots were used to detect a bioanalyte glucose. When glucose was added to the carbon dots, the luminescence intensity increased, which means the developed luminescent carbon dots can be utilized as a turn-on sensor for the detection of glucose. To 3 mL of carbon dots taken in a cuvette, a variable amount of glucose was added. The solutions were mixed and shaken for 3 seconds and then excited at 240 nm. The corresponding emission values during each addition were recorded. The stock solution of glucose (0.1 M) was prepared. Solutions of diluted concentration of glucose (0.0001 M) were then prepared by diluting the stock solution using distilled water. The gradual addition of increasing concentration of glucose solution to boric acid capped carbon dots, the luminescence intensity was found to be slightly enhanced. It is due to the formation of a complex between boric acid conjugated carbon dots and glucose.[52].

## CHAPTER 5

### CONCLUSION

The carbon dots were synthesized by hydrothermal method from orange fruit extract. The synthesized carbon dots were characterised by UV visible spectroscopy, Photoluminescence spectroscopy, Dynamic Light Scattering, Transmission Electron Microscopy. Antimicrobial properties were studied by agar well diffusion method. From the UV visible spectra analysis an absorption peak was centered at 400 nm which originates from the n-  $\pi^*$  transition of C=O bond. From PL studies the highest PL intensity was observed at an excitation wavelength of 240 nm. A strong excitation band centered at around 550 nm was obtained for synthesized carbon dots. From DLS studies, the synthesized carbon dots have size less than 100nm. The Zeta potential value of the carbon dots were found to be positively charged. pH value of carbon dot solution was determined. When alkali added pH of C dot was increased. C dot were used to detect bioanalyte glucose. High resolution Transmission Electron Microscopy analysis carried out to find particle size. From antimicrobial studies it was evident that the carbon dots does not exhibit significant antimicrobial activity. Gram positive organism *Staphylococcus aureus* was used as the test pathogens. The synthesized carbon dots have strong luminescence intensity. Hence they can be developed to suitable probes which may find significant applications in chemical, biological, medical and many other fields.

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