SUGAR DERIVED CARBON DOTS AS SENSOR, LUMINESCENT MARKER AND REDUCING AGENT

Thesis submitted to
the university of Calicut in partial fulfilment of
the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY
in Chemistry under the Faculty of Sciences

by

ANSI V.A.

Under the guidance of
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DEPARTMENT OF CHEMISTRY
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KERALA-673635
MARCH-2021

CERTIFICATE

This is to certify that the thesis entitled “**SUGAR DERIVED CARBON DOTS AS SENSOR, LUMINESCENT MARKER AND REDUCING AGENT**"submitted by Ansi.V.A to the University of Calicut for the award for the degree of Doctor of Philosophy in Chemistry under the faculty of Science, is an authentic record of research work carried out at the Department of Chemistry, University of Calicut under my guidance and supervision. The contents of the thesis have been checked for plagiarism using the software ‘Urkund’ and similarity index falls under permissible limit. I further certify that the content of this thesis has not been submitted elsewhere for the award of any Degree/ Diploma.

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ACKNOWLEDGEMENT

I would like to put on record my sincere gratitude to a number of people, whose continuous support and encouragement helped me a lot to complete my doctoral dissertation.

First and foremost, I would like to express my sincere gratitude to my Research Supervisor, Dr. Renuka N.K, Associate Professor, Department of chemistry, University of Calicut for the intellectual guidance and valuable advices she had given throughout my research period. Despite her busy schedules, she always made herself available for discussing experimental observations and also for clarifying my doubts. I adore her immensely for the timely advice, scholarly inputs, and meticulous scrutiny during the time of research and thesis writing. I'm extremely grateful to my guide for being a patient listener and also for responding promptly to all my queries. I shall be forever obliged for her guidance, patience and continuous encouragement.

I would like to thank Dr. A.I Yahya, Head of the Department Of Chemistry, University of Calicut and former HODs, Dr. P. Raveendran, Dr. K. Muraleedharan and Dr. V.M Abdul Mujeeb for providing necessary facilities for my work. My sincere gratitude goes to all the faculty members of this department for their support. I'm also sincerely grateful to Dr.Sindhu, Head of department of Nano-science and Technology, University of Calicut for giving permission me to conduct various characterisation analyses in their department. I' would like to thank Jyothilakshmi, research scholar Department of *nano-science and technology, University of Calicut for helping me to conduct various characterization analysis. I would like to mention the name of professor D. Bahulayan, Senior Scientist, Energy Research Institute at Nanyang Technological University (ERI@N), Singapore, my teacher, with immense gratitude. I'm extremely thankful for his valuable academic suggestions and also respect him a lot for his unparalleled benevolentness. It is a great pleasure to acknowledge the expeditious service received from the librarian, instrument technician and the present and former office staffs of our department.*

Without the financial support provided by kerala government through post metric E-grantz scholarship for my research work, this work would not have been possible. I'm extremely grateful to the funding authority.

I'm extremely grateful to Prof. P.Raveendran, Dept. of Chemistry, University of Calicut, Prof. Aji A. Anappara, Dept. of Physics, NIT-Calicut and Prof. K.Muraleedharan, Dept. of Chemistry, University of Calicut for their valuable suggestions and support during the research work. I extend my gratitude to CSIF –University of Calicut, NIIST-Trivandrum, STIC- CUSAT,Centre for Nanosciences, Amrita Institute of Medical Sciences, Kochi and NIT Calicut for providing the necessary characterization facilities for my work.

I'm extremely thankful to the members of my research team, A.K. Akhila, Varsha Raveendran P.T, Anjali. C, Nidhisha V and Amrutha T.P, for those motivating discussions, boundless love and especially for all the fun we have had in the last four years of research life.

Words fall too short when it comes to mention my love and affection to Anjali.C, my little sister. It is also a great pleasure to acknowledge the enthusiastic support and valuable suggestions given to me by my seniors, Dr. T. Divya, Dr. M.P. Nikhila, Dr. Anju Mohan and Dr. Arsha Kusumam T.V. I would like to express my sincere gratitude to all the researchers and M.Phil students for all your care and constant support during my research period.

Even the most beautiful words fall short when I'm trying to describe my affection towards my bosom friends Dr. Anju Ajayan and Nadira P.P. They simply transformed those stressed moments of research in to most beautiful pages of my life filled with everlasting memories of bliss, care and trust. I express my heartfelt affection to Dr. Safna Hussain (Department of Physics), an amazing person carved with innocence and extreme intelligence. Thank you Safnatha for making those research days most beautiful and I'm extremely grateful for your academic instructions and those interesting scientific discussions. I would like to extend my thanks to Jinitha T.V, Soufeena P.P, Anupama R. Prasad, Deepak Joshy, Lijin Rajan, Anjitha T, Roopashree R, Dipin T, Siva and Ritu for all your support and help during the research period. I express my immense love and affection to Thasleena panakkal who always bestowed positive radiance to me with her inherent innocence and care.

I would like to reserve in memory lane my post graduation days at Sree Kerala Varma College, Thrissur, kerala, where I met true friends and most affectionate teachers in my life. I'm gratefully remembering my teachers Dr. V.Indira, Dr. V.S. Eswari, Dr. T.D. Sobha, Dr. K.M.

Geetha and Dr. P.V. Divya for their keen interest and continuous support in my academic life.

Words fall short to express my love and affection towards my family, especially to my 'AMMA', to whom I'm deeply indebted. She gave me immense support, warmth and strength to chase all my dreams. Thank you my father for always standing by my side and also for placing your trust and confidence in my ability from the very first day we met. I would like to extend my love and affection to my little brothers kannan and unni for their support and care. Without the eternal affection and pampering from my family, this task would not have been completed like this.

I sincerely thank each and every person with open arms for giving me the opportunities and experiences that have made me who I am.

Above all, praises and thanks to the god, for his mercy and boundless blessing upon me throughout my research period. No words can sum up my gratitude and love towards the almighty that always holds my hand and cares me like a child during each moments of my life.

ANSI V.A

Incredibly Indebted To..

“LORD KRISHNA” -** My Mentor & Best friend**”

PREFACE

The surprising entry of carbon dots, bright luminescent nano carbon has revolutionized the traditional paradigm regarding the black material, carbon. Carbon dots, quasi-spherical carbon particles with diameter below 10 nm, are grabbing immense attention in the scientific world. Owing to their intriguing features including intense fluorescence, excitation tunability, low-toxicity, commendable photo and thermal stability and easy functionalisation, carbon dots has bloomed in every branch of applied sciences. Aforementioned features of carbon dots tunes them as most potent competitive to conventional organic fluorescent dyes and heavy metal based quantum dots in various fields including sensing, imaging, photo-catalysis, drug delivery, fabrication of opto-electronic devices etc.

But, unfortunately most of the conventional synthesis techniques to carbon dots involves the usage of toxic materials, demands some cumbersome post-chemical treatments for ensuring the purity of the product and also requires the assistance of expensive instrumentation facilities. These widely practiced methods to carbon dots were definitely believed to raise some threatening issues related to global energy conservation and environmentally benign developments. Hence, designing of an entirely facile, environmentally benign, cost-effective strategy for the bulk production of carbon dots, one of the protagonists of carbon nano science, is having great deal of research interest.

Gradually, scientists used to overcome these demerits associated with conventionally practiced synthesis protocols by the judicious implementation of core principles of green chemistry while designing the synthesis route. Hence, realizing the pulse of recent research interest, we have decided to focus our area of interest in to the development of fluorescent carbon dots from different naturally available precursors, upholding almost all the core-concepts of green and sustainable development.

Herein, we have chosen commercially available table sugar as the raw material for the fabrication of fluorescent carbon dots, considerably cheaper and non-toxic precursor. Very facile synthesis strategy was adopted with consumption of minimum amount of time and energy. Thus obtained carbon dot was found to possess diameter below 4 nm and also exhibit bright cyan colour fluorescence emission. Table sugar derived carbon dots were adequately characterized by various techniques and subjected for several interesting applications.

Present thesis consists of 7 chapters

First chapter provides a general introduction to the present work. After a detailed introduction to carbon family, this chapter mainly emphasize the details regarding surprising invention of fluorescent carbon nano dots, various synthesis methodologies, several applications and their inherent physico-chemical characteristics. Along with exhaustive review of literature, this section also provides details regarding the motivation/inspiration for conducting the present research work.

Chapter 2 provides details regarding the experimental methods. Fundamental features of adopted characterization techniques including High-Resolution Transmission Electron Microscopy(HRTEM) Analysis, X-Ray Diffraction (XRD) analysis, Atomic Force Microscopy (AFM) analysis, Energy Dispersive X-ray analysis (EDX), X-ray photoelectron spectroscopy (XPS) analysis, Fourier Transform Infra-Red (FT-IR) spectroscopy, Raman spectroscopy analysis Zeta potential analysis, Dynamic light scattering analysis , Ultra Violet-Visible absorbance spectroscopy, Fluorescence spectroscopy and Fluorescence Decay (Lifeime measurement) are also discussed in second chapter. Chapter 3 comprises detailed discussion on the results of the aforementioned characterization analysis and thereby providing the physico-chemical features of obtained carbon dot.

Chapter 4 presents the utilization of carbon dot as a promising fluorescent marker, when mixed with naturally available gum , Gum accacia as a binder. Fluorescent markers with two different consistencies were prepared by simply altering the concentration of binder, gum accacia. Construction and practical application of the fluorescent marker for patterning and coating applications were discussed in detail. Chapter 5 discusses the sensing application of prepared carbon dots. This section is divided into two parts. First part presents the visual/naked eye sensing of toxic heavy metal, Pb^{2+} with higher selectivity and sensitivity. This visual sensing of metal ion was enabled via selective aggregation of carbon dots. Second part of chapter 5 deals with fluorescence sensing of analytes, in which fluorescence intensity of carbon dots were selectively disturbed/quenched by the presence of external analytes. Here, we have prepared a carbon dot- Rhodamine dye FRET (Fluorescence Resonance Energy Transfer) unit, which could selectively detect trace amounts of extremely toxic Hg^{2+} ions. Fluorescence intensity of carbon dots was also found to be quenched with the introduction of very small amounts of a set of Nitro Aromatic Compounds (NACs) having nitrobenzene parental frame work. This sensing trend was further verified with the aid of some computational analytical techniques including Global reactive descriptors and donor acceptor mapping (DAM).

Sixth chapter discusses the utilization of carbon dot as a promising green reducing agent. Reducing ability of the carbon dot was employed for effortless extraction of bulk metallic silver from silver salt solution as well as for the instantaneous preparation of gold nano particles from chloroauric acid. Seventh chapter summarizes the major findings and highlights of the present work and also outlines the scope of future aspect.

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CD(s)	*Carbon Dot(s)*
CQD(s)	*Carbon Quantum Dot(s)*
GQD(s)	*Graphene Quantum Dot(s)*
CNT(s)	*Carbon Nano tube(s)*
QCE	*Quantum Confinement effect*
TS-CD(s)	*Table sugar derived Carbon Dot(s)*
HR-TEM	*High-Resolution Transmission Electron Microscopy*
XRD	*X-Ray Diffraction*
AFM	*Atomic Force Microscopy*
EDX	*Energy Dispersive X-ray*
XPS	*X-ray photoelectron spectroscopy*
FT-IR	*Fourier Transform Infra-Red*
DLS	*Dynamic light scattering*
UV-Vis	*Ultra Violet-Visible*
IFE	*Inner filter effect*
PET	*Photo electron transfer*
EPA	*Environment protection Agency*
ICH	*International Conference on Harmonization*
FDA	*Food and Drug Administration*
GA	*Gum accacia/Gum Arabic*
PL	*Photo-Luminescent*

LOD	*Limit of detection*
ppm	*parts per million*
Ppb	*Parts per billion*
Ppt	*Parts per trillion*
HEG	*Highly Exfoliated Graphite*
FRET	*Fluorescence resonance energy transfer*
NB	*Nitro Benzene*
4-NA	*4-Nitro Aniline*
4-NP	*4-Nitro phenol*
4-NT	*4-Nitro toluene*
4-CNB	*4-Chloro nitrobenzene*
4-NBA	*4-nitro benzoic acid*
4-NBAl	*4-nitro benzaldehyde*
NAC(s)	*Nitro Aromatic Compound(s)*
DAM	*Donor Acceptor Map*
Au-NP(s)	*Gold nano particle(s)*
4-AP	*4-Amino phenol*

Chapter 1

INTRODUCTION

- *This chapter provides general introduction to the carbon family as well as the surprising invention of a new member, fluorescent carbon dots (CDs). Details on inherent physico-chemical features, various synthesis methodologies along with several important applications of carbon dots are also provided here. In brief, this section outlines the wide acceptance of the material in the nano-world and also simply narrates the motivation/inspiration for conducting the present research work.*

1.1 CARBON - Historical Importance

Carbon, the most promising tool for next-generation tech materials, was acknowledged as an element in the second half of the 18th century. In 1789, A.L. Lavoisier proposed the name "“carbon” for the material, *inspired* from the Latin word “*carbo*” meaning "charcoal". In French the name is "charbone" and in German language "Kohlenstoff" which literally means “coal-stuff”. Carbon is placed in the periodic table with atomic symbol ‘C’ as element number 6 (Atomic number=6) with electronic configuration $1s^2$ $2s^2$ $2px^1$ $2py^1$. It exists in solid phase at room temperature, identified as the 6th most profuse element in the universe.Succeeding to the element oxygen, Carbon constitutes the major portion of human body [1]. It is observed that about 18 percent of human body weight is ascribed to this wonder element carbon. Dealing with the solar system, carbon is counted as the 4th most abundant element in it. Elemental carbon has got atomic weight of 12.01 (average mass) units, density 2.26 g/cm^3. Carbon has got an astounding ability for making bonds with one another as well as a wide range of other elements in different hybridization states (sp, sp^2 and sp^3), that is crucial for the existence of life on earth.

The discovery of this incredible element is marked nowhere in history. Primitive humans never mark the sole identification and purification of carbon like the majority of the elements in periodic table. Before 1 million years ago, ancient life frequently included this wonder black material as soot and cinder during their daily tasks. For them, whatever blacksubstance remains after a casual burning of any organic matter was counted as un-clean carbon. The element was very familiar among common men form olden time in the form of “charcoal”, the global resource of fuel. Charcoal plays a decisive role in several important processes including Smelting of different metals from their ores, metal casting assisted with melting process of the smelted metals, forging of iron and steel (non meltable), glass and advanced ceramic objects manufacturing etc. All these processes are believed to be inevitable for moulding the economic sustainability and maintaining the cultural harmony.

Activated charcoal is another class of carbon which includes finely grounded charcoal, potent enough to remove pollutants from liquid samples in to which it is added. When charcoal is subjected to so call ‘activation’ and heating, it is found to be able to purify contaminated solution (water and oil) by removing its unwanted colour and odour. Carbon has three naturally occurring isotopes C-12, C-13 and C-14, among which the later is radio-active by nature. In addition to these three natural isotopes, it has also got five artificial radio-active isotopes. C-14 isotope is a much celebrated radio-active agent by archaeologists, used to date once- living materials and relics. Atomic carbon forms various allotropes, simply by the rearrangements of bonded carbon atoms in different configuration, for stabilizing its short lived structure [1-6]. This unique feature makes carbon imperative and leaves this ‘carbon family’ as a perpetual stuff to study and explore.

1.2 Allotropes of carbon

Carbon constitutes an astonishing collection of allotropes with every doable dimension. Aforesaid collection of allotropes includes amorphous carbon, diamond (3D), graphite (3D), fullerenes (0D), carbon nanotubes (1D) and graphene (2D). Along with these prominent allotropes, this broadest chemical family is abundantly blessed with several other significant nano-allotropes such as nano diamonds (0D), single walled nano horns (1D), graphene nano ribbons(2D), onion like carbon spheres (0D) .

(I) Amorphous carbon

Highly reactive and free standing allotrope of carbon, lacking any crystalline structure is collectively termed as amorphous carbon. Coal, carbon/lamp-black and soot comprise the series. This class of materials lacks long range atomic arrangements. They are characterised with short range pattern of atomic positions in it. Amorphous forms of carbon mainly found their applications in the production of black colour inks, paints, synthetic petrol, stove polishers, water gas, rubber tires, gramophone records and typewriter ribbons. They are extensively used in engines, furnaces and gas mask.

(II) Diamond-3D allotrope

This allotrope of carbon is the hardest natural mineral known, with perfect crystalline arrangement. Diamond is the three dimensional metastable allotrope of carbon, where sp^3 hybridised carbon atoms are arranged tetrahedrally in a face cantered cubical structure. The name

‘Diamond’ is a combination of two greek words meaning "see-through" and "invincible."The superlative properties of diamond can be accredited to the covalent bonding between the individual carbon atoms. Owing to the commendable hardness, diamond is widely used as an excellent abrasive in various industrial applications including polishing, grinding and cutting of hard materials like glass and metals. Diamond also possesses higher light dispersion ability which tunes this carbon allotrope as the most alluring and expensive jewel stone.

(III) Graphite-3D allotrope

Graphite, very soft and flexible carbon allotrope, consists of sp^2 hybridised carbon atoms packed up in a honey comb like lattice. It is the most stable allotrope of carbon. Graphite occurs naturally in metamorphic rocks. It is often used in the manufacture of lead pencils. Scientists proposed the name ’Graphite’ from greek word ‘grafo’ meaning ‘to-write’, indicating ‘pencils lead’. Unlike diamond, graphite conducts electricity due to the delocalization of π bond electrons present above and below the planes of the carbon atoms. These electrons are highly mobile in nature and hence graphite behaves as a good conductor. However, the electricity is only conducted along the plane of the layers. Graphite widely found its application as lubricant as well as in the fabrication of high temperature bearing crucibles, as it does not melt upon heating. This well-known allotrope of carbon is commonly used as a neutron moderator within nuclear reactors. They also found their applications in the fabrication of black-paint, explosive materials and television set cathode ray tubes etc.

(IV) Fullerenes-0D allotrope

Fullerenes are the zero-dimensional allotrope of carbon having a soccer-ball-shaped geometry. They basically consist of hollow spheres, tubes, ellipsoid with the general formula C_{2n} (n=30-40), whose identification grabbed the Nobel Prize in chemistry in 1996. They are named after an American architect, Buckminster Fuller who designed the well known geodesic domes, greatly resembling to the allotrope arrangement. Fullerenes are prepared by passing high voltage current through graphite in an inert atmosphere. The most common fullerene is C_{60}, consisting of twenty hexagons and twelve pentagons of carbon atoms fused together in the form of a spherical ball. Spherical fullerenes are also called Buckminster fullerene/buckyballs. Generally, fullerenes are structurally similar to graphite. Fullerenes find applications in the field of material science, electronics and nano technology. This interesting allotrope of carbon progressively finds its place in the field of medical research for attaching drug molecules. Fullerenes can also result molecule-by-molecule incorporation for achieving effective delivery of medicines to the targeted body sites.

(V) Carbon nanotube-1D allotrope

Carbon nano tube (CNT) is a renowned allotrope of carbon with its cylindrical nano structure, invented after the efforts of Lijima. They have been synthesised with length to diameter ratio of up to 132,000,000:1, much larger than for any other material. For this, carbon sheets are rolled at specific angles resulting in the formation of Carbon nano tubes. They can be single walled or multi walled by structure. These nano tubes are composed entirely of sp^2 hybridised carbon atoms. CNTs are the strongest and stiffest material ever known in terms of tensile strength and elastic modulus respectively. They are found to be almost 100 times stronger than steel, though they have light weight feature which is only one-sixth of that of steel. Hence they can be used as additives to any materials to reinforce their strength. CNTs are also believed to be better conductors of heat and electricity than the well-known metal copper. Due to the unusual properties of carbon nano tubes such as high thermal conductivity, mechanical strength, and commendable magnetic property, these structures have been widely utilised in the field of nano technology, electronics, optics etc.

(VI) Graphene-2D allotrope

Graphene, the legendary material raised so far in the scientific community, is a hexagonal array of carbon atoms with one atom thickness. The term “graphene” is first referred in 1987 to describe single sheets of graphite. This allotrope holds an elegant position amongst all discovered materials owing to its exciting properties and enormous applications. World acknowledged scientists Andre Geim and kostya Novoselov by Nobel Prize for the year 2010 (physics) for their consummate contributions in the field of graphene. This monolayer hexagonal carbon is counted as the thinnest material reported till date. Though graphene is ultra-light and too flexible as rubber, surprisingly it was found to be potent enough to compete with a series of materials in terms of its strength. This single sheet of graphene can be converted in to other allotropes of carbon with entirely different dimensionalities via effortless physical actions such as folding (fullerene – 0D), rolling (carbon nanotube- 1D) and stacking (graphite-3D). The much celebrated graphene family includes three important members, graphite oxide, graphene oxide and reduced graphene oxide, the materials evolved during the synthesis of graphene. All these three forms strictly differ in the number of layers and the levels of oxidation of carbon atom. Graphene is the purest form of carbon; it has got commendably large theoretical specific area 2360 m^2/g, extremely high Young's modulus (~1.0 TPa), great optical transmittance (97.7%), high intrinsic mobility (200,000 cm^2 $v–^1$ $s–^1$) and unbeatable thermal conductivity (~5000$Wm^{-1}K^{-1}$). Graphene and its composites find applications in energy storage, sensors, light emitting diodes, super-capacitors, solar cellsenvironmental applications, transistors etc.

1.2.1 Extension to carbon nano allotropes

Carbon family possesses a vast collection of interesting nano carbon forms which should be counted as an extension to the aforementioned main members of carbon family. The identification of following nano forms of carbon was less revelatory compared to the prominent allotropes (CNTs, fullerene, graphene etc.). But it is believed to be quite note-worthy to mention about their names and whereabouts at this context, as it further demonstrates the amazing capability of carbon to form distinctive nano-structures which are matchless while comparing with the nano structures of other classes reported so far.

(I) Nano diamonds

Nano sized crystalline allotrope of carbon with sp^3 hybridization having diamondoid topology was widely recognized as nano diamond. They possess diameters greater than 2 nm, and not exceed 20 nm in size. Nano structures of this class having diameters greater than 20 nm act exactly as bulk diamonds. Lower limit of diameter of nano diamond is not prescribed yet. Nano diamonds are usually obtained through abrasion of microdiamonds or through processes like jet milling. They were found to be non- dispersible in nature. Detailed investigation on their structural frame works reveals that about fifteen percent of carbon atoms are situated at the nano diamond surfaces. They were found to be involved in bond formation with hydrogen/other atoms, thereby ensuring more stabilization to the structure. Nano diamond possesses features of organic molecules rather than bulk diamond. Depending upon the chemical conditions adopted for the purification process, various organic functional groups will be deposited over the nano diamond surfaces. Nano diamonds widely found their applications in photo-detectors, Raman lasers, micro lenses, quantum information, communication technologies etc,.

(II) Onion-like carbon spheres

Onion-like carbon structures possess various giant fullerenes, concentric graphenic shell, which encircles a series of gradually smaller fullerenes. These multi shell spherical hollow allotrope of carbon were first discovered by Ugarte from a mixture of CNTs after prolonged irradiation of strong electron beam to it. This nano form of carbon with interesting construction is the least studied member in the carbon family tree. Compared to the other nano-allotropes of carbon, Onion-like carbon structures are found to possess greater capability for energy storage applications. Thus, these allotropes may have decisive role in the construction of super capacitors and efficient electrodes.

(III) Single Walled Nano Horns

Nanohorns, another interesting class of carbon nano-allotrope, are the tubule or cone-like constructions made by mono graphenic layer. During 1999, the scientist Lijima met this structure while conducting detailed investigation on carbon nano tube structure as well as its formation mechanism. It was reported that the structure of single walled nano horns strongly resembles that of dahlia flower, as they appears as collectively large spherical aggregates with 80-100 nm diameter size. Among this collective aggregation of spheres, individual nano horns can be identified. These individual nano horns possess diameters about 1-2 nm at their tips and almost 4- 5nm diameters around the base of cone structure. They usually have 0.4 nm distance between adjacent walls of each single walled nano horn structures. Other than these commonly observed single walled nano horns, there occurs different varieties of nano horn aggregates which mimic buds and seeds in all aspects.

(IV) Graphene nano ribbons

Graphene nano ribbons are thin strips of graphene single layer, formed by the ‘chemical unzipping’ of conventional 1 dimensional carbon nano tubes. They are currently grabbing much research attention as they can be re-framed as a potent alternative of graphene. Combined action of strong oxidizing agents such as potassium permanganate and sulphuric acid results in successive unfolding of individual graphitic layers of nanotubes. This unfolding leads to the formation of thin graphene nano ribbons decorated with excess amount of oxygen containing functional groups such as carboxyl, carbonyl, hydroxyl groups at the corners and the entire surfaces. This 1 Dimensional carbon nano form consists of sp^2 hybridized carbon atoms. As the oxidized sites were marked with disruption of aromatic frame work, graphene nano ribbons exhibit very poor electrical conductivity. They have been widely used as efficient contrast agents for photo/thermo acoustic imaging and also for tomography imaging. Nano ribbons as well as their corresponding oxidized equivalents are successfully employed as potent nano-fillers to reinforce the mechanical features of polymeric nano hybrids. Based on their edge termination, graphene nano ribbons are classified in to three; zigzag nanoribbons (i), chiral nanoribbons (ii) and armchair nanoribbons (iii).Theoreticians utilized graphene nanoribbons as an appropriate model tool to probe its interesting magnetic nature and also to recognize each parameter that decides the magnetic features of pristine graphene and its derivatives.

For the last few decades, researchers mainly focus on this prolific non-toxic element, carbon, and the wide spectrum of its family members. Countless efforts by these research communities make the carbon vocabulary affluent.

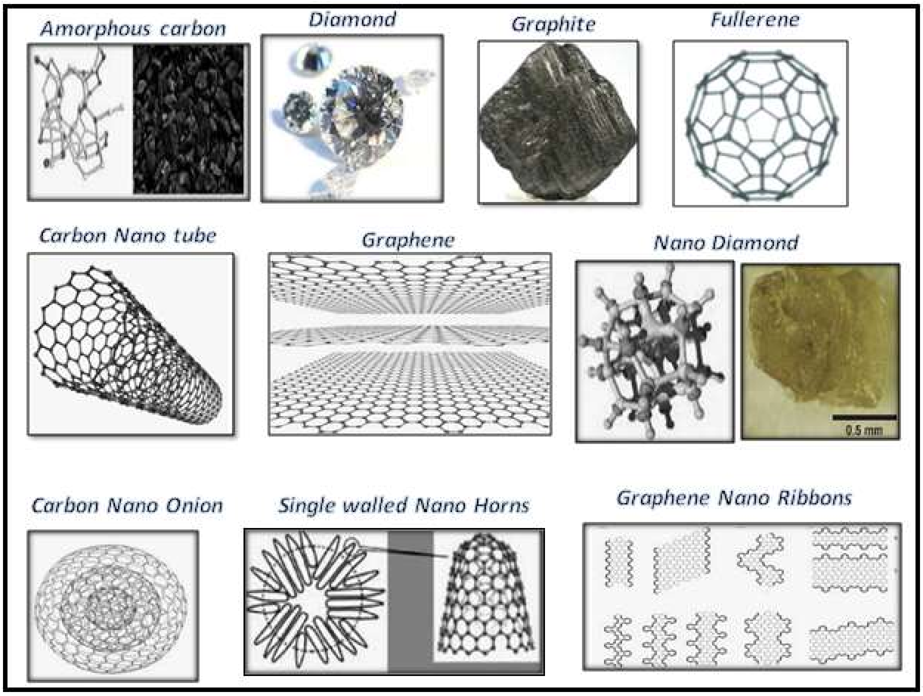


Figure 1.1 : Various allotropes of carbon

1.3 Carbon quantum dots -The surprise entry of bright fluorescent nano sized carbon

As carbon has got a wide spectrum of allotropes with promising applications in various fields of science and technologies, it always upholds the supreme rank among the elements with the inestimable designation, "Carbon - king of the elements"[2]. Pathways of carbon research were strictly focussed on the peerless features of the element including higher surface area, mechanical strength, thermal conductivity and commendable electronic properties. Till the early stages of 20th century, the scientific scenarios were in such a way that the entire possibilities for the utilization of properties of this element are strictly restricted to the aforementioned class. Due to its black colour and reluctance to aqueous dispersibility, it was rather far beyond imaginations / expectations to correlate the word “carbon” with the alluring world of “colour and light”-optics.

It was in 2004, contradictory to the formal scientific paradigms and observations, there occurred the accidental birth of a bright fluorescent carbon while conducting a routine laborious practice, which were finally termed as carbon quantum dots [7]. This serendipitous identification of fluorescent carbon has become a ground breaking discovery as it triggered a great deal of research efforts and left the entire scientific world restless for probing deeper in to their origin and structure.

1.3.1 Serendipity- Building block of scientific inventions

Though majority of the scientific inventions happened as an outcome of painstaking zealous goal oriented laborious experiments, it has to be admitted that a great deal of incredible innovations in the world were always accompanied with “luckily happened accidents“ when somebody found out something that they weren’t in search of. The most important fact about this kind of surprising incidents was, none of these “unexpected” observations would have been the world changing invention, if they take place without the presence of such brilliant research minds/brain, capable enough to recognize their implications. Hence, it should be admitted that basic inquisitiveness and observation skill are the indispensable precursors to scientific inventions.

It was reported in literature that most of the researchers discarded numerous findings and ideas as they were belonging to the "unsought" category [8]. This strong competition between 'sought' versus 'unsought' forces the research community to leave certain novel observations as worthless which may perhaps become major ground-breaking discovery that the entire world was longing for several decades. Detailed analysis of several un-anticipated findings has revolutionised the entire world as most of them were essential for the well-being of layman life. History provides most suitable pieces of evidences for illustrating the aforementioned fact [https://www.sciencealert.com/these-eighteen-accidental-scientific-discoveries-changed-the-world].

- In 1895, Wilhelm Roentogen, German physicist, while working with a cathode ray tube and a crystal, noticed that though the cathode ray tube was covered, it produces a bright glow on the chemical coated screen placed in front of the apparatus in that dark room. He was enthusiastic with this particular observation and started to do several experiments using the rays that caused this particular glow under different scientific circumstances. For better understanding of this finding, he duplicated the whole experimental set-up by blocking the pathway between ray tube and screen by placing several materials within the space. He collected several data to correlate with the observations. Finally, he found out the fact that irrespective of the material placed between the light source and screen, the glow remains unaltered. Surprisingly he noticed that while placing his own hand in front of the tube, he could clearly see the image of bones of his hands on the screen. This is further termed as a medical miracle as it is offering clear photographs of human bones without undergoing any kind of surgery which could serve as most anticipated boon in the field of medical science. Roentogen was recognized with Nobel Prize in 1901 (first Nobel prize in physics) for this invention of X-rays.

- One of the most popular Nobel Prize winning inventions, penicillin, was also born from such a “by-chance” observation. The well known Scotish biologist Alexander Fleming was busy with the preparation of paper discussing biological actions of several harmful bacteria. As a part of this study, he had placed a number of pertidishes in his lab in which staphylococcus bacteria colonies were grown. After few days of vacation he visited his lab and found out something intriguing in the pertidish, which he has forgotten to cover with a lid. The petridish that left uncovered possessed a green mold over the bacterial colony. This green mold was identified as a collection of “pennicillium notatum”, which was considered as a contaminant for the preserved colony of staphylococcus bacteria. The action of this green mold appeared over the uncovered petridish was something interesting, the harmful colonies of staphylococcus bacteria wasn’t growing around this green mold. This observation provoked his research mind and he duplicated the entire experimental setup with and without the green mold for further confirming the authenticity of this particular observation. After undergoing detailed scientific analysis he strengthened his hypothesis that the green mold of penicillium notatum is secreting an ‘antibacterial’ substance capable enough to kill/destruct the vast collection of toxic staphylococcus bacteria. He extracted this peniccillium mold and subjected to various prolonged scientific assays, and finally he named the first modern antibiotic as “penicillin” in 1928. This antibiotic has saved the lives of million from the vicious face of world wars. He received the most prestigious recognition from the scientific word, Nobel Prize in 1945 for his imperative contribution to the field of medicine, and thereby saving mankind.

- An engineer at Roythean Corporation, Percy Spencer, who was engaged in radar based projects and experiments discovered the presence of a strong heat energy source which could melt any substance placed at the premises of the experimental arrangement. During his radar assisted experiments, surprisingly it was noted that a chocolate bar placed in his pocket was melted so quickly while he was operating the vacuum tube apparatus. This unexpected observation made him restless and he was continuously engaged in experiments with the same vacuum tube to probe much deeper in to the cause. After this detailed investigations, he concluded that the heat which is able to melt all those nearby substances was coming from microwave energy. Finally Spencer filed patent for the 'first micro-wave" source, which further revolutionized the energy concept of scientific world.

- Safety glasses that are widely used in safety goggles and car wind shields were also originated from such an un-expected observation. A French scientist, Benedictus accidently dropped a flask made out of glass which is filled with cellulose nitrate solution, a kind of liquid plastic. Definitely, the glass was broken and the liquid has evaporated. Surprisingly, the broken glass pieces were never got shattered. The glass pieces remains in place and they maintained the exact shape of the container. Upon detailed study the scientist found out that, perhaps the plastic coating inside the glass flask (container) had prevented the glass pieces from shattering and helped them to stay together even after breaking, leading to the concept of safety glasses.

- In this similar manner, history had seen numerous un-expected incidents that pave way to adorable innovations, which are capable enough to change the world. The discovery of excellent adhesive-'superglue' , heat resistant and non-corrosive 'Teflon materials' , mouldable clay for making profitable toys - 'play-doh' , Successful implantation of first 'pacemaker', the artificial sweetener- 'Saccharin', origin of the most significant reaction process-'Radio activity' etc., belong to this interesting category.

In brief, it is clearly evident that such unforeseen discoveries are of considerable importance in the progression of science and technology. These kinds of unsought, yet vital observations met with scheduled practices, often provide strong foundation for essential academic leaps of understanding. In literature, it was better summarized by the legendary writer Lawrence Block, who discusses this conspicuous idea in very simple words as follows [8].

"One aspect of serendipity to bear in mind is that you have to be looking for something in order to find something else" (Block 2004).

World of carbon research has also met this type of serendipity in the year 2004. Unexpected evolution of a small portion of bright light emitting material along with the expected bulk product has astonished the researchers, as they were dealing with the member of dark community – carbon.

1.3.2 Preliminary Identification of fluorescent carbon - Electrophoretic purification of Single Walled Carbon Nanotubes (SWCNTs)

In 2004, Xiaoyou Xu, Robert Ray, Yunlong Gu, Harry J. Ploehn, Latha Gearheart, Kyle Raker and Walter A. Scrivens, a group of researchers in the Departments of Chemistry, Bio-chemistry and Chemical Engineering from the University of South Carolina and Presbyterian College- south Carolina were attempting the perfect purification of single walled carbon nanotubes (SWCNTs) obtained through different synthesis techniques [7]. SWNTs obtained through arc-discharge method are found to have more crude soot than that from vapour-deposition and laser ablation techniques. Owing to the excess quantities of impurities, arc-discharged soot were subjected to series of purification techniques including oxidative cutting, micro-filtration process etc. Arc-discharged SWCNTs were found to be characterized by unique structures of graphitic sheets. Hence, they were found to be strongly resistant towards oxidative purification process due to the strong oxidative stability of graphitic layers within the CNT structure. Micro-filtration also stood as an ineffective purification technique, as the excess amounts of particles in the crude soot quickly block the pores of filters. Hence, the existence of graphitic sheets within the SWCNTs made the purification of arc-discharged soot more complicated.

Xu and co-workers successfully overcome this issue regarding the effective purification of arc-discharged SWCNT soot, by conducting gel-electrophoresis of the crude soot. It was reported in literature that single walled nano-tubes may undergo migrations towards electrodes and the thus obtained sample containing nanotubes can be separated using capillary electrophoresis. But unfortunately the experimental techniques failed to do such a preparative electrophoresis purification of SWCNTs. Hence, this research team took an innovative effort for the purification of SWCNTs obtained through arc-discharge method, by conducting preparative electrophoresis of crude soot using agarose gel and glass bead columns. This gel-based electrophoresis proceeds through sieve effects of individual components of discharged soot under the influence of the electric field. The components of the analyte sample get fractionated according to their different migration abilities upon the application of particular electric field towards the crude soot.

For the preparation of charged CNTs, the arc-discharged soot of SWCNTs were treated with nitric acid (3.3 Normal) followed by the extraction with aqueous sodium hydroxide solution having pH 8.4. Thus obtained aqueous extract is the crude suspension of SWCNTs, as it contains majority of the formed SWNTs and other soluble impurities that may be present within the arc-discharged SWCNT structures (30 Wt %). Further characterization confirms the fact that the sediment left after consecutive washes of crude suspension of SWCNTs possesses large amounts of graphitic sheets and bulk carbonaceous aggregates. This crude SWCNT suspension was subjected to the conventional gel-electrophoresis using 1% agarose gel slab by applying 600 Volts maintaining the reaction temperature at 55°C inside the unit. It was surprisingly observed that the crude SWCNT suspension was separated into three distinct classes of nanomaterials: (i) collection of long nanotubes which did not pierce the gel matrix, (ii) slowly moving dark bands corresponding to the collection of short tubular carbon and (iii) quickly moving multi-coloured bands indicating the mixture of highly fluorescent carbon nano particles. The Electrophoretic profile of crude sample under UV light illumination (365nm excitation) is displayed as well A in Figure 1.2. Well B represents the mixture of multi-colour emitting fluorescent carbon nano materials. It is further separated in to another three discrete bands showing three different coloured emissions upon 365 nm excitation of UV light. The digital image displaying the aforementioned multicoloured emission by the three distinct bands of fluorescent nano carbon is provided below as Figure 1.2 (ii). Well C corresponding to mixture of short tubular carbon is further fractionated into two distinct classes of nanomaterials moving with two different electrophoretic mobility as wells D and E. As mentioned previously, the remaining set of long tubes of carbon agglomerates that are unable to penetrate the gel matrix was further subjected to mild acid assisted treatment for ensuring the purity of the crude soot. The long nanotube agglomerates were chopped in to nanotubes of desired size via harsh oxidative cutting. Thus obtained material was found to be able to penetrate the gel matrix when it was run on the gel. Well F of Figure 1.2 (i) denotes this chopped short carbon nanotubes. A small fraction of fluorescent carbon material was produced during the chopping process. Thus isolated fluorescent nano materials were further subjected to various characterization techniques for confirming the formation of homogenous carbon nano particles. FT- IR spectral data show the presence of carboxyl functionalities within the carbon nano particle.

In the year 2000, four years earlier to this unexpected discovery, Riggs and co-workers identified strong luminescent CNTs while dispersing and cutting down the polymer bound CNTs in homogenous organic solvents [30]. Regardless of this early identification by Riggs team, the aforementioned gel-electrophoretic purification by Xu et al. in 2004 was recognized as the serendipitous invention of fluorescent carbon nano dots, as they were able to perform successful isolation, detection and analysis of bright emitting multicoloured fluorescent carbon nano materials for the first time.

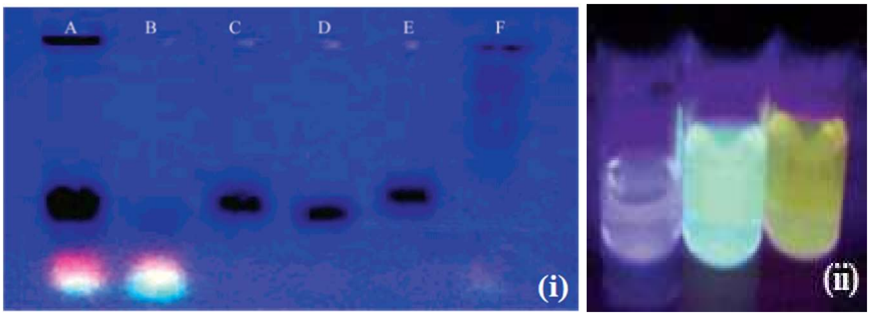


Figure 1.2 : Primary identification of Carbon dots; (i) Electrophoretic profile of SWCNT suspension in 1% agarose gel matrix under UV light of 365 nm wavelength; Well A- Crude suspension of SWNTs, Well B- Bright emitting fluorescent carbon materials, Well C- collection of short tubular carbon, Wells D & E – further separation of Well C and Well F- Chopped SWNTs. (ii) Digital image of the three different fractions of bright fluorescent carbon materials (well B of Figure i)

This unintentional birth of an elegant material, comprising a wide range of bright colours, tempted the researchers for executing conscientious efforts for recreating the formation of this light emitting carbon (fluorescent). This in turn offered a vast platform for probing out the hidden mechanistic route for their formation, and also detailed evaluation of its structure and features. From its surprising evolution on 2004, a series of sincere endeavours were reported in literature for making the picture of this “fascinating material” more evident , by formulating certain scientifically acceptable mechanism to explain its formation and properties. In spite of these meticulous researches, the scientific world still lacks an exact mechanism for explaining the evolution of such a colour emitting carbon material and satisfactory explanations to define its excellent fluorescent nature. This debatable circumstance regarding the existence, detailed structure and properties of this fluorescent carbon can be attributed to the words of great science philosopher Emmanuel Kant, who wrote ***"Accidental observations, made in obedience to no previously thought-out plan, can never be made to yield a necessary law, which reason alone is concerned to discover . . ." (Kant 1929/1965)*** *while referring to classic experimental discoveries in Kant's vision of science* [8].

1.3.3 Different kinds of fluorescent carbon nanodots

Though there occurs a continuous leap in the synthesis of different types of fluorescent carbon nano particles, scientific community strictly lacks a general nomenclature which is capable enough to provide an indisputable understanding about their structure and features. As mentioned previously, during the first identification of fluorescent carbon by Xu et al, they coined the name "carbon quantum dot" without giving any satisfactory scientific evidences/clarifications to be called as "quantum dots"[7,9]. For them, any bright emitting particle in nano range might term as "quantum dot", no matter whether they possess quantum confinement or not. The title "quantum dot" was first used by the scientist Mark Reed, to define semiconductor nano materials with quantum confinement effect; size less than Bohr exciton radius / particles within a few nanometer range [10]. Excitons of these semiconductor nano particles were strictly confined to quantized energy levels, thereby named as semiconductor quantum dots (SQDs). Owing to the similarities of the particles with semiconductor quantum dots in its fluorescence and nano scale dimensions, Xu and co-workers simply called the obtained luminescent by-product as “carbon quantum dots”. Generally, all carbon nano particle surfaces contain a considerable percentage of oxygen atoms, making them water soluble and tunes them highly bio-compatible and less toxic. Though semiconductor quantum dots have received huge amounts of attention and practical applications, they were associated with certain drawbacks related to environmental and health issues. Majority of the semiconductor quantum dots were highly toxic and they tend to aggregate upon storage and release noxious heavy metal ions from the core to the exterior. This demerit was soon after overcome with the introduction of non-heavy metal based semiconductor quantum dots such as Zns, ZnO, ZnSe etc,.This situation enhances the demand ofcarbon-based nano dots, as they offer environmentally benign and user-friendly fluorescence.

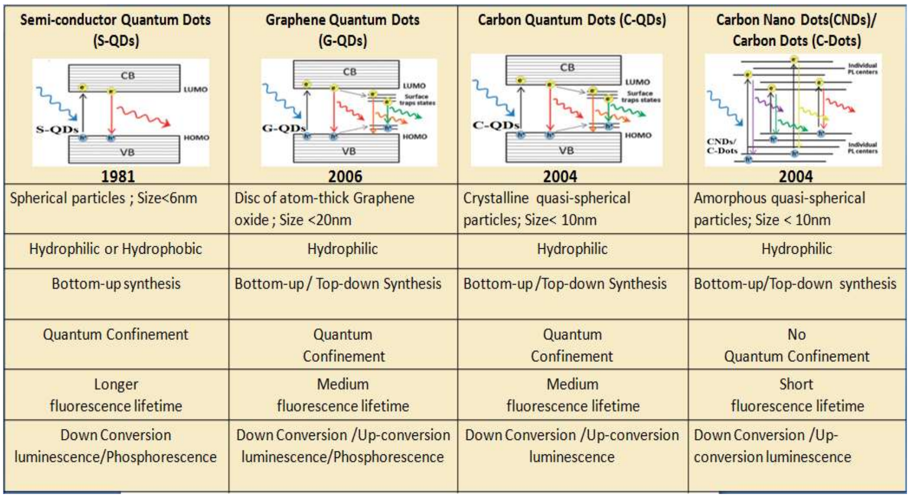
This intense urge for non-toxic fluorescent nano dots creates an exponential increase in the number of such “carbon quantum dots” in literature [11-22]. As earlier, scientists never probed deeper in to these materials to collect any scientific information regarding the structure and particle orientations to frame a universally acceptable theory to explain the mechanism and existence. Hence, it has to be admitted that majority of the fluorescent nano carbons were improperly labelled as “Quantum dots”. This lack of precision about the description should be overcome by getting clear picture about both the concepts; “Quantum” and “dots”. For this, one has to be well aware of the characteristics of carbon dots including orientation of carbon atoms, structural pattern-crystalline/amorphous, and also the dimensionality. Based on this, the wide collection of carbonaceous fluorescent nano dots can be simply classified into three main categories, as detailed below.

Carbon Quantum Dots (CQDs): Fluorescent quasi-spherical carbon nano particles with well defined crystalline structure and quantum confinement can be termed as "carbon quantum dots". As carbon quantum dots are having crystalline arrangement, carbon nanostructures with perfect crystalline orientation are the most eligible precursors for them [21]. Majority of the researchers utilized carbon nano tube, the carbon nano material with perfect crystalline arrangement, as the excellent precursor to carbon quantum dots. Higher temperature assisted pyrolysis of bulk organic molecules can also yield CQDs. Being a carbon based material, CQD surface is abundantly decorated with oxygen containing functional groups. This makes them highly vulnerable towards functionalisation with several organic molecules and hetero atom insertions. The aforesaid surface functionalisation will enhance the properties of pristine CQD structure, thereby creating surface defects or excess amount of surface functional groups. Presence of these types of surface defects, impurities, functional groups etc, is counted as trap states of this material. The spherical nano carbon strictly follows "quantum confinement effect" akin to that of semiconductor quantum dots. But, unlike the case of the later, surface traps buried within the structure of former also plays a vital role in deciding the photo-luminescence mechanism along with the quantum confinement effect. Upon light irradiation, the aforesaid surface trap states result in entrapment of photo-excited electrons/holes, followed by consequent recombination of these charge carriers leading to emission of radiation with lower energy / higher wavelength. Hence, the resulting fluorescence is not form a sole source. The synergistic action of carbon core, inherent quantization effect, as well as surface trap states are serving as the photo-luminescence origin of carbon quantum dots [23,24]. CQDs are characterized by comparatively broad fluorescence emission bands. They exhibit excitation dependant fluorescence emission. For them, continuous increment in the excitation wavelength results gradual shifting of fluorescence emission curve to higher wavelength, with successive reduction in its intensity. This excitation dependent fluorescence emission is an interesting observation, as semiconductor quantum dots, exhibit fixed emission curve irrespective of the excitation wavelength changes [21].

Carbon nanodots (C-dot, CDs): These are amorphous carbon structures having quasi-spherical morphology with particle size between 2 to 10 nm [21]. Carbon nanodots completely lack "Quantum confinement". Any organic material upon carbonization will yield carbon nanodots. They always exhibit wide fluorescence emission band, and emission curve of this material shifts gradually to the higher wavelengths while successively increasing the excitation wavelength. All carbon nanodots are hydrophilic and environmentally benign by nature. Researchers usually abbreviated this material as "C-dot", "CDs" and sometimes as C-nano clusters. As they are having nanometer diameter, they deserve the label "dots". But we cannot use the word “quantum” to describe these materials, as they were devoid of quantum confinement effect. Due to the absence of quantization of excitons, their photo-luminescence emission cannot be connected with the general fluorescence mechanisms including collective excitonic effect and quantum confinement effect. It was found out that the fluorescence of these carbon nanodots arises to the superposition of assembled individual fluorophore units/emitters situated on the surface [25]. If the abovementioned individual emitter units were blocked/ attached with external agents including metal ions, organic molecules, bio-molecules etc., the inherent fluorescence intensity of the system will be suppressed/ quenched.

Graphene Quantum Dots (GQDs): π conjugated single sheets of carbon in nano scale dimensions with well defined quantum confinement effect are termed as graphene quantum dots. In simple words, they are disk of graphene having size in the range of 2 to 20 nm [21]. This laminar carbon quantum dots were identified in 2006, immediately after the appearance of the interesting class of fluorescent carbon nano dots. Graphene quantum dots are abbreviated as ’GQDs’. GQDs were generally synthesised from graphene based materials. Majority of the research works yield graphene quantum dots through oxidative cutting of carbonaceous materials including graphite, carbon fibres and carbon nanotubes via modified Hummer’s method, oxygen plasma treatment, hydrothermal methods, organic synthesis using tiny fragments of molecules as precursors and electrochemical methods [26]. Graphene quantum dots possess an intact graphene structure as their carbon cores are decorated well with oxygen bearing functionalities. The formation of GQDs, miniaturization of graphene, induces band gaps to the well exploited carbon structure ‘graphene’. This is due to its innate quantum confinement effect, which further highlights the credibility of this invention. The aforementioned structural band gap created within the graphene core of GQDs serves as the intrinsic photoluminescence emission centre. As GQDs are considered to be zero-dimensional graphene with non-zero band gap, the inherent light emitting feature of this nano material can be attributed to the conjugated π states of their core structure. Similar to the case of CQDs, along with the quantum confinement effect of excitons, there exist other vital fluorescent centres within the GQD structure. Light irradiation of GQDs results in charge carrier excitations, followed by the radiative recombination of excited electron/hole pairs within the conjugated π / sp^2 states of its graphene core. Simultaneous to this intrinsic core-state emission, the surface defects as well as the surface functionalities together contribute towards the bright emission of GQDs [27-29]. Graphene quantum dots possess wide spectrum of applications including construction of bright light emitting diodes, solar cells, bio-sensors and molecular sensors, photo-detector manufacturing and organic photovoltaic diode fabrication etc.

As GQDs possess much lighter atoms than that of metal based quantum structures, they possess comparably low dielectric constant and very weak spin-orbit coupling. Regardless of these features, GQDs are marked with admirable charge carrier interactions, and thereby possess discrete electronic levels with strictly defined spin multiplicity. This leads to the creation of comparatively larger sized energy bands within GQD structure than that of conventional semiconductor quantum dots of similar size. Literature also shows that the absorption of a single photon results excess amount of exciton generation within GQDs [28,29,18]. This enhanced carrier-carrier interactions will gradually increase the number of carrier excitons, which further improves the efficacy of photo-excited carriers. Similar to CQDS, GQDs also possess comparatively broad emission bands with excitation wavelength dependant fluorescence emission curves [21].



Semi-conductor Quantum Dots (S-QDs)	Graphene Quantum Dots (G-QDs)	Carbon Quantum Dots (C-QDs)	Carbon Nano Dots(CNDs)/ Carbon Dots (C-Dots)
1981	2006	2004	2004
Spherical particles ; Size<6nm	Disc of atom-thick Graphene oxide ; Size <20nm	Crystalline quasi-spherical particles; Size< 10nm	Amorphous quasi-spherical particles; Size < 10nm
Hydrophilic or Hydrophobic	Hydrophilic	Hydrophilic	Hydrophilic
Bottom-up synthesis	Bottom-up / Top-down Synthesis	Bottom-up /Top-down Synthesis	Bottom-up/Top-down synthesis
Quantum Confinement	Quantum Confinement	Quantum Confinement	No Quantum Confinement
Longer fluorescence lifetime	Medium fluorescence lifetime	Medium fluorescence lifetime	Short fluorescence lifetime
Down Conversion luminescence/Phosphorescence	Down Conversion /Up-conversion luminescence/Phosphorescence	Down Conversion /Up-conversion luminescence	Down Conversion /Up-conversion luminescence

Figure 1.3: Classification of various fluorescent nano dots structures

The basic differences between carbon dots and semiconductor quantum dots are summarized in Figure 1.3 [21].

1.4 Carbon dots- Features

As mentioned earlier, the comprehensive term “carbon nanodots” (CDs) is widely used for describing all fluorescent quasi-spherical carbon structures with diameter below 10 nm which lacks any necessary scientific proof of quantum confinement effects. Owing to their exciting features, CDs grabbed immense attention in scientific world. Majority of the research works are focused towards the fabrication of bright emitting CDs from various precursors adopting different synthesis routes [15,20,22].This immensely growing interest produced numerous publications emphasizing the fabrication of CDs with significantly improved/tailored features, which tune these tiny carbon fragments as the multidisciplinary explored material during recent years. Fascinating fluorescence shown by the CDs makes them strong competitor for the commercially available organic fluorescent dyes and conventional semiconductor quantum dots. But, in contrast to metal based quantum dots and organic dyes, CDs possess a series of credible attributes including non-toxicity, good bio-compatibility, water solubility, temperature resistant non-blinking fluorescence and cost effective simple synthetic methods etc. [20,31, 32]. All these factors make them superior in position compared to those aforementioned conventional fluorescent analogies.

1.4.1 Plausible structure of fluorescent CDs

The diversity of precursors used, methods adopted and post-chemical treatments make the precise determination of chemical structure of CDs is almost ambiguous. Generally, the structure of CDs is believed to be composed of a carbogenic core hosting several functional groups, mainly of oxygen bearing groups, including carboxyl, carbonyl and epoxyl functionalities. As the core structure and the surface shells are relatively prone to the synthesis routes and the reaction parameters, aforesaid diversity leads to entirely different intrinsic architecture for CDs. This in-turn makes a qualitative comparison of different CDs practically impossible [18,23,25,33]. Regardless of this confusion, there are some interesting efforts in literature which involve systematic classification of the most probable core structures and morphologies of CDs [21,34]. In general, the core structure of CDs are evaluated with the aid of certain characterization tools including High-Resolution Transmission Electron Microscopy (HRTEM), X-ray Diffraction analysis (XRD), Atomic Force Microscopy (AFM) and Raman spectroscopy, whereas the connected surface groups were mainly analysed via Fourier-Transform Infra Red spectroscopy (FTIR spectroscopy) and X-ray Photoelectron Spectroscopy (XPS).

Literature presents some vague detailing related to the possible core structures of CDs, as follows**.** Hu and colleagues assigned a diamond like orientation for the core structure of CDs, based on the SAED pattern and ring radius ratio [35]. Some works reports sp^2 hybridized graphitic core domains with sp^3 defects. Valid inferences from the lattice spacing data from XRD analysis, C1s XPS details, Raman spectral profiles as well as the FTIR spectral bands clearly confirm this observation [36-42].Thermal decomposition of ammonium citrate salts yields blue emitting CDs, whose XRD pattern displays two broad peaks that are superimposed (one at 0.35 nm and the other at 0.49 nm), demonstrating defective carbon frame work [43]. For them, the core of CDs possesses carbonized components with highly disordered structural format comprising both aromatic as well as aliphatic moieties. This structural pattern strongly resembles the graphite oxide structure or interrelated networks of carbogenic materials including coal, lignite and humic contents.

Numerous reports were there in literature, suggesting extraordinary amorphous core structure for CDs via meticulous analysis of their XRD pattern and Raman spectral profiles [44-49,19].In addition, Liu and co-workers provided HR-TEM images of corresponding CDs, which lacks apparent lattice fringe patterns for illustrating the aforesaid amorphous nature [44].

Some authors report the presence of sp^2 graphitic clusters within the oxygen containing amorphous carbon matrix of CDs, which is further scientifically backed by the HR-TEM images and Raman spectrum with more evident D and G bands (slightly different from pure amorphous carbon)[50-54]. They also point out the fact that the presence of more evident D band as well as the absence of 2D peak was solid enough to ensure the formation of partially graphitized sp^2 clusters (other than the pure graphite form) embedded within the amorphous core [50]. Hence, these works classify CDs as a multi-crystalline/amorphous carbon form bearing considerable quantity of sp^2 clusters. For Zhu and group, the HRTEM image analysis was adequate enough to confirm the formation of amorphous particles lacking any distinct lattice fringes. This observation was further illustrated using XRD pattern having distinct bands around 25° indicating highly disordered carbon frame with lattice spacing ~0.34 nm [51]. They also confirmed the presence of sp^3 aliphatic carbons and sp^2 clusters within the hydrothermally synthesied CDs by analysing ^{13}C NMR signals located around 30 ppm-45 ppm and 100 ppm- 185 ppm, respectively.

As mentioned earlier, the sp^2-sp^3 mixed core was believed to be decorated with certain functional groups ranging from small groups to longer molecular chains, which is generally termed as surface functionalities. Owing to the lack of any kind of systematic awareness on the formation mechanism of such CDs structure, the Predictability of their chemical compositions still remains debatable. Irrespective of the enthusiastic efforts for unveiling the core structure details of CDs, attempts for identifying and classifying their surface structure was seldom reported. In 2017, an interesting investigation was done by Aji and co-worker which shed insight in to the surface moiety attached into the core structure of tannic acid derived CDs (acid catalyzed ester hydrolysis of tannic acid) [55]. Through this study, these authors portray a plausible formation mechanism for this CDs, which ends up with the formation of partially graphitized sp^2 (cluster) core domains decorated with ellagic acid derivatives. This chemical structure was believed to be formed during the synthesis, which proceeds through the depolymerisation of precursor (tannic acid). The depolymerisation leads to the closure of two tannic acid moieties, that makes ellagic acid derivative .They also illustrate the presence of ellagic acid derivative by the systematic analysis of Electro spray ionization mass spectroscopy (ESI-MS) signals, FTIR bands and XPS peaks of the CDs. As this work suggests, comprehensive understanding on the formation mechanism of the obtained CDs is inevitable for identifying the attached surface moieties, which may further help to predict the underlying fluorescence mechanism. Hence, based on careful analysis of the aforementioned class of experimental observations, one can definitely suggest a plausible architecture (general) for CDs that comprising a sp^2 domain core decorated with oxygen rich functionalities (Figure 1.4).

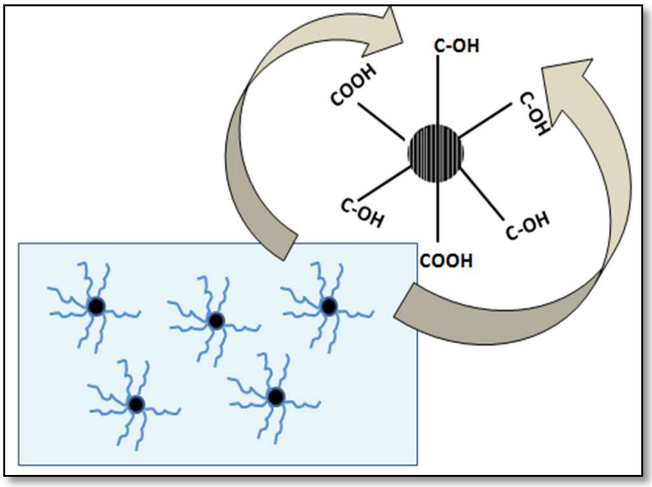


Figure 1.4 : Plausible General Structural Framework of Fluorescent CDs.

1.4.2 Synthesis methods of CDs

From the time of its first identification itself, these fluorescent carbon nano particles have gained remarkable attention among the research community, which subsequently resulted in enormous amount of efforts for their successful fabrication. Generally, the available synthesis pathways for CDs are classified into two categories: (i) Top-down approach and (ii) Bottom-up approach.

(a) Top-down synthesis approach for CDs

In simple words, Top-down synthesis techniques can be described as the process of nano-cutting, which involves dispersion/ destruction of macrostructures of carbon into desired nano dimensions via different chemical processes. Commonly, large carbon structures with sp^2 hybridization that lack efficient energy gaps/band gaps to exhibit fluorescence feature, were selected as the starting materials for the top-down processes. The precursors with aforesaid requirements include carbon nano tubes (CNTs) [7, 56] candle soot [57], carbon rods [58] carbon black [59] carbon fibres [60] and graphite powder [61]. These non-fluorescent bulk carbon structures were made fluorescent, by fine-tuning the surface chemistry and the size of the precursors. For this, the bulk precursor materials were subjected to various chemical and physical processes. Chemical methods include acid treatments, electrochemical methods, metal-graphite intercalation and oxidation processes [61]. Physical methods involve laser ablation, arc-discharge, ultra-sound assisted sonication etc. [61-66]

Laser Ablation Method

In Laser ablation technique, precursor is subjected to intense laser pulse irradiation creating higher temperature-pressure thermodynamic environment within the surface. This will lead to rapid heating of target material, which subsequently crystallizes to nanoparticles of desired dimension after transforming (vaporizes) through plasma state. [67-70]. The first report on laser ablation mediated synthesis of fluorescent CDs was presented by sun et al.,[9], who used laser beams from Nd:YAG (1064 nm, 10 Hz) source maintaining argon atmosphere under 900°C and pressure of 75 kPa. Being one of the most efficient synthesis route to CDs, numerous scientists have adopted this laser assisted ablation process which ensures good aqueous dispersibility, narrow size distribution as well as fine fluorescence features. In 2009, Hu and co-workers demonstrated that the proper choice of organic solvent used during the laser operation would vary the surface state/ functionalities of CDs, thereby enabling fine-tuning of its luminescence properties [35]. Later in 2011, Li and group used laser ablation technique by altering the organic solvents in which the used carbonaceous target was dispersed, yielding CDs with interesting features including visible luminescence with tunable emission [71]. Despite the credibility and effectiveness of this method, the complicated operation and higher expense limit the wide usage of laser ablation synthesis of CDs.

Arc Discharge Method

As mentioned in the previous section, these fluorescent carbon particles were first identified by Xu and group quite unexpectedly during the Electrophoretic arc discharge purification of multi-walled carbon nano tubes (MWCNTs) [7]. The experimental observations were discussed in detail in the previous section. Various research groups have tried the arc discharge method, which yielded CDs attached with majority of hydrophilic functionalities which exhibit excellent water solubility [72]. Authors also reported that the arc-discharge synthesis of CDs ensures better reorganization of carbon atoms that decomposed from the macro/bulk precursor structure in the anodic electrode. During this process the carbon vapour assembles to form CDs in the cathode electrode [73,74]. Such arc discharge treatments were carried out inside a sealed reactor marinating very high temperature ambience (~ 4000 K) so as to produce high energy plasma for driving the entire reaction process. Bottini and group prepared bright fluorescent CDs via arc discharge treatment of pristine and oxidized SWCNTs, exhibiting violet-blue emission and blue to green emission respectively [75].

Electrochemical Method

In electrochemical synthesis methods, bulk carbon structures including CNTs, carbon fibres, graphite etc., are commonly used as the precursor (electrode) in presence of appropriate electrolyte solution. Electro-chemistry assisted fabrication is considered as one of the widely practised synthesis techniques to achieve ultrapure CDs. This is the most facile and convenient route to CDs, which can be performed under ambient temperature-pressure environment [76-79]. Zhou and group first reported an electrochemical avenue for the fabrication of blue luminescent CDs from multi-walled CNTs using tetra-butyl ammonium perchlorate solution as the electrolyte [80]. Later, Zheng and group produced water soluble CDs with tunable luminescence using graphite as the electrode material maintaining neutral pH with phosphate buffer [65]. Thus obtained CDs were found to be a promising candidate for bio-imaging applications. Crystalline CDs which can act as an efficient photocatalyst were prepared by Li and co-workers via electrochemical treatment of graphite [58]. These CDs were found to exhibit size tunable up-conversion luminescence, a note-worthy photoluminescence behaviour. In 2015, Hou and colleagues fabricated bright blue emitting CDs by electrochemical treatment (carbonization) of urea and sodium.citrate in de-ionized water [81]. These blue emitting CDs find their application as an efficient optical sensor for toxic mercury ions with commendable selectivity and sensitivity. Though, electrochemical methods were strongly believed to construct highly efficient electrocatalytic materials, CDs processed through one-pot electrochemical treatments of bulk carbon structures seldom found their application as efficient electro-catalysts.

Acidic Oxidation

Researchers mainly adopt acid treatments/ acidic oxidation method for exfoliating and further decomposing large carbon structures into luminescent carbon particles with nano-scale size. Along with the production of carbon nanoparticles, this method further ensures the simultaneous introduction of numerous hydrophilic groups (mainly carboxyl / hydroxyl) over the surface. This in-turn increases the aqueous solubility as well as luminescence features of the synthesised CDs [5,82-85]. Owing to their surface modifications and commendable aqueous stability, CDs obtained from such acidic oxidation processes were found to act as potent electro-catalyst [85]. In addition, Kim et al fabricated promising near-infra red emitting CDs via acidic oxidation treatment of tire soot, which found their application as efficient in-vitro and in-vivo bio-imaging tool [86].

(b) Bottom-Up synthesis Approach for CDs.

Bottom-up synthesis of CDs involves simple polymerization or carbonization of various small molecules via different chemical reactions yielding small clusters of reacting species which finally burst out into nanoparticles of desired dimensions (below 10 nm). In brief, bottom-up methodologies generally involve carbonization/pyrolysis of carbohydrates, followed by organic synthesis of molecular species and self-assembling of several aromatic (polycyclic) hydrocarbon compounds. Contrary to the top-down synthesis methodologies, bottom-up methods do not demand any specific requirement for the precursors. Any carbonaceous material can be tuned in to bright emitting CDs via bottom-up route. Owing to this flexibility, bottom-up methods were considered as the most effective synthesis strategy for the large scale production of fluorescent CDs [87,88].

Solvothermal Method

Solvent mediated synthesis of carbon nano dots are generally termed as solvothermal route. Hydrothermal synthesis methods usually involve the chemical processing of carbon nano particles in aqueous environment (in presence of solvents) under superheated conditions, i.e., greater than 100°C in sealed reaction containers/ vessels. This superheated condition imparts some favourable changes to the physical and chemical properties of water. Temperature between 150°C to 300°C (subcritical region) makes water more favourable for the dissolution of organic compounds [89,90]. Hydrothermal method generally involves comparatively simple experimental set-up. Majority of the hydrothermal reactions yield highly fluorescent carbon nano particles with commendably high quantum yield and almost uniform size distribution [91-94]. In brief, a typical hydrothermal approach involves dissolution of small organic moieties or polymeric materials in water or common organic solvents. This will subsequently lead to the formation of appropriate precursor, that assembles together to produce carbon seeding cores, which finally mature in to CDs of dimension below 10 nm [79]. One-pot hydrothermal synthesis strategy was first introduced by Zhang and group, which yielded CDs with 2 nm average particle size with quantum yield around 6.79 % from ascorbic acid in ethanol (solvent) medium [95].In 2013, Zhu and co-workers fabricated highly fluorescent CDs from citric acid and ethylene diamine via hydrothermal route with considerably high production yield and was found to be potent biosensor , for tracing out ferric ion (Fe^{3+}) in body cells [51].Full colour emitting CDs with controllable luminescence were synthesised by Hola and group via hydrothermal route, which exhibit tunable emission profile in accordance with alteration in the graphitic nitrogen content [96]. Recently, Lu and colleagues proved that hydrothermal condensation of bio-molecules with higher carbon and nitrogen content results in effective fine tuning of inner structures of CDs [97].

Micro-Wave treatment

Being one of the most established green alternative energy source, utilization of micro-wave radiation for the fabrication of CDs is grabbing considerable amount of attention. In addition to the eco-friendliness or green nature, commendable time-saving efficiency, excellent reaction rate/ faster reaction and energy saving efficiency increases the credibility of this synthesis method. Generally, microwave assisted reactions involve simple carbonization of tiny organic molecules via microwave irradiation/heating for very short time period leading to the formation of carbon nano structures with size below 10 nm. First report on microwave synthesis of CDs with fine electrochemiluminescence features was made by Zhu and co-workers in 2009, using carbohydrates as the starting material [98]. Followed by this, numerous synthesis efforts were reported in this direction, yielding CDs with promising applications in various fields [99-104].

Pyrolysis Method

Pyrolysis is considered as one of the facile reaction routes to luminescent CDs, utilizing several organic compounds as the starting material under very high temperature conditions in strongly acidic/alkaline medium. Guo and colleagues presented a very facile route to fluorescent CDs, which involves one-pot pyrolysis of human hair (keratin). Thus obtained CDs were able to serve as a potent sensing tool for one of the most noxious heavy metal ion, Hg^{2+} with commendable selectivity and sensitivity. [105]. Similarly, another group developed promising fluorescent sensing tool for biologically relevant ion, Fe^{3+}, through simple pyrolysis of guanidinium chloride and citric acid. These Nitrogen doped CDs found their application in fields including metal sensing as well as bio-imaging and therapeutic purposes [106].

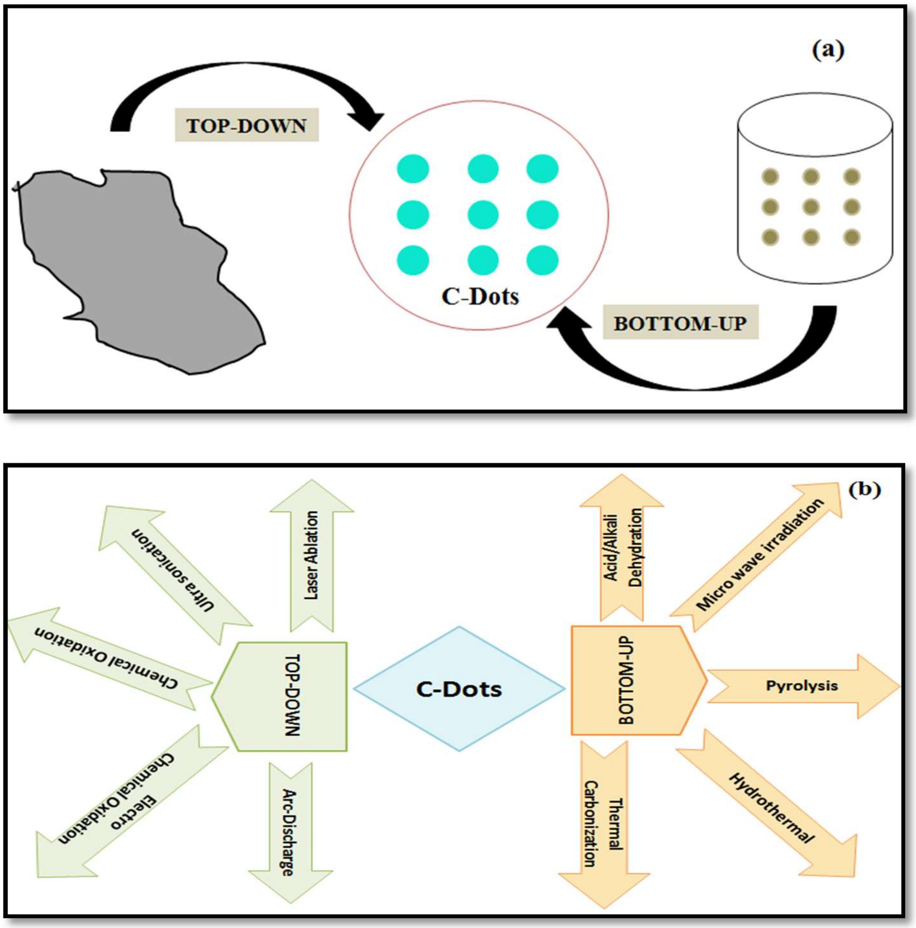


Figure 1.5: Schematic representation of Top-Down and Bottom-Up techniques to fluorescent CDs (a), List of various Top-down and Bottom-up synthesis methods commonly used to produce CDs (b).

1.4.3 Physico-chemical features of CDs

In spite of the extensive exploration in the field of fluorescent carbon nano dots, the systematic investigation, design and control of the precise structure still remains incomplete. This intricacy in defining the structural aspects along with the wide diversity of CDs makes the quantitative elucidation of their physiochemical properties more difficult. Nano-sized fluorescent carbon particles can be considered as the superposition of carbonaceous core (bulk) and numerous surface states, joined together in different proportions. Among the common features of CDs, few of them including toxicity, and photo-stability are primarily dictated by the carbon core. Some other properties such as dispersibility and luminescence intensities were exclusively derived from attached surface functional groups. It was suggested that majority of physico-chemical features were determined by the combined action of bulk and surface functional groups [107,108].Due to their very small size as well as the high aspect ratio, this collective effect of core and attached surface functionalities enables the understanding of the exact origins of physico-chemical features of CDs challenging. It was already established that the starting materials do have a decisive role in determining the specific functionalities enables on the carbonaceous core structure of CDs. Thus, carbon dots synthesised from different precursors via different reaction procedures possess entirely different physico-chemical features. Owing to the existence of diversified classes of CDs, a comprehensive understanding about their fundamental structure- physicochemical property relationship is very important. The following section of this dissertation is entirely devoted for the meticulous discussion on their decisive features.

(I) Low- toxicity, Bio-compatibility and water solubility

These fluorescent nanomaterials possess numerous unique features which make CDs as a potent nano-probe in the field of science and engineering. One of the most interesting property associated with CDs are their commendable environmental friendly nature, whereas the conventionally used fluorescent probes such as organic dyes and semiconductor quantum dots are highly toxic and environment deteriorating. The inherent hazardous nature of metal based quantum dots can be connected with toxic elements including cadmium, lead and some rare earth metals present within their structure. Commercially available fluorescent organic dyes are considered as the major pollutant in industrial waste water. They were found to be extremely lethal, mutagenic and to some extent were reported to be highly carcinogenic towards several aquatic organisms and living body cells. It was scientifically proven that the light enhanced reactions within the widely used xanthene dyes may result acute proliferations of body tissues of vital organs. This will subsequently destruct the functioning of concerned body part. Contrary to these conventional fluorescent entities, the new entry, CDs are marked with commendable environmentally benign nature as they are composed of inherently non-toxic element “carbon”. This admirable non-toxic nature as well as its bio-compatibility pave way to tremendous progression in engineering bright emitting CDs based bio-probes with considerably high stability.

Apart from the aforementioned bio-compatible nature, carbon nanodots are characterised by excellent water solubility, which further enhances the biological sustainability of CDs. This can be ascribed to the oxygen bearing functional groups, either derived from the starting material or produced during the synthesis procedure [109, 110]. Hence, these fluorescent carbon structures can serves as fundamental building blocks for developing diagnostic tools for monitoring living body cells and organisms (both in-vivo and in-vitro analysis). Several works were conducted on evaluating the cytotoxicity of bare CDs and doped CDs. Sun and co-workers that the arc-discharge derived CDs after 12 hr reflexing with nitric acid appeared to be non-toxic to living body tissues while conducting cytotoxicity assay. Most of the biological assay confirms that the cell viability of living body tissues of both humans and animals were do not affected by even under relatively higher concentration of bare CDs. This is achieved due to the combined effect of non-toxicity, bio-compatibility and water solubility of CDs. The commendable eco-friendliness of CDs was further substantiated by numerous research works in literature. In 2018, H. Li and colleagues developed extremely bio-compatible and non-toxic carbon dots which are able to enhance the growth of rice plants along with boosting its disease resistance capacity [111]. Owing to their inherent excellent hydrophilicity, these CDs increase the water absorption ability of rice plant seeds thereby accelerate the seed germination as well as ensure elongation of plant roots. According to this report, these graphite derived carbon dots enhance the plant life by providing increased diseases resistance capacity to the rice plant. This is due to the augmentation of thionin (Os06g32600) gene expression by means of insertion into the DNA grooves. All together these CDs offer enhanced plant growth rate and better yield [figure 1.6].

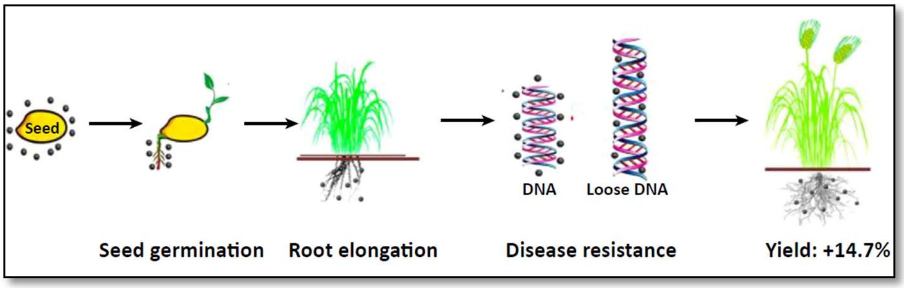


Figure 1.6: Bio- compatibility of CDs; Bio-compatible CDs boosting the growth rate of rice plants by accelerating the seed germination, root elongation and enhancing their disease resistance capacity thereby ensuring better yield.

(II) Optical features of CDs

The most intriguing feature that sets the position of CDs as the extensively explored carbon nano material, photoluminescence, remains as a hot area to debate. Researchers constantly put their efforts for unravelling the formation mechanism of CDs which may subsequently resolve the mystery regarding the understanding of the exact root cause of its photo-physical features. Meticulous investigation regarding the photo-physical properties of various CDs strongly suggests the participation of three different photoluminescence mechanisms as “plausible fluorescence origins”. This includes (i) Quantum confinement effect, (ii) surface state effect and (iii) molecular fluorescence.

The ‘photo-luminescence’ can be defined in simple words as “the process of emission of light by system as a result of excitation using a collection of photons”. In general, electron from the lowest energy level (ground state) of the system will be transported to higher energy level (excited state) utilizing the energy absorbed from the incident radiation source, upon excitation with an energy source.

The excited electron dissipates its stored energy in different manner, and finally returns to the lower energy ground level via two different modes of transition. This excited electron can either returns to the ground level by emitting radiation of definite energy (Radiative return) or it may return to the ground state without emitting any radiation (Non-radiative return). The former, transition of electron into the ground energy state with radiative emission always counts for the observed photo-luminescence of a particular system. Generally, non-radiative transitions to the lower energy levels do not play any role in the photo-luminescence behaviour. It accompanies the radiative transition upon photon-excitation due to the action of phonons that are converted into lattice vibrations which transfer energy as heat. For an efficient photo-luminescent material, radiative transitions should dominate over the transition of excited electron to the lower level without radiation emission.

The term photo-luminescence generally involves both fluorescence and phosphorescence, which can only differentiated based on electronic states of excited energy levels. To get a more precise understanding on the fate of photo-excited electron and the mechanistic route to luminescence emission, we have to use an energy state diagram, jablonski diagram. It is named after a polish academic, Aleksander Jablonski, who dedicated his entire life for the study about molecular absorbance and the process of light emission[112].

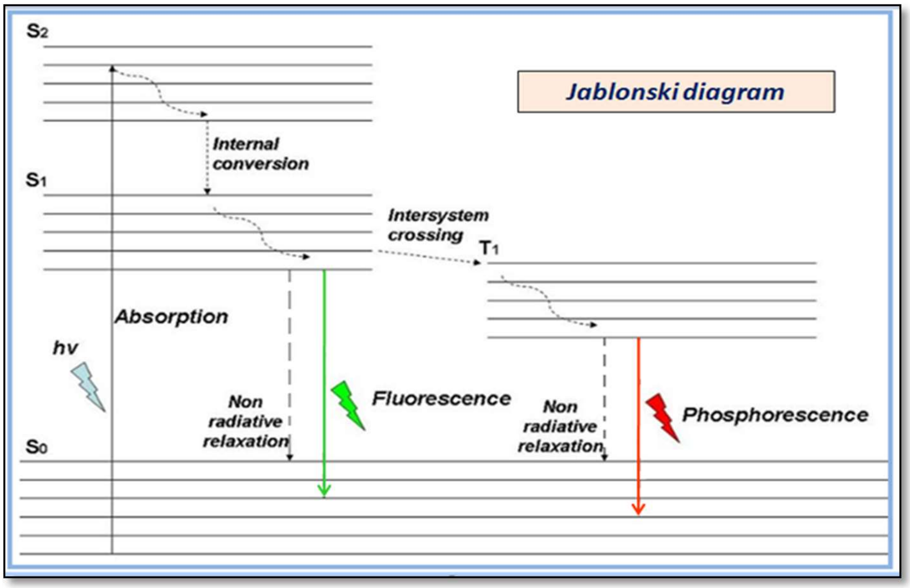


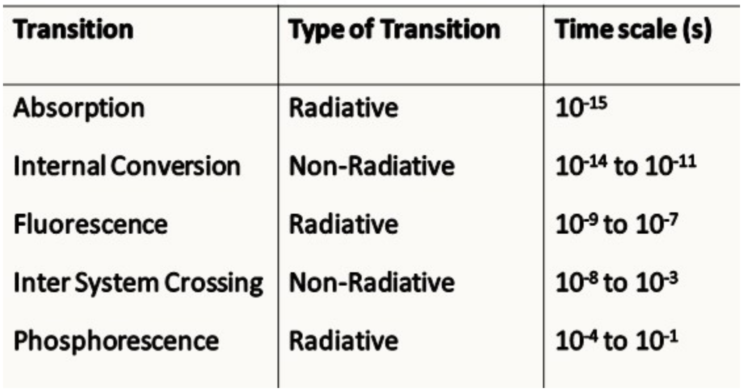
Figure 1.7: Jablonski diagram describing photoluminescence of molecules

Generally, jablonski diagram arranges electronic levels in terms of energy and spin multiplicity; singlet ground state as S_0, singlet first state as S_1, singlet second state as S_2, triplet first state as T_1 etc. It was established that each electronic energy levels of the fluorophore possess numerous vibrational energy levels (displayed in Figure 1.7). In a typical jablonski diagram, electronic transitions between each energy states upon absorption of light of definite energy are represented using vertical arrows. These arrows indicate the direction of electron movement which is commendably instantaneous in nature.

There is a thumb rule that both absorption and emission occur from the lowest vibrational energy levels of molecules of each electronic states (n=0). This is because at the room temperature the available thermal energy is not capable enough to considerably populate the vibrational levels of higher energy (n=1). Usually, it was observed that in accordance with the absorbed energy from the incident photon, the fluorophore will move in to higher order vibrational levels of electronic states either S_1 or S_2. As evident from the Figure, the excited electron staying at higher energy state dissipates its stored energy in the form of several electronic relaxation processes between the electronic energy levels. The excited fluorophore may undergo non-radiative transition from a vibrational level of a particular electronic state towards another vibrational level of electronic state of lower order of same multiplicity (say, S2 to S1 non-radiative transition). This aforementioned non-radiative electronic transition is generally termed as internal conversion (IC), indicated as dotted lines in the jablonski diagram. Internal conversion arises due to the strong overlap between the electronic and vibrational energy levels. It was observed that as the energy of the system increases, the manifold of electronic energy states and vibrational energy states become closer. This in turn enhances the degree of possibility for the electronic movement between the strongly overlapped energy bands. The time scale for this internal conversion was found to be 10^{-14} to 10^{-11} s.

The photo-excited electron can release the stored energy through a radiative emission process from higher electronic level to lower electronic level of the same multiplicity, fluorescence, a slow process characterized with time scale around 10^{-9} to 10^{-7} s. Electron staying at higher energy states most often dissipates the energy via non-radiative relaxation processes including internal conversion. But, majority of the excited electrons residing at first excited states generally prefer fluorescence transitions to the energy states of ground electronic level. For the first excited electronic state, fluorescence may compete with other non-radiative electronic transitions. Though, internal conversion is much faster than fluorescence, generally the former seems to be less efficient for dissipating energy for an electron staying at S_1 level. Vibrational and electronic bands of S_1 level possess larger energy separation than that of higher energy electronic levels (S_2, S_3 etc.,), where manifolds of vibrational levels closely overlap with the electronic levels. Hence, larger energy gap between the electronic and vibrational levels most often prefer radiative emission process, fluorescence rather than the non-radiative emission internal conversion for transitions from S_1 to S_0. For fluorescence, energy associated with the emitted photon will be exactly same as the difference in energy between the electronic states of transition. But for electrons at higher electronic energy levels, some of the absorbed energy definitely lose through few non-radiative processes such as internal conversion during its transition to S_1 level. Hence, fluorescent photons always possess lower energy than that of exciting/incident photons. Owing to the presence of strongly overlapped numerous vibrational levels, which can couple with the aforesaid electronic transition, the observed fluorescence emission is usually arranged over a range of wavelengths.

The excited electron can proceed through the slowest electronic transition in the jablonski diagram, defined as inter system crossing (ISC). It is denoted using the horizontal dotted arrow directing from one column to the other. As the name indicates, this particular transition involves the movement of excited electron between two different systems designated with different electronic arrangements. Different from the aforementioned electronic transitions, internal conversion(IC) and fluorescence, this inter system crossing (ISC) undergoes spin conversion from singlet excited states to triplet first state (T_1). This transition between two electronic states of different spin multiplicity is a kind of forbidden transition by selection rules. Hence, this may subsequently become weakly allowed electronic transition via incorporating vibrational factors with electronic selection rule. Due to this fact, rate constants associated with triplet emissions were found to be very smaller in magnitude than that of emissions conserving spin multiplicity (singlet to singlet transitions). Electron residing at the excited triplet level dissipates the stored energy by a radiative process while travelling to the ground level singlet state, phosphorescence. Phosphorescence was found to be generally shifted in to lower energy/longer wavelengths compared to fluorescence. It was noted that majority of the fluid solutions never exhibit phosphorescence, as there occurs a struggle between this radiative triplet emission with non-radiative deactivation/ quenching processes at room temperature. According to the selection rules, faster transitions were found to be more likely to happen than that of electronic movements with slower rate constants. Hence, time scales associated with each possible electronic transition within the excited fluorophore system play a crucial role in determining the feasibility of concerned transition. General time scale values related with fundamental radiative and non-radiative transition processes are tabulated in table 1.1. Time scale values illustrate the fact that, the photo-excited electron more likely dissipates its energy via the spin conserving radiative transition, fluorescence, than that of triplet to singlet radiative transition, phosphorescence.



Transition	Type of Transition	Time scale (s)
Absorption	Radiative	10^{-15}
Internal Conversion	Non-Radiative	10^{-14} to 10^{-11}
Fluorescence	Radiative	10^{-9} to 10^{-7}
Inter System Crossing	Non-Radiative	10^{-8} to 10^{-3}
Phosphorescence	Radiative	10^{-4} to 10^{-1}

Table 1.1 :Time scale values of basic electronic transitions of a photo-excited electron.

Photoluminescence lifetime (τ)

Photoluminescence lifetime of a fluorophore reveals the average time duration that an excited molecule spends at the excited energy levels of the system for interacting with the environments prior to the its subsequent return to ground state. Lifeime can be expressed using the following equation,

$$\tau = \frac{1}{\Gamma + K_{nr}}$$

τ= photoluminescence lifeime

Γ= Rate of radiative decay

K_{nr}= Rate of non-radiative decay

If all the probable non-radiative decays were restricted, $\mathbf{K_{nr=0}}$, lifeime of the fluorophore can be termed as natural lifeime (τ_n).

$$\tau_n = \frac{1}{\Gamma}$$

Photoluminescence Quantum yield (Φ)

For a fluorophore unit, one of the most important quantities that is used to characterize its fluorescence efficacy is its quantum yield. In simple words, photoluminescence quantum yield denotes the relative ratio of number of emitted photons to the number of absorbed photons.

$$\textbf{Quantum yield, } \Phi = \frac{\textbf{Number of emitted photons}}{\textbf{Number of absorbed photons}} = \frac{N_E}{N_A}$$

Generally, quantum yield serves as a significant index for deciding the practical applicability of the fluorophore unit as photo-devices, as it provides exact picture about the fluorescence efficiency of the system.

Quantum yield gives direct indication about the luminescence intensity of the system, greater the Q.Y value of a fluorophore, brighter will be its emission. Surface functional groups present in the carbon dot structure plays a decisive role in determining its quantum yield. On examining the previous works, it can be stated that appropriate surface modifications can enhance the fluorescence intensity, and thereby the quantum yield of the system [32,113,114]. For example, for most of the CDs prepared from precursors possessing excess amount of electron withdrawing functionalities including carboxylic and epoxy groups, corresponding fluorescence quantum yield will be relatively low. This is due to the presence of electron withdrawing groups on the surface of CDs, which strongly reduces the electron density [115]. When this electron withdrawing groups were converted in to electron donating functionalities including hydroxyl group via surface engineering, electron density over the CDs surface will increase. This will in turn enhances the photoluminescence quantum yield of the system and consequently results brighter emission [116]. Along with this surface group conversion, hetero atom doping also serves as an efficient approach for enhancing the features of CDs, especially the Quantum Yield. It was noted that, Nitrogen, Sulphur co-doped CDs with orange emission prepared by Y.Zeng etal., and Sulphur doped highly fluorescent carbon dots synthesised by Q.Xu and co-workers are exhibiting very high quantum yield, greater than 65% . This hetero atom insertion results in considerable alteration in electronic arrangements and band gap of CDs. This results in drastic enhancement in the photo luminescent events of the excited fluorophore, thereby achieving commendably high quantum yield [117,118]. Recently, researchers could fabricate CDs with very high Quantum yield value greater than 80% [119].

Generally, quantum yield of fluorescent sample is calculated in accordance with the following equation (1). Quantum yield calculations of fluorescent samples are always conducted with the aid of a standard fluorescent solution as reference. Quinine sulphate and Rhodamine dye solutions were commonly used as reference.

$$Q_s = Q_R \frac{I_s}{I_R} \frac{A_R}{A_s} \frac{n_s^2}{n_R^2} \times 100$$

Q_s : Quantum yield of sample
Q_R : Quantum yield of reference
I_s : Integrated emission intensity of sample
I_R : Integrated emission intensity of reference
A_S : Optical density of sample
A_R : Optical density of reference
n_S : Refractive index of sample
n_R : Refractive index of reference

Basically, irrespective of the diversified synthesis routes and starting materials, CDs exhibit broad spectrum of absorbance maxima in the ultra-violet region between 220-300 nm which can be connected with π

- π* electronic transitions corresponding to -C=C- bonds (carbon frame work of core). Along with this absorbance maximum, there will be a small tail extending to the proximity of visible region, denoting the n - π* transitions of –C=O groups within CD structure [11,120].

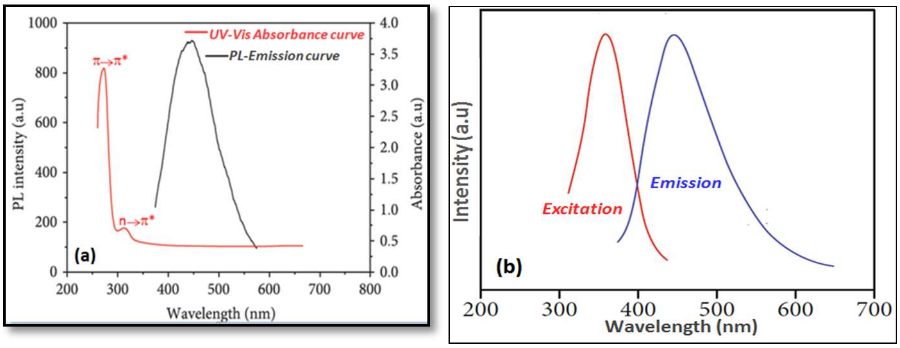


Figure 1.8 : General optical response of CDs under excitation at 360 nm; (a) UV-Visible absorbance spectra (red line) and photo-luminescence emission spectra (black line), (b) typical excitation (red line) and emission curves (blue line) of CDs.

Chemical and mechanical alterations including doping, surface functionalisation and size/ defect site controlling may cause slight shift in the aforesaid optical trend (positions of absorbance curve). These modifications strongly influence the electronic nature of bare CDs, thereby resulting in significant alterations in the *π*-*π** energy states [121]. It was found that the surface defects/surface traps present within the CDs structure are responsible for the broad nature of its absorption spectral curves [122]. If the surface functional groups of CDs are substituted with hetero atom (nitrogen, sulphur and phosphorus) bearing organic groups, significant variations within the energy gap (HOMO-LUMO energy levels will be resulted. This will definitely cause considerable shifts of band maxima in the UV-visible absorbance spectra.

Different from the UV absorbance spectra, photo-luminescence emission curve will vary in accordance with the starting materials and synthesis methods. Fluorescence emission maxima may shift either to the lower wavelength or to the higher wavelength, even with minute changes in the reaction parameters. These fluorescent CDs possess commendable resistance towards photo-bleaching. Majority of the CDs so far synthesised CDs withstand prolonged irradiation of UV rays without any remarkable reduction in their photo-luminescence intensity (3 to 5 hrs). This interesting feature of CDs further highlights the credibility of this non-toxic fluorescent carbon over the conventional fluorescent organic dyes which were reported to be highly susceptible towards photo-bleaching. Typical luminescence emission spectra of CDs cover wide range of wavelengths. Majority of the emission curves are situated in the blue-green wavelength region (450-520 nm). Optimal emission at longer wavelength region beyond 550 nm is exhibited by small fraction of synthesised CDs under excitation at 350 nm. This excitation and emission photoluminescence curves of CDs displayed below as Figure 1.8(b).

Few CDs were found to possess an interesting behaviour, up-conversion luminescence. This is found to be highly desirable for a fluorophore entity to render itself as a potent candidate for a wide spectrum of applications in fields including energy harvesting, light display devices, bio-imaging and medical therapy etc,.[43,123,124].

Fundamentally, up-conversion photoluminescence is considered as a non-linear effect in which manifolds of excitation photons with lower energy results emission of higher energy photons. Due to this interesting feature, the emission maxima of CDs will be situated at shorter wavelength region even under excitation at longer wavelength. CDs with this up-conversion luminescence property were marked with inherent capacity to penetrate deeper in to body tissues without resulting any harmful effects to living cells. Owing to this aforesaid ability, many CDs successfully find their role in sensing of several body tissues including HeLa cells [20,22,125]. Recently, hydrothermal treatment of citric acid yielded bright emitting CDs with up-conversion fluorescence feature .Photoluminescence emission curve of these CDs appears at UV-visible range upon excitation with near infrared (NIR) wavelength [126].

Tunable photoluminescence – Excitation dependant fluorescence emission

One of the most interesting observations about CDs is the tunable fluorescence emission, the continuous shifting of emission curves upon gradual change in excitation wavelength. This tunability in fluorescence emission maxima serves as the unique feature of carbon nano dots, which render them potent candidates in various fields of analytical chemistry [34,20,22,127].The digital image showing the aforesaid excitation wavelength dependent multicolour fluorescence emission of carbon nano dots is displayed in Figure 1.9. CDs could exhibit fluorescence emission over a wide range of wavelength, across the entire visible spectral region, covering blue to red with the gradual increment in the excitation wavelength. As evident from the bottom panel of Figure, gradual increment in excitation wavelength results continuous shift in the position of emission peaks, though the intensity was found to be gradually reducing upon increase in the excitation wavelength. This is due to the decreased optical absorption in visible spectral range under this condition.

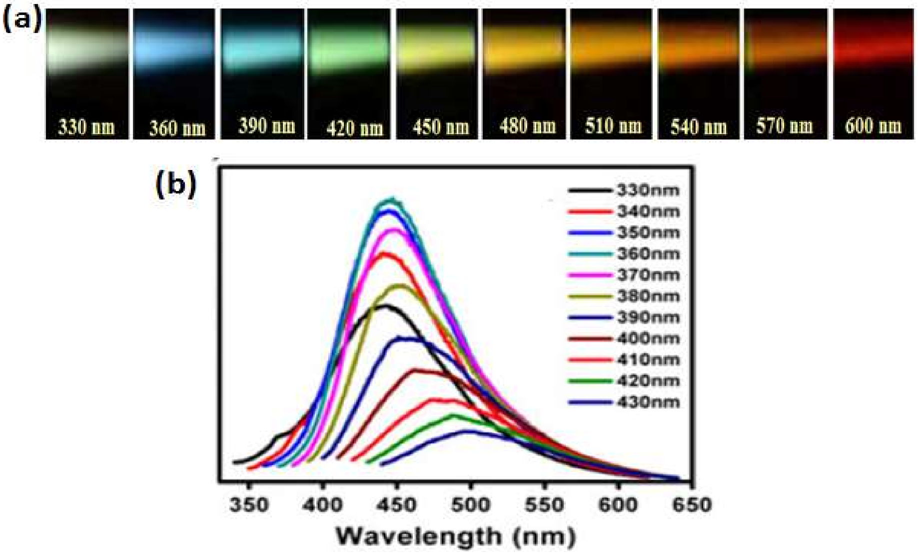


Figure 1.9 : Excitation dependant fluorescence emission of CDs; Digital photographs showing multicolour fluorescence emission by CDs in accordance with gradual change in excitation wavelength (excitation wavelengths corresponding to each emission is labelled in the image) (a). Typical photoluminescence spectra of CDs displaying excitation wavelength dependant fluorescence emission (excitation wavelength is labelled in the inset) (b).

Though tunable photoluminescence emission serves as the most intriguing feature of CDs, researchers still do not have a scientifically acceptable explanation about its origin and mechanism. Based on detailed literature survey, majority of the authors suggest it is the structural “inhomogeneity” of CDs derived from different precursors via extremely different reaction conditions, that is responsible for the excitation dependant emission behaviour. This wide range of diversity existing in the structural pattern of CDs brings several disorders, which play a decisive role in tunable fluorescence emission,that the other conventional fluorescent analogies lack. Even though researchers suggest the observed structural heterogeneity (disorder/ defect) as the core reason for the excitation dependant fluorescence emission, indisputable explanations and evidences for corroborating this proposal are still lacking. This is mainly because of the ambiguity regarding the exact structure and the underlying photoluminescence mechanism of CDs. On one hand, scientists still fails to design a synthesis procedure yielding CDs having adequate homogeneous structural features including narrow and uniform size distribution and precise formula of chemical composition. On the other hand, the exact root cause of the observed fluorescence still remains a hot topic for debate, which makes the efforts for explaining the tunable fluorescence more challenging. This fact strongly demands systematic and highly focused attempts to specifically pinpoint the structural features that necessitate detailed tuning for obtaining CDs with spectroscopically uniform optical properties. Though there are more than thousand publications discussing the excitation dependent fluorescence emission of CDs, none of the studies suggest a widely acceptable conclusion. Majority of the attempts produce highly contrast observations and justifications regarding the tunable emission of CDs [34]. Hence, scientific explanation and systematic controlling of tunable fluorescence emission of CDs over the wide range of visible spectrum remains as a great irony in literature. On the ground of fundamental concepts on photoluminescence emission, this fluorescence tunability is believed to be arising from the emission form various electronic states with single excitation, where each electronic state possesses different emission behaviour. This aforementioned statement is just a hypothesis, which requires intense experimental efforts and systematic approaches for proving the authenticity.

1.4.4 Brief discussion on possible origin of photoluminescence

Despite the intense research regarding CDs, the precise mechanism or root cause for their observed fluorescence still remains a topic of debate. Wide range of diversity among the precursors used, synthesis methods adopted and post-synthetic treatments tunes the system as the most intricate one. This will impart different optical features to the system. This complexity regarding the structural aspects and the optical features restrict the formulation of a unanimous fluorescence mechanism in CDs. Hence, the origin of unique photoluminescence of these nano particles still remains mysterious. As mentioned in the previous section, the currently suggested root causes of fluorescence include (i) Quantum confinement effect/ core state emission (ii) surface state emission and (iii) Molecular fluorescence.

(I) Quantum Confinement Effect

It was already established that, when the size of a particle approaches smaller dimensions, below exciton Bohr radius, quasi-continuous electronic level adjacent to Fermi energy level shifts into the discrete levels of energy. This may leads to the strictly confined motion of carriers, thereby exhibiting size dependant optical and electronic features, the Quantum Confinement Effect (QCE). Hence, being a quasi spherical nano material with very small size of the order < 10 nm, the unique emission process by carbon quantum dots can be indisputably connected with the aforesaid QCE associated with the extensively conjugated carbon core. Several research works were there in the literature, endorsing this size dependant optical features/ quantum confinement as the major root cause for fluorescence of CDs [58,60,128,129].

Some of the reports suggest that, it was the graphene like structural orientation of carbon quantum dots, which is solely responsible for this intrinsic fluorescence emission related with the extensively conjugated π domain of carbon core. This tiny carbon form exhibits interesting optical and electronic properties extremely different from the bulk counterparts. Generally, bulk graphene is never expected to exhibit any kind of luminescence behaviour because of their zero optical band gap [130]. But the energy of excitons in graphene strictly follows scaling laws corresponding to considerable alterations in size. Hence, conversion of graphene fragments into few nanometre scale will results zero-dimensional quantum confined system. This aforesaid size reduction imparts non-zero band gap to the graphene structure, thereby facilitating size-dependant optical features, which are extremely different from their bulk analogies. As mentioned earlier, owing to their zero dimensionality and conjugated core structure connected with light constituent elements, several investigations on fluorescence mechanism of CDs strongly emphasize on this QCE.

Normally, this size dependent / band-gap dependent energy relaxation dynamics of zero-dimensional nano carbon can be depicted as shown in Figure 1.10. As evident from the Figure, decrease in particle size brings more confined orientation of excitons which consequently enhances the recombination energy (increase in energy gap). Hence, based on QCE, size reduction tunes emission into lower wavelength region/ higher energy region. As the Figure suggests, gradual decrease in the size of nanoparticle shifts the emission from higher wavelength region (red) to lower wavelength region (blue).

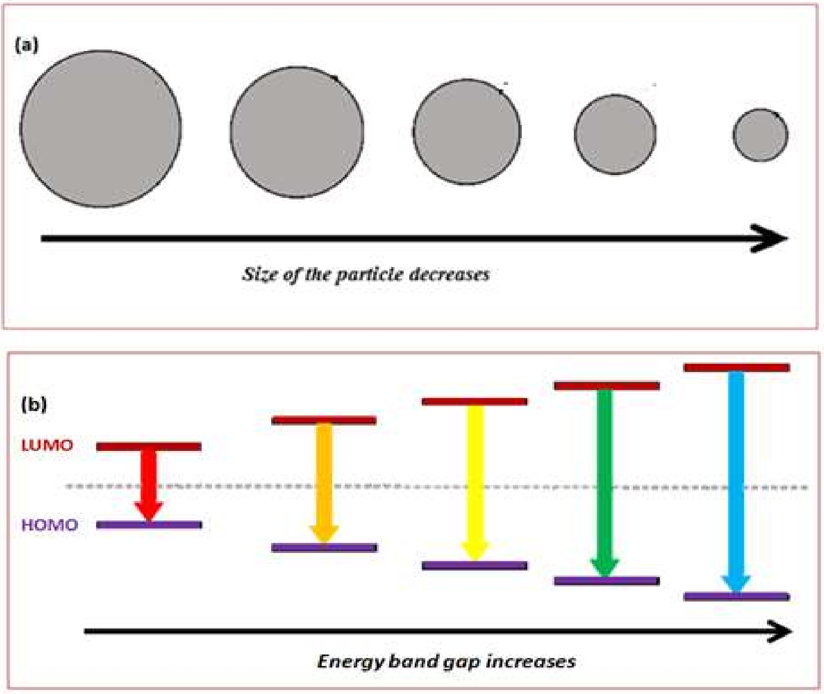


Figure 1.10 : Illustrating the concept of Quantum Confinement Effect: (a) as the nanoparticle becomes smaller in size, more confined will be the orientation of electron-hole pair (excitons) (b) Further spatial confinement of excitons by the size reduction increases the recombination energy, thereby the energy gap.

Literature witnessed lot of evidences for the occurrence of QCE within the system. Kim and group investigated the influence of carbon dot's size on their optical features by conducting fluorescence and absorbance measurements of a series of CDs with different sizes, ranging from 5 nm to 35 nm having different morphologies [131].Their studies revealed the fact that the absorption peak energy of CDs decreases with increase in their size and corroborates the occurrence of QCE. Li and co-workers further states the aforesaid size-dependent fluorescence emission through their research work involving optical studies of different sized CDs (1.2 to 4 nm) separated via column chromatography technique[58]. In this work, the smallest CDs having size ~ 1.2 nm exhibit ultraviolet light emission, CDs with intermediate size, i.e., between 1.2 to 3 nm emit visible light and the larger CDs (greater than 3.5 nm) results near-infra red emission. This observation indicates the band gap reduction upon enlargement of particle. In addition, numerous works are there in literature demonstrating the size dependant absorbance and emission profiles of CDs [122,131]. These bunches of works presenting multi-colour emission by different sized CDs having identical chemical composition tentatively recommend the QCE as one of the major causative of observed fluorescence feature of CDs.

Despite this suggestion, various experimental evidences substantiating the influence of several extrinsic factors (surface functional groups, surface defects/ charge traps, extent of functionalisation) on the emission of CDs [9,128,132] strongly recommend another probable mechanistic route for the observed luminescence feature of these nano materials. Furthermore, studies by Wen and group as well as the recent observations made by Sciortino and co-workers [133,134] reveal that upon size reduction, CDs exhibit noticeable red-shift in the electronic transition energy, strictly offending the concept of QCE. Hence, for better understanding of the fluorescence behaviour of CDs, along with the intrinsic energy states, the vital role of extrinsic factors in determining their optical properties should be considered

(II) Surface State Emission

As stated above, majority of the research works demonstrated that the fluorescence emission feature of CDs could be appreciably fine-tuned by altering their surface states. Since the invention of these fluorescent nano carbon fragments in 2004, the most widely accepted origin of their luminescence was connected to their unique surface features. This was further endorsed by numerous research works in the absence of valid convincing experimental proofs [22,34]. According to this concept, sp^2 domain core of the system serves as the receptor/ antenna of incident photon and then transfers the absorbed photons to the surface of CDs. This will initiate surface mediated emission processes, involving the contribution of surface functionalities, surface defects/ extent of surface oxidation. The significant role of surface functional groups was successfully manifested through several literature reports. This includes reports on surface passivation mediated fluorescence intensity enhancement, pH dependant emission behaviour, solvent controlled luminescence and the strong influence of extent of surface oxidation in determining the emission efficiency, thereby the quantum yield [22, 32, 34].

Role of surface functional groups in determining fluorescence emission of CDs

Various studies on surface mediated luminescence tuning suggests the possible interference of core state derived QCE by surface oriented factors such as traps/defects/chemical conversions of functional groups/insertion of hetero atoms [34,36]. In such cases, the so-called surface traps serve as the effective centre for radiative/non-radiative recombination of excitons, thereby causing photo-luminescence. Despite the intense research regarding the surface oriented exciton transitions of CDs, fundamental awareness on the nature of surface traps/defects and the extent of contribution from each surface moiety towards the shifting of emission wavelengths still remains rather unclear. Surface functional groups present on CDs play decisive role in determining surface energy level as well as the level gap. During the early periods of first identification of CDs, a group of researchers provided experimental evidences confirming the significant role of surface passivation for tuning the emission process. This was done by Sun and colleagues, who submitted proofs claiming appreciable enhancement in fluorescence quantum yield by surface passivation using the chemical agent, PEG-1500N [9]. Later, works by Bourlinos et al and Yang and colleagues scientifically back-up this aforesaid observation through solid evidences showing improved photoluminescence features assisted by surface modifications/ passivation [43, 135]. Wang and colleagues demonstrated that the fluorescence emission wavelength can be effectively fine-tuned by proper alteration of surface functional groups [136]. They manifested that the fluorescence centres were evenly distributed over certain special edge states (especially carbonyls). As the aforesaid functional groups strongly influence the emission behaviour, the proper engineering of CDs surface eventually tunes their fluorescence profile. Research work byTetsuka et al. showed that CDs decorated with nitrogen (N) atoms bearing functional groups obtained via dehydration reactions and nucleophilic substitutions possess tunable energy gap/band gap spanning from 1.30 to 2.23 eV. This will subsequently result in wide range tunable multi-colour emission from blue to red. It can be attributed to the orbital resonance of N moieties with CDs [137]. The inevitable role of surface functional groups on deciding the fluorescence pattern of CDs was well demonstrated by Zheng and co-workers. They report that the presence of a reducing agent could shift the emission wavelength as well as the fluorescence efficiency [138]. As evident from Figure 1.11 (a), feeble green emission by original CDs shifts in to bright blue emission upon chemical treatment using the reducing agent, Sodium borohydride. According to this report, quantum yield of original CDs increases from 2 to 24% upon the reduction process, strictly confirming their surface controlled luminescence feature. The significant role of surface functional groups was further established through the repeated cyclic shifting of emission maximum wavelengths of CDs between green (~ 520 nm) to blue (~ 450 nm) region upon consecutive switching of reduction and oxidation processes. Immediately after this work, Zhu et al. further illustrated the crucial role of surface oxidation process on deciding the fluorescence emission colour and intensity [139]. Similar to the action of oxidation-reduction treatments, presence of nitrogen containing functional moieties also results effective surface passivation, providing enhanced photo-luminescence features [140].Theoretical evidence illustrating the significant role of Nitrogen bearing surface groups for determining the emission characteristics of CDs were provided by Jin and group in 2013. They investigated the dramatic influence of –NH_2 groups causing red-shift of luminescence emission. As evident from Figure 1.11 (b) gradual increment in the number of –NH_2 group results continuous decrease in the band gap, thereby red-shift of luminescence wavelength [141].

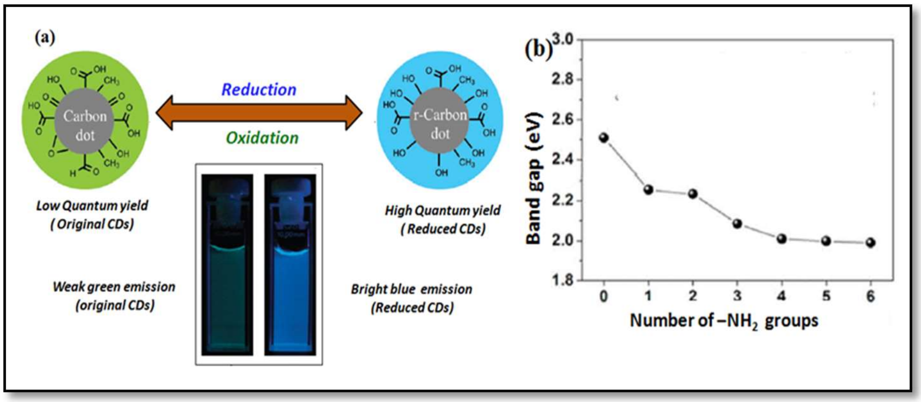


Figure 1.11: Role of surface functional groups in determining fluorescence emission of CDs; (a) Pictorial representation of reduction induced shifting of luminescence emission colour, inset: original CDs with green emission (left) and blue emission by reduced CDs (right).(b) Graphical representation indicating relationship between number of –NH2 groups and band gap of CDs.

Moreover, research work by Hu and colleagues provided effective experimental proofs illustrating the surface functional group controlled fluorescence features of CDs. Results of this work corroborate well with theoretical calculations [142]. Through this report they demonstrated the fact that the insertion of functional groups like oxygen into the surface structure of CDs introduces several electronic levels/states within the bandgap. This will leads to red-shifted emission with low quantum yield. Reduction induced blue-shift and oxidation induced red-shift by CDs were also successfully presented by the authors using sodium borohydride and ortho-phosphoric acid as reducing and oxidizing agents respectively. A group of scientists ascribed the red-shifting of fluorescence emission to the presence of electron withdrawing surface groups, especially -COO$^{(-)}$ groups. Such systems are generally marked with decreased electron density and comparatively low quantum efficiency [37]. In addition to this observation, the fluorescence efficiency/ quantum yield of CDs was found to be almost doubled via simple chemical surface modification involving conversion of electron withdrawing groups into electron donating groups. This observation further illustrates dramatic influence of chemical characteristics of surface functionalities for designing luminescence profile of CDs [116].

Influence of degree of surface oxidation

Many studies are there in literature, that emphasises the finding that the luminescence feature of CDs was exclusively determined by the extent/degree of surface oxidation [22,45,122]. These works suggests that the number of surface traps/defects will increase with increase in the degree of surface oxidation. This will enhance the entrapping of excitons and subsequently ends up with red-shifted emission while recombination of these trapped excitons [122]. Xu et al., presented TEM images of two different CDs having variable degrees of oxidation, as evidence to illustrate the crucial role of surface oxygen content in deciding the structural pattern and carbon core design. The report demonstrates that this may subsequently leads to extremely different photo-luminescence characteristics for CDs [143].This oxidation controlled red-shifting of luminescence emission was further cited by several authors. Xiong and group reports that the blue emitting single CDs exhibits multi-colour emission spanning around green-yellow-orange to red colour region while gradually increasing the degree of surface oxidation [22, 122, 142,144]. According to an interesting work by Hu and colleagues, the oxygen species incorporated via surface oxidation process is closely related to the alignment of energy levels or band gaps of the system. These reports also suggest that the increase in the amount of surface oxygen content/ degree of surface oxidation tremendously reduce the band gap of CDs, thereby causing red-shifting of luminescence emission [142]. Research work by Bao and group strongly supports this observation, in which they synthesised two different CDs with variable oxygen content adopting electrochemical procedures. They noted blue colour emission for the one with lower degree of oxidation whereas the highly oxidized CDs have green colour fluorescence [122]. Schematic presentation of fluorescence emission profile of CDs related to the degree of surface oxidation is provided in Figure 1.12.

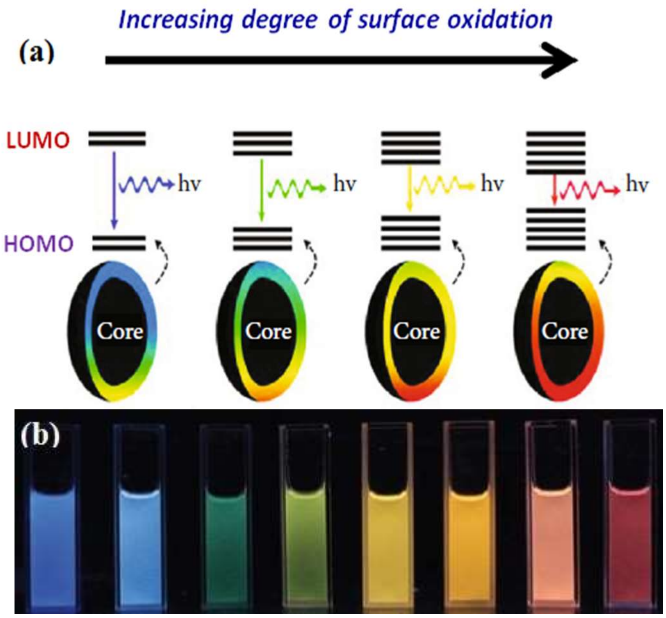


Figure 1.12 : Fluorescence emission profile of CDs based on the extent of surface oxidation.(a) schematic representation indicating reduction of band gap of CDs with increasing oxygen content,(b) digital images showing multicolour emission upon gradual increment in the degree of surface oxidation.

In addition to these findings, there are some recent works proposing the synergistic action of carbon core oriented QCE and surface state emission for determining the fluorescence behaviour of CDs [36,145]. Systematic investigation on the contribution of structural orientation and surface characteristics was done with the aid of several advanced characterisation tools like time-correlated single photon counting measurements. These attempts revealed that fluorescence features of some CDs could be adjusted by fine-tuning the size of carbon core (QCE) as well as by the chemical conversion of surface groups (alterations to the alignment and number of surface traps). Currently, Liu and group presented a series of full colour emitting CDs obtained via hydrothermal process followed by column chromatographic purification. These CDs were found to exhibit red-shifted fluorescence emission, which can be strongly attributed to the synergistic action of increment in particle size and extent of surface oxidation/ modification [146].

(III) Molecular Fluorescence

In addition to the aforementioned reasons of luminescence, current research works propose an alternative suggestion for the observed fluorescence behaviour, molecular fluorescence. This is believed to be arising from simple organic molecules existing in the reaction mixture as an impurity/ side-product or may found to be connected with the dot structure itself as an undesired part of the product [147,148]. Along with this, some research groups attribute the dual emission profiles of the CDs to the aggregates of number of molecular chromophores [149,150]. Some other reports even declare the crucial role of freely existing molecules that are diffused in the solution for determining their fluorescence characteristics [151].

Quantum of research works published recently claims that the small luminescent molecular derivatives produced alongside with the synthesis process, i.e., during the carbonization of precursors both in the aqueous medium and organic solvents , were able to serve as the major source of the observed fluorescence of CDs [22].As a strong supporting experimental evidence, one can present the interesting research work done by song and co-workers, discussing the fabrication of two types of blue emitting CDs, one with excitation dependant emission characteristic and the other with non-tunable fluorescence emission profile [24]. According to this report, excitation dependant luminescence activity of former set of CDs can be ascribed to the size-dependant luminescence mechanism involving the core structure of CDs, QCE. The non-tunable fluorescence emission was fully devoted towards the presence of organic molecules in the medium either existing as free molecules or firmly connected to the CDs surface.

Investigation on emission behaviour of CDs obtained from citric acid as well as the Nitrogen containing precursors suggests that the incomplete carbonization/ partial decomposition of precursors definitely ends up with the production of some inherently fluorescent molecular fragments such as 2-pyridone derivative [147,152] including citrazinic acid [148, 150, 153] which further alters the core structure under higher temperature conditions[24,154,155]. According to the established results, these luminescent by-products (citrazinic acid) conventionally exhibit blue emission around 420-440 nm [156], which is perfectly merged with the expected emission range of blue emitting CDs. Hence, it was found to be very difficult to discriminate the emission by the molecular fluorophore from the emission exerted by the dot structure itself. The only striking difference was the emission by such by-products (chromophore molecules) found to be entirely independent of excitation wavelength alterations, whereas the CDs when excited under lower energies, exhibit strongly tunable emission characteristics [24]. Righetto and co-workers clearly demonstrated the free molecule oriented luminescence emission process with the aid of advanced characterization techniques including time-resolved electron paramagnetic resonance spectroscopy and fluorescence correlation spectroscopy [151]. Schneider and group provided experimental evidence demonstrating the existence of single chromophore unit in two different ways; one set of molecules attached to the surface and the remaining set as free molecules in the solution medium, as the detailed analysis depicts bi-exponential decay kinetics for the CDs emission peak at 430 nm [156].

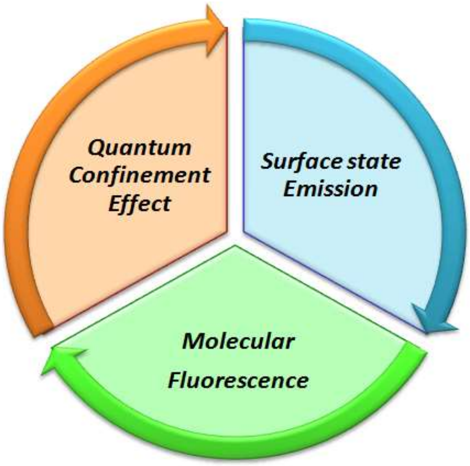


Figure 1.13 Diagrammatic presentation comprising the major sources of luminescence of CDs.

<u>Concluding Remarks</u>

As mentioned previously, wide range of diversity among the structure and synthesis methods of CDs hinder the predictability of exact structure , fluorescence mechanism and thereby their particular photo-physical features. With these, we wind up the discussion on the current understanding on possible fluorescence origins of CDs. This area still remains as a hot topic for debate due to the lack of comprehensive methods for the fabrication of CDs with desired structural pattern and functional orientations.

Surface passivation/modification induced fluorescence enhancement

Since the time of discovery, it was observed that bare surface structure of CDs was very much prone to external impurities/contaminants, which may render them defenceless and consequently reduce their photoluminescence efficiency. In addition, presence of dangling bonds, radicals as well as non-radiative centres may also result in fading of optoelectronic characteristics of bare CDs. Hence, researchers spend their time for introducing a protection layer over the surface of CDs, for ensuring long term stability and enhanced fluorescence features. Insertion of such passivating layers result in the formation of an insulating capping shield, ensuring protection from adhesion of contaminants / impurities. This will bring remarkable enhancement in the photoluminescence quantum yield and photo-stability of CDs. Scientists generally chose the surface passivating agents in such a way that they should not bear any visible/near-UV chromophore units so that the surface introduced protecting agents will not interfere with the emission profile of CDs. Commonly used surface passivating agents were polymers.

Surface passivation was first reported in 2006, in which usage of diamine-terminated oligomeric poly(ethylene glycol) (PEG1500N) as surface protecting agent yielded water soluble CDs with remarkably high quantum yield and commendable photostability for several hour [32]. Followed by this, several similar attempts were made frequently by the researchers, for passivating the surface layers of CDs using chemical agents including Polyethyleneglycol(PEG), polyethylenimine (PEI) [157], PEG 1500N [44,61,135,158], PEG 200N [98,159], PEG 600N[160],PEG 2000N [161], poly-(propionylethylenimine-*co*ethylenimine) (PPEI-EI) [17] and PEG 4000N [162], 1-hexadecylamine [163] and 4,7,10-trioxa-1,13-tridecanediamine [61, 82]. Lu et al, presented credible enhancement of quantum yield of CDs (electrochemical exfoliation of graphite electrodes) by means of passivation using ionic liquid[64].

Along with the surface passivation process, the optoelectronic features of CDs were also found to be strictly fine-tuned by the modification of surface functionalities. This can be achieved by chemical conversions using acid treatments/ addition of reducing agents/ alkali treatments/ pyrolysis/insertion of chemical groups / oxidation processes etc [43,57,164]. This process of tailoring functional features of CDs is generally termed as surface functionalisation and assures improved optical characteristics including higher quantum yield, commendable photo and thermal stability and fluorescence intensity. Hence, surface functionalisation tunes them as potent candidates in the field of sensing, bio-medical diagnosis, photo-catalytic devices, energy structure and mechanical property engineering [36].

Photo induced redox property- Carbon dots as electron donor/acceptor

In addition to the interesting features discussed so-far, CDs could also act as excellent redox agents. This feature of CDs can be ascribed to the spontaneous charge separation that occurs within the structure upon light irradiation. There are numerous reports in the literature, exploiting the aforesaid photo-induced electron donor as well as electron acceptor character of carbon dots [58,165-167].CDs when subjected to light irradiation, the charge carriers (electrons and holes) will be generated within the system. These carriers will be transferred to the species introduced externally to the system, the analytes. This results in quenched fluorescence intensity of CDs when combined with electron-donating/electron-accepting molecules. The first report on such a photo-excited electron donating/accepting carbon dot was by X.wang and group in 2009. They presented electron donating action of CDs by discussing the efficient quenching of its fluorescence intensity while interacting with strong electron acceptors like 4-nitrotoluene and 2, 4-dinitrotoluene. At the same time, they also reported sensitive fluorescence quenching with the introduction of electron donors like N,N-diethyl-aniline (DEA), illustrating the electron accepting nature of CDs. In the absence of a precise scientific photoluminescence mechanism, authors propose an assumption that the introduction of such an electron donor/acceptor analyte molecule may induce strong disruption to the radiative recombination on the functionalized surface of CDs. This will definitely fade its fluorescence efficiency, resulting selective and sensitive quenching of their luminescence intensity [168]. This dual role of CDs enhances the scope of their applicability in wide spectrum of areas including photo harvesting studies, light driven instruments, photocatalytic units when coupled with potent semiconductor surfaces for hydrogen evolution and pollution abatement [169,170].

As this unique feature tunes CDs as a promising candidate in various light energy conversion processes, researchers invest more time for probing the fundamental cause of this redox behaviour, which is still at its infancy. It was found that the dynamics of this interaction between the quencher and the CDs cannot be monitored with standard spectral tools, as they are in the order of picoseconds or less. Therefore, detailed investigation on the underlying mechanism of this electron-donor acceptor interaction between the CDs and the analytes strictly demands the aid of ultrafast spectroscopic techniques. As the usage of such ultrafast studies was still rare in the field, it restricts the progress of this investigation. Fortunately, some ultrafast spectral techniques are being used for analysing the precise mechanism of such interactions, recently. which is believed to shed light into the underlying physical and chemical interactions between the molecules. Xie and co-workers designed carbon dot sensitized TiO_2 which can act as an excellent photo-catalytic device as well as a good photo-sensitizer, owing to their commendable electron accepting and donating nature ,respectively[171]. As displayed in Figure 1.14 (a), carbon dots (pictured as yellow seeds) accept charge carriers originated from photo excited TiO_2 surface, which slows down/suppress charge carrier recombination. This will enhance the rate of chemical processes within the hybrid and tunes it as an excellent photo-catalytic device. Authors also discuss the electron donor action of carbon dots (Figure 1.14 b), in which carbon dots successfully transfer the photo-excited electrons to the coupled TiO_2 surface, thereby extending the catalytic performance of the composite in the entire visible region.

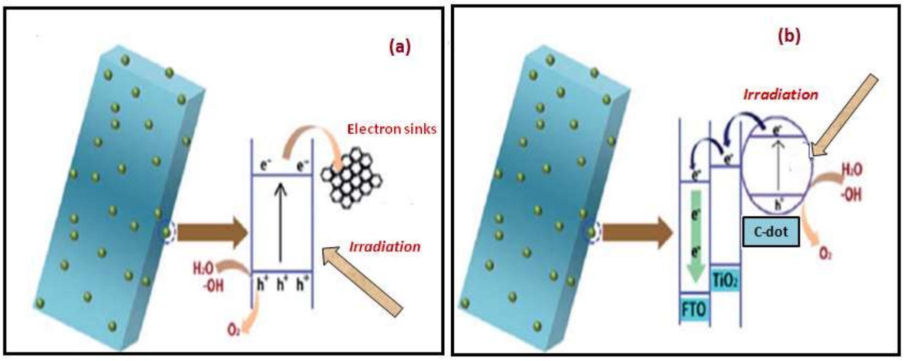


Figure 1.14: Dual photo-electronic action of carbon dot (yellow seeds) when coupled with TiO2 semiconductor (blue block); (a) CDs as an electron acceptor (electron sink) from the TiO2 surface enhancing the catalytic activity of semiconductor material, (b) CDs as an electron donor-generating a cascade of electrons from Lowest Un-occupied Molecular Orbital(LUMO) of carbon dot to the semiconductor surface.

Most of the reports relate the observed electron donor/acceptor capacity of CDs with their redox potential, which is strongly determined by the core and surface structure [134, 171,172]. For a better understanding of their redox property, and thereby designing photo-catalytic/optoelectronic devices with better performances, Barman and co-workers conducted an experiment using CDs–Nickel (II) Phthalocyanine Conjugates. Through the report, they propose a suggestion that the electron donor and acceptor behaviour of CDs can be compared with the performance of n-type and p-type doping respectively [173]. According to them, the flow of electron from CD surface to the analyte can be related to the chemical environment of phosphorus doping (n-type doping), whereas electron accepting CDs can be equated with boron doped environment (p-type doping). In addition, Rigodanza and group demonstrated the vital role of surface functionalities of CDs in controlling the redox potential. They tailored the surface of bottom-up synthesized CDs with different kind of quinones and demonstrate the promising electron transfer behaviour of CDs under different surface environments [172].

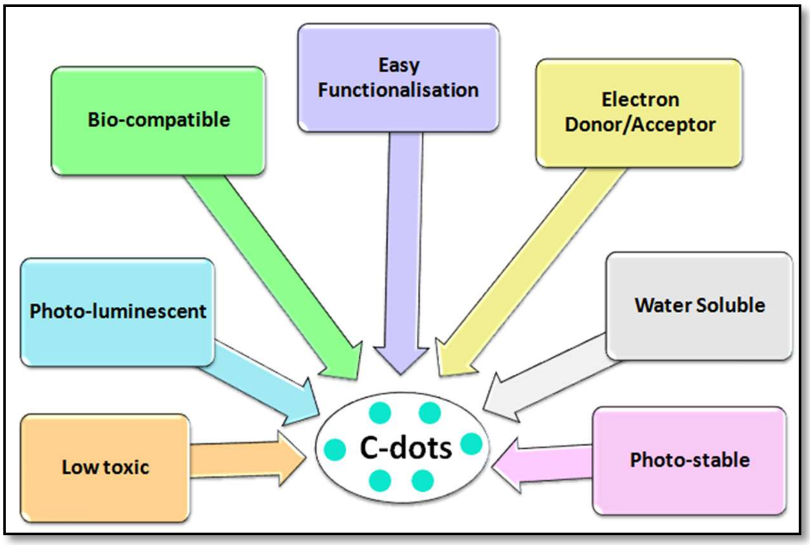


Figure 1.15: Different properties of Carbon nanodots

1.4.5 Applications of CDs.

Intriguing features of CDs render them one of the most potent candidates for wide range of applications in various fields, including optical sensors, bio-imaging , disease therapy , patterning/ luminescent markers , food safety, energy conversion and photocatalysis [20,22,175].

Optical Sensors

Fluorescence, the most interesting feature of CDs was found to be extremely inclined towards small alterations of surrounding environments. This will result in noticeable changes in their luminescence intensity, i.e., quenched/enhanced intensity. This aforesaid change in their luminescence emission intensity in presence of external analytes is used for tracing the presence of corresponding analyte. In this manner CDs could serve as an efficient sensing / quantification tool towards a set of analytes. This externally introduced analyte, which is capable enough to cause significant fluorescence intensity alterations, includes metal ions, bio-molecules, explosives, parameters including pH of the medium, temperature, humidity content etc. Majority of the CDs based sensing tools exhibit extraordinary selectivity and sensitivity towards the corresponding analyte.

CDs based Metal ion Sensors

Fluorescence intensities of CDs were effectively disturbed, more frequently quenched, by the addition of several electron acceptors, thereby tracing out quantitative tracing of metal ions in solution. Owing to the extreme lethal nature, quantitative tracing of Hg^{2+} ions is having considerable attention among the researchers. Literature presents a large number of interesting efforts for designing promising Hg^{2+} sensors marked with commendable selectivity and detection range [175-179]. Lu and group synthesised fluorescent CDs from grape peels adopting hydrothermal method, offering effective detection of minute quantity of toxic Hg^{2+} ions with detection limit 0.23 nM. Practical applicability of the system was confirmed in mercury contaminated river water sample, with excellent reproducibility of results [176]. Polyethylene glycol derived CDs also exhibit ultra-sensitive tracing of toxic Hg^{2+} ions with commendable detection range, 1 femto molar [177]. In addition to Hg^{2+} ions, researchers also designed various sensing devices to trace the presence of other relevant ions including Fe^{3+}, Cu^{2+}, Al^{3+}, Pb^{2+}, Ag^{+} and Zn^{2+} [20,22,34,174,175].

CDs based anion and small molecule sensors.

Along with the fabrication of metal ion sensor, researchers also put their efforts for developing efficient sensors for tracing the presence of various anions and small molecules. Different from the mechanism of metal ion /cation detection proceeding through quenching of their fluorescence intensity, anion/ small molecule sensing involves the restoration/regaining of the reduced intensity of a quenched system, realised using some other agent. In 2014, Zong and colleagues conducted an experimental demonstration showcasing the aforesaid sensing mechanism, in which the quenched luminescence of silica derived CDs-Cu^{2+} unit successfully regained by the addition of L-cysteine, a small bio-molecule [180]. In this work, typical CDs structure express specific binding affinity towards Cu^{2+} ions leading to considerable reduction in their luminescence intensity. The introduction of bio-molecule, L-cysteine to this CDs/Cu^{2+} system selectively displaces the attached Cu^{2+} ion from the CDs surface, thereby restoring the luminescence intensity of the system. Hence, this single system can serve as effective sensing device for both Cu^{2+} and L-cysteine with excellent selectivity and detection limit of 2.3×10^{-8}mol/L and 3.4×10^{-10}mol/L respectively. Similarly, Du and group designed CDs for tracing iodide ion and Hg^{2+} ions, in which iodide ion replacing the attached Hg^{2+} ions from the CDs surface [181].

CDs as Bio-sensor

Fluorescence intensity of CDs was best utilized by several researchers for the detection of various bio-molecules including ascorbic acid, glucose, enzyme (e.g. Thioredoxin Reductase -TrxR), dopamine, Intracellular Lysine, guanosine 3′-diphosphate- 5′-diphosphate (ppGpp) and DNA [22,175]. It was by Bai and group, the very first fabrication of an excellent bio-molecule sensor was reported in 2011, which detect DNA while joining ct-DNA in solution. This system was found to exhibit detection limit of $1.0 \times 10-6$ mol/L (linear ranging from $3.0 \times 10-6$mol/L to $8.0 \times 10-5$ mol/L) [182].Later, Xu and colleagues developed an efficient system for detecting thrombin, an important protein via formation of sandwich like structure marked with extraordinary specificity towards thrombin and possess commendable sensitivity. [183]. Aforementioned sensor exhibits detection limit 1 nM (nano Mol), highly appreciable while comparing with the results of previous works in this direction.

CDs as Photo-catalyst.

As the absorption range of conventional photo-catalytic materials including TiO_2 and ZnO is restricted to the ultraviolet light region, full advantage of visible spectrum is beyond the scope for several practical applications. Hence, design of nanomaterial platforms with commendable stability and excellent visible light composite catalytic efficiency is having considerable amount of attention for the abatement of environmental problems and energy issues [20,34,174,]. Generally, utilization of CDs based photocatalytic systems can be classified in to two. First category involves the application of CDs based systems as photo-sensitizer. In this, light energy irradiation causes shifting of photo-generated charge carriers (electrons) towards the semiconductor unit (absorbance of which is strictly restricted to UV region in the absence of CDs), which is coupled with the CDs system, thus offering catalytic activity extending throughout the visible light region. Second category involves the utilization of CDs based photo-catalytic materials as excellent charge carrier acceptors when coupled with photo-excited semiconductor materials. This will results in retarded or suppressed recombination of charge carriers (electron-holes), thereby offering more possibility for the occurrence of chemical reactions within the composite which in turn enhances the rate of photo-catalytic performance [58,167,165,171]. In addition to the CDs- semiconductor hybrid systems, some authors report simple or raw CDs with abundantly functionalized surface for applications involving water splitting/ CO_2 conversion [169]. In this work, Cao and group successfully conducted the CO_2 reduction/ release of H_2 molecules via water splitting using highly functionalized CDs blended with platinum or gold shell, highly efficient in photon harvesting. In 2007, Chen and group successfully devised a TiO_2-CDs composite. This system offers wide spectrum of visible light absorption/solar energy source utilization, thereby enhance the optical features of titania, the most widely used photocatalyst [184]. In addition, several efforts were there in literature that improve the photo-response of semiconductor photocatalysts by coupling with fluorescent CDs. These hybrids possess promising applications including organic pollution abatement, water splitting/ hydrogen production, energy saving purposes etc,.[34,174,185].

CDs based optical devices-LEDs

Fluorescent CDs also widely find their application in electronic sector. Fabrication of various promising light emitting devices are reported in literature [20,34,]. Guo and team developed a series of CDs with multicolor emitting feature, conducting thermal pyrolysis of polystyrene microspheres decorated with epoxyl groups [186]. In this work, CDs produce blue colour emission when they are processed under 200° C, whereas pyrolysis at 300 and 400°C yields orange and white emitting CDs respectively, when exciting under single wavelength UV radiation. These CDs were marked with highly commendable quantum yield (greater than 47%), combined with note-worthy optical features, which strictly tune them as LEDs exhibiting the aforesaid emission properties. Then, white light emission was reported by Wang's group using CDs derived from citric acid using octadecene coupled with surface passivator,1-cetylamine [187].Recently, J. Joseph and A.A Anappara have fabricated white emitting LEDs via esterification followed by polymerisation of the precursors, EDTA and ethyleneglycol in H_3PO_{4s} medium. This system exhibited broad range of emission under single wavelength excitation, which was ascribed to the presence of various functionalities and emissive traps on the surface of CDs [188].

CDs as medium for patterning and printing applications

Several conventional nano-materials were utilized for the designing of efficient luminescent inks and patterning agents for anti-counterfeiting applications [189, 190]. Being less toxic, highly stable and easily obtainable, fluorescent CDs could effectively replace these afore said nano-materials for various anti-forgery applications and patterning. CDs was effectively utilized for several security purposes, mainly in anti-counterfeiting of valuable documents including bank notes, legal files, electronic components and various branded products of important companies. Luminescent CDs were also successfully utilized for the fabrication of fluorescent markers and ink, which can be served as an effective medium for information communication. They are mainly utilized for secret data encryption of paramilitary and defence sector, thereby ensuring the safety of the whole nation. Numerous works are there in literature, discussing the significant role of CDs for fingerprint sensing and various criminal investigations. Visibly unclear latent fingerprints were found to be more evident with the introduction of these fluorescent entities [191]. The first report on such latent fingerprint enhancement visualisation by CDs was made by Kelarakis and team, with the aid of CDs grinded with silica nano particle [192]. Y. Fang et al presented large scale preparation of bright fluorescent green emissive hollow CDs through facile method, exhibiting excellent printing and patterning ability when applied to water mark ink [189]. Songnan and colleagues developed highly promising fluorescent labels via facile and economic synthesis route. These systems were able to replace conventional inks / markers and offer permanent patterning and printing applications. This further forecast the significant role of the synthesised CDs in anti-counterfeiting applications of valuable assets, data encryption and secret information storage and other security applications. [193]. An interesting work was done by Wang's group, involving plasma induced fabrication of fluorescent CDs from egg yolk, which can be utilized for silk-screen printing of variety highly stable multi colour patterns [190].

CDs medium for Bio-imaging and Drug delivery applications

Owing to the excellent bio-compatibility, low-toxicity, photo-stability and aqueous dispersibility, fluorescent CDs are extensively used as potent candidates in medicinal and health sector [20, 22, 34, 174, 175]. It was clearly established that, fluorescent CDs can be effectively utilized for tracing/detecting and imaging of living body cells, via conducting in vitro and in vivo clinical studies. These fluorescent systems effectively differentiate normal healthy cells from affected/ diseased body cells. Being the emerging fluorescent two-photon candidate, CDs could provide efficient mapping/ imaging of deep body tissues with the aid of confocal microscopy technique [194]. Fu and colleagues designed excellent fluorescent probe from arginine for in vitro cytotoxicity assays on various body cells including MCF-7, N1H3T3 and HeLa cells with extra ordinary cell viability, above 90% [195]. In addition, numerous works were reported in literature, discussing the in vitro and in vivo mapping of different living cell lines using fluorescent CDs probes [20,22,174]. Other than this bio-imaging application, CDs also serve as efficient carriers of drugs to the concerned body parts. Singh et al., demonstrated that fluorescent CDs can serve as excellent drug carrier, which successfully interacted with single stranded DNA phosphoramidate linkage enriched with cytosine and then considerable variation in electrostatic interaction with DNA results successful release of drug. Most of the works concluded that, unlike the conventional nanomaterials used for drug carrier applications, it was the emissive character associated with CDs that serves as the sole factor for tuning them as super drug carrier. They enable effective tracing/ placing of drugs in normal cell lines as well as affected/ abnormal cell lines. A group of researchers reported that modified CDs can effectively release cancer drugs (optimal cell environment) and then show its activities in the concerned affected area of cell lines. Curcumin- ionic liquid based CDs assembly was designed by Shu and group, which can serve as a promising anti-cancer drug with extraordinary drug carrier capacity. This system was marked with commendable cell-penetration ability along with excellent drug-loading power. The study revealed the interesting observation that the aforesaid combination of CDs and curcumin exhibits about 70 % drug loading power and cell viability approximately 87.5 % towards HeLa cell lines [196].

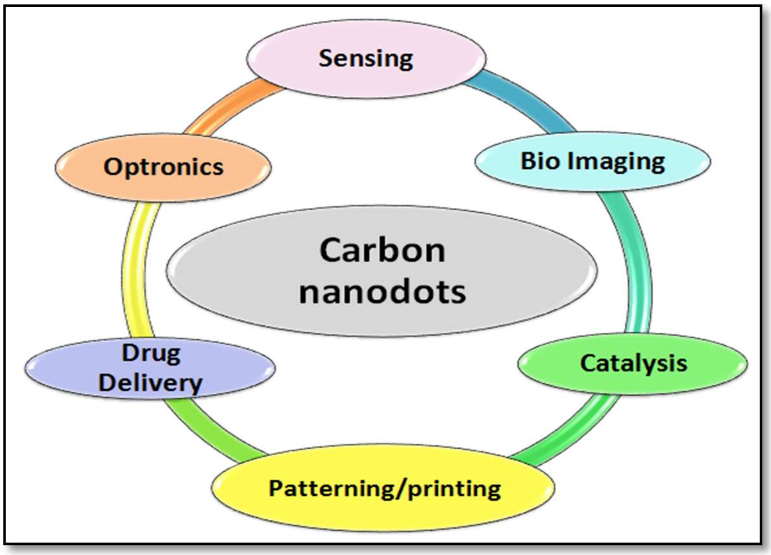


Figure 1.16 : Different Applications of Fluorescent CDs

1.5 Motivation for the present work.

Owing to the intriguing (electrical/thermal/surface/mechanical/optical) features, common allotropes as well as the conjugated nano forms of carbon, more precisely graphene, carbon nano tubes, fullerenes and most recently carbon nano dots are grabbing considerable amount of attention. Though, conventional synthesis routes adopted for the fabrication of these nano carbons brought several promising components to the broad spectrum of nano-technology and engineering, unfortunately majority of these methods were found to be highly expensive and tedious. Most of them demand cumbersome post-chemical treatments for ensuring the purity of the product and frequently requires the usage of toxic chemicals as the precursor as well as the un-wanted side products. Hence, these conventional approaches to nano-carbons definitely raise threatening issues pertaining to global energy conservation and environmentally benign developments. Thus, while considering the increasing demand of these materials, there is an intense urge for the bulk production of the aforesaid carbon structures with extreme purity through entirely facile, environmentally benign and cost-effective strategy. The current scientific scenario strongly insists the incorporation of the core concepts of “green chemistry” while designing the synthesis procedure. Implementation of green chemistry concepts ensures complete protection and benefit to the world economy, population, and the planets. This attempt facilitates the practice of creative and novel methods to reduce/eliminate hazardous materials during the designing, synthesis and applications of chemical products. Green chemistry assisted reactions offers energy conservation as well as efficient replacement of toxic chemicals with suitable bio-compatible natural materials.

Among the promising nano-carbon members, fluorescent carbon nano dots (CDs), the potent alternatives to conventional semiconductor quantum dots, are grabbing the top-notch rank in scientific world. As mentioned earlier, most of the conventional routes to CDs including laser ablation, electrochemical methods, arc-discharge, electro-beam lithography, hydrothermal method and solvothermal methods, have certain disadvantageous in several aspects. Many of these approaches are marked with expensive synthesis route, increased energy requirements, usage of toxic precursors and solvents, as well as tedious post-chemical treatments for the separation and purification procedures. These demerits encountered while attempting the conventionally practiced synthesis protocols are gradually overcome by the judicious implementation of core principles of green chemistry during the designing of CDs. On this ground, usage of hazardous and expensive chemical precursors is successfully replaced by extremely environment friendly and inexpensive substitute, bio-mass. Numerous efforts were reported in the literature, which fruitfully converts cheaply available biological wastes, fruits, vegetables, leaves, fishes, meat, paper, several food materials etc., into highly efficient CDs having commendable fluorescence nature. Along with the suggestion of green raw materials, greener solvents which are non-toxic and highly economical were also prescribed and utilized for ensuring the maximum perpetuation of 'safer- cheaper- easier' motto for upholding economic and sustainable production.

In addition to the selection of greener raw materials and cheaper solvents, the concept of sustainability and global energy concern also should be extended to the matter of 'synthetic techniques'. It was noted that, majority of conventional hydrothermal and solvothermal reactions progress by maintaining comparatively higher temperatures for longer durations for the completion. Besides, the most traditional production strategies, electro chemical synthesis and electron-beam lithography techniques consume commendably higher amount of electrical energy for the fabrication of CDs with desired features. The method strongly conflicts the energy conservation and cost-effective policies of green concepts. Consequently, for maintaining the aforesaid energy sustainable synthetic techniques, one should frame a reaction route to CDs, which necessitate low temperature, as well as time requirements. Literature reports several such attempts to highly fluorescent CDs, which meet the soul of sustainable development by completely preserving the core concepts of 'Green chemistry'.

Scientific world has witnessed zealous efforts for fulfilling all the norms of sustainability by successfully suggesting potent green raw materials, safer solvents and sustainable synthesis techniques confirming the effective maintenance of global energy and monetary safety. The articles detailing various aspects of bio-mass derived/energy conserved carbon dot synthesis are capable to provide a clear picture to realize the significance of such efforts in reducing the global energy and economic crisis at least in the science and engineering field. Observations from such green synthesis of carbon dots are appealing enough to provoke the research mind to design bright emitting CDs, upholding almost all the core-concepts of green and sustainable development.

Keeping this spark as a strong incentive, the present work is designed for developing efficient multifunctional fluorescent CDs form a cheaper non-toxic raw material, adopting very facile, cost-effective and sustainable synthesis strategy. For this, we have selected commercially available edible table sugar (sucrose) as the green precursor, which costs only about 4 paisa per gram.

1.6 Outline of the present study

The entire theses is divided in to seven chapters

- ***Chapter 1* :Introduction** *:* The present chapter as it elaborates, is a detailed introduction to the carbon family as well as the entry of a new member, fluorescent carbon dots (CDs)to the carbon nano world is presented . This chapter also describes various synthesis methodologies and several applications of CDs, along with a detailed discussion on their inherent physico-chemical characteristics. This section was also utilized for presenting the motivation/inspiration for conducting the present research work.

- ***Chapter 2:*Experimental** : Present chapter gives detailed description on various materials and experimental aspects for the fabrication of fluorescent CDs. This section also discusses different characterization techniques employed for the research work.

- ***Chapter 3:*Characterization of the Carbon Dot:** The present chapter is fully devoted for the meticulous analysis of various characterisation results, thereby confirming the formation of bright emitting CDs with desirable properties.

- ***Chapter 4:*Table Sugar Derived Carbon Dot-Gum Arabic hybrid as a fluorescent marker:** This chapter deals with the fabrication of promising luminescent markers using the present CDs by mixing with naturally available binder, gum acacia/ gum arabic. Luminescent markers with two different consistencies (Luminescent Ink and Gel) were fabricated and subjected for patterning and coating applications.

- ***Chapter 5:* Table Sugar Derived Carbon Dot For Sensing Applications*:*** This chapter presents the utilization of bright fluorescence feature of CDs for tracing/sensing of different significant external analytes including metal ions and organic molecules. This section is divided into two parts.

- **PART A**: Utilization of the prepared CDs for visual/naked eye monitoring of the toxic metal ion, Pb^{2+} with higher selectivity and sensitivity.
- **Part B**: Fluorescence intensity of CDs was found to be quenched by the introduction of few analytes, thereby enabling detection of analytes including metal ion and organic molecules, through fluorimetry.

(i) Synthesis of FRET based sensor: Fabrication of CDs- dye hybrid that offers effective sensing of toxic heavy metal ion, Hg^{2+}. Rhodamine-B is serving as the model dye.

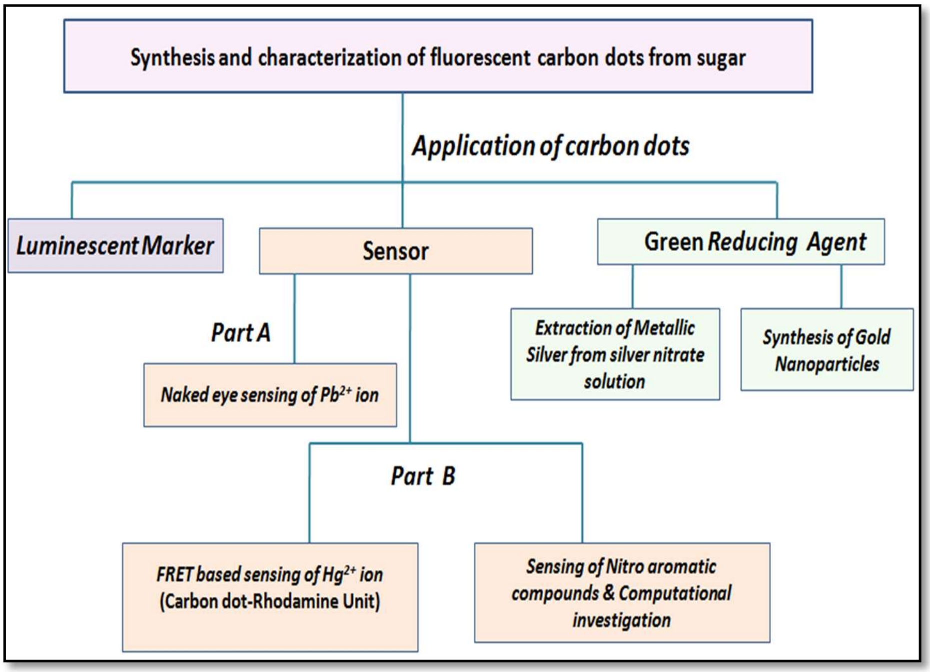
(ii) Fluorescence intensity of CDs was found to be quenched with the addition of a set of Nitro Aromatic Compounds (NACs).This section also discusses structure–activity relationship of the synthesised CDs with detailed analysis using computational methods. This sensing trend was substantiated with the aid of some computational analytical techniques including Global reactive descriptors and donor acceptor mapping (DAM).

- ***Chapter 6:*****Table Sugar Derived Carbon Dot As A Green Reducing Agent:** This chapter presents the reducing nature of the prepared CDs.

(i) The first section presents the reducing power of CDs for the extraction of metallic silver from silver nitrate solution via very facile and ingenious route.

(ii) Second section discusses the instantaneous preparation of gold nano particles from Chloroauric acid ($HAuCl_4.3H_2O$) solution via a very facile route. The catalytic activity of as–synthesised gold nano particles is illustrated by conducting hydrogenation of 4-Nitrophenol to 4-Aminophenol.

- **Chapter 7: Summary, Highlights and Future Perspectives of Work :**This section summarizes the major findings of the research work and also outlines the scope of the study as well as the future perspective.



Frame work of the thesis

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Chapter 2

EXPERIMENTAL

- *This chapter presents the details of used materials, synthesis procedures and various characterization techniques adopted for the analysis of prepared sample.*

2.1 Materials Used

Commercially available Table sugar of edible grade was collected from local market, precursor for carbon dot. Ammonia solution (90 %) was purchased from Merck. All the dilution processes were carried out by using double distilled water as the solvent throughout the entire experimental procedure.

2.2. Experimental Details

(a) Microwave radiation assisted synthesis

As mentioned earlier, this research work is wholly intended to design a highly reliable economic, facile and non-toxic approach to bright emitting carbon dots, the most promising nano material with fine features. We have selected commercially available Table sugar as the precursor, which is non-toxic and highly economic by nature. As well as the choice of non-toxic raw material, the selection of appropriate synthesis technique is also playing a crucial role for determining the sustainability of designed reaction procedure. For fulfilling this norm, one should select a particular mode of synthesis, which is commendably facile, scalable, and above all energy conserving, by nature.

Present work projects the utilization of 'Microwave energy' for the efficient conversion of raw material table sugar into highly fluorescent carbon nano particles. Microwave energy is recently recognized as the potent green alternative to conventional energy sources used for heating, such as hot-plates and oil-baths. The introduction of microwave irradiation in chemical synthesis is a major breakthrough as it is ensuring product formation through safer and convenient reaction conditions with commendable increment in the reaction rate and product yield. Microwaves are arranged in between infra-red radiation and radio waves of electromagnetic spectrum. These rays are characterized by wavelength of 1 mm to 1 m analogous to frequencies in the range of 0.3 to 300 GHz.

As discussed earlier, majority of the conventional heating practices like heating mantles, furnaces and oil-baths will always result in slow and energy inefficient synthetic approach. These ordinary heating sources are associated with convection currents/conduction of reaction vessel walls. Major portion of the heat energy of hot plates and mantles is utilized for heating the container walls (conduction/convection currents) instead of transferring the appropriate energy to the sample in the container. Hence, during the course of heating, these conventional sources induce elevated temperature for the reaction vessel than the reaction sample inside it. To be more specific, reflux reaction conditions results notably higher temperature gradient in the synthetic platform, leading to the decomposition of substrates/reagents and may also yield undesirable side products. Thus, for the reaction strategies involving conventional energy sources, the core of reaction mixture took much longer time to attain the target temperature. In this manner, these ordinary energy sources may result energy inefficient and time-consuming reaction routes with considerable amount of waste formation, strictly contradictory to sustainable production.

Thus for ensuring sustainable nano particle synthesis, the reaction pathway should proceed through energy efficient and facile route. Utilization of microwave energy as an external energy source for a chemical synthesis offers volumetric heating. Hence, microwave assisted reactions will leads to simultaneous temperature rise in the bulk of reaction container, thereby ensuring efficient internal heating of the reaction mixture. Microwave radiation penetrates deeply in to the reaction chamber without resulting any unnecessary heating of reaction vessel via conduction/convection currents as in the case of conventional heating. The whole energy of the external microwave source is perfectly transferred to the reactants without any delay, offering spontaneous chemical reaction with enhanced reaction rate. Thus, microwave assisted nanoparticle synthesis shortens the time needed for the completion of reaction by minimizing the possibility for unwanted by-product formation. This is a great advantage form the green chemistry perspective. In short, microwave assisted carbonization of green raw materials strictly meet almost all the postulates of green and sustainable development.

- *The main advantageous of Microwave assisted chemical reactions over conventional heating practices can be summarized as below*

- ***Faster reactions:*** As microwave radiations are associated with higher temperatures than conventional heating sources, rate of the reaction will be 10000 fold faster than that of conventional heating methods.

- ***Energy conserving:*** Microwaves affect efficient heating of the core of reaction mixture. No energy is wasted for heating of vessel walls, ensuring considerable energy saving.

- ***Improved product yield and cleanness:*** As microwave radiations results in prompt chemical reactions, the synthesis proceeds through the absence of reactants/solvents disintegration. It assures almost perfect conversion of starting materials in to the target product. On account of this enhanced product formation, micro wave synthesis require rapid and negligible purification steps in the absence of unwanted side products. Hence, it offers clean synthesis of nanoparticles.

- ***Better Reproducibility:*** Microwave radiations offer homogenous heating of reaction mixture and better control over reaction parameters such as temperature, pressure and time. These parameters can be monitored within the Microwave reactor, where conventional heating sources fail to do so. Owing to this scalable operation methodology all the microwave assisted reactions are found to be reproducible.

- ***Green synthesis:***Microwave radiation causes direct heating of the sample without dissipating the energy for superheating of surroundings, there by suggesting a reaction protocol which either eliminates or reduces the usage of solvents in chemical reactions. As majority of the microwave assisted reactions avoid the usage of hazardous reagents/solvents, there will be a tremendous decrease in efforts required for purification of final products. Hence, microwave assisted reactions strictly follow all the core-concepts of green chemistry such as clean and safe production of materials form environmental benign precursors in a facile manner.

All these facts highlight the credibility of microwave assisted synthesis method over usual heating methods. These incredible achievements excited the researchers to swiftly switch from conventional heating practices to microwave assisted reactions. Hence, one can confidently approach microwave assisted synthesis method for the large scale production of brightly luminescent carbon nano particles with interesting features which are distinct and reproducible in a cost-effective route and negligible environmental footprint.

(b) Apparatus and reaction procedure

Anton paar Monowave-300 synthesis microwave oven was utilized for the synthesis of fluorescent CDs. This is a single –mode microwave synthesis reactor, which offers intense microwave radiations towards a single reaction vessel at a time, resulting enhanced reaction rate with higher product yield. This monowave 300 synthesis reactor provides simultaneous monitoring and controlling of the reaction parameters including temperature, time and pressure. It also ensures necessary safety measures against undesirable pressure variations during the course of reaction.

In accordance with the total quantity of the reacting sample, there will be three different types of reaction vials with volume capacity ranging from 5 ml to 30 ml**.** For heating the sample with monowave radiation we have to put the sample containing sealed vial inside the microwave reactor cavity and run the following programmes in accordance with the with the preset reaction conditions of temperature, pressure and time.

1) Select the vial type and enter the sample code
2) Open the chamber lid and place the sealed vial with magnetic needle inside it
3) Click heat as fast as possible option, enter the required reaction temperature
4) Select the option hold temperature, enter a fixed time period.
5) Select the required stirring speed (rpm)
6) Click cool as fast as possible, enter temperature ~55 ºC and less.
7) After verifying all the digital displays of parameter inputs, close the chamber lid and click start option.

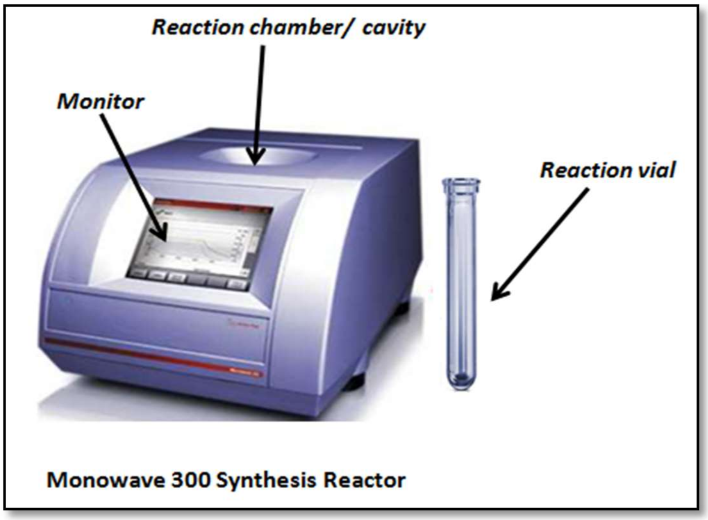


Figure 2.1: Microwave reactor (Anton Paar monowave-300)

2.3 Synthesis of Table sugar Derived Carbon Dots

About 1 g of table sugar taken in a silica crucible was heated in a muffle furnace at 150 °C for about 3 minutes. The pale yellow sugar slurry obtained was transferred in to a 50 mL beaker. To this caramelized table sugar slurry, very dilute ammonia solution (0.25 weight % in double distilled water) was added and mixed well for obtaining homogenous solution. This caramelized sugar-ammonia mixture was carefully transferred to a sealed 30 mL microwave reaction vial. The reaction vial was then placed inside the microwave reactor (Monowave 300- synthesis microwave oven), which enables simultaneous monitoring and control of critical reaction parameters such as temperature, time and pressure. The reaction temperature was adjusted to 120 °C, amicable while considering the permissible temperature range postulated by green chemistry. The reaction mixture was subjected to microwave assisted carbonization for 3 minutes by maintaining all the reaction parameters constant throughout the synthesis process. The resulting carbon dot solution was then cooled, filtered and subjected to ultra-centrifugation for 20 minutes at a speed of 12,000 rpm. Small portion of the solution was freeze-dried to yield carbon dot in powder form and was subjected to various analysis for characterization.

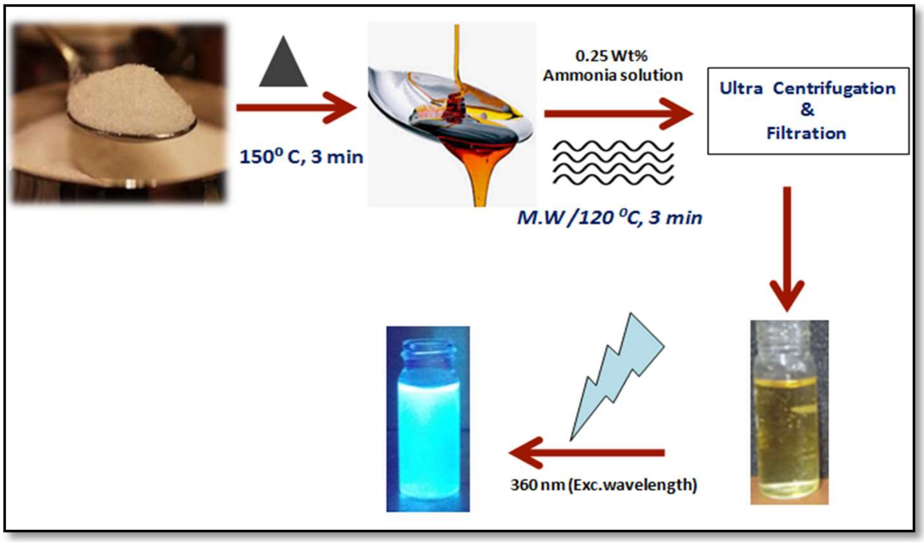


Figure 2.2: Schematic representation of microwave assisted synthesis of fluorescent carbon nano dots from table sugar

In brief, the present work demonstrates a facile route to bright emitting CDs exploiting cheaply available table sugar, a common component in the daily routine of layman, a highly cost-effective course of action preserving environmental friendly concepts. As we have utilized microwave radiation as the energy source, the synthesis procedure also ensures energy conservation and better yield without the formation of any kind of unwanted side products. In the absence of considerable amount of impurities we don’t have to spend more time, money and energy for purification processes compared to the majority of previous reports, impart remarkable benefit on the overall cost of final product. Hence, the present work offers easier, cheaper and safer synthesis protocol, assuring almost all the suggested norms of sustainable development, most anticipated modus operandi in nano-science and engineering.

As-synthesised carbon dots were subjected to further post-synthesis treatment, ultra centrifugation with speed rate, 12,000 rotations per minute for removing unwanted carbonaceous precipitates from the final solution. This will ensure purity of the product. Carbon dot solution is filled inside 15 mL centrifuging tubes and then subjected to continuous ultra-centrifugation for about 20 minutes. Brownish black precipitate collected over the solution was decanted carefully, leaving pale yellow solution of carbon dots.

Figure 2.3 : Digital image of Ultra-centrifugation chamber

2.4 Plausible Mechanistic route to the formation of Table sugar derived Carbon Dots (TS-CDs) - Maillard Reaction

As mentioned in the previous chapter, scientists strictly lack a unified theory for explaining the formation mechanism of fluorescent CDs. There are no generally acceptable mechanistic routes for the formation of CDs, leaving each system unique in their formation process and thereby in their structure. Herein, based on several scientific inferences and detailed literature review, we could suggest Maillard reaction, as the chemical pathway for the synthesis procedure.

(a) Brief introduction to Maillard Reaction

In 1912, a French chemist Louis-Camille Maillard has made a significant discovery while reproducing a biological protein synthesis between a sugar moiety and amino acid. Chemistry Nobel laureate Jean-Marie addressed Maillard reaction as the most widely practiced chemical reaction so far in the world. Instead of a single final product the aforesaid reaction tentatively forms a massive collection of chemical products (several thousand Daltons) during the course of interaction between carbonyl group (reducing sugar moiety) and amino acid. This haziness about the exact Maillard chemistry and thereby the final Maillard product triggered several enthusiastic research minds, note-worthy efforts and findings in this direction were spotted through thousands of publications and conferences. Maillard reaction celebrates its 109th year in 2021 with 5000 publications and 11 international symposia on Maillard reaction.

As Maillard reaction is fully devoted for reducing sugar amino acid condensation, the formed products will vary in accordance with the reacting sugar species and amino acid side-chain there by resulting heterogeneous products including Schiff base imines, melanoidins, amadori reaction products along with some probable chemicals such as 5-Hydroxy Methyl Furfural and acrylamide with a huge massive collection of products which are collectively termed as "HUMIC materials". Due to this uncertainty regarding the final product, numerous investigations were conducted by researchers for probing a definite structure by means of Fourier Transform Infra Red assisted studies on mixture of Maillard products. These efforts in turn end up with certain conclusions regarding final products, which are strictly exclusive for specific starting carbohydrates and amino acids.

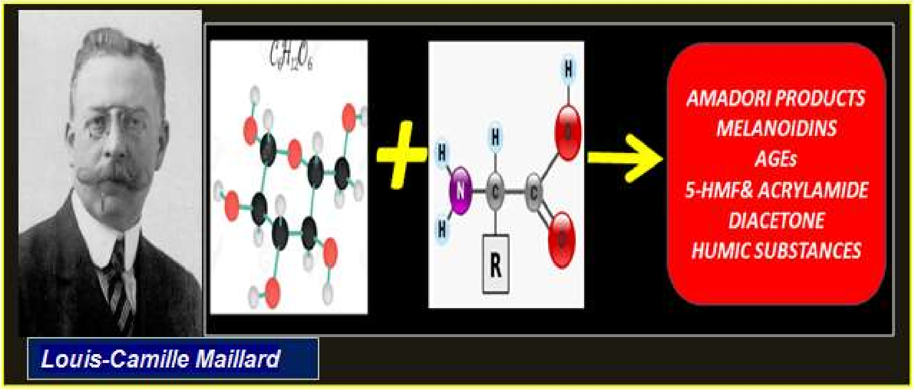


Figure 2.4 : Schematic representation of Maillard Reaction

In 1953, Hodges suggested the possible chemical routes of Maillard reaction, which leads to the conclusion that carbohydrate-amino condensation may furnish a series of products including Ketosamine, Reductones, N-glycosides and dehydro reductones along with some unknown aroma compounds. According to Hodges, the Maillard reaction may result in the formation of plenty of dehydration products including acetol/pyruvaldehyde, diacetyl etc,. Evolution of buttery like odour during the synthesis of table sugar derived carbon dots (carbonization stage) indicate the presence of diacetyl moiety at the early stage of reaction, which is a major bi-product of Maillard reaction. These observations clearly substantiate the Maillard assisted formation of fluorescent carbon dots, rather than normal caramelization of sugar (Saccharose).

(b) Maillard chemistry to the formation of Table sugar derived carbon dots.

During the fabrication of fluorescent carbon dots, pyrolysis of table sugar results in decomposition of saccharose moiety in to individual units, glucose and fructose. After this heating process, the sugar caramel formed is treated with diluted ammonia solution. These sugar molecules will drive Maillard browning when comes in contact with any amino acid/ammonia moiety and furnish a collection of heterogeneous products with structural ambiguity.

Ammonia mediated sugar browning is a rarely discussed branch of Maillard reaction which may proceed through synthetic routes which are entirely different from general Maillard pathway that ends up with exclusively new product. Unlike amino acids, the added dilute ammonia solution in the present case is inept to carry out the complicated chemical reaction in general Maillard reaction scheme like amadori rearrangement, strecker degradation, hydrolysis of Schiff bases etc,. Hence, the probability for the formation of suggested Maillard products (outcome of afore said complicated rearrangements) like imines, Amides, Advanced Glycation End products, Diacetones, 5-HMF, Acrylamides and melanoidins can be completely ruled out during the course of ammonia assisted carbon dot synthesis.

The series of products formed during Maillard reaction with known and unknown structural compositions may undergo series of polymerization reactions followed by condensation processes. These condensation/polymerization products when subjected to continuous exposure of micro wave radiation, will undergo aromatization, subsequently results the formation of several aromatic clusters. Finally when the concentration of formed aromatic clusters exceeds a particular critical super saturation level, all the formed carbon nuclei/ clusters will burst out and results the formation of fluorescent carbon nano dots with desired size (below 10 nm) , attached with several surface functional groups.

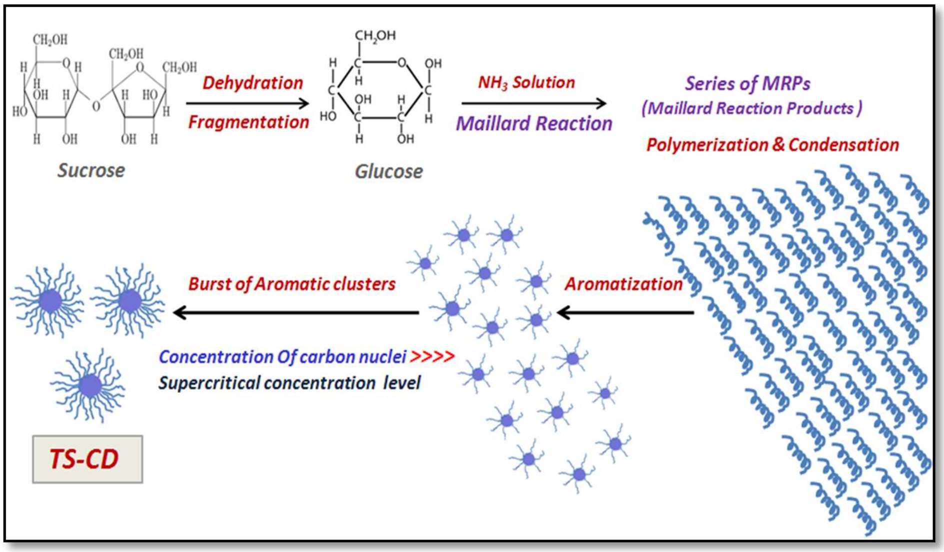


Figure 2.5: Schematic representation of reaction pathway to Table sugar derived carbon dots (TS-CDs).

2.5 Experimental Techniques

Synthesised carbon dot solution is adequately characterised by various experimental techniques including X-Ray Diffraction analysis (XRD analysis), Energy dispersive X-ray analysis (EDAX), High Resolution Transmission Electron Microscopy (HRTEM), Atomic Force Microscopy (AFM), Fourier Transform Infra Red spectroscopy (FTIR), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), UV-Visible absorbance spectroscopy, and Fluorescence spectroscopy. These characterization techniques provide detailed description about the structure and optical features of the system.

2.5.1 Structural Characterization of Carbon Dots

- **High-Resolution Transmission Electron Microscopy(HRTEM) Analysis**

Morphology of the synthesised carbon dots were investigated using High Resolution Transmission Electron Microscopy (HRTEM). Tecnai G2 F30 S-Twin microscope was utilized for recording the HR-TEM image of carbon dots. Sample preparation for this analysis was done by dropping aqueous solution of carbon dot on carbon coated copper grid.

Digital image of HRTEM instrument

- **X-Ray Diffraction (XRD) analysis**

Crystalline/amorphous nature of the system is traced using XRD analysis. Rigaku MiniFlex-II diffractometer with Cu Kα (λ=0.154 nm) radiation source in the range from 0^o to 90^o was adopted for the XRD analysis of the carbon dot sample.

Digital image of XRD instrument

- **Atomic Force Microscopy (AFM) analysis**

Surface topography of carbon dot sample was analyzed using Atomic force microscope (NTEGRE-NT-MDT) equipped with

NOVA software in semi-contact mode by recording 2-Dimensional images. For AFM analysis, droplet of carbon dot solution was placed on the surface of freshly cleaved mica substrate.

Digital image of AFM instrument

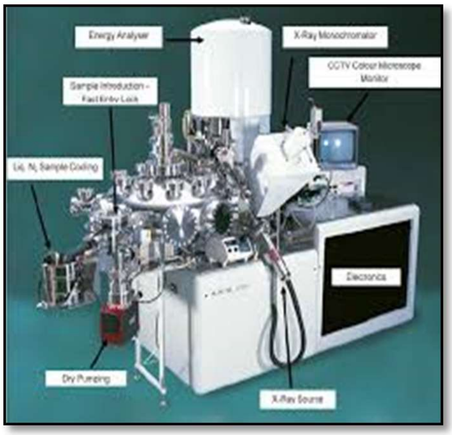
- **Energy Dispersive X-ray analysis (EDX)**

Energy Dispersive X-ray analysis (EDX) was done using FEI SEM QUANTA 200 3D instrument. EDX analysis provides clear idea about the elemental composition of the synthesised carbon dot.

Digital image of EDAX instrument

➢ X-ray photoelectron spectroscopy (XPS) analysis

Surface functional groups of carbon dots were studied with XPS analysis. kratos Axis Ultra DLD spectrometer with Kα X-ray source is used for the XPS analysis.



Digital image of XPS instrument

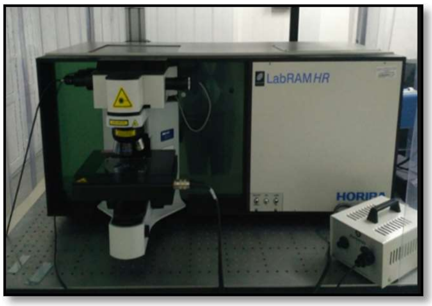
➢ Fourier Transform Infra-Red (FT-IR) spectroscopy

Detailed information about the functional groups of carbon dots was obtained by FTIR analysis. FT-IR spectroscopy was carried out via employing KBr disc method in the range of 4000 cm^{-1} to 400 cm^{-1}, using JASCO FTIR-4100 instrument.

Digital image of FT-IR instrument

➢ Raman spectroscopy analysis

Lab Ram HR-Horiba Jobinyvon Spectrometer with Raman Microprobe with 532 nm Nd: YAG excitation source was adopted for recording the Raman spectrum of prepared carbon dot solution, for achieving detailed information about structural aspects/ surface functionalities of the system.



Digital image of
Raman spectrophotometer

➢ Zeta potential analyzer

Surface charge of the system was recorded with the help of Anton paar Litesizer^{TM} 500 light-scattering instrument.

Digital image of
Zeta potential analyzer

- **Dynamic light scattering analysis**

Particle size of carbon dot can be identified from dynamic light scattering analysis of the sample using Zetasizer Nano ZS-90; Malvern Instruments.

Digital image of DLS analyzer

2.5.2 Optical Characterization of Carbon Dots

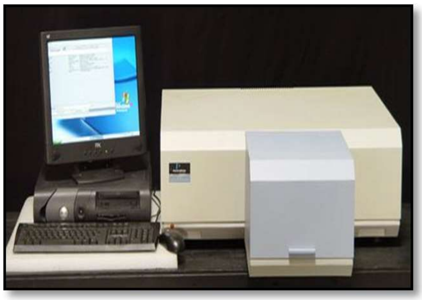
- **Ultra Violet-Visible absorbance spectroscopy**

Ultra-violet visible absorbance spectrum of the carbon dot solution was recorded using JASCO V-550 spectrophotometer. 1 cm X 1 cm PMMA cuvette was used for recording the UV-Visible spectrum. For all the measurements, corresponding solvents were used as the reference against each sample. Necessary dilutions were made for the carbon dot samples for obtaining well-defined UV-Visible absorbance spectrum of the sample.

Digital image of UV-Visible absorbance spectrophotometer

➢ Fluorescence spectroscopy

Fluorescence, the unique feature of carbon dots was recorded using Perkin Elmer Fluorescence spectrometer LS 55. 1 cm X 1 cm PMMA cuvette was used for recording the fluorescence spectrum of the sample.



Digital image of Fluorescence spectrophotometer

➢ Fluorescence Decay (Lifeime measurement)

Fluorescence decay time was monitored using Fluoro Log-3 fluorescence spectrophotometer (Jobin Yvon Inc., USA), by maintaining the excitation wavelength as 370 nm.

Digital image of fluorescence spectrophotometer for life-time measurements

➢ Photoluminescence quantum yield measurements

Photoluminescence quantum yield of carbon dots was measured from integrated photoluminescence intensities (excited at 360 nm) and UV-Visible absorbance of sample and reference solutions. Quinine sulphate in 0.1 M sulphuric acid was used as the reference solution. Quantum yield of reference was found to be 54 % and the absorbance spectrum was measured below 0.05 units (Y-axis) for avoiding the chance of re-absorption. Quantum yield can be measured using the following equation,

$$\mathbf{QY_{CDs} = QY_{Ref}(I_{CDs}/I_{Ref})\,(A_{Ref}/A_{CDs})\,(\eta_{CDs}/\eta_{Ref})^2}$$

- **QY** = Quantum yield
- I = Integrated fluorescence intensity
- **A**= Absorbance value
- η = Refractive index
- Subscripts **CDs** and **Ref** represents sample (Carbon dot) and reference solution (Quinine sulphate in H_2SO_4) respectively.

2.6 Concluding Remarks

- ✓ Commercially available table sugar was selected as the precursor for carbon dot synthesis
- ✓ Used precursor is highly economic in nature
- ✓ Pyrolized table sugar was treated with very dilute ammonia solution
- ✓ Microwave radiation is used as external energy source for few minutes
- ✓ Microwave assisted synthesis strictly conserves energy
- ✓ Formation of carbon dot follows Maillard like reaction pathway yielding collection of heterogeneous products with structural ambiguity
- ✓ Plausible formation mechanism is suggested for table sugar derived carbon dots (TS-CDs)
- ✓ Prepared TS-CDs were subjected to various analysis techniques for characterization

Chapter 3

CHARACTERIZATION OF CARBON DOT

- *This chapter provides detailed information on results of various characterization techniques, thereby discussing the important characteristics of the prepared sample.*

3.1 Introduction

Carbon dots, the latest entry to the carbon nano world are the tiny carbon particles with size below 10 nm, and considered to a most promising nanomaterial, both in the scientific and technological perspective. As mentioned in the first chapter, entry of such a fluorescent carbon member revolutionize the whole scientific world, which thereby prompted scientists to devote their efforts for fabricating promising carbon dos for numerous applications in various fields. Before proceeding to designing a promising carbon dot based material, it is highly desirable to investigate all their inherent physico-chemical features using adequate characterization techniques. Detailed discussion and inferences about the results of all the characterisation techniques were provided in this chapter.

3.2 High Resolution Transmission Electron Microscopy (HR-TEM) analysis

As discussed previously, the morphology of the prepared system can be investigated by conducting HRTEM analysis.

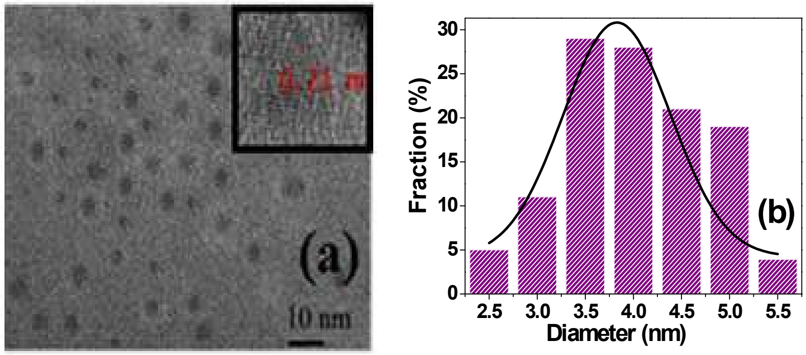


Figure 3.1: (a) HRTEM image of carbon dots with lattice fringe pattern in the inset and (b) Histogram profile showing particle size distribution.

HRTEM image of table sugar derived CDs is provided below as Figure 3.1 (a), indicating the formation of quasi-spherical particles with size below 5 nm. Inset of Figure 3.1 (a) shows the corresponding lattice fringe pattern having interplanar distance of 0.21 nm [1]. Through the histogram profile (Figure 3.1b) of CDs, average particle size was identified as 3.8 nm. All these results strongly confirm the successful formation of carbon dots.

3.3 X-Ray Diffraction (XRD) analysis

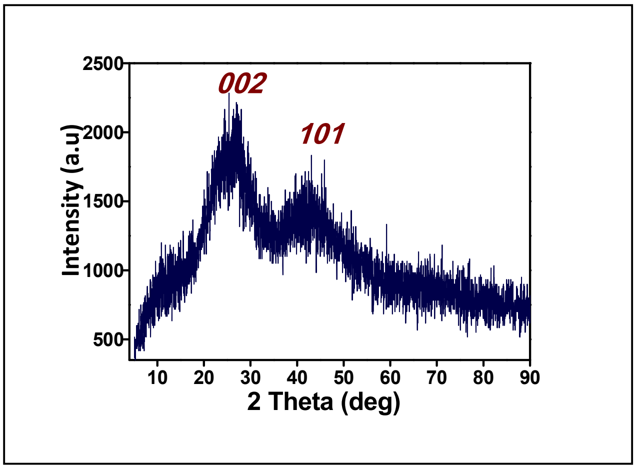


Figure 3.2: XRD pattern of table sugar derived carbon dots.

XRD analysis provides clear picture about the crystalline/amorphous nature of the materials. As evident from the figure, XRD pattern of carbon dots displays two distinct peaks, at 24^{0} and 44.5^{0} owing to the diffraction from (002) and (101) planes respectively (Figure 3.2). The peak at 24^{0} originating from the (002) plane diffraction can be ascribed to the inherent graphitic nature of the system with interplanar spacing of 0.37 nm, significantly greater than pristine graphitic system (0.34

nm) [1].This increased 'd' spacing of the system indicates highly exfoliated graphite layers imparting more graphenic nature to the present system [2]. The second peak at 44.5° corresponding to (101) planes with spacing of 0.2 nm, indicating higher order of the 2D array in vertical direction, which is highly comparable with that of HEG (Highly Exfoliate Graphite) units [3].

3.4 Atomic Force Microscopy (AFM) Analysis

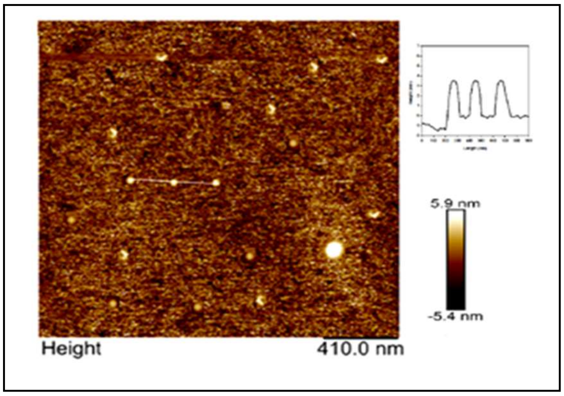


Figure 3.3: AFM image of TS-CDs.

The surface morphology of the prepared system was further confirmed from AFM analysis results (Figure 3.3). Formation of spherical particles with average size of 3.5 nm is clearly evident from the AFM image and the height profile (inset) of diluted solution of TS-CDs. This observation is found to be in good agreement with the conclusion from HRTEM analysis [1].

3.5 Energy Dispersive X-Ray (EDX) Analysis

Elemental composition of the synthesised carbon dot sample was investigated using EDX analysis (Figure 3.4). As evident from the

EDX pattern, table sugar derived carbon dots were composed of higher concentration of Carbon atoms (73.04 %) along with Oxygen atoms (26.96 %). This analysis result confirms the presence of carbon within the prepared system.

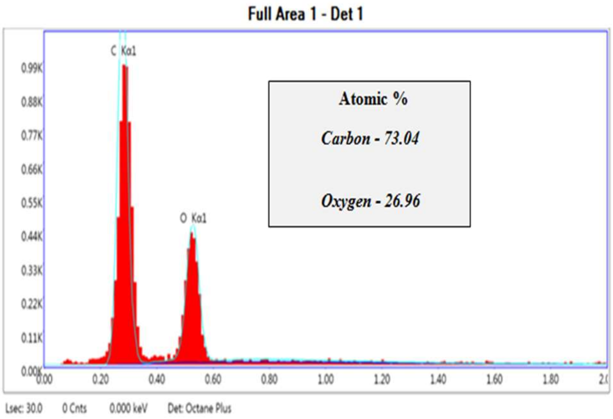


Figure 3.4: Elemental analysis of sample: EDX pattern of TS-CDs with atomic percentages of Carbon and Oxygen (inset).

3.6 X-ray Photoelectron spectroscopy (XPS) analysis

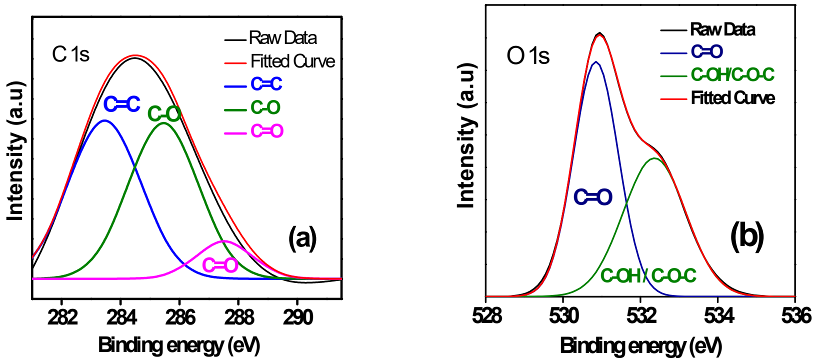


Figure 3.5: XPS profiles (a) C1S spectrum and (b) O1S spectrum of TS-CDs.

Functional groups of the prepared system can be identified by conducting XPS analysis of the sample. The XPS profiles of the carbon dots are displayed in Figure 3.5, confirming the presence of numerous oxygen containing functionalities within the system. C1s spectrum (Figure 3.5 a) possess three distinct peaks at 283.5 eV, 285.6 eV and 287.3 eV, corresponding to C=C, C-O and C=O functional groups respectively. O1s spectral profile (Figure 3.5 b) of the CDs contains two different peaks, at 530.8 eV and 532.4 eV, corresponding to C= O and C -OH / C- O- C respectively. Hence, XPS analysis confirms the presence of enormous amount of carboxyl and carbonyl groups over the CDs structure [4,5].

3.7 Fourier Transform Infra-Red (FTIR) spectroscopy analysis

Detailed information about the functional groups of the sample can be obtained by FTIR spectral analysis. FTIR spectral profile of the prepared system is provided below (Figure 3.6). The signal situated at 3440 cm^{-1} can be ascribed to the O-H stretching vibrations that may arise due to the presence of carboxyl and hydroxyl groups. FTIR spectral band centered at 2930 cm^{-1} indicate asymmetric C-H stretching vibrations, whereas symmetric C-H stretching vibrations leads to weak signal at 2840 cm^{-1}. Broad spectral band near 1625 cm^{-1} denoting aromatic C=C bond conjugated to C=O/COO^- is found to be fused with C=O asymmetric stretching vibration at 1540 cm^{-1}. The intense signal at 1400 cm^{-1} represents C=O symmetric stretching which justifies the presence of COO^- group vibration. Presence of intense band at 1035 cm^{-1} confirms C-O stretching and O-H deformations by alcoholic and polysaccharide units. Hence, FTIR spectral profile of the prepared CDs indicates that the system is well decorated with carboxylate, hydroxyl and epoxy functionalities, carboxylate being the majority [6,7].

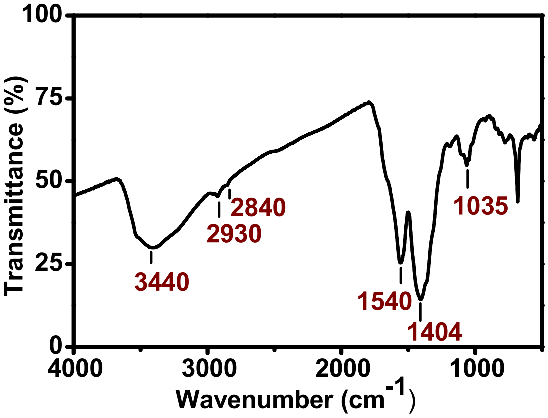


Figure 3.6: FTIR spectrum of TS-CDs

3.8 Raman spectroscopy analysis

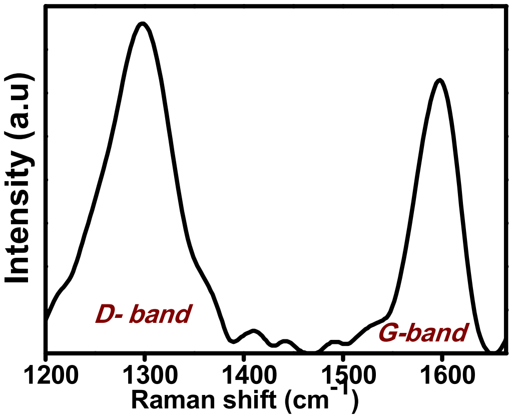


Figure 3.7: Raman spectral profile of TS-CDs.

Structural information of the system can be gathered from the Raman spectral profile (Figure 3.7). [8]. As evident from Figure 3.7, TS-CDs possesses two distinct bands, centred at 1298 cm^{-1}and 1597 cm^{-1},denoting the characteristic 'D' and 'G' bands respectively. D band provides information about the sp^3 hybridized carbon frameworks of the system, whereas details' regarding sp^2 carbons of the system can be obtained from the G band. Intensity ratio of these bands, ID/IG was found to exceed unity (I$_D$/I$_G$= 1.2), indicating more defective carbon sites/ presence of abundant functional groups [9] Observations obtained from Raman spectral analysis strongly substantiate the information from FTIR and XPS analysis of the sample.

3.9 Zeta potential measurement

Surface charge of the system was analyzed by conducting zeta potential analysis. As evident from Figure 3.8, sample possesses surface charge of -2.38 mV. Negative zeta potential value of the sample further confirms the presence of excess amount of oxygen rich functionalities including hydroxyl and carboxyl groups.

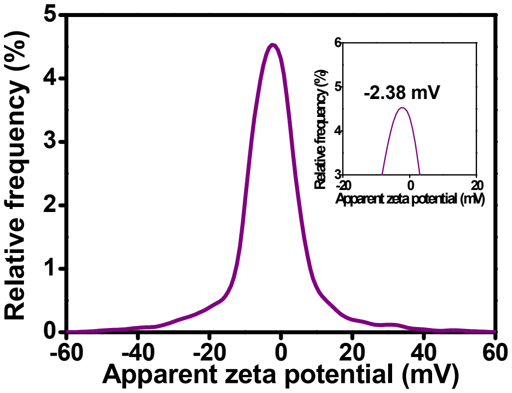


Figure 3.8: Zeta potential curve of TS-CDs.

3.10 Dynamic Light Scattering (DLS) analysis

Dynamic light scattering analysis was performed for obtaining the average hydrodynamic diameter of the sample.

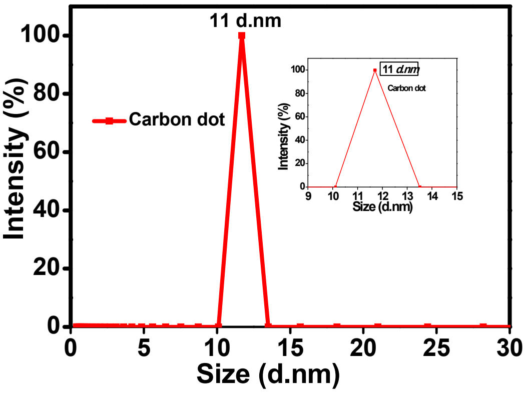


Figure 3.9: Particle size analysis: Hydro dynamic particle size measurement from DLS analysis

From Figure 3.9, the average particle size of TS-CDs was identified as 11nm. Poly dispersity index (PDI) of this measurement was found to be 0.349. Results from DLS analysis was found to be larger, when compared with the particle size obtained from HRTEM and AFM analysis, which is usually expected. This deviation in particle size (anomalous observation) can be ascribed to the formation of electrical double layer on the carbon dot surface as well as may due to the hindrance in path created by the overlapped nanoparticles while conducting the scattering experiment.

3.11 UV-Visible absorbance spectroscopy analysis

Optical features of the prepared system were analyzed using UV-Visible absorbance spectrophotometer. The sample exhibits characteristic UV-Visible absorbance curve of carbon dot units (Figure 3.10). As evident from the spectrum, TS-CDs possess a prominent absorbance at 280 nm denoting π-π* transitions of aromatic sp^2 domains, substantiating the suggested mechanistic route of carbon dot formation involving aromatization of individual polymerized/condensed fragments. In addition, a shoulder peak corresponding to n-π* transition of lone pair containing surface functionalities was observed around 300-320 nm [10-13].

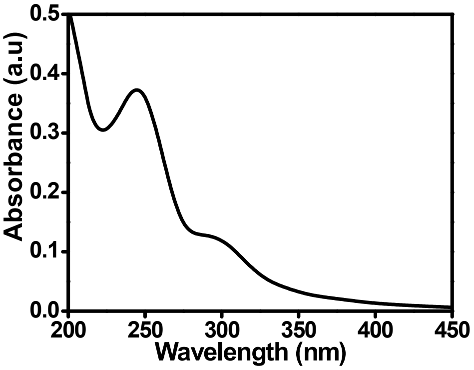


Figure 3.10: UV-Visible absorbance curve of TS-CDs

3.12 Fluorescence spectroscopy analysis

Fluorescence, the unique feature of carbon dots can be displayed through fluorescence spectral analysis of the sample. Fluorescence of the prepared TS-CDs was measured under excitation wavelength of 350 nm (Figure 3.11 a). The sample solution was found to emit bright cyan colour emission when placed under UV radiation of 360 nm wavelength (inset of Figure 3.11 a). As evident from Figure 3.11(a), TS-CDs exhibits intense fluorescence emission at 450 nm (λ_{exc}: 350

nm). Fluorescence quantum yield of the system was measured to be 2.5 % against quinine sulphate as reference solution, using the equation mentioned in chapter 2. It was interesting to note that, present carbon dot system is able to exhibit excitation dependant fluorescence emission feature. As evident from the tunable emission profile (Figure 3.11 b), fluorescence emission wavelength of the system shifts to higher values with gradual increment in excitation wavelength [13-16].

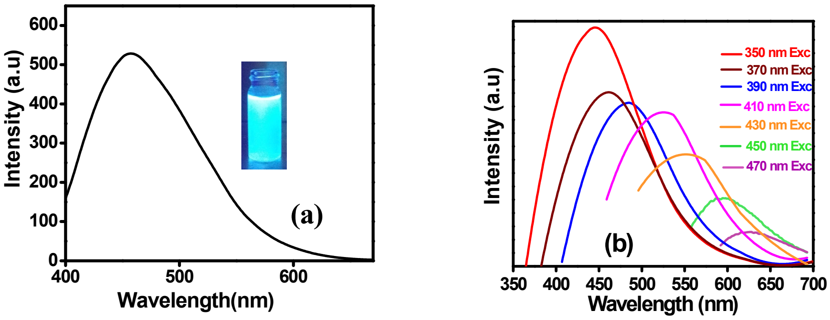


Figure 3.11: (a) Fluorescence emission curve of TS-CDs, digital image of cyan emitting carbon dot (inset), (b) Excitation dependant fluorescence emission of TS-CDs.

3.13 Fluorescence lifeime measurement (fluorescence decay profile)

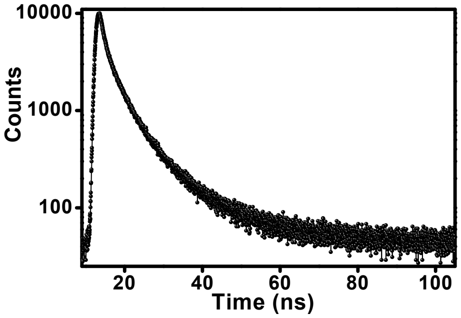


Figure 3.12: Fluorescence decay profile of the sample.

Fluorescence decayprofile of the prepared sample was recorded under excitation wavelength of 370 nm (Figure 3.12). The average fluorescence lifetime of the TS-CD system was measured to be 4.9 ns.

3.14 Summary and Highlights

- Morphology, structural characteristics, chemical composition, particle size, physico-chemical features and the non-toxic nature of the prepared sample was well understood from the results of employed characterization techniques. Formation of quasi-spherical nanoparticles with average diameter ~ 4 nm is evident from the HRTEM image. This observation is further backup by the AFM analysis results. Results of DLS measurement also substantiate the formation of nanoparticles in the aforesaid diameter range.XRD pattern of the system reveals the presence of two distinct peaks at 24^{o} and 44.5^{o} indicates X-ray diffraction from (002) and (101) planes respectively. Increased lattice spacing of former suggests highly exfoliated graphitic (HEG) nature to the system. Elemental analysis results (EDX pattern) confirm the presence of higher amount of carbon (73.04 %) along with Oxygen (26.96 %).XPS and FTIR studies reveal the presence of carboxyl and hydroxyl groups over the carbon core of TS-CDs. Functional group analysis confirms that the sample is abundantly decorated with various oxygen containing functionalities (C=O, -COOH, -OH etc.,).Raman spectral profile of the system indicates more defective carbon sites/ presence of abundant functional groups, substantiating the results from FTIR and XPS analysis of the sample. Zeta potential of TS-CDs was found to be -2.38, confirming excess amount of oxygen rich functional groups over the surface (carboxyl and hydroxyl). TS-CDs exhibits characteristic UV-Visible absorbance spectrum with distinct bands corresponding to π-π* and n-π* transitions within the system. System also exhibits intense fluorescence emission at 450 nm (excitation wavelength: 350 nm). Sample shows intense cyan emission when placed inside a UV chamber (excited using UV lamp with wavelength of 364 nm). System exhibits excitation dependant fluorescence emission over a wide range of wavelength (emission ranging from 450 nm to 650 nm in accordance with excitation ranging from 350 nm to 470 nm). Photo-luminescence quantum yield of the sample was found to be 2.5 % against quinine sulphate as reference solution. Fluorescence lifeime of the system was found to be 4.9 ns from the fluorescence decay profile.

☞

✓ Quasi-spherical particles with diameter below 4 nm is formed.

✓ System exhibits bright cyan colour fluorescence

✓ XRD pattern of the system suggests highly exfoliated graphitic (HEG) nature

- ✓ Spectral analysis confirms abundantly available oxygen containing surface functional groups
- ✓ Present system exhibits tunable fluorescence emission over a wide range of wavelength by changing the excitation wavelength
- ✓ TS-CDs are extremely bio-compatible and environmentally benign, with commendable anti-oxidant nature.

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Chapter 4

TABLE SUGAR DERIVED CARBON DOT-GUM ARABIC HYBRID AS FLUORESCENT MARKER

- *This chapter discusses the fabrication of a stable fluorescent marker for patterning and coating applications by mixing bright cyan emitting TSCDs with naturally available accacia gum (Gum Arabic). Fluorescent markers with two different consistencies, ink and gel, are prepared by altering the concentration of gum arabic, the binding agent. Utilization of accacia gum as a binding agent for making fluorescent markers is a novel attempt. Practical applicability of the markers is also confirmed using substrates of extremely different textures.*

4.1 Introduction

Drastic growth of technology has the way for the intensification of cyber crimes and counterfeiting, challenging national economies, deteriorating financial organizations and jeopardizings public life. Despite the developed sophisticated security taglines/tools, such forgery/ counterfeiting crimes are growing like anything in the recent time [1].Fraudulent usage of authorized documents/ files/ products, which are forged /counterfeited, will definitely become a great threat to the government affairs as well as the well-being of individual's life. Among the valuable assets, currencies/ bank notes were found to be most vulnerable towards forgery/counterfeiting. There are numerous reports on media about the printing and circulation of such counterfeited currencies by various illegal firms, capable of destroying the economical balance of one nation .Other than bank notes/currency, various valuable assets including immigration documents, credit cards, identity cards, digital files, data encryption by military forces/defence service and commodities owned by branded/authorized companies were also found to be duplicated /forged frequently by criminals. As evident from the statistics (Figure 4.2), Asia is much frequently haunted by such forgery/counterfeiting crimes compared to the other continents so far As evident from the statistics (Figure 4.2), Asia is much frequently haunted by such forgery/counterfeiting crimes compared to the other continents so far. On this ground, wide usage of sophisticated reference tools and comprehensive database such as EdisonTD, DISCS enclosing images of genuine documents were also issued by the concerned authority for ensuring the security of such valuable assets [2]. This current scientific scenario urgently needs the development of some promising security tags/markers for preventing such counterfeiting actions [1-3].

Figure 4.1: Examples of various issues regarding the forgery/duplication attempts and global rise in the number of counterfeiting/forgery of various documents/assets.

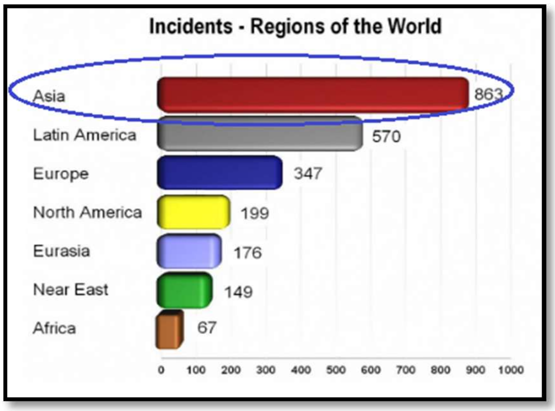

Figure 4.2: Statistics showing the extent of forgery crimes all over the world.

Numerous efforts were there in literature, projecting the development of various markers/labels as potent anti-forgery tools [4-13]. Among the conventionally practised security markers, luminescent paints/inks/coatings have grabbed significant amount of attention, owing to the excellent practicability towards wide range of products/documents/data. Any fluorescent material possessing commendable adherence over wide range of surfaces can be tuned as an efficient labelling agent for assuring the security of valuable materials form counterfeiting/duplicating actions. Fluorescent inks/paints are having immense applications as anti-forgery agents, the general composition of which involves a colouring agent (pigment/dye) and vehicle/carrier (binder). The medium of dispersion (binder) has to be highly viscous enough to impart sticky nature to the luminescent marker for facilitating better adherence over a range of surfaces. Conventionally, chemicals such as Xylene, Methyl benzoate, Toluene and some polymeric materials like Poly Vinyl Alcohol and Poly Vinyl Chloride were used as binding agents for fluorescent markers. Gold and aqueous solution of Sodium Hexa Meta Phosphate was frequently selected as dispersion medium for the fabrication of such markers [4,9,10]. Though, the aforesaid materials could offer better applicability for a wide range of substrates, most often they were met with issues including (i) increased expense associated with fabrication process (ii) lack of stickiness/stability of the marker (iii) higher volatility and decreased viscosity of the used solvent medium (iv) poor adhesive power over various surfaces and (v) decreased resistance towards external stimuli like acids, alkali and oil. As printability of these markers over any substrate is considered as an essential criterion for its acceptability/commercialization, poor adherence capacity and decreased stability are the major challenges in developing such anti-forgery labels/tools. It is also highly desirable to achieve such efficient fluorescent markers via an economic as well as facile synthesis process.

Herein, the bright fluorescence feature of TS-CDs is well exploited for making promising fluorescent markers. Admirable photo-stability exhibited by TS-CDs tunes them as excellent colouring agent/ pigment in inks/paints. We have synthesised two types of highly fluorescent markers with entirely different consistencies; namely photo-luminescent ink (PL-Ink) with semi fluid nature and gel like fluorescent marker photo-luminescent gel (PL-Gel). These fluorescent markers were obtained by altering the composition of the binding agent, i.e., gum arabic (gum accacia), which is extracted from the exterior of tree accacia. Excellent adhesive power as well as commendable viscous nature of gum arabic/ gum accacia offers better adaptability as a binding component for making ink/gel. Though Gum accacia is widely used for several applications in industrial fields, its utilization as a binding component in fluorescent marker was never reported before. Practical utility of the prepared system is examined by coating the fluorescent sample over various substrates including fabrics, plastic and glass plate.

4.2 Fluorescent carbon dot as colorant/pigment in luminescent marker

Electronic era extensively depends on carbon based printing materials (inks/gels) for obtaining better printing and reading experiences [14-17].For making anti-counterfeiting/ anti-forgery tools one should ensure the excellent fluorescence feature of the pigment/colorant used, which exhibits strong resistance towards various external factors, thereby offering improved security for the authorized documents under a prescribed illumination wavelength. As mentioned in the previous chapters, the fluorescence feature of carbon nano dots were abundantly exploited for the sensing/tracing of significant analytes, based on notable alterations in their fluorescence intensity upon the introduction of various external agents [18-20]. As carbon dots offer commendable luminescence feature, they can definitely serve as colouring agents/pigments in markers. Moreover, compared to the conventional colouring agents, majority of carbon dots can be achieved from environmental friendly materials/ cheap precursors via easy synthesis procedures. Hence, intense fluorescence emission, improved water dispersibility as well as significant photo stability of these fluorescent carbon particles tune them as a potent candidate for pigmenting agents in marker. Except a few, the fluorescence emission feature of these materials were rarely utilized for the fabrication of such markers/labelling agents [21,22].

4.3 Introduction of a novel natural binder/carrier, GUM accacia (Gum arabic) for luminescent marker

Gum Arabic /Gum Accacia is the edible natural gummy exudates harvested from the exterior (bark and stem) of accacia tree as dry, hard nodules, ranging from almost colorless to brown. During 1982, JECFA categorized Gum Arabic as 'ADI not specified' [23]. But, as a consequence of subsequent research, its specifications have been revised on several occasions [24,25]. Chemically, Gum arabic can be described as branched chain complex polysaccharide, the backbone of which is made of 1,3-linked β - D- galactopyranosyl units. Main chain is linked to two to five 1,3- linked b- D- galactopyranosyl units through 1,6-linkages. Gum arabic is characterized by high water dispersibility, better emulsifying actions and more over, they exhibit relatively very low viscosity, even at comparably higher concentration ratios. Gum arabic is widely used as emulsifier, stabilizer, thickening agent etc,. It also finds role in food and beverages industry for making soft drinks, marshmallows and gummy candies, as well as in cosmetics, handicrafts/pottery, textiles, lithography and pharmaceuticals [26]. Data related to folk medicines reveal the fact that they have got excellent medicinal properties (anti-oxidant), including treatment of intestinal inflammations, some chronic renal diseases, wound healing and drug carrier.

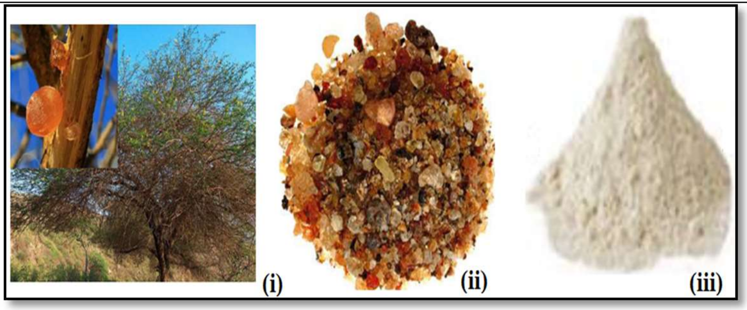

Figure 4.3: Images of (i) Accacia tree (enlarged image of Accacia gum nodules at the exterior bark of tree in inset), (ii) Hard nodules of sticky Gum Arabic and (iii) dried powder of Gum Arabic.

Owing to the enhanced stabilizing as well as suspending ability of Gum arabic, it can play a significant role in industrial manufacturing of emulsions and beverages. Gum arabic is widely utilized for encapsulation of essential oils, plant essences, vitamins and aromatic compositions. As they were characterized by very low calorie as well as higher fibre percentage, Gum arabic acts is the first choice for the production of diet products, functional beverages and other drinks with nutritional claims. Gum arabic provides excellent flavour retention, homogenous texture, shelf life, pleasant mouthfeel and homogenous texture in oil-water/ water-oil suspensions by coating fat/oil droplets. As Gum arabic can serves as an excellent binder and low calorie fiber, it can replace bulk sugar while combining with other sweetening agents. It is also widely utilized for making dietetic and diabetic products like sugarless candies and other confectionery. They can be chosen as an ideal solution for reduced-fat creams and milky desserts, as GA replaces fat content by preserving the creamy nature of products.

Features of Gum arabic including emulsification, binding, demulcent action, encapsulation, stabilization, suspending property and film forming ability are well exploited in pharmaceutical industry. Gum arabic widely finds application in medicated cough-drops/syrups, owing to the enhanced stabilization and suspending action along with the ability to keep away from sugar crystallization and thereby ensuring better texture. Moreover, the commendable adhesive feature of GA tunes them as a promising agent for manufacturing compressed tablets pill coatings. Increased viscous nature, film forming ability, binding action, stabilizing power and emulsification nature tunes Gum arabic as a common ingredient for preparing cosmetic materials such as body care lotions, creams and mascaras. Gum arabic can also serves as a protective colloid in cake cosmetics.

Various other Industrial applications of Gum arabic

- Glaze thickener In ceramics and Porcelain manufacturing
- Binder and adhesive agent in fire-works and explosive manufacturing.
- Suspending action is well utilized in pesticides and insecticide synthesis.
- Transparent adhesive films in stamps and cigarette packets by utilizing its enhanced adhesive nature.

- Natural ingredient in shoe polishing agents along with other commonly used synthetic materials.

- In historically significant Gum bichromate photography, where Gum arabic mixed with ammonium/ potassium dichromate and a pigment/coloring agent form a coloured photographic suspension, comparatively insoluble in water under UV irradiation. Fine quality print is achieved, as Gum arabic exerts commendable binding action towards the paper, thereby permanently fixing the colorant to the print

- Serves as an important component in special purpose inks manufacturing. Suspending and binding features of Gum arabic were well exploited for making specially designed dry inks. Gum arabic also finds its applicability in the production of various water colours, pastels and quick water paints.

- Widely utilized as an efficient sensitizer for lithographic plates. It acts as the light sensitive element as well as the major ingredient of the fountain solution.

4.4 Synthesis of Anti-forgery/Anti-counterfeiting Agents from Carbon Dot-Gum Arabic Combination - Photo-luminescent INK and GEL

As-synthesised cyan emitting carbon dot solution was chosen as the colouring agent/pigment and the finely grounded Gum arabic powder was utilized as the corresponding binding material for making photo-luminescent markers with different consistencies, fluorescent ink and fluorescent gel. Consistency of these two systems was tuned by altering the quantity of binding agent, Gum arabic.

(a) Fabrication of Photo-luminescent ink

For making photo-luminescent ink, 1 mL of carbon dot solution was mixed with 10 mg of finely grinded gum arabic powder, which thereby results in the formation of a semi-fluid suspension with bright cyan fluorescence under UV light irradiation.

(b) Fabrication of Photo-luminescent gel

For making photo-luminescent gel, 1 mL of carbon dot solution was mixed with 20 mg of finely ground gum arabic powder, which results in the formation of cyan emitting photo-luminescent gel with very thick consistency.

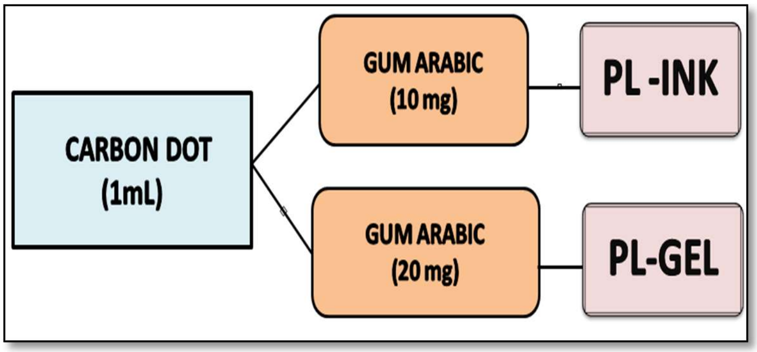

Figure 4.4: Schematic representation of synthesis of photo-luminescent Ink (PL-INK) and photo-luminescent Gel (PL-GEL).

4.5 Practical Application of the Prepared Luminescent Markers

- **Security marker - Patterning and labelling over various substrate surfaces**

Practical applicability of the fluorescent markers was investigated by filling the PL-Ink in the empty cartilage of commercially available gel pen and by coating PL-Gel at various substrate surfaces. As Figure 4.5 (a) displays, the prepared bright cyan emitting fluorescent ink with semi fluid consistency could be successfully filled inside the cartilage of a gel pen. The strong luminescence is visible, when placed under UV chamber with excitation at 364 nm (Figure 4.5 b). This photo-luminescent ink was used for writing and drawing on a commercially available non-luminescent paper. The letters and the symbol drawn was found to be invisible under day light and highly fluorescent upon UV irradiation (Figure 4.5 c and d). As evident from Figure 4.6, the prepared fluorescent gel was successfully utilized for coating over various substrates including cotton thread (b), glass plate (c), plastic sheet (d) and paper (e). All the substrate surfaces coated with the fluorescent gel was found to be highly luminescent when placed inside UV chamber with desired wavelength. All these observations suggest the promising future of prepared luminescent markers for patterning and coating purposes.

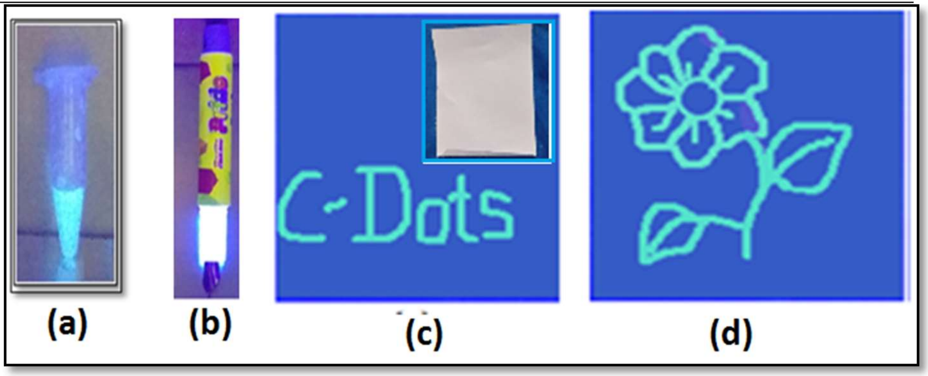

Figure 4.5: Digital image of photo-luminescent ink (a), pen cartilage filled with photo-luminescent ink (b),letters (c) and symbol (d) drawn using the pen on non-luminescent paper under UV light irradiation (image of corresponding paper under visible light in the inset).

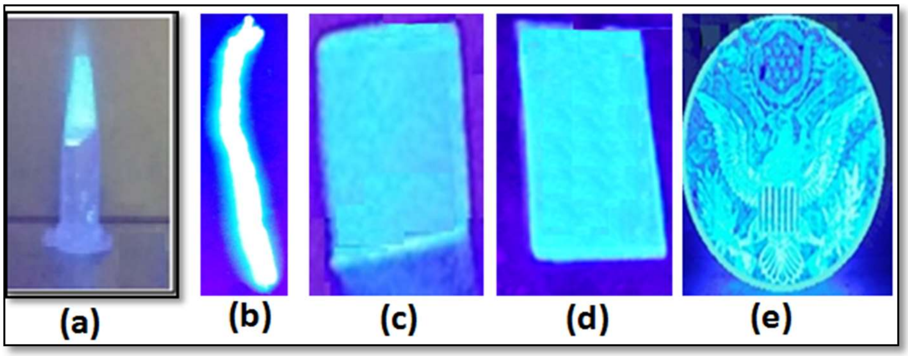

Figure 4.6: Digital image of Photo-luminescent Gel inside the vessel (a), photo-luminescent Gel coated over different substrates under UV light - Cotton thread (b), glass plate (c), plastic sheet (d) and hologram sticker imprinted on paper (e).

4.6. Summary and Highlights

- Fluorescent markers with two different consistency, fluorescent ink and fluorescent gel were prepared using TS-CDs as colouring agent and naturally available Gum arabic as the binding component. Two different concentrations of this natural binder (Gum arabic/ Gum accacia) are mixed with fixed amounts of fluorescent carbon dots. Mixing of 10 and 20 mg of gum accacia with 1 mL of carbon dot solution yielded PL-Ink and PL-Gel respectively. Prepared PL-Ink was filled inside the cartilage of gel pen and successfully employed for writing and patterning purposes, invisible under day light and extremely fluorescent upon UV light irradiation with fixed excitation wavelength. Both the prepared fluorescent markers finds excellent adherence over variety of substrates including cotton thread, plastic sheet and glass plates. It also exhibits excellent printability on non-fluorescent paper imprinted with hologram sticker.

✓ Gum arabic is introduced for the first time as an efficient binder for making fluorescent marker.

Suggestion of gum arabic as a green and cheap alternative for commercially used toxic, expensive binding agents for the fabrication of markers/ paints.

✓ All the gel coated substrates exhibit excellent stable fluorescence under UV chamber of fixed excitation wavelength (360 nm).

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Chapter 5

TABLE SUGAR DERIVED CARBON DOTS FOR SENSING APPLICATIONS

- *This chapter discusses the utilization of TSCDs for monitoring the presence of trace amounts of various significant analytes. TSCDs enabled naked eye monitoring of extremely lethal metal ion, Pb^{2+} with excellent selectivity and sensitivity. Quantification of Pb^{2+} ions was achieved by simple turbidimeter and was found to be in the range of parts per billion. TSCD - Rhodamine dye hybrid constituted an excellent FRET unit, which enabled the selective monitoring of toxic metal ion, Hg^{2+} in picomolar concentration range. Apart from these, carbon dots could trace the presence of a set of nitro aromatic compounds having nitrobenzene parental frame work, with very low detection limit.*

5.1 Introduction

Fascinating features of carbon dots tune the system as a potent candidate in many fields, which in turn resulted in a great leap in the number of publications related to the preparation and applications of carbon dots. As evident from the graphical representation, there was a tremendous hike in the number of published works from 2008 to 2019. Most of the works dealt with the applications of carbon dots in optics, catalytic and electro-chemical fields. It is noted that the utilization of fluorescence feature of carbon dots for monitoring of various significant stimuli, Vis; optical sensing, has grabbed much attention, which is growing rapidly for the last ten years. As depicted in Figure 5.1, around 89.9% of the research works is devoted to the sensing application of carbon dots. 3.54% discussed imaging, 2.02 % provided catalytic studies, and 1.52% dealt with light-energy conversion devices. The remaining 3.03%, presents various important reviews and investigations about significant features of CDs. More precisely, fabrication of sensing units utilizing the change in fluorescence intensity with the introduction of external analyte is grabbing more attention. Studies reveal that, owing to the ease of preparation, credibility and reliability for real sample analysis, optical sensors possess prevailing positions (Figure 5.1 c). Design of promising optical sensing units is still of increasing interest, which is reflected in the constant growth in the number of published works in this direction [1-6]. These sensors find their application in various fields such as environmental protection, bio-medical diagnosis, therapeutic purposes etc. Various analytes comprising significant ions (anions and cations), organic molecules, bio-molecules, proteins etc., are also monitored with admirable selectivity and sensitivity, using these particles.

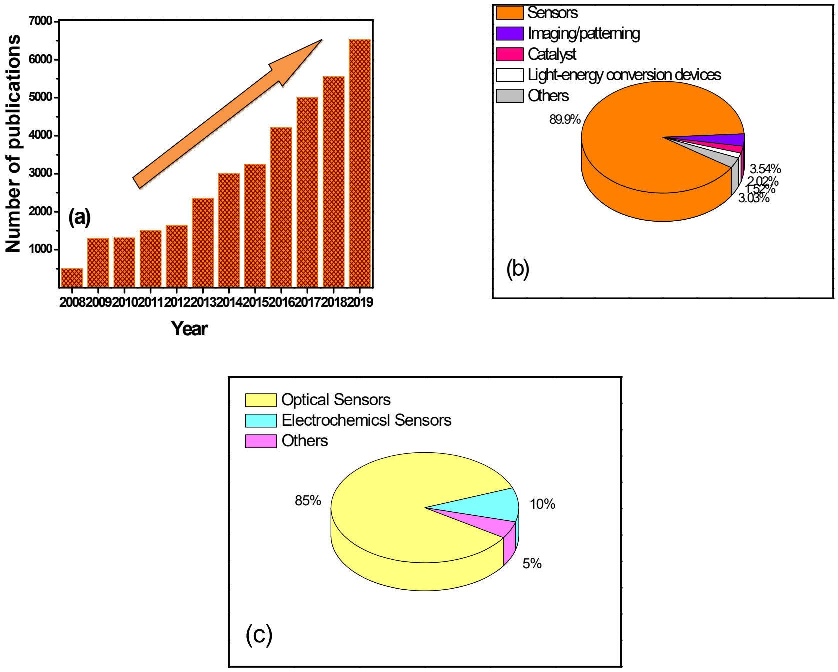

Figure 5.1 : Statistical profile of publications related to carbon dots; (a) sudden increase in the number of publications for the last ten years , (b) statistical estimate of publications related to various applications of carbon dots and (c) statistical evaluation of publications related to various sensing applications of carbon dots

5.2 Sensors: In general, any portable miniaturized entity, which is able to provide real-time as well as on-line information in presence of specific external agent/stimuli can be termed as a chemical sensor [7]. Depending upon the types of transducers, sensing devices can be classified into (i) Electro-chemical sensor (ii) Optical sensor and (iii) mass/heat/pH sensor [8]. Physical sensors are basically found to be sensitive towards physical parameters such as pressure, force, temperature, magnetic field etc, and they strictly lack a distinct chemical interface. Another important category of sensing devices is biosensors. Generally, bio-sensors utilize bio-molecules/ biologically active structures to monitor something with bioactivity / biological significance, more precisely they target bio-molecules including proteins, living tissues, vitamins etc. Chemical sensors basically respond to specific external agents by means of a chemical reaction and thereby provide quantitative and qualitative measurement of corresponding analyte.

Among the chemical sensors, optical sensors are getting tremendous attention. Fabrication of such sensing tools by exploiting the significant optical features, more precisely fluorescence property, is growing like anything [1,2,6]. Unlike the conventionally adopted analytical methods employed for tracing the presence of external agent, fluorescence based sensors possess excellent sensitivity and selectivity. Fluorimetric sensors are also characterized with facile operation, admirable response time and ease of tunability of the sensing action by adequate modifications of receptor-target interaction. All the aforementioned conventional analytical techniques are highly expensive and most of them fail to achieve continuous monitoring of analytes. Thus, owing to the excellent features and response ability, fluorimetric sensors grab the supreme position among the research community. Generally, these sensing tools measure the variation in photo-physical properties of the molecule when interacting with an analyte like metal ions, organic molecules, bio-molecules etc., through a fluorescent signal. Upon interaction with particular analyte of interest, their output signals are generally displayed as noticeable alterations in its luminescence intensity/ emission wavelength shifts/ fluorescence decay lifeime.

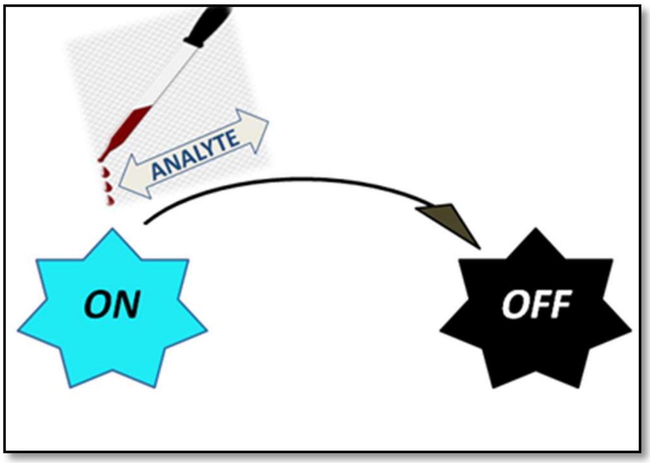

Figure 5.2 : Schematic depiction of basic principle of fluorescence based sensing of external analytes

The fluorescence intensity of sensing unit was most probably noticed to be decreased / quenched with the addition of foreign molecule, which is able to exert adequate chemical interactions with the fluorescent system and in-turn disturb its luminescent centres. Majority of the reports present the fabrication and applications of such 'on-off' sensing probes for monitoring analytes based on quenched fluorescence as output signal. Few fluorescent sensors trace the presence of analyte molecules via enhancement of their inherent fluorescence intensity due to the interaction of latter. "On-off-on" strategy in luminescence response is also exhibited by same system, i.e., the output signal involves the regaining of quenched fluorescence of fluorophore-analyte unit with the addition of another specific external entity with selectivity and sensitivity. Development of fluorescent sensors based on the aforementioned sensing strategies is flourishing like anything and majority of the research works are exclusively devoted for the fabrication of sensors with commendable detection limit and practical utility. An ideal sensor has to be extremely selective towards a particular analyte in presence of mixture of analytes of comparable origin or structures. Besides, it should be able to quantify the target entity even at trace amounts. It is also highly desirable that the sensor should exhibit useful dynamic range, so that it can function successfully in a wide range of concentrations.

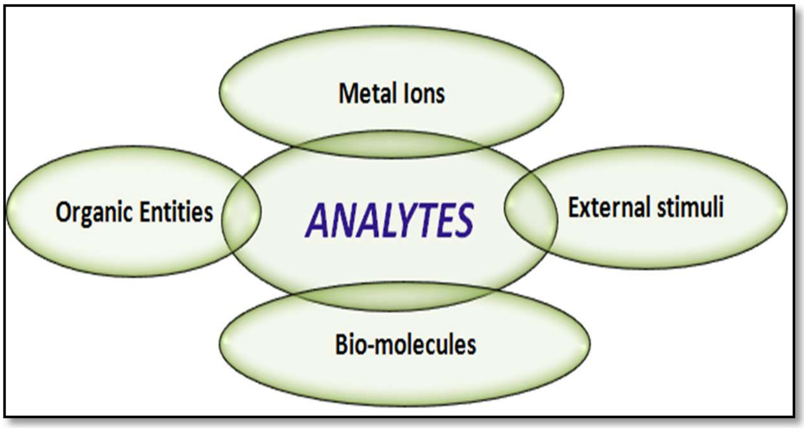

Figure 5.3 : Different types of analytes monitored by fluorescent sensors.

Design of an efficient sensor for monitoring the presence of significant heavy metals are considered to be a challenging task, while considering the concentration limits, which is too low, as prescribed by different standards/ authorized guidelines concerning the toxicity. Numerous analytical tools fulfilling this criterion, including flame/graphite furnace assisted AAS analysis, photometry, inductively coupled plasma emission/ mass (ICPES/ICPMS) spectral studies, anodic stripping Voltammetry and X-ray fluorimetric analysis are in practice extensively. But it was found that, among these methods, most of them fail when it comes in to the matter of routine analysis. In addition, majority of them are highly expensive and time-consuming, and may frequently meet with errors while considering the basic assortment/transportation and pre-treatment of samples. Hence, despite the commendable detection limit and broad linear ranges, the aforementioned demerits strongly suggest the necessity of a portable, reliable, less expensive robust sensing tool for tracing the presence of such heavy metal ions. Hence, ideal sensing tools offering commendable selective and sensitive nature, fast response time, excellent stability as well as facile, economic and safe fabrication methodologies are highly demanded. Though, extensive research works have been in progress, in this line, poor selectivity as well as inefficient reversibility are met with many cases. Recently, scientists have exploited the advantage of chemo-metric sensing tools, combinatorial methods such as artificial neuronal networks/ regression models etc., which in-turn offer selectivity from a response signal of unselective sensors. Unfortunately, practical utility of most of the techniques were found to be non-reliable, which frequently met with some error judgement. Few of them were highly expensive also. Thus, owing to facile and economic synthesis procedure, fluorimetric sensors serve as the most versatile candidates for identification as well as quantification of heavy metal ions.

Fluorescence of CDs are extensively utilized for qualitative as well as quantitative identification of numerous metal ions via distinct interactions like coordination/hydrogen bond formation with their surface functionalities (carboxyl, hydroxyl, amino etc,) [1,2,4]. Synthesis and working of such CDs basedfluorescent sensors were discussed in detail in section 1.4.5 (chapter 1).

(a) Possible interactions between CDs and analyte molecules:

(i) **Static quenching**: If the fluorescent CDs interact with a quencher and forms non-fluorescent ground state intermediate, the quenching mechanism can be termed as by means of static interactions. Thus the formed complex returns immediately to the ground level by a radiation less transfer upon light absorption [9]. Certain criteria should be met with for static quenching interactions to exist between the fluorophore and external agent. (a) The relative lifeime ratio should be unity ($\tau_0/\tau = 1$), (b) ground state complex formation should result considerable alterations in the absorbance spectrum of CDs and (c) temperature increment has profound effect on the stability of thus formed ground state complex, rise in temperature may sometimes retards the effect of static interactions [10-12]

(ii) **Dynamic quenching**: If the excited fluorophore returns to the ground energy state by means of collision with analyte (quencher molecule) on account of energy/charge transfer interactions, the quenching mechanism can be termed as dynamic quenching. The entire mechanism can be represented as follows:

$$\mathbf{A^{*}+Q \longrightarrow A+Q}$$

; A-CDs, Q- Quencher and * denotes excited state

Kinetics of dynamic quenching interactions is usually described by stern-Volmer equation,

$$F_0/F = 1+ k_q\tau_0\,[Q]$$

; F_0 and F denote fluorescence intensities of CDs in the absence and presence of quencher, k_q : Quencher rate co-efficient,τ_0 : lifetime of the excited state of fluorescent CDs in the absence of quencherand [Q] : Concentration of the quencher.

In contrast to the static quenching process, dynamic quenching only influences the excited levels of fluorescent CDs, hence it never leads to any changes to the UV-visible absorbance spectrum. Lifeime of the CDs would be changed during this quenching process. Different from static process, the temperature increment in dynamic quenching interactions would enhance the quenching effect [10-12].

(iii) **Photo-induced Electron Transfer (PET) Mechanism:** If the interactions between CDs and a quencher involve electron transfer, in which they act as donor and receptor entities according to the reaction parameters, give rise to the formation of cation and anion radicals. This photo-induced electron transfer may result in the formation of complex between the interacting donor-acceptor moieties (CDs-Quencher unit), which can return to the ground state through a radiation less transition [12]. PET can be a reductive PET or an oxidative PET, in which CDs acts as electron donor and acceptor respectively. In reductive PET, electron transfers from donor (analyte) molecule to CDs (acceptor), whereas oxidative PET involves shifting of electron from CDs (donor) to the acceptor analyte molecule. It was established that, the energy gap between Lowest Un-occupied Molecular Orbitals (LUMO) of quencher and Highest Occupied Molecular Orbitals (HOMO) of CDs are acting as the driving force for the occurrence of reductive PET, whereas the oxidative PET is decided by the energy gap between LUMO's of CDs and quencher (both donor and acceptor. For PET interactions, the lifetime of CDs were found to be decreased with the process. If there exists considerable energy gap between HOMO-LUMO / LUMO-LUMO of interacting molecules (CDs and quencher), the fluorescence interaction will be definitely based on PET mechanism.

(iv) **Inner Filter Effect (IFE)**: inner filter effect or apparent quenching will occur in a fluorophore-quencher unit, when there is considerable overlapping between the absorbance curve of quencher and excitation/emission curve of fluorophore (CDs). Hence, the emission intensity of CDs can be modulated by the absorber (quencher), and the absorbance of the quencher molecule was found to be sensitive and selective to the concentration of analyte in the unit. One of the essential criteria for IFE within a sensing unit is that the fluorescence emission by the fluorophore system (CDs) has to be perfectly independent of the concentration and all the other properties of analyte of interest [12]. This phenomenon is not at all associated with a perfect quenching process. But to a certain extent, this can be due to attenuation of excitation source or absorption of emitted rays by the CDs or quencher molecule present in the system in excess amount. Though, IFE results reduction in the intensity of fluorophore unit (CDs) with the introduction of quencher, this cannot be termed as quenching as there was no considerable reduction in the fluorescence decay time. Rather than a quenching interaction, IFE simply involves the action of a second absorber molecule, that filters off the emission of CDs. IFE is not a distance dependant interaction. This will happen even if the distance between emitter and the re-absorber exceeds the prescribed distance, 10 nm. IFE is not associated with the formation of any new material during the interaction. As IFE doesn't follows static or dynamic quenching criteria, absorbance curve of fluorophore (CDs) would not get altered during the process. It will not result any considerable change in the fluorescence lifetime of CDs. It was found that, different from the other quenching mechanisms in fluorescent sensing, IFE never demands any kind of electron/energy transfer between the sensing unit and also does not require any chemical modifications of CDs. IFE always results facile and easier operating sensing mechanism. A correction factor should be incorporated for validating the IFE mechanistic interactions within the system. This deviation of IFE can be calculated with the help of following equation,

$$\frac{I_{Corr}}{I_{abs}} = 10\ (\ A_{ex}x1 + A_{em}y1)$$

:**A_{ex}**and **A_{em}** denotes the absorbance values (per cm) at excitation and emission wavelengths respectively, whereas x1 and y1 are the optical path length of excitation and emission respectively

(v) Fluorescence Resonance Energy Transfer (FRET) :

Resonance Energy Transfer (RET) generally involves non-radiative energy transfer between donor-acceptor pair, when they are in contact within 10 nm distance. This interaction is a kind of dipole-dipole interaction, and this can be classifieds into three (i) Chemi-luminescence Resonance Energy Transfer (CRET) (ii) Bio-luminescence Resonance Energy Transfer (BRET) and (iii) Fluorescence Resonance Energy Transfer (FRET), if the donor entities interacting with the fluorophore are chemi-luminescent, bio-luminescent and fluorescent in nature respectively [12,13].FRET is found to be highly sensitive and the efficiency was inversely related to the sixth power of distance of separation between the donor and acceptor. FRET occurs when the absorbance curve of acceptor is perfectly overlapped with emission curve of fluorophore (donor). FRET efficiency for a particular donor-acceptor pair can be calculated using the following equation

$$E = \frac{R_0^{6}}{R_0^{6} + r^{6}}$$

; R_0– forster distance between CDs and quencher, r-distance between the acceptor and donor.

In FRET units, this interaction between two fluorescent components situated within a distance below 10 nm with overlapped absorbance and emission spectral curves results in transfer of energy from the donor molecule of higher energy to the acceptor fluorescent system with lower energy, which further leads to the enhancement of luminescence intensity of the acceptor by sacrificing that of donor fluorophore system. In other words, this FRET interaction leads to quenching of donor's fluorescence along with enhanced luminescence of acceptor molecule. Addition of external agent, analyte, capable enough to disturb the orientation of FRET pair, will definitely result in regaining of emission intensity of CDs by competitive displacement of quencher with the particular analyte (target), thereby offering selective or sensitive monitoring of analyte molecules.

Conventionally used organic dyes and luminescent proteins may sometimes fade the efficiency of the FRET pair in terms of their photo-stability, absorption and emission characteristics etc,. Owing to their appreciable photo-stability, high values of molar extinction coefficients, commendable quantum yield, symmetric narrow emission signals comprising UV to infra red region, water dispersibility, extended fluorescence lifeime and broad absorbance curve, CDs can serve as excellent alternative for the aforesaid molecules for bringing perfect FRET interactions. The enhanced emission rate results in 20 times brighter fluorescence emission for CDs when compared to organic dye molecules. Besides, appreciably high fluorescence lifeime of CDs tunes them as efficient FRET donors than the aforesaid conventional candidates.

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PART A- Carbon Dot Based Naked Eye Sensor

Table Sugar Derived Carbon Dot- A Naked Eye Sensor for Toxic Pb^{2+} Ions

5.4 Introduction and Relevance of Pb^{2+} Sensing

Among the various heavy metal ions, efficient monitoring and quantification of Pb^{2+} ions is having significant interest on considering the lethal action of the said ion in living tissues and environment even in trace quantities [1-4]. It was established that exposure to minute amount of Pb^{2+}ions may cause various health issues related to the functioning of reproductive, neurological and cardiovascular systems. It may also result in severe retardation in mental health of children along with retardation of intelligence, delayed motor response, developmental disorder and hypertension. European Union's Restriction on Hazardous Substances directive ordered to ban the usage of Pb^{2+} ion in various electrical and electronic devices considering its adverse effect towards both flora and fauna [2]. Owing to the non-biodegradable nature, Pb^{2+} ions will easily accumulate within the contacted surface [1-5].Being the major component in water taps/service pipes, the odourless, tasteless and colourless metal, Pb^{2+} can easily enter in to the water and can remain as undetected for a long time, increasing the risk, most probably when contact with acidic water or water with low mineral content. Excess concentration of Pb^{2+} ions often lead to higher possibility for cancer, renal disorders, stroke and memory loss in adult .Various authorized agencies prescribed a particular permissible concentration for Pb^{2+} ions in drinking water. According to Lead and Copper rule by U.S Environment protection

Agency (EPA), the permissible amount of Pb^{2+}ion in drinking water is 15 parts per billion (ppb) [5]. Allowed concentration for Pb^{2+} ions prescribed by International Conference on Harmonization (ICH) and Food and Drug Administration (FDA) for oral administration, inhalation exposure and parenteral exposure is 0.5 parts per million (ppm) and 0.1 ppm respectively [3,5].

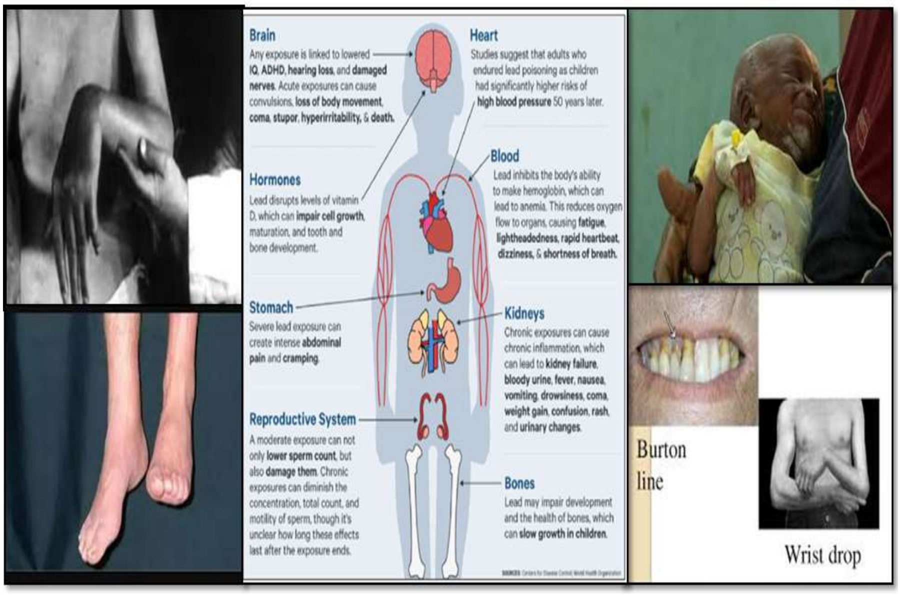

Figure 5.4: Adverse effects of Pb 2+ ions in human body (images collected from internet)

Scientists have developed numerous devices for tracing the presence of Pb^{2+} ions which also assures quantification of the ion with admirable selectivity. Owing to the ease of operation and reliability of the results, majority of scientists utilized colorimetric or fluorimetric methods for the metal detection [7-13]. Colorimetric methods were used by Chen and team, which involves the utilization of gold nanoparticles [14]. Appreciable detection limit was reported by Chai and group in this regard, using glutathione functionalized gold nano particles based on colorimetric route [15]. Various electrochemical sensing devices and several analysis methods are also suggested for monitoring the presence of heavy metal ions because of the merits of small size, low cost, facile mode of operation and high sensitivity [8, 16-18].

There are certain interesting efforts in literature, which report admirable sensing efficiency towards Pb $^{2+}$ ions using fluorescent CDs [7]. In 2013, Wee and group fabricated fluorescent carbon dot based efficient sensing tool by hydrolysis of bovine serum albumin, which offers monitoring of trace quantity of Pb^{2+} ion with admirable efficiency based on fluorometry assay [9]. The system offers excellent sensing action with detection range in nanomolar concentration. An interesting fluorescent sensor was suggested by Liu et al, in 2016 via very facile method using commercially available chocolate as the starting material offering admirable sensitive and selective monitoring of Pb^{2+} ions with limit of detection in the range of 12 nM [10]. In 2017, a group of scientists developed fluorescent CDs from naturally available ocimum sanctum which offers efficient sensing of Pb $^{2+}$ ions along with effective imaging of live body cells [11]. Fu and co-workers synthesised gold nanoparticle decorated graphene for fluorimetric detection of Pb^{2+} ions with detection limit 10 nM, based on accelerated leaching of gold nanoparticles [12].Though, 2-(2-hydroxybenzylamino) propanoic acid derived CDs prepared by Vinod kumar and co-workers offer excellent sensing of Pb^{2+} ions with admirable detection range, Its sensing ability was found to be hindered in presence of other cations, which may retard the efficiency of the system for practical applications [13]. Majority of the previous efforts invariably marked by the usage of toxic materials as well as tedious and expensive analysis techniques for detection and quantification of the ion.

It is interesting to state that, herein, table sugar derived CDs could enable easy monitoring of toxic ion, Pb^{2+}, with commendable selectivity and sensitivity using naked eye. As the present system allows visual monitoring of Pb^{2+} ions with appreciable detection limit, this can be considered as a novel achievement established via a highly facile, economic and green route. Table sugar derived CDs enable naked eye monitoring of Pb^{2+} ions through aggregation induced precipitation of individual carbon dot units in presence of these ions, which is further substantiated by various characterisation techniques and detailed analysis of the results. As the crux of the sensing strategy is aggregation of individual carbon dot units, presence of Pb^{2+} ions is quantified by means of simple turbidimetry, which also highlights the facile mode of operation of the system.

5.4.1 Results and discussion

Presence of abundant oxygen rich surface functionalities (hydroxyl, carboxyl, carbonyl etc.) in the system was clearly manifested through the results of concerned characterization techniques in chapter 3. These surface moieties are the suspected root cause of aforesaid interesting observation, Vis; Pb^{2+} ion assisted aggregation of TS-CDs solution. This section is exclusively meant for the discussion on the role of TS-CD surface structure in the aggregation process

(a) Aggregation assisted precipitation; visual sensing of Pb^{2+} ion in parts per billion (ppb) levels

The introduction of parts per billion amounts of Pb^{2+} ion induces the formation of turbidity in the solution of TS-CDs. Digital images indicating the aforesaid precipitation is provided in Figure 5.5. Unlike the previous works, presence of Pb^{2+} ions can be identified with naked eye, without the assistance of any equipment.

TSCD solution (left) and aggregated TSCD particles (right)

Figure 5.5 : Visual aggregation of TS-CD units with the addition of Pb^{2+} ions

As mentioned previously in this chapter, for a sensor to be claimed as an ideal one, selectivity of the system should be investigated. For this, a series of metal ion solutions were prepared and their interaction with the TS-CD solution was examined. Small amounts of the test solutions including Cd^{2+} , Hg^{2+}, Cu^{2+}, Fe^{3+},K^{+}, Na^{+},Ni^{2+},Co^{2+},Cr^{6+},Mn^{2+}, Ca^{2+} and Zn^{2+} were introduced in to the pale yellow solution of TS-CDs. Digital images displaying the response of TS-CD solution towards each metal ion solution is provided in Figure 5.6 (a). As evident from Figure 5.6 (a), all the other metal ions except Pb^{2+} fail to bring the particular aggregation assisted precipitation of TS-CDs under similar conditions. Hence, it is clear that the prepared fluorescent CDs can serve as a promising tool for sensing Pb^{2+} ions with commendable selectivity and appreciable detection limit.

Sensing efficiency of the system has been verified by investigating whether any external metal ion can interfere with the visible detection of Pb^{2+} ions. For this interference assay, mixture of metal ions (Cd^{2+} , Hg^{2+}, Cu^{2+}, Fe^{3+},K^{+}, Na^{+},Ni^{2+},Co^{2+},Cr^{6+},Mn^{2+}, Ca^{2+} and Zn^{2+}) with and without Pb^{2+} ion was introduced in to the TS-CD solution (Figure 5.6 b). It was noticed that, metal ion solution mixture without Pb^{2+} ion failed to evoke the aggregation process. But the addition of metal ion mixture containing Pb^{2+} ions brings momentous change to the solution, i.e., instantaneous formation of turbidity was observed. This nil interference by the external analytes on the detection performance confirms the credibility of the present system as an ideal sensing tool for Pb^{2+} ions.

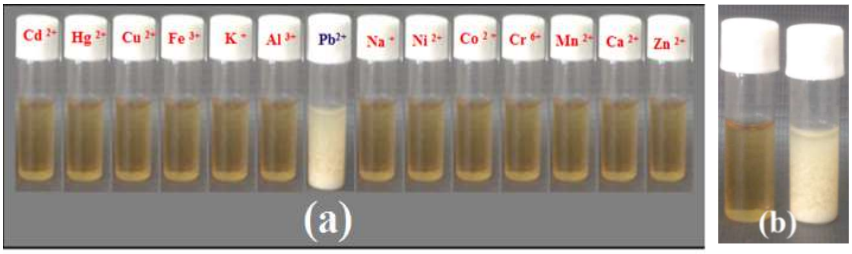

Figure 5.6 : Investigation of Selectivity and sensitivity of the TS-CD solution: (a) all the tested metal ions except Pb2+ produce negative response under similar reaction conditions(selective sensing), and (b) TS-CD solution after the addition of mixture of metal ions (Cd2+, Hg2+, Cu2+ , Fe3+, K+ , Na+, Ni2+, Co2+ , Cr6+ , Mn2+ , Ca2+ and Zn2+ except Pb2+ ions) (left) ; after the addition of Pb2+ ions to this mixture (right)

(b) Aggregation of TS-CD system- Verification through characterisation result.

The authenticity of the suspected reason for the observed turbidity, Vis; aggregation of individual CDs, is investigated by the thorough analysis of the collected precipitate. As displayed in Figure 5.7 (a), TEM image of the collected precipitate clearly indicates the formation of aggregates of individual CDs. A comparison of the image with that of pure TS-CDs shows (Figure 3.1 (a)- chapter3), that the introduction of Pb^{2+} ions brings each carbon dot particle close enough to cause aggregation and the size of the aggregated units become large enough to scatter the incident light, which in turn imparts turbidity/precipitate formation within the solution. Figure 5.7 (b) presents the EDX pattern of the precipitate, which emphasizes the incorporation of added Pb^{2+} ions in to the surface of TS-CD system. The precipitate was filtered off and the remaining filtrate solution was collected for fluorescence spectral analysis. Fluorescence emission characteristics of filtrate obtained after the removal of aggregated precipitate further substantiate the suggested aggregation of CDs (Figure 5.7c). As the figure suggests, almost complete suppression of fluorescence was observed for the collected filtrate, confirming the fact that the removed part (precipitated portion) is the sole source of luminescence, which is nothing but fluorescent TS-CDs. XRD analysis of collected precipitate is conducted to verify this. Figure 5.7 (d) clearly confirms that the XRD pattern of precipitated portion resembles that of TS-CD solution (Figure 3.2, chapter 3). The only difference noted was in terms of their peak intensity. XRD peaks of the precipitated portion were little bit less intense than that of TS-CD system. All these results further validate the suspected aggregation of individual TS-CDs upon the addition of trace quantities of Pb^{2+} ions.

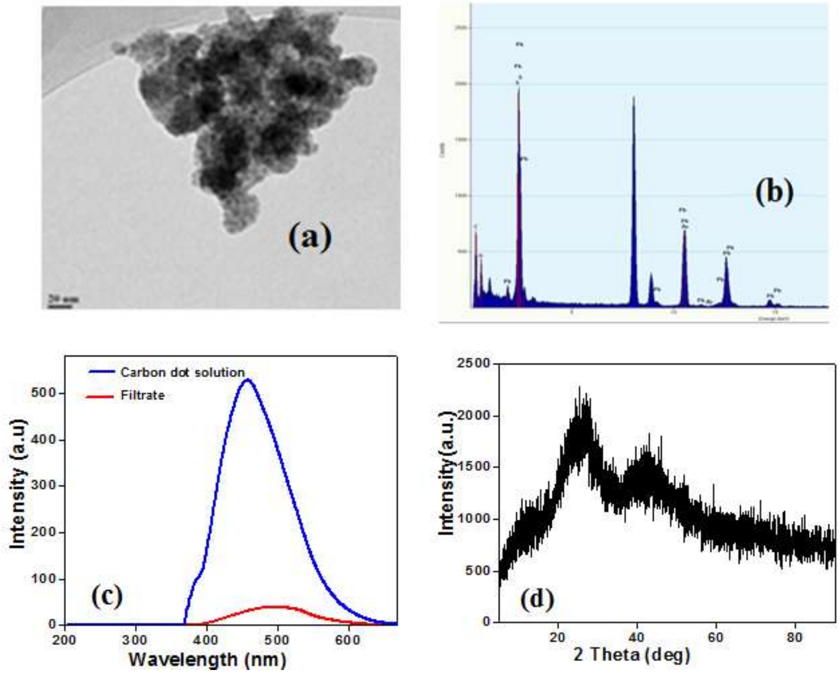

Figure 5.7: Characterisation of precipitate and filtrate: (a) TEM image, (b) EDAX pattern of aggregated system, (c) Fluorescence emission spectra of TS-CD solution and filtrate obtained after the removal of precipitated portion.(d)XRD pattern of precipitated portion.

(c) Involvement of carboxylate functionalities in the aggregation process

As the crux behind the visual monitoring of Pb^{2+} ions by the TS-CD solution was proved to be the aggregation of individual CDs, introduction of the said ions is expected to make the system unstable. Presence of abundant carboxylate groups imparts excess negative charge to the TS- CD system. The addition of Pb^{2+} ions most probably nullifies this excess negative charge of TS-CD surface via significant interaction of this metal ion with the carboxylate surface functionalities. Previous reports reveal the extra-ordinary affinity of Pb^{2+} ions towards the carboxylate functionalities, with commendable selectivity [19].

The collected precipitate (aggregated TS-CDs) was subjected to FTIR spectral analysis, to gather information regarding the structural aspect of the system. FTIR spectral profile of precipitate was compared with that of TS-CD system (Figure 5.8). As the figure reveals, there is a noticeable reduction in the intensity of FTIR spectral signals of aggregated system while comparing with that of pure TS-CDs. The reduced intensity of spectral bands corresponding to carboxylate groups around 1560 cm^{-1} (asymmetric stretching) and 1410 cm^{-1} (symmetric stretching) strongly substantiates the involvement of carboxylate functionalities in the aggregation process.

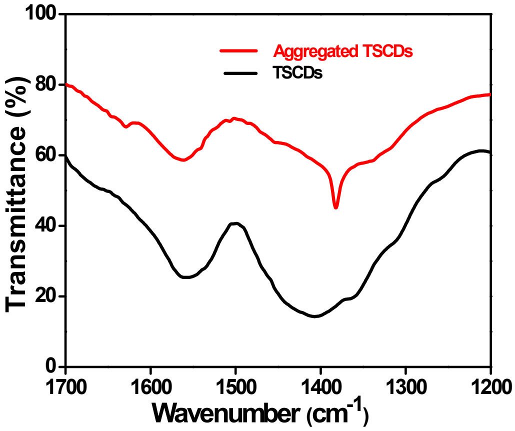

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Figure 5.8: FTIR spectrum showing direct involvement of carboxylate groups in the aggregation process.

As mentioned in the previous chapter, XRD pattern of TS-CD system clearly illustrated well separated graphitic units approaching more graphenic nature, which has proven to be highly susceptible for aggregation in presence of Pb^{2+} ions with extreme selectivity [20,21].

(d) Quantitative sensing of Pb^{2+} ion –Turbidimetric analysis

As the presence of Pb^{2+} ion is identified by turbidity formation, the concentration of Pb^{2+} ion is quantified with the aid of a simple turbidimeter.

Figure 5.9: Image of Turbidimeter used for quantification of Pb2+ ions(LABTRONICS LT-33 Digital Turbidity Meter)

During the Turbidimetric analysis, it was noticed that the introduction of increased amounts of metal ions into the TS-CD solution results progressive increment in turbidity/precipitate formation. The variation of turbidity with the concentration of Pb^{2+} ion was found to be perfectly linear with regression coefficient of 0.996 (Figure 5.10 a). The limit of detection was calculated using 3σ/slope method and was found to be 14 ppb, highly commendable in view of the permissible amount of lead in drinking water (15 ppb) [3,5].

For analysing the accuracy and reliability of this sensing method, the influence of time in the aggregation process was monitored using 14 ppb of Pb (II) solution. Corresponding result is presented in Figure. 5.10 (b). As indicated, the formed aggregates were stable for a long time period. All these findings uphold the observation that the present TS-CD system offers accurate and reliable sensing of this toxic ion via highly economic and facile route, using naked eye, suggesting that the sensor has great potential for application in fast, sensitive and low-cost detection of Pb^{2+} in water. The requirement of a simple turbidimeter for quantification of the analyte as well as the highly admirable detection limit further enhances the significance of this work.

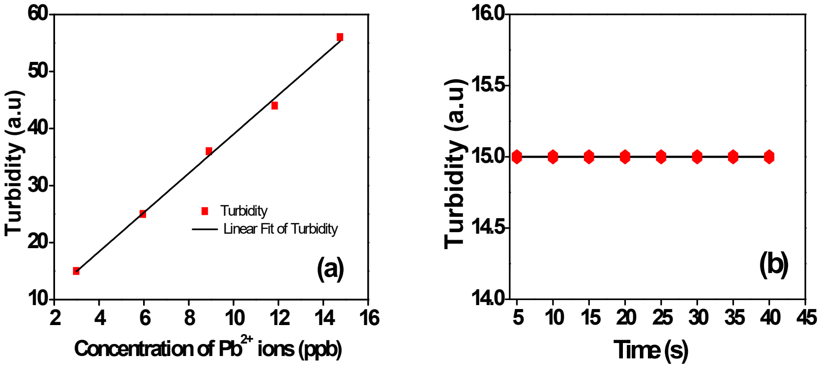

Figure 5.10 : Quantification of Pb2+ ion using Turbidimetric analysis: (a) quantitative variation of turbidity with concentration of Pb2+ ions and (b) Time dependence study of carbon dot aggregation monitored using turbidimetry.

(e) Practical applicability of the present system for real sample analysis

Viability of the visual sensor as an efficient layman's tool for the detection of Pb^{2+} ions is analysed using three real water samples collected from local premises. For testing the analytical capability, the 3 samples collected from Tap water (university of Calicut), well water (Calicut) and lake water (Calicut) were labelled as sample A, B and C respectively. All the collected real samples were spiked with known concentration of Pb^{2+} ions. Blank samples and the spiked samples were subjected to Pb^{2+} monitoring using the TS-CD solution. The data in this regard is presented in table 5.1.

Sample code	***Spiked concentration (ppb)***	***Detected concentration (ppb)***	***Spiked recovery (%)***
A	*(i) 20* *(ii) 25* *(iii) 30*	*(i) 19.9* *(ii) 24.7* *(iii) 29.9*	*(i) 99.5* *(ii) 98.8* *(iii) 99.7*
B	*(i) 20* *(ii) 25* *(iii) 30*	*(i) 19.7* *(ii) 24.8* *(iii) 29.9*	*(i) 98.5* *(ii) 99.2* *(iii) 99.7*
C	*(i) 20* *(ii) 25* *(iii) 30*	*(i) 19.6* *(ii) 24.6* *(iii) 29.5*	*(i) 98* *(ii) 98.4* *(iii) 98.3*

Table 5.1: Spiked recovery calculation for real-water samples using TS-CD solution

As the results indicate, TS-CD solution enables excellent tracing of Pb^{2+} ion in all the tested samples. The appreciable spiked recovery rate exhibited by the system highlights its efficiency as a real time sensing tool. Each sample was spiked with 20, 25 and 30 μ g/L of analyte ion. The corresponding spiked recoveries of samples A, B and C were found to be in the range 98 to 99.7 %. These observations ensure the practical applicability of the present TS-CD solution as a potent alternative for conventional Pb^{2+} sensing devices.

An analysis on the performance of various established Pb^{2+} ion sensors suggests the supremacy of the TS-CD system over the others, in terms of selectivity as well as sensitivity towards the target ion (Table 5.2). Though the ocimum sanctum derived carbon dots, chocolate derived carbon dots and gold nanoparticle decorated graphene sheets show lower detection limits than the present system, the requirement of an analytical instrument and the utilization of expensive chemicals are suggested as their demerits.

System	**LOD**	**Sensing strategy**	**Reference**
DNAzyme-Directed Assembly of Gold Nanoparticles	*100 nM*	*Colorimetry*	*[7]*
Unmodified AuNPs and lead-stabilized G-quartet formation	*5000 nM*	*Colorimetry*	*[8]*
Bovine Serum Albumin (BSA) derived carbon dot	*5050 nM*	*Fluorometry*	*[9]*
Chocolate derived carbon dot	*12.7 nM*	*Fluorometry*	*[10]*
Ocimum sanctum derived carbon dot	*0.59 nM*	*Fluorometry*	*[11]*
2-(2-hydroxybenzylamino) propanoic acid derived carbon dot	*100 nM*	*Fluorometry*	*[13]*
Table sugar derived carbon dots	*67 nM (14 ppb)*	*Turbidimetry*	*Present work*

Table 5.2: Comparison of TS-CDs over some established Pb2+ sensors.

5.4.2 Summary and Highlights

- The prepared TS-CD solution becomes turbid upon the addition of parts per billion levels of Pb^{2+} ion, paving way for naked eye monitoring of the said ion. This turbidity formation was found to be extremely selective and sensitive. As the presence of Pb^{2+} ions was identified by the appearance of turbidity, quantification of target ion is easily achieved with the assistance of simple turbidimeter. The lower detection limit was found to be 14 ppb, which is highly commendable compared to the permissible amounts of Pb^{2+} ion prescribed by various agencies (US EPA- 15ppb) and authorized guidelines FDA (0.1ppm) and ICH (0.5ppm).. Appearance of turbidity was found to vary linearly with the concentration of added Pb^{2+} ion (regression coefficient 0.99). The underlying reason for this commendable selectivity of TS-CD solution towards the Pb^{2+} ions was confirmed to be the extraordinary affinity of Pb^{2+} ions towards carboxylate functional groups enriched over the surface of TS-CDs. Practical applicability and analytical capability of the present sensing unit was investigated by performing sensing assay with different real water samples collected from local water bodies (tap and lake).The spiked recovery rate for real sample analysis was found to be in the range of 98 to 99.7 %.

✓ Naked eye monitoring of extremely toxic Pb^{2+} ion through visible aggregation of carbon dots.

✓ Interference free detection of Pb^{2+} ions with high selectivity and sensitivity.

✓ Demonstration of practical applicability through real sample analysis

5.5 References

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PART B- Carbon Dot Based Optical Sensors

(I) TS-CD-Rhodamine dye array for FRET based sensing of Hg^{2+} ion

5.6 Introduction and Relevance of Hg^{2+} sensing

Hg^{2+}, ranked as the sixth most poisonous heavy metal, is a cumulative toxicant due to the non-biodegradability and fast transport to remote areas from the point source[1-6]. The most dangerous factor concerning Hg^{2+} toxicity is its bio-magnification through food chain and aquatic surfaces. According to available statistics, around 75% of individual mercury exposure is resulted from amalgams. Most important source of non-occupational human Hg exposure is methyl mercury deposited in sea foods, especially in fishes. In addition, there are various sources which results intense release of toxic Hg^{2+} , such as agricultural industries, manufacturing of electrical commodities, paper making industries, fabrication of paints, photography field etc,. Generally, Hg^{2+} is released in to atmosphere in two forms (i) methyl mercury and (ii) metallic mercury. As mentioned earlier, the most noxious form of Hg^{2+}, methyl mercury is mainly transported to human body through the consumption of sea food. Studies reveal that, human body has inherent capacity to resist the adverse effects exerted by metallic mercury to a certain extent. But the administration of methyl mercury badly affects the working of Central Nervous System (CNS) and may cause severe damage to certain areas of brain.

The most dangerous fact connected with Hg^{2+}contamination of aquatic life is that once introduced in to the cycle, Hg^{2+} ions will be instantaneously solvated to form stable inorganic mercury (solvated Hg^{2+}). Continuous exposures to Hg^{2+} ions cause several physiological problems to human, including neurological dysfunctioning, autoimmune disease and dementia. Various scientific studies suggest that intense exposure to Hg^{2+} ions may cause several health issues including memory loss, heart problems, mental stress, irritations, cold feet and digestion problems. Researchers suggest that Hg^{2+} toxicity leads to health conditions similar to symptoms of autism. There are several Hg^{2+} induced disasters reported in history. Minamata disease, the most notorious event, witnessed the death of many people, severe damage to environment and exhaustion of a local population because of the strong discrimination towards the victims and conflicts between neighbouring inhabitants. Minamata disease also referred as Chisso-Minamata disease, results in muscle weakness. In acute poisoning it may cause paralysis , coma and even death of the victim. This may also lead to visual, hearing and speech impairment. Acrodynia (pink disease) also results from chronic exposure to Hg^{2+} ions. It is generally characterized by the appearance of pink coloured rashes over the body. The victims may suffer watery and weeping skin lesions [1,4,5].

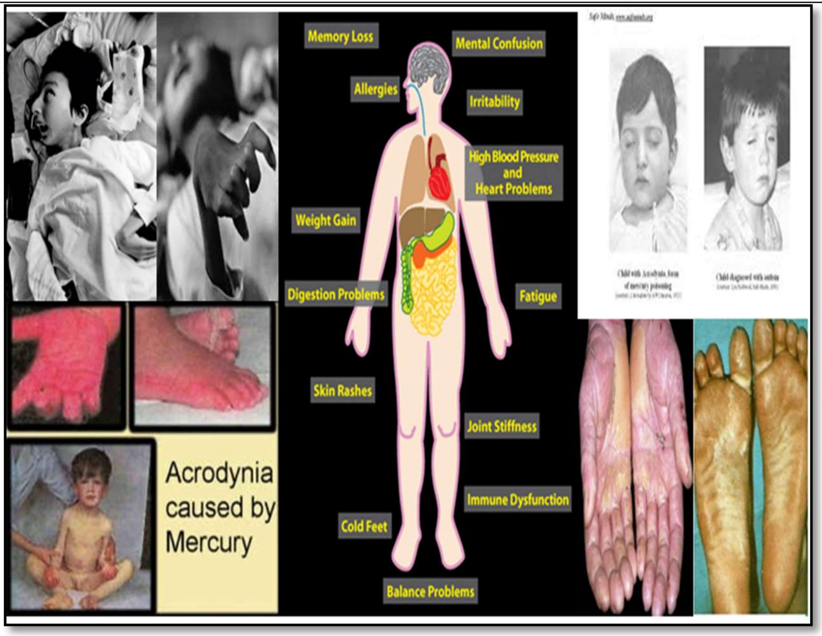


Figure 5.11 : Adverse effects of Hg2+ ions

All the perils by Hg^{2+} ions strongly demand the development of a proper sensing device, which enables efficient monitoring of even trace amounts of the said ion. According to U.S Environment Protection Agency rules and W.H.O (World Health Organization), the permissible concentration of Hg^{2+} ions in drinking water is restricted to 2 ppb. The closed d-shell configuration of Hg^{2+} ion restricts the metal from significant optical interactions, which in-turn strictly limits the direct detection of the said ions with the aid of spectroscopic tools. Literature witnessed a series of research efforts for designing a potent sensing tool that offering admirable sensing efficiency towards Hg^{2+} ions [7-22]. Among the established sensing tools, majority of them were based on colorimetric, electrochemical and fluorescent techniques [7-10, 14-16, 19-21]. Scientists mainly used several organic molecules [11,12], polymeric substances [17] and noble metal nanostructures including gold and silver for tracing Hg^{2+} [13,18,22]. Detailed survey on previous efforts suggests that utilization of organic molecules as well as polymeric materials suffers from problems including poor aqueous dispersibility and tedious fabrication and purification procedures. Though, the efficiency offered by noble metal based Hg sensors is note-worthy, comparatively high cost associated with the fabrication of sensing device restricts the wide range applicability of those sensing gadgets. For the past few years, graphene is widely used as the promising platform for fabricating efficient Hg^{2+}sensors [23-25]. Rather than modified graphene structures, graphene-organic dye hybrids has carved a novel formula for monitoring the presence of Hg^{2+} ions [14]. Among such graphene- dye hybrids, an interesting report was made by Zhang and colleagues discussing the non-covalent interactions between graphene surface and xanthene structured dye molecules [26].According to them, these xanthene dyes and graphene structure will result considerable FRET interactions between themselves. These selective non-covalent interaction between the two will leads to the formation of supramolecular assemblies that offer efficient tracing of various relevant analytes. Apart from these, recently, scientists extensively exploit fluorescent carbon nano dots for designing promising Hg^{2+} sensors [20,27-31]. Majority of such sensors function based on the noticeable variation in the optical response of fluorescent carbon dot systems upon the addition of target ion .

As mentioned previously, the presence of highly exfoliated graphitic core structure may impart more graphene like features to the present system. Hence, motivated from the aforesaid report of Zhang's group, TS-CD is utilized as a donor fluorophore moiety for forming a FRET pair, which could definitely effect efficient FRET interaction with a xanthene dye, Rhodamine 6G. Herein, FRET from TS-CD to xanthene dye is utilized to design an efficient Hg^{2+} sensor. It was noted that the FRET process increases the luminescence intensity of the dye. This TS-CD-dye couple act as a selective optical sensor for Hg^{2+}, which is manifested as fluorescence turn off in presence of the analyte ions.

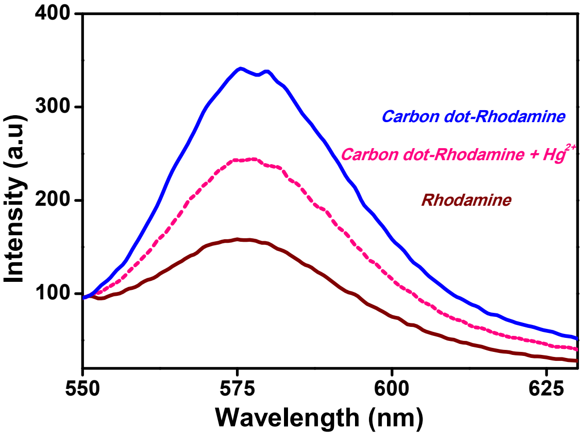


Figure 5.12 : Fluorescence spectra demonstrating Hg2+ sensing action of carbon dot-Rhodamine FRET unit

5.6.1 Results and discussion

(a) Carbon dot-Rhodamine dye combination as FRET pair

Non-radiative quantum mechanical energy transfer, fluorescence resonance energy transfer (FRET) may occur between the carbon dots and the dye molecule when they are placed in close proximity, less than 10 nm. Generally, in FRET process, one entity acts as donor and the other one assumes the role of acceptor molecule via dipole-dipole interaction. This leads to excitation energy transfer from donor to acceptor entity resulting in the enhanced emission intensity of the latter. As discussed earlier, such FRET pairs pave way for quantitative detection of various analytes with commendable selectivity [14,32-34].

sp^2 domains in the core structure of carbon dots exert strong π-π interaction with the xanthene units of Rhodamine molecule. In addition, the π-π interaction, presence of considerable amount of dispersive forces between the donor-acceptor pair also bring them within a desirable FRET distance [14,26]. The probable interactive modes between the FRET pair are displayed below in Figure 5.13.

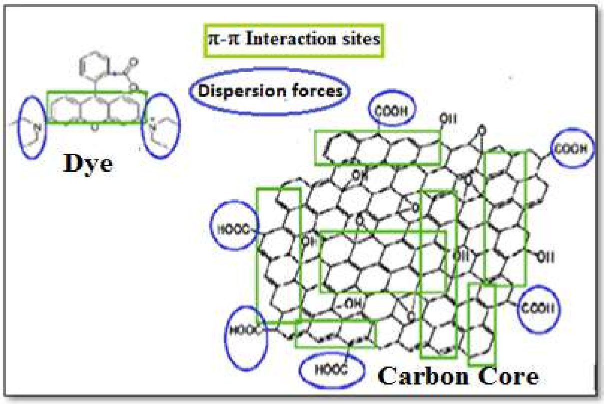


Figure 5.13 : Schematic representation of possible interactive modes of carbon dot-Dye unit.

Fluorescence emission curve of carbon dot solution exhibits commendable overlap with the absorbance curve of Rhodamine dye molecule (Figure 5.14), confirming the increased probability of FRET interaction within this pair. Similar to the observation made by Chao and colleagues, here also the prepared carbon dots act as a donor molecule, thereby resulting excitation energy transfer to the Rhodamine dye. The aforesaid paper reports the case of the acceptor molecule fluorescein [35]. Corresponding mechanistic route describing the excitation energy transfer from donor carbon dot system to the acceptor xanthene dye (Rhodamine) is displayed in Figure 5.14(b).

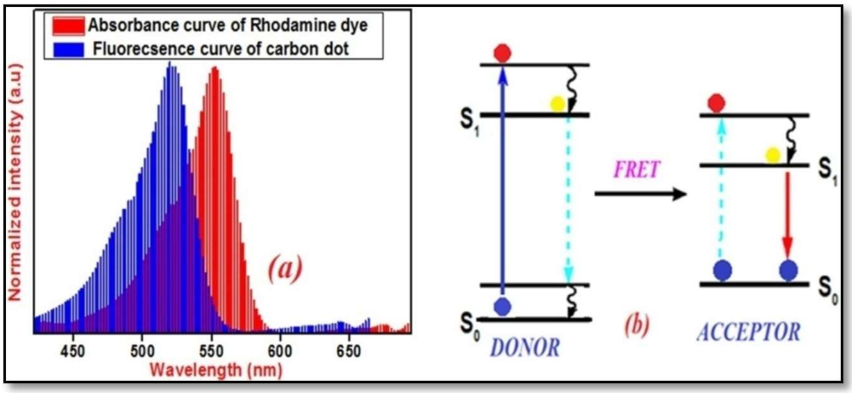


Figure 5.14: Overlapping between fluorescence emission curve of donor and absorbance curve of acceptor (a), Jablonski diagram displaying FRET mechanism between donor-acceptor molecules (b).

The donor role of TS-CDs is clearly evident from the enhanced fluorescence emission of the dye molecule upon the addition of carbon dot solution into it. Corresponding optical behaviour of Rhodamine dye molecule is displayed in Figure 5.15 (a). Digital image confirming the enhanced fluorescence intensity is incorporated in the inset. Thus, Figure 5.15 (b) is sufficient enough to illustrate the acceptor role of Rhodamine dye. Concomitant reduction of carbon dot emission intensity with the introduction of dye molecule further substantiates the proposed donor action of TS-CDs. This observation is further substantiated by the lifetime measurement of pure carbon dot solution and carbon dot-dye unit. As Figure 5.15 (c) indicate, fluorescence lifeime of pure carbon dot solution was observed to be 3.49 ns, which gradually decreases to 2.43 ns upon the addition of Rhodamine dye solution into it, illustrating the excitation energy transfer from carbon dot solution to the dye molecule.

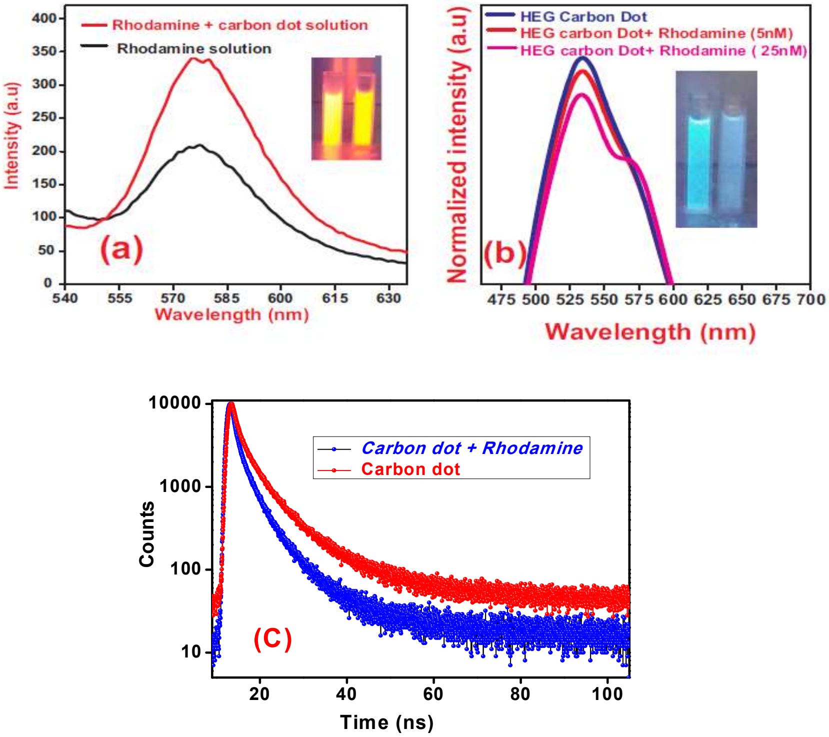


Figure 5.15 : Confirmation of acceptor role of Rhodamine dye and donor role of carbon dot solution in the FRET pair; FRET induced increment of luminescence emission intensity of dye (a), reduction of fluorescence emission intensity of carbon dot (b) and Fluorescence lifeime curve of carbon dot solution before and after the addition of dye solution.

The role of FRET in the process was further validated by duplicating the entire experimental procedure using another fluorescent carbon dots of different origin, instead of sugar derived carbon dot solution. For this, cyan emitting carbon dots from commercially available honey was prepared via hydrothermal treatment. About 2 gm honey was mixed with 8 mL of distilled water and stirred for few minutes for obtaining a homogenous solution. To this, 3 mL of hydrogen peroxide solution was added, transferred to teflon sealed autoclave, and placed in a muffle furnace for 2 hrs at 200 °C. Purification steps were also conducted. The obtained powder was subjected to XRD analysis. Figure 5.16 (a) displays the XRD pattern of the sample. It can be seen that there is no overlap between the emission spectral profile of honey derived carbon dots and Rhodamine dye absorbance spectral curve (Figure 5.16 b). Because of the absence of FRET interaction, the addition of honey derived carbon dot does not brings any noticeable change to the fluorescence emission intensity of Rhodamine solution (Figure 5.16 c), as expected.

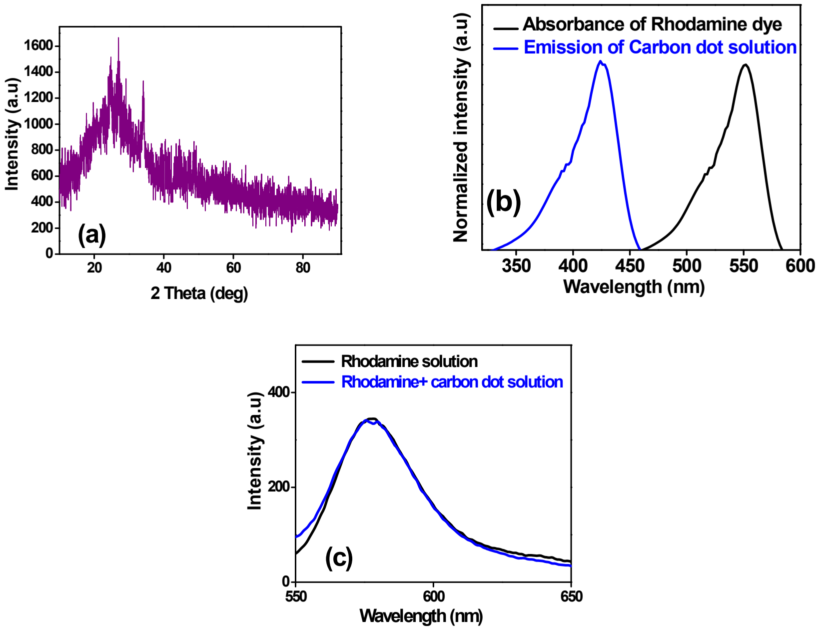


Figure 5.16: XRD pattern of honey derived carbon dot (a); illustration of the absence of FRET interaction from honey derived Carbon dot to Rhodamine dye (b) and Fluorescence emission profile of Rhodamine dye solution with the addition of honey derived carbon dot solution (c).

(b) FRET Assisted Monitoring of Toxic Hg^{2+} ions

As mentioned earlier, FRET units enable efficient monitoring of external analytes, which is capable of perturbing/disturbing their orientation. Herein, stock solutions of different metal ions were prepared at fixed concentrations for examining whether any of them evoke such noticeable variation in the fluorescence intensity of carbon dot-Rhodamine FRET pair. As can be noted (Figure 5.17 a), introduction of picomolar concentration of toxic Hg^{2+} ion solution produces considerable reduction in the fluorescence response of carbon dot- dye FRET pair. The enhanced fluorescence of carbon dot-dye solution unit is gradually decreased with the addition of increased amount of Hg^{2+} ions into it. Digital image showing the aforesaid observation is also incorporated inset. The fluorescence intensity variation was found to be linear with the concentration of analyte ion, with regression coefficient 0.985 (Figure 5.17 b). This assures quantification of the analyte using the FRET pair.

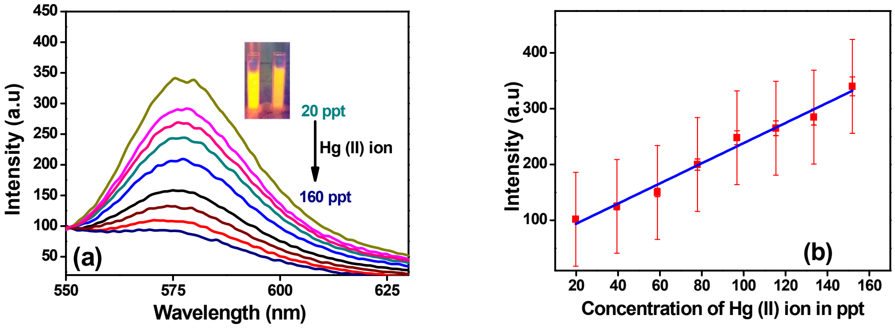


Figure.5.17 : Fluorescence emission profile of FRET pair upon the addition of increased amounts of Hg2+ ion (digital image showing the decreased intensity of FRET pair with the addition of Hg2+ ion solution in the inset) (a) Quantitative variation of fluorescence response with the concentration of Hg (II) ions (b).

Standard deviation method, (3σ/m method) was employed for determining the lower limit of detection of Hg^{2+} ions and was found to be 98 picomolar, equivalent to 20 parts per trillion levels of analyte ion. This detection limit is note-worthy to mention, as most of the reported Hg^{2+} sensors possess LOD values which are higher. Among the reported Hg^{2+} sensors, inherent surface Plasmon resonance tunes gold nanoparticle based systems efficient in detecting toxic Hg^{2+} ions. Supremacy of the resent sensing device over other established Hg^{2+} sensors is displayed in table 5.3. It is noted that the LOD achieved herein is significant while comparing the performance of these systems.

System	*LOD*	*Reference*
Quaternary Ammonium Group-Capped Gold Nanoparticles	*30 nM*	*[11]*
Gold nanoparticles functionalized by dithio carbamate derivative of calixarene	*40 ppb*	*[12]*
Au-Ag core-shell nano particles	*5 nM*	*[13]*
Gold nanoparticles (AuNPs) and a paper-based analytical platform	*50 nM*	*[16]*
Gold nanoparticles stabilized with a dithia-diaza ligand	*35 nM*	*[18]*
Pomelo peel derived carbon nano particles	*0.23 nM*	*[20]*
Carbon dot- Rhodamine dye unit	*0.098 nM*	*Present study*

Table 5.3 : Comparison of carbon dot- dye unit over few established Hg2+ sensing tools.

Sensitivity as well as selectivity of this FRET pair towards the analyte ion (Hg^{2+}) is investigated using a set of metal ion solutions of fixed concentration. As Figure 5.18 displays, the present system offers extreme selectivity as well as sensitivity towards Hg^{2+} ion. No other metal ion except Hg^{2+} produces any noticeable change in the optical response of FRET pair. The optical response of FRET pair towards two set of test solutions (one set having mixture of all test ions except Hg^{2+} ions and the other set containing all test ions including Hg^{2+} ions) were screened for analysing its selectivity. As evident from Figure 5.18, test solution containing all metal ions including Hg^{2+} ion was able to reduce the fluorescence intensity of FRET pair, whereas the other mixture of test ions excluding Hg^{2+} ions fails to bring any noticeable change to the optical response. The reliability of the present system as an interference free, selective and sensitive Hg^{2+} sensor is thus assured.

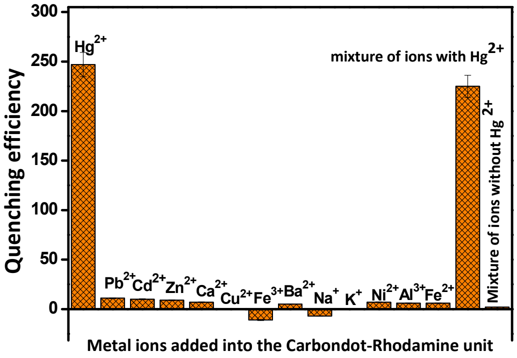


Figure 5.18: Selective and sensitive monitoring of Hg2+ ion; quenching profile of carbon dot-Dye combination luminescence in presence of various metal ions

(c) Metal ion assisted hindrance in FRET- Principle behind the sensing strategy

As mentioned earlier, Hg^{2+} ions are capable of disturbing the orientation of FRET, paving way for selective monitoring of said ion even under trace quantities of addition. This selective action of Hg^{2+} ions towards the carbon dot- Rodamine unit can be ascribed to their extraordinary affinity towards the core structure of carbon dot system. Owing to this commendable affinity of Hg^{2+} ions to the carbon core structure of donor moiety, introduction of even small amounts of said ion will disturb the well overlapped curves of dye (acceptor) and TS-CD (donor). Hence, it is suggested that the Hg^{2+} addition results displacement of FRET pair, thereby causing considerable decrease in the FRET interaction. This will definitely leads to significant reduction in the fluorescence intensity of TS-CD-Dye unit.

Figure 5.19 substantiates this concept of Hg^{2+} induced hindrance in FRET between carbon dot-dye unit, as it manifests the considerable reduction in the overlapping area of FRET pair with the addition of sufficient amount of metal ion. The decreased efficiency of excitation energy transfer between donor-acceptor molecules is clearly evident, which confirms the metal ion induced quenched emission intensity of the FRET pair.

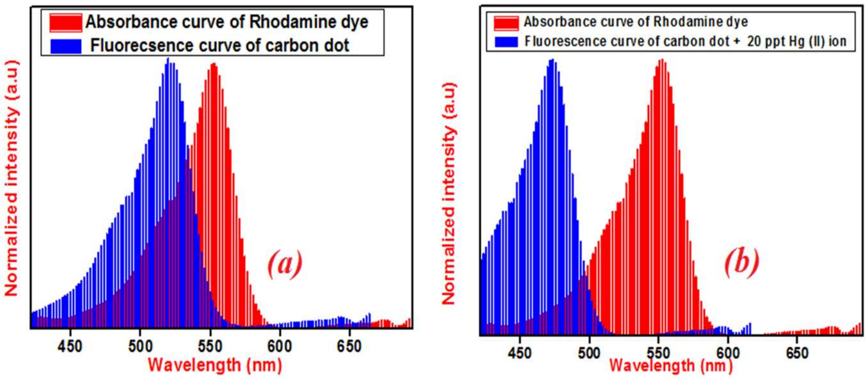


Figure 5.19: Illustration of metal ion induced hindrance in FRET between carbon dot and Rhodamine dye; Excellent overlap between absorbance curve of Rhodamine dye and fluorescence emission curve of carbon dot solution before Hg^{2+} addition (a), Decreased FRET efficiency with the addition of sufficient amount of Hg^{2+} ions (20 ppt)

This observed Hg^{2+} induced selective displacement of FRET pair can be scientifically connected with the commendable affinity of mercury ions towards the various surface oxygen functionalities as well as the π cloud of the carbon core (donor) [14]. Plausible selective interaction between the metal ion and graphenic units of carbon dot core is represented in Figure 5.20 (a). Hence, addition of Hg^{2+} ions results in interaction with the carboxyl (-COOH), carbonyl (C=O) and hydroxyl (-OH) functionalities on the graphenic layers, which successfully displaces the donor carbon dot moiety form the acceptor dye molecule , thereby reducing the excitation energy transfer efficiency, as obvious from the quenched emission of FRET unit. The concept of Hg^{2+} induced selective displacement of carbon dot molecule is further confirmed by recording the corresponding UV-Visible absorbance curve of carbon dot solution upon the addition of mercury ions. As evident from Figure 5.20 (b), absorbance indicating n-π* electronic transition corresponding to oxygenous surface groups undergo sequential decrease with increase in the amount of added metal ion (Hg^{2+}) solution, corroborating well with the proposed chemical interaction between Hg^{2+} ions and oxygen bearing surface groups of carbon dots.

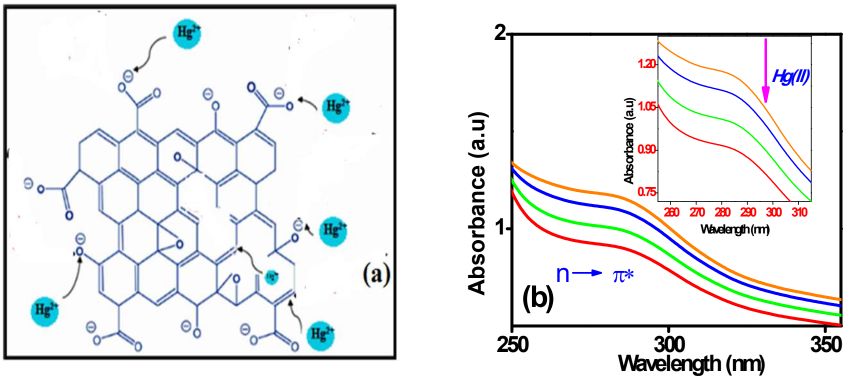


Figure 5.20 : Illustration of specific interaction of Hg2+ ions with the surface oxygen functionalities; (a), UV-Visible absorbance characteristics of carbon dot solution with the addition of increased amount of mercury ion solution (decreased n-π* absorbance with increased concentration of ions) (b)

(d). Practical applicability of TS-CD based Hg^{2+} sensor

Reliability of the present sensing unit (carbon dot-Rhodamine pair) for monitoring Hg^{2+} ions is investigated by analyzing real water samples collected from local premises. For this, two sets of real samples were used and the corresponding optical response of FRET unit was monitored with the aid of fluorescence spectroscopy. Contaminated water sample was prepared by spiking known concentration of Hg^{2+}

ions (50 ppt) to one of the water sample. As evident from Figure 5.21(a), fluorescence emission intensity of FRET unit was found to be quenched upon the addition of contaminated water. The undisturbed fluorescence emission curve in Figure 5.21 (b) confirms that the sample collected from nearby well is completely free from Hg^{2+} ions, showing the reliability of the present sensor.

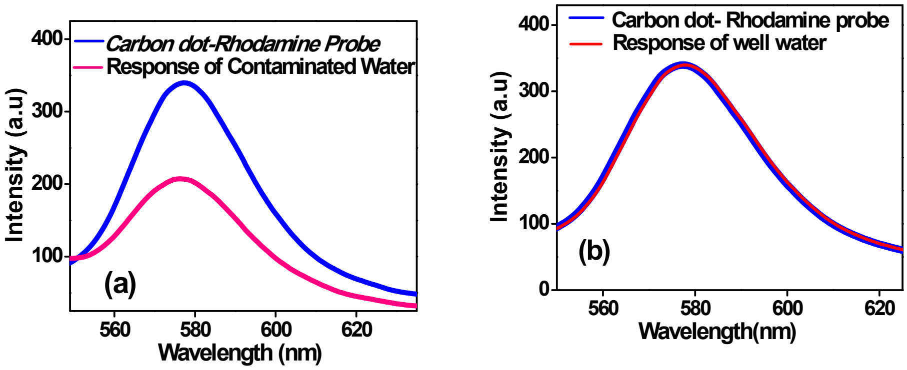


Figure 5.21: Fluorescence emission profile of FRET unit towards (a) real water sample contaminated with Hg^{2+} ions and (b) well water free from Hg^{2+} ions

5.6.2Summary and Highlights

- Fluorescence emission curve of TS-CDs exhibits commendable overlapping with the absorbance curve of Rhodamine-6G dye molecule. Owing to the existence of significant amount of π-π interactions and dispersive forces between xanthene dye structure of Rhodamine and aromatic cloud of carbon dot core structure, this combination could form an excellent FRET pair. Carbon dots serve as donor and the dye, as acceptor. As a consequence of excitation energy transfer from carbon dot solution (donor) to the Rhodamine dye (acceptor), fluorescence intensity of carbon dot was found to be quenched, whereas that of dye molecule was enhanced. Introduction of trace amount of Hg^{2+} ion into the FRET unit selectively displaces the donor carbon dot molecule, thereby reducing the extent of FRET overlapping with the acceptor dye molecule. This Hg^{2+} assisted disturbance in the FRET process results quenched emission intensity of TS-CD-dye pair. Optical response, quenching of emission intensity of FRET unit was found to vary in a linear fashion with increasing concentration of Hg^{2+} ions. This observation was found to extremely selective and sensitive towards the Hg^{2+} ions. Limit of detection offered by the present sensing tool was found to be 98 picomolar, highly commendable, while considering the permitted concentration of Hg^{2+} ions in water bodies, by various organizations. Extra-ordinary affinity of Hg^{2+} ions towards carbon dot was illustrated using various characterization techniques.

✓ Excellent sensitivity of the FRET pair in detecting Hg^{2+} ions, which is superior to gold based sensors.

✓ Commendable practical applicability (reliability) of the present sensing unit, as evident from the negligible error percentage associated with real sample analysis.

(II) Table Sugar Derived Carbon Dot- Sensor for Nitro Aromatic Compounds

5.7 Introduction and Relevance of Nitro Aromatic Compound Sensing

Nitro Aromatic Compounds were easily absorbed by human body upon oral/dermal exposure as well as by inhalation of vapours [36]. It was observed that administration of NACs, especially nitro benzene and its corresponding derivatives, in to the blood stream induces metabolism of injected NACs, which converts them into aniline, leading to methemoglobinemia (MetHb).This health condition reduces oxygen transport capacity of blood leading to blue colour in various body parts. Several health issues including headache, sleepiness, dizziness, vomiting, weakness and grouchiness are also observed [36-40]. This impaired oxygen delivery may result in acute cyanosis along with neural illnesses such as headache, confusion, nausea, dizziness, unconsciousness, coma and may even lead to death. The methemoglobin produced in the body will impart chocolate colour to human blood, which will never regain redness even on air exposure. The formation of 20 % of methemoglobin was reported to be well tolerable by human body, whereas the increase of methemoglobin above 40 % will result several cardio-respiratory weaknesses. When the amount of methemoglobin produced exceeds 70%, the condition will be fatal. Hence, accurate monitoring/ analytical quantification of toxic NACs is of increased demand.

Damage to male reproductive system is also reported due to exposure to NACs. According to International Agency for Research on Cancer, Nitrobenzene derivatives are classified as most dangerous human carcinogen. Through this report authors proved the adverse effect of NACs on various body cells, illustrating higher probability for various tissue specific tumors (benign and malignant). As per this study, administration of excess amount of NACs will cause lung tumors, Kidney tumour, liver tumour and benign mammary-gland tumors in different experimental animals such as mice and rats [41]. It was also suggested that oral administartion/ inhalation of chloro-nitro benzene will result abdominal, vascular and splenic tumors in animals . Moreover, these nitrobenzene derivatives have been listed as a strong genotoxic substance, as they cause considerable mutation in various bacteria.

Generally, excess amount of NACs are involved in the manufacture of various products including plastics, textiles, insecticides, elastomers, dyes, fuel additives, plant growth regulators and various pharmaceuticals. Wide usage of some nitrobenzene derivatives is banned by various recognized authorities such as Food Protection Quality Act (1996), Australian Pesticides and Veterinary Medicines Authority (2010) and United State Environmental Protection Agency (2006), by considering high risk associated with their bio-accumulative nature and toxic effects in living organisms. Owing to the afore said health issues caused by inhalation/ administration of NACs, environmental pollution prevention/control concepts strongly urges the detection of even trace quantities of NACs in aqueous bodies as they dose threat to the well-being of flora and fauna of our eco system. Various analytical screening techniques such as combined utilization of gas chromatography- mass spectrometry (GC-MS) and gas chromatography with flame ionization detector (GC-FID) were practiced for the tracing of these toxic NACs.

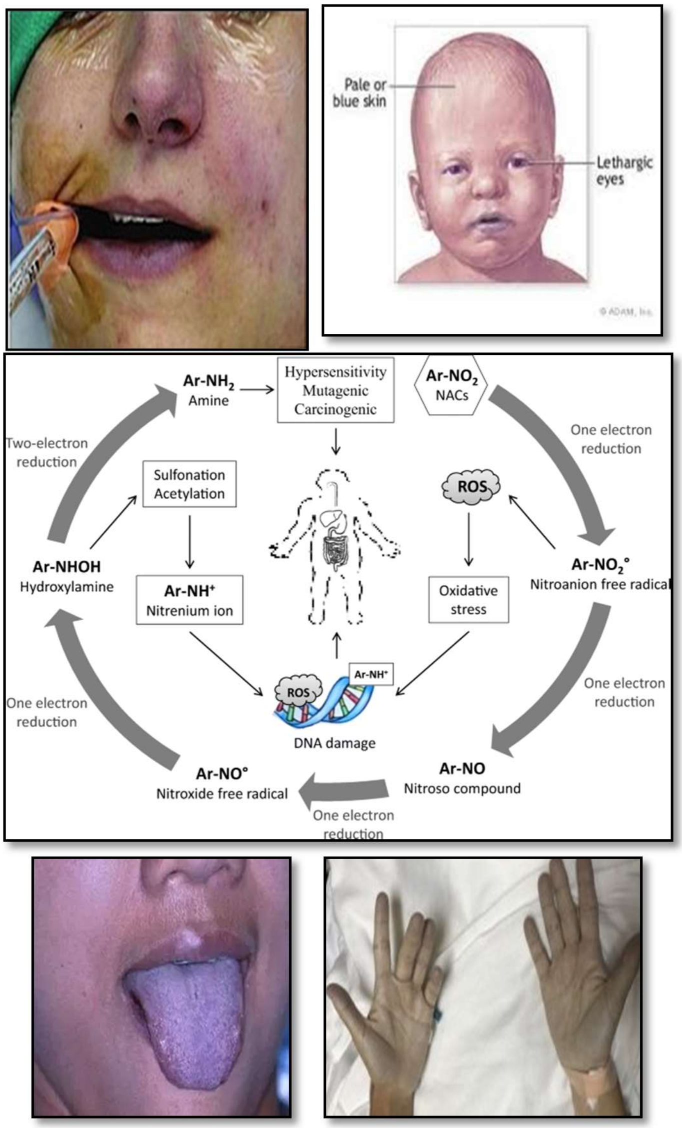
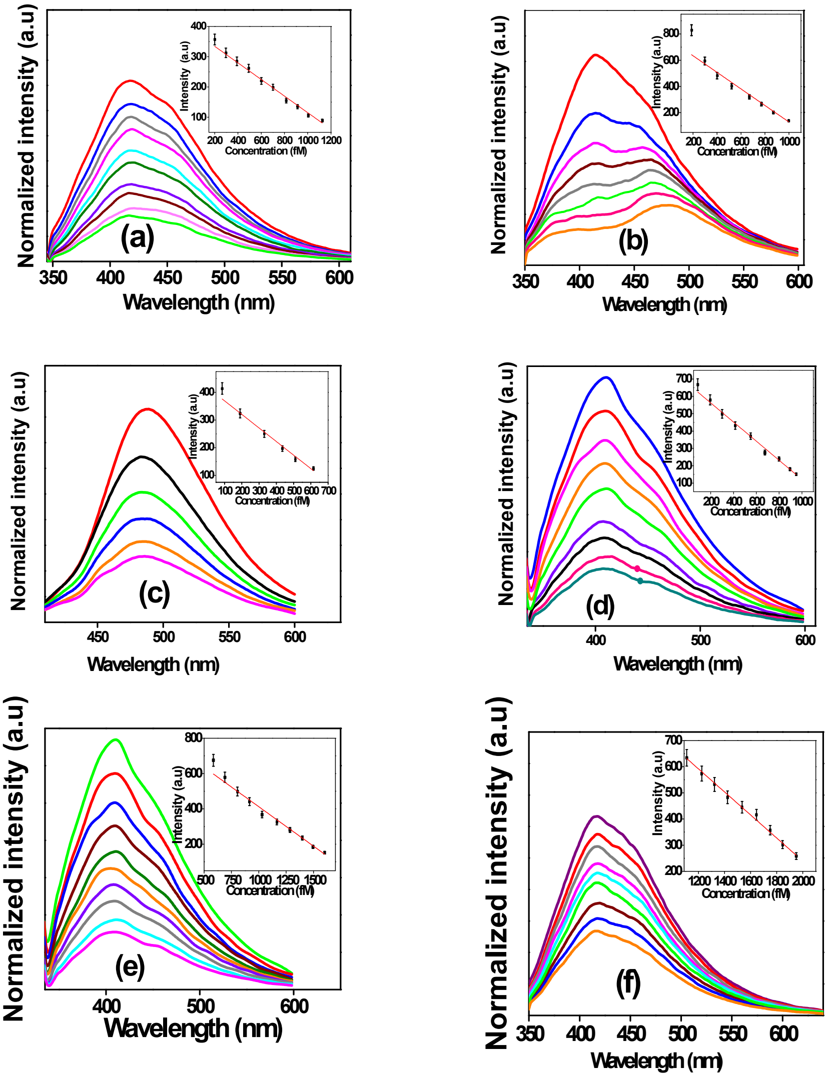


Figure 5.22 : Toxic effects of various Nitro Aromatic Compounds in human body (images collected from various internet sources)

5.7.1 Results and Discussion

(a) Sugar Derived Carbon Dot as a Efficient Nitro Aromatic Sensor

Bright fluorescence of carbon dots is utilized for investigating its efficiency for monitoring various nitrogen containing molecules, including both aromatic and aliphatic nitro-compounds of micro molar level. Among the tested analytes, a set of nitro aromatic compounds sharing similar parental aromatic frame work (nitrobenzene) could effectively quench the fluorescence intensity of carbon dot solution with commendable selectivity and sensitivity. Aromatic Nitro compounds exhibiting this aforesaid response includes Nitro Benzene (NB), 4- Nitro Phenol (4-NP), 4-Nitro Aniline (4-NA), 4- Nitro Toluene (4- NT) ,4- Nitro Benzoic acid (4- NBA), 4- Chloro Nitro Benzene (4-CNB) and 4- Nitro Benzaldehyde (4-NBAl). It is very interesting to note that, no other tested analytes except these nitro benzene structures, impart such a noticeable change in the fluorescence intensity of carbon dots. The fluorescence response of present carbon dot solution towards different NACs is provided in Figure 5.23. Inset of each spectral profile bears the linear plot portraying the relationship between luminescence intensity and the added concentration of NACs. As evident from figure, the luminescence intensity of carbon dot solution varies linearly with the addition of analyte NACs. 3σ/slope method was employed for calculating the detection limit for each NACs solution. Surprisingly, carbon dot solution enables femtomolar molar (fM) level tracing of all the tested analytes, which is appreciable while considering their threshold levels prescribed by various environmental agencies.


(a)
Normalized intensity (a.u)
Wavelength (nm)
350 400 450 500 550 600
Intensity (a.u)
100 200 300 400
Concentration (fM)
200 400 600 800 1000 1200
(b)
Normalized intensity (a.u)
Wavelength (nm)
350 400 450 500 550 600
Intensity (a.u)
200 400 600 800
Concentration (fM)
200 400 600 800 1000
(c)
Normalized intensity (a.u)
Wavelength (nm)
450 500 550 600
Intensity (a.u)
100 200 300 400
Concentration (fM)
100 200 300 400 500 600 700
(d)
Normalized intensity (a.u)
Wavelength (nm)
400 500 600
Intensity (a.u)
100 200 300 400 500 600 700
Concentration (fM)
200 400 600 800 1000
(e)
Normalized intensity (a.u)
Wavelength (nm)
400 500 600
Intensity (a.u)
200 400 600 800
Concentration (fM)
500 750 1000 1250 1500
(f)
Normalized intensity (a.u)
Wavelength (nm)
350 400 450 500 550 600
Intensity (a.u)
200 300 400 500 600 700
Concentration (fM)
1200 1400 1600 1800 2000

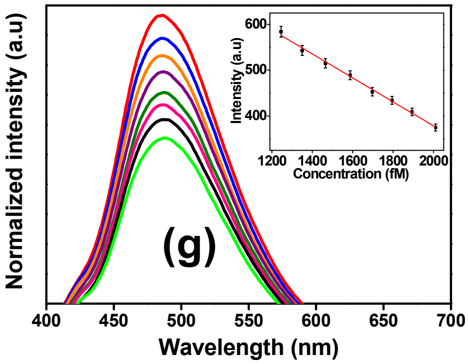


Figure 5.23 : Fluorescence quenching of carbon dot upon the addition of different Nitro Aromatic compounds (NACs) : variation of luminescence intensity with added concentration of concerned NACs is provided in the insets. (a) Nitro Benzene (NB); (b) 4- Nitro aniline (4-NA); (c) 4-Nitro Toluene (4-NT); (d) 4-Nitro Phenol (4-NP); (e) 4- Nitro Benzoic acid (4-NBA); (f) 4-Nitro Benzaldehyde (4-NBAl) and (g) 4-Chloro Nitrobenzene(4-CNB).;

Among the analytes, TS-CDs show extreme sensitivity towards 4-Nitroaniline solution with highly commendable detection range, 27 femtomolar molar (fM). Present carbon dot solution exhibits sensitivity towards the remaining tested analytes in the order, 4-Nitro Toluene ≈ 4-Nitro Phenol > Nitro Benzene> 4-Nitro Benzoic acid> 4-Nitro Benzaldehyde ≈ 4- Chloro Nitrobenzene. Detection limits correspond to 4-Nitro Toluene, 4-Nitro Phenol, Nitro Benzene, 4-Nitro Benzoic acid, 4-Nitro Benzaldehyde, 4- Chloro Nitrobenzene were found to be 370, 390, 700, 1200, 1600 and 1720 * 10^{-15} molar (femtomolar molar-fM) respectively. Figure 5.24 displays the corresponding data.

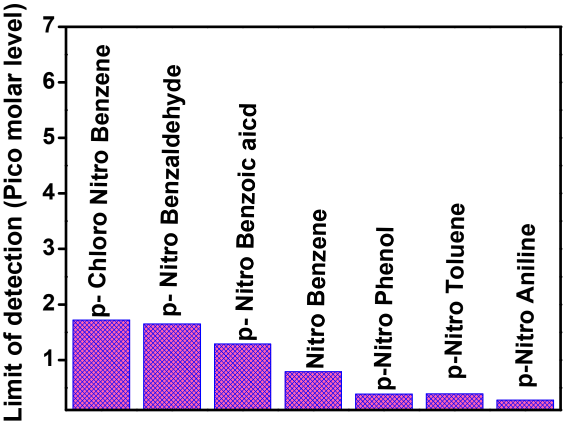


Figure 5.24 : Detection limit corresponding to various analytes

(b) Plausible Mechanism of NACs sensing

This observed quenching of carbon dot fluorescence intensity is suggested to be due to the transport of electrons from the ground/ excited state of CDs in to the ground electronic level of NACs (analyte). Electron deficient nature associated with each NACs will monopolize the probability of strong binding interaction with electron rich surface of sensor entity (oxygen rich CDs). Schematic representation showing possible interaction between the entities is provided in Figure 5.25.

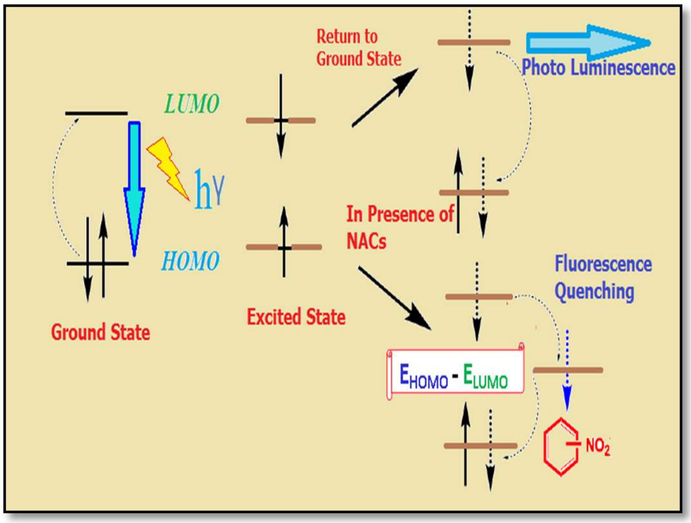


Figure 5.25: Schematic representation showing plausible mechanism involved in NACs sensing using carbon dot.

Owing to the uncertainty regarding the exact chemical structure of CDs, suggestion of a unified mechanistic route for explaining all probable chemical interactions between carbon dot solution and the added analytes (NACs) is relatively difficult. As illustrated in the previous section, presence of myriad graphenic like structures provides excellent π cloud orientation within the core structure. Hence, addition of increased amount of NACs causes strong π-π bonding with the aromatic cloud of the analyte. In addition, existence of profound dispersion forces between the surface functional group of carbon dot and NACs also facilitates the interaction between the sensor and analyte molecule. These two interactive forces bring the sensing unit (carbon dot) and analyte molecule (NACs) in close proximity, which in turn facilitates easy transport of electrons between them. It was observed that, stronger the π cloud interaction, more intense will be the extent of photo-induced electron transfer (PET) between the carbon dot and NACs.

Extent of π electron cloud availability is solely determined by inductive effect of para substituent of parent nitro aromatic frame work of analyte. Presence of electron releasing substituent at the para position will increase the availability of π electron cloud for NACs, compared to the same with electron withdrawing para substituent. Availability of increased π electron cloud at the analyte structure results in enhanced rate of non-covalent interaction with π frame work of carbon dot core structure. This increased rate of non-covalent interactions, in turn results in quenching of the fluorescence of carbon dots upon the introduction of adequate amount of NACs. Hence, extreme selectivity of 4-Nitro Aniline (4-NA) towards the optical response of carbon dot solution is well corroborated with the presence of most electron releasing $-NH_2$ groups at the para position of analyte. This finding is further substantiated on examining the reactivity order of each analyte molecule. There occurs a particular relationship between the order of values of detection limit corresponding to each NACs molecule with the electron releasing/withdrawing efficiency of its para substituent.

It was observed that, analyte molecule with higher electron withdrawing para substituent, herein 4-chloro Nitro Benzene (4-CNB), is having the least sensitivity to the fluorescence response of carbon dot solution, as indicated by the higher detection limit. This observation can be correlated with the least availability of π electron cloud of the analyte compared to the remaining tested NACs. Least availability of electron cloud may lead to lesser interaction with TS-CDs and thereby the higher value of detection limit. Thus, in brief, the possible interaction between the fluorophore molecule and analyte NACs can be explained in a superficial way, as mentioned above. But the uncertainty related to the origin of fluorescence of CDs limits further authentic justification on the observations made herein.

(c) Selective and Sensitive monitoring of NACs

Practical applicability of the present sensing unit under complicated chemical environment is investigated by examining the influence of interference from external analytes including various metal ions and significant molecules in presence of NACs. For this, the entire experimental procedure is carried out in presence of various possible interfering analytes such as metal ions (Fe^{2+},Fe^{3+},Cd^{2+},Ca^{2+},Cu^{2+},Ni^{2+},Al^{3+},Zn^{2+},Hg^{2+},K^{+}, Na^{+}, Mg^{2+}, Mn^{2+}, $Co^{2+}$and Ba^{2+}) as well as bio-molecules including glucose, folic acid, urea, ascorbic acid and methionine. Results in this regard are provided in Figure 5.26, which is expressed in terms of relative reduction in fluorescence intensity I_0-I/I_0 (I_o and I are the luminescence intensity of carbon dots before and after the introduction of analytes, respectively).

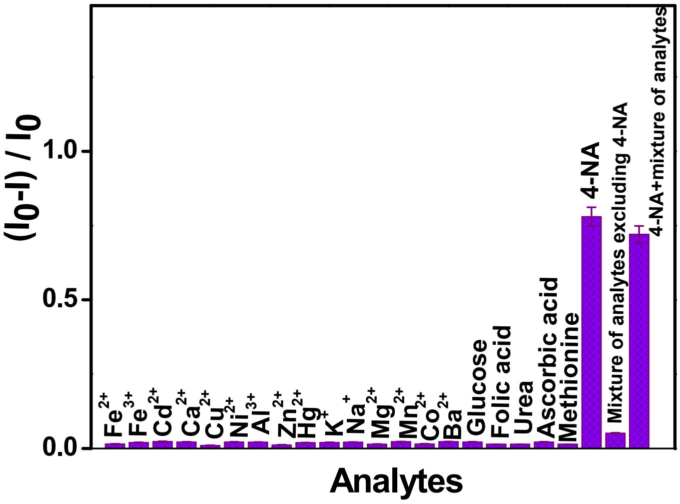


Figure 5.26: Illustration of selective and interference free monitoring of NACs by TS-CDs.

As evident from the plot, present carbon dot solution offers excellent selectivity and sensitivity towards the NACs. All the other foreign analytes including metal ions and bio-molecules exhibit negligible interference with NACs detection, as illustrated by the nil influence of theses moieties on luminescence of carbon dots.

5.7.2 Computational Investigation on Structure – Activity Relationship in NACs sensing

Comprehensive information on electronic properties as well as structural reactivities of all the analyte molecules (NACs) is gained by conducting various computational calculations/operations. Most widely used computational tool (mainly for organic molecules), density functional theory (DFT) has been utilized for this purpose [42]. All the computational calculations have been carried out using DFT-

B3LYP/6-31+ G (d,p) [43,44], through Gaussian 09 software package [45]. For having systematic comparison of structural aspects as well as extent of reactivity of all the analytes (NACs), Global reactive descriptors have been computed and corresponding donor-acceptor map (DAM) was also plotted [46-48].

(a) Global reactive Parameters

Global descriptive parameters of a particular molecule give information on the relation between its chemical reactivity and sensitiveness to structural perturbations and corresponding responses to the alterations in external conditions. Chemical potential, electro negativity, hardness, softness, electrophilicity index, etc together constitute the aforesaid global descriptive parameters of a molecule. These parameters can be related to the linear responses of the electron density with respect to the variations in external potential as well as the number of electrons [49].

Accordingly, global descriptive parameters are of significant relevance in comparing various features of different molecules. Among these parameters, overall stability of a molecule is determined by the global hardness value [50].Chemical hardness value of a particular molecule fundamentally indicates its reluctance towards the deformation/ polarization of electron cloud of the atoms (ions/ molecules) under small perturbation encountered during chemical processes. Capacity of a molecule to receive electrons is measured by chemical Softness, which is precisely related to the groups / atoms present in that molecule. This softness value is found to be inversely related to chemical hardness [51]. Escaping tendency of an electron from equilibrium is indicated by chemical potential, which is represented by the first derivative of energy with respect to the number of electrons [42]. This is found to be the negative of the parameter, electronegativity, indicator of tendency to attract electrons in a chemical bond [51]. Electrophilicity strength of the species is generally indicated by the electrophilicity index value [49]. Two different calculation strategies were generally adopted for measuring the global reactive descriptors of a molecule. One method involves the calculation based on total electronic energy difference of neutral molecule and its corresponding anion and cation analogue. This calculation mode is generally termed as 'energy vertical' method, which is based on the geometry of the neutral molecule, so as to keep the external potential constant [52]. The second calculation method is by Koopmans's theorem. As the former computation strategy, energy vertical method, offers a more accurate computing of global reactive parameters, we employed the same method for having comprehensive idea on structure-activity relation of all the NACs. These global descriptors are computed based on the equations 1-7 [53].

Ionisation Potential (IP) = $E_{\text{Cation}} - E_{\text{neutral}}$ (1)

Electron Affinity (EA) = $E_{\text{neutral}} - E_{\text{anion}}$ (2)

Hardness (η) $\approx \frac{IP-EA}{2}$ (3)

Electro negativity (χ) $\approx \frac{IP+EA}{2}$ (4)

Softness(S) $\approx \frac{1}{2\eta}$ (5)

Chemical potential (μ) $\approx -\chi$ (6)

Electrophilicity index (ω) $\approx \frac{\mu^2}{2\eta}$ (7)

(b) Donor-Acceptor Map

Electron acceptor/donor character of a molecule can be understood by constructing donor-acceptor map based on various parameters. The parameters ω+ and ω- were used to describe the tendency of a particular compound to accept/donate charge/electrons. Decrease in ω- denotes strong tendency of a compound to donate charge/electrons. A compound with excellent electron accepting nature is always marked with higher ω+ value. Generally used parameters for describing electron donor / acceptor character of a compound including ionization potential and electron affinity were significant only when related with complete transfer of electrons/charge between two systems. But this may not be the case always. Sometimes, the interaction between two systems may involve only fractional transfer of electrons/charges. Hence, it was found out that, more accurate explanation about the charge transfer interactions/mechanism between two entities can be provided by means of ω+ and ω- values, other than the conventionally used parameters such as ionization potential and electron affinity [54]. ω- and ω+ can be calculated using following equations (8) and (9).

$$\omega^{-} = (3IE + EA)^{2} / 16(IE - EA) \qquad (8)$$

$$\omega^{+} = (IE + 3EA)^{2} / 16(IE - EA) \qquad (9)$$

Simultaneous representation of electron acceptance and electron donation ability of compounds of interest can be provided by means of Donor Acceptor Map (DAM plot. [24-26]. For constructing this DAM plot, electron accepting index with respect to fluorine (Ra) and electron donating index with respect to Sodium (Rd) were used as the respective parameters [54].

5.7.3 Analysis of Computational Results

For computational studies, optimized geometries of all the tested analytes were prepared. Optimized geometries of each nitro aromatic molecule are displayed in Figure 5.27. It is further utilized for obtaining computational perspective of NACs sensing mechanism by computing corresponding global reactive descriptors and thereby to construct Donor Acceptor Map. Observed trend among the NACs is carefully analyzed using the information obtained from aforesaid computational tools so as to frame a solid scientific backup to this sensing strategy.

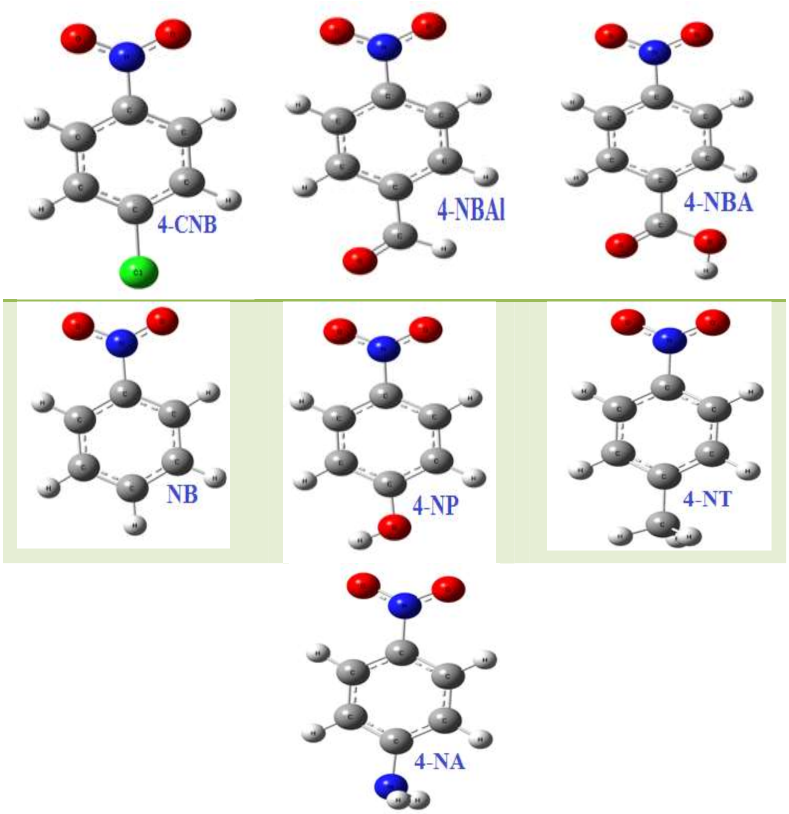
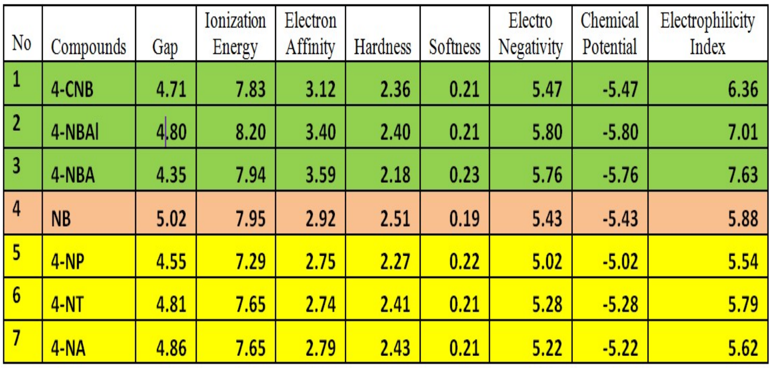


Figure 5.27 : Optimized geometries of NACs.

(a) Global reactive parameters of analyte molecules

As mentioned in previous section, structural reactivities of the tested NACs is systematically analysed from the global descriptors of each system. Computed global reactive descriptors for all the NACs are provided in Table 5.4.



No	Compounds	Gap	Ionization Energy	Electron Affinity	Hardness	Softness	Electro Negativity	Chemical Potential	Electrophilicity Index
1	4-CNB	4.71	7.83	3.12	2.36	0.21	5.47	-5.47	6.36
2	4-NBAl	4.80	8.20	3.40	2.40	0.21	5.80	-5.80	7.01
3	4-NBA	4.35	7.94	3.59	2.18	0.23	5.76	-5.76	7.63
4	NB	5.02	7.95	2.92	2.51	0.19	5.43	-5.43	5.88
5	4-NP	4.55	7.29	2.75	2.27	0.22	5.02	-5.02	5.54
6	4-NT	4.81	7.65	2.74	2.41	0.21	5.28	-5.28	5.79
7	4-NA	4.86	7.65	2.79	2.43	0.21	5.22	-5.22	5.62

Table 5.4: Global reactive descriptors (eV) of studied compounds

As evident from the table, introduction of substituent at the para position of nitrobenzene ring decreases the energy gap values of each system, indicating enhanced reactivity of the parental aromatic frame work (NB) due to the electron releasing or electron withdrawing inductive effects of para substituent. This observation was further confirmed by the corresponding softness values (S) of each analyte molecule. Greater the S value, more reactive will be the molecule. It is also noticed that the introduction of para substituent with electron withdrawing nature increases the electron affinity of the corresponding NACs. This observation is true, as such NACs will become good electron acceptors owing to the electron withdrawing para substituent.

The corresponding ‘χ’ as well as ‘ω’ values for those NACs also confirms their greater electron accepting ability/ electrophilic character. While considering the case of NACs with electron donating para substituent, electron affinity values corresponding to such systems decrease owing to their electron rich nature so that they need not accept electrons. For them, significant reduction in their ionization values compared to the parent nitro benzene molecule was noticed, further substantiating the commendable electron donating tendency of these NACs. Considerable decrease in the ‘ω’ values of electron donating para substituted NACs compared to the parent nitrobenzene molecule suggests that they are exhibiting nucleophilic character different from remaining set of NACs with electron withdrawing para substituent.

(b) Donor-Acceptor Map (DAM) of NACs

As mentioned in the previous section, we can simultaneously monitor the electron donating and accepting power of all the tested analytes (NACs) in a single frame by constructing DAM plot of concerned molecules. Electron accepting/ donating abilities of all the NACs can be easily understood by computing electron donating index with respect to Sodium (Rd) and electron accepting index with respect to fluorine (Ra) values and there by plotting corresponding donor-acceptor map. Based on this, all the analyte NACs can be categorized in to good, bad and best electron donor/acceptor molecules (Figure 5.28).

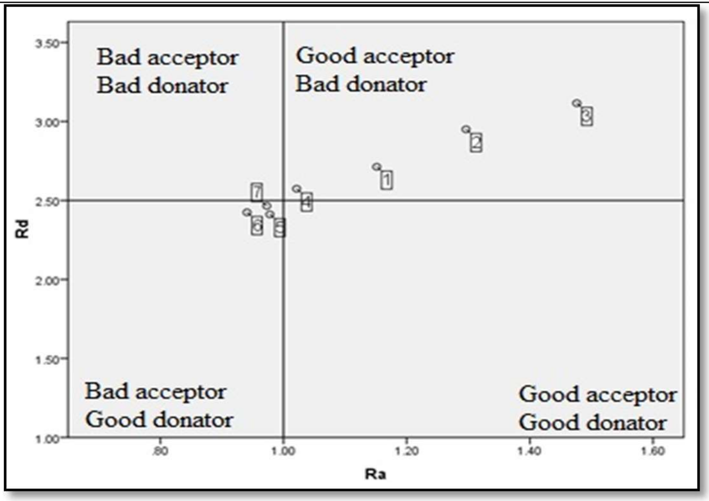


Figure 5.28 : Donor-Acceptor Map (DAM) plot demonstrating the electron donor andacceptor ability of all the tested NACs.

Based on the table showing global descriptor values, seven NACs can be numbered as 1-7, in which 1, 2, and 3 includes 4-CNB, 4-NBAl, and 4-NBA respectively, electron withdrawing para substituted NACs. Parental aromatic compound NB is designated as 4. Whereas, electron donating para substituted NACs such as 4-NP, 4-NT and 4-NA were denoted as compounds 5,6, and 7 respectively.

According to Figure 5.28, compounds 1-4 can serve as good electron acceptor, whereas NACs 5-7 can act as good electron donors. This observation further substantiates the proposed mechanistic route of NACs monitoring. Hence, owing to the presence of electron withdrawing substituent, analytes 1-4 become electron deficient which in turn tunes them good electron acceptors. These four NACs follow the electron acceptance ability order 4-CNB > 4-NBAl>4-NBA > NB. For the remaining class of NACs, i.e, NACs with electron releasing para substituent, order of electron acceptance ability on account of the electron rich nature of these analytes will be as follows 4-NP>4-NT> 4-NA.

Thus, based on the combined effect of computed global descriptors and DAM analysis findings, reactivity of all these seven NACs follows the order 4-NA>4-NT≈4-NP>NB>4-NBA>4-NBAl≈4-CNB, as displayed in Figure 5.29. Hence, computational analysis results also corroborate well with the observed experimental trend in sensing NACs.

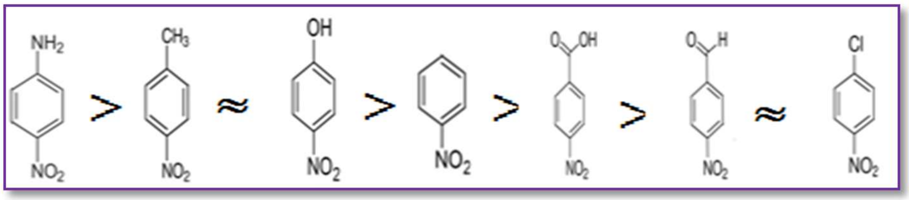


Figure 5.29: Activity order of NACs combining the value of global descriptive parameters and the DAM analysis results.

5.7.4 Summary and Highlights

- Fluorescence intensity of TS-CDs was found to be quenched in presence of a set of nitro aromatic compounds having nitrobenzene parental frame works. TS-CDs solution could sense Nitro benzene (NB),4- Chloro Nitro Benzene (4-CNB), 4-Nitro benzoic acid (4-NBA), 4-Nitro benzaldehyde (4-NBAl), 4-Nitro Phenol (4-NP), 4-Nitro Toluene (4-NT) and 4-Nitro Aniline (4-NA). System offers femtomolar molar level tracing of all the NACs with excellent selectivity and sensitivity. Selectivity/ reactivity of NACs towards the luminescence quenching of carbon dot solution follows the order 4-NA>4-NT>4-NP>NB>4-NBA>4-NBAl>4-CNB. A plausible mechanism for explaining the sensing action is suggested. According to the suggested mechanism, presence of para substituted electron withdrawing functional groups (in 4-CNB, 4-NBA and 4-NBA) reduces the π cloud availability of NACs ,whereas electron releasing para substituent enhances the electron availability at the π cloud of those NACs (4- NA,4-NT and 4-NP). Hence, electron releasing para substituent imparts more reactivity to the NACs towards optical response of carbon dot solution. Presence of the most efficient electron releasing para substituent (NH_2 group) makes 4- Nitro Anile (4-NA) as the most sensitive analyte among the selected molecules. Similarly, most efficient electron withdrawing group (Cl group) makes 4- Chloro Nitro Benzene (4-CNB) as the least responding NACs among the seven analytes. In addition to the sensing assay, we have made a novel attempt to interpret the sensing action through computational perspective. Results were examined and scientifically analyzed by computing global descriptive parameters and Donor Acceptor Map (DAM) method.

- ✓ First report on sensing a set of nitro aromatic molecules sharing similar parental frame work using a single fluorescent carbon dot system
- ✓ First report on utilization of computational tools including global descriptors and donor-acceptor mapping for analysing the sensing action of carbon dot system.
- ✓ Suggestion of a plausible mechanism for explaining the observed sensing trend.

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Chapter 6

TABLE SUGAR DERIVED CARBON DOT AS A GREEN REDUCING AGENT

At the end of the day, the goals are simple: safety and security.

— Jodi Rell —

- *This chapter presents the excellent reducing nature of TSCDs. Owing to their bio-compatibility and non-toxic nature, TSCDs can serve as a promising green alternative for conventionally used hazardous reducing agents. Commendable reducing ability of the system is manifested through the effortless extraction of metallic silver form silver salt solution as well as by the instantaneous preparation of gold nano particles from chloroauric acid.*

6.1 Introduction

Various authorised environmental organizations/pollution prevention bodies including United States Environmental Protection Agency stated that intense chemical reactions by both academical and industrial institutions result in dumping of enormous amounts of hazardous materials in to the environment, which may become fatal to the existence of life on earth. Hence, regardless of the enthralling profits gained by these chemical reactions, the aforementioned safety/security concern encouraged the whole world to reduce such experimental efforts and frame some environmentally compatible chemical formulae for the future practices. This intense urge for such an eco-friendly reaction scheme ensuring sustainability, safety as well as economic viability successfully ends up with the emergence of a highly promising transformative chemical synthesis strategy, "Green Chemistry". This concept of green chemistry, the careful utilization of fundamental sciences for the well-being of mankind, the environment as well as economy, was initially originated as a response to the well-known pollution prevention Act on 1990. According to this significant pollution control act, one nation should follow a policy that strictly eliminate contamination/ pollution of ecosystem by practising improved synthesis design comprising cost-effective alterations in final products, reaction method, raw materials, reagents and post-purification methods instead of seeking help for waste management and removal after a chemical reaction. Eventually, U.S government strictly proclaimed the aforesaid benign reaction strategy as "green chemistry [1]. From this nascent phase of development, this widely focused area flourished like anything owing to the relevance of the projected principles as well as the reliability of previous research efforts. Since from the implementation of this concept in 90's, the augmented pulse of green chemistry continued to grab attention even in the middle of 20's too. Wide acceptance to the concept and practices of this benign science was obvious from the citation for 2005 Chemistry Nobel prize to great scientists Chauvin, Schorck and Grubbs as an honour to their work "a great step forward for green chemistry".

It was in 1998, Paul T. Anastas and J.C Warner formulated 12 principles, that served as the core factor for understanding and worldwide implementation of the concept of green chemistry [2]. In general, by definition, green chemistry involves the utilization of these 12 principles for reducing/eliminating the usage/ generation of hazardous materials during the designing, manufacturing and application of chemical products. These 12 principles strictly insist the implementation of following ideas in to the conventional chemical synthesis strategy;

1. Prevention to waste/by-products formation
2. Conserve Atom Economy
3. Synthesis of less hazardous chemical products
4. Designing of safer/bio-compatible chemicals
5. Usage of safer/non-toxic reagents and auxiliary substances
6. Designing of energy efficiency methods
7. Usage of renewable feedstock
8. Strictly reduce derivative formation
9. Promote catalysis

10. Designing of degradation
11. Practice real-time pollution prevention
12. Practice safer chemistry for accident prevention

Scientists, Paul T Anastas and Evan S. Beach presented a review report in 2007, discussing sampling of research achievements for last 20 years of practices, involving progress in synthetic efficiency, utilization of alternative synthetic methods/ less hazardous reagents (auxiliary substances) as well as advancement in sustainable developments [3]. Through this aforementioned review report, they expressed their anxiety towards the future of green chemistry by stating that *"The future of green chemistry will depend on innovations that consolidate and integrate these achievements that have been made, using all Twelve Principles as a framework for intentional design"*.

Figure 6.1: Core concepts of Green Chemistry (images collected from various internet sources)

6.1.1 Usage of Alternative Non-Toxic/ Less Hazardous Reagents During Chemical Synthesis

As mentioned in the previous section, implementation of green practices in chemical reactions for eliminating or reducing the usage of hazardous chemicals has become a pressing requirement of high priority for sustainable living and healthy life. Literature indicates numerous efforts in this direction, involving successful replacement of every toxic/hazardous chemical reagent by an alternative, less toxic one, for maintaining the aforesaid green protocol. Majority of the chemical reactions involve the utilization of certain common reagents/solvents including fuming acids/various halogenated organic compounds/ highly volatile petroleum based compounds/ aromatic compounds etc,. For keeping green and safer synthesis protocol, these conventional hazardous reagents were found to be frequently replaced by various harmless/mild reagents such as glycerol, mild esters (ethyl acetate) and most desirably by water [4]. Apart from the usage of alternative innocuous reagents/ solvents, scientists completely assure harmless and safe reaction strategy by practising Ionic-liquid mediated organic reactions as well as liquid phase supported synthesis and most preferably water based reactions.

(a) A glance in to the world of green reducing agents

Design and applications of metal nanoparticles have caught immense attention owing to the unique and interesting features possessed by nano sized metal particles compared to their macro-scaled counterparts [5]. Most of the metal nanoparticles with promising applications were synthesised by adopting various chemical reaction methods involving harsh chemical conditions such as utilization of strong reducing/stabilizing agents as well as toxic solvents. In addition, conventional reducing conditions in metal nano particle synthesis are often associated with the usage of expensive metal catalysts such as Pt and Pd. All these aforesaid factors necessitate ceaseless attempts to green up the reduction process by introducing more effective and benign/bio-compatible reducing agents which are also cost-effective by nature. Metal nanoparticle synthesis via green chemistry assisted reducing reactions induces less harmful effects on the ecosystem and human health than that of chemically derived systems. Hence, research community has extensively devoted their time and skill for building eco-friendly criteria to yield promising metal nano particles by means of environmental benign reducing as well as capping agents in greener solvents via highly facile and cheaper route [6-9]. Generally, different microbial systems (bacteria, algae, fungi, yeasts etc,.), plants and several plants parts (flower, bark, seed, stem, leaf etc,.), leaf extracts, natural polymers, amino acids, vitamins and proteins/enzymes were utilized as bio-reducing agents for making various metal nanoparticles [5]. Among these green methods, utilization of plants (plant origin)/ leaf extracts and micro-organisms are found to be the best choices as green reducing agents on account of their non-toxicity, reaction efficiency as well as cost-effectiveness, compared to their conventional chemical rivals. Scientists utilized wide range of bacteria including Bacillus subtilis, Escherichia coli, Bacillus mycoides, Enterococcus sp, Bacillus methylotrophicus, Bacillus cereus, Bacillus sp, Lactobacillus acidophilus , Bacillus licheniformis and Bacillus flexus as microbial agents for obtaining promising nanoparticles including Au, Ag, TiO2, ZnO, CdS etc,. Fungi such as Aspergillus sp., Aspergillus niger , Hypocrea lixii ,Penicillium sp., Fusarium sp ., Trichoderma sp., Phaenerochaete Verticillium sp. and Trichothecium sp. were widely utilized as microbial sources for metal nanoparticle synthesis. Saccharomyces cerevisiae, Yarrowia lipolytica, Candida sp., Saccharomyces boulardii, Rhodotorula sp. Schwanniomyces occidentalis and Pichia pastoris were the widely utilized yeast as a microbial sources for producing various metal nano particles[5]. On the basis of various characterization results, presence of reductase enzymes, various ploysaccharide units, reducing enzymes and proteins etc, within these aforementioned micro-organisms were believed to be the inherent reducing units that mould them as promising microbial sources for metal nano particle.

Recently, plants and different plant organs were extensively utilized for fabricating metal nano structures with promising applications, owing to various advantages offered by this method over micro-organism assisted synthesis. Plant derived syntheses were extensively marked with various benefits such as the enhancement in the reaction rate, improved monodispersity, increased product stability and moreover facile experimental procedures. Commendable reducing ability associated with plants and plant derived materials (organs and extracts) is corroborated with the abundantly available reactive components such as polyphenols, terpenoids, flavanoids, polysaccharides, proteins, carbonyl, hydroxyl , carboxylate, amino groups ,amino acids and reducing sugars within their structure.Metal nanoparticles including Au, Ag, Cu, Pd, Cu, Ni, Fe, Al_2O_3, Cr_2O_3, CuO *etc,. with* interesting features were prepared from various plants and plant derived green sources such as Hibiscus rosa sinensis (leaf extract), Mimosa pudica (leaf/stem/root extracts) Green tea Vitis californica (leaf extract) Daucus carota extract Olive (leaf extract) Camellia sinensis (leaf extract) Heliotropium indicum (leaf extract) Barberry (fruit extract) Euphorbia wallichii (leaf extract), lemon grass (leaf extract) ,Ocimum sanctum (leafextract) Citrus sinensis, Citrusreticulate, Citrus aurantifolia (peel extract), Morinda morindoides (leaf extract) Eucalyptus (leaf extract), Euphorbia thymifolia L. (leaf extract),Cavendish banana peel extract ,Abutilon hirtum (leaf extract), *Arachis hypogaea (leaf extract),* Silybum marianum L. (seedextract),Ginkgo biloba L. (leaf extract) etc,.[5]. Figure 6.2 provides statistical estimate of the widely used green sources for metal nanoparticle synthesis.

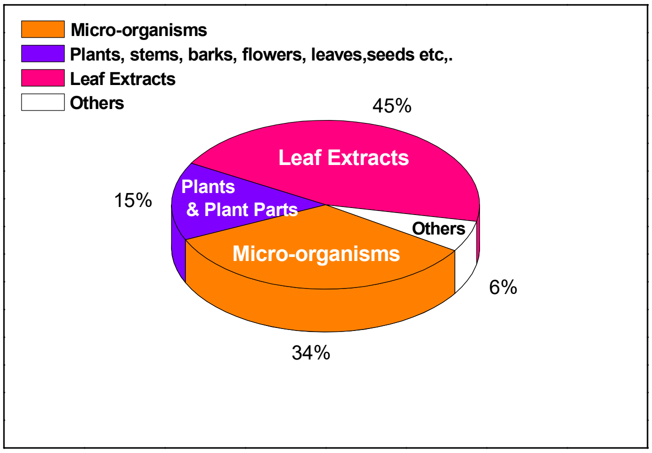

Figure 6.2: Statistical representation of widely used green reducing/capping agents (data collected via literature survey)

This area of metal nanoparticle synthesis using various plant extracts and plant derived materials is still under exploration. Regardless of this extensive green synthesis using plants and micro-organisms, many issues remain unresolved, especially related to the exact functional group/ chemical origin responsible for the observed bio-reduction ability. The precise mechanism involved in the reduction process is also thus under shadow. Detailed literature survey conducted in this regard also ends up with the absence of scientifically acceptable specification for this bio-synthesis, though it is still remaining as the most interesting and relevant practice to metal nanoparticles. The phytochemical compositions may differ according to seasons and locations, which strictly demands the identification of secondary metabolites of plants (chemical components) for further progression of biosynthesis of promising metal nanoparticles.

(b) Previous efforts involving carbon dots as green reducing agent.

As the previous results of biosynthesised metal nanoparticles using the aforesaid plants, microbials and several other potent green sources (vitamins, amino acids, proteins etc,.) strongly substantiates the instrumental role of various oxygen bearing functionalities, scientists made extensive research on the reducing ability of fluorescent carbon dots enriched with oxygen containing surface functionalities. The role of these fluorescent carbon nanoparticles as a reducing agent is less explored area compared to the widely exploited fields such as sensing, bio-imaging etc. However, some interesting works are there in literature, discussing their successful application as potent green agent for the production of metal nanoparticles with promising applications. Many of the works successfully correlate the observed reducing power with the presence of various oxygen containing surface groups of fluorescent carbon dots [10-19]. Research report by Masoud et al., scientifically back up the suggestion that hydroxyl functionalities on carbon dot structure are the sole source for the conversion of Fe^{3+} ions into Fe^{2+}, which then simultaneously converted into carbonyl groups (C=O) [20]. Very recently, Ghosal and colleagues also substantiated the crucial role of surface functionalities (hydroxyl and carboxyl) for the formation of silver nano particles (reduction of Ag^{+} to Ag^{0} nano particles) [21]. Reducing ability of fluorescent carbon dots were also effectively utilized for synthesising gold nano particles with commendable features [22 -24]. Research work by Wang's group presented successful formation of gold nanoparticles from chloroauric acid using highly reducing carbon dots, in presence of reducing agent $NaBH_4$ [24]. This report substantiates the crucial role of excess amount of hydroxyl (-OH) surface functionalities in imparting this excellent reducing ability to the system.

In this context, owing to the presence of abundantly available oxygen bearing surface functionalities as confirmed through adequate characterization techniques (chapter 3), it is highly desirable to inspect the reducing property of the present carbon dots. Surprisingly, the as-synthesised carbon dot solution offers excellent reducing ability while attempting metal nanoparticle synthesis. Environmentally friendly as well as economically viable synthesis route along with the commendable bio-compatibility further highlight the credibility of the

TS-CDs as a promising green alternative to the conventionally used chemically derived hazardous reducing reagents such as hydrazine, sodium borohydride etc,. Herein, we could successfully demonstrate the excellent reducing nature of this benign system (TS-CDs) via two significant reduction reactions ;

I. ***Successful extraction of bulk metallic silver from Ag (I) solution.***

II. ***Instantaneous preparation of gold nanoparticles from Chloroauric acid.***

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(I) Table Sugar Derived Carbon Dots for the Extraction of Bulk Silver

6.3 Introduction

Herein, table sugar derived carbon dots serve as an excellent benign reducing agent/medium for the bulk production of very precious and shiny metal, silver via a very facile procedure. Several experimental efforts were reported earlier for the bulk production/extraction of this metal, which is counted as one of the seven metals of antiquity. Metallic silver is grabbing enhanced world-wide interest owing to its multifaceted applications in various fields. Metallic silver generally finds applications in the manufacturing of coins, jewellery, dining wares, wood preservatives, surgical equipments, mirrors, touch screens, water purification, photography and photo-electronic devices etc., This lustrous metal is marked with commendable thermal and electrical conductivity. Conventionally adopted experimental procedures for the bulk preparation of shiny silver involves metallurgical processes, mainly smelting or chemical leaching of silver ores [1]. Other than these conventionally practiced metallurgical operations, electrolytic refining of metallic copper, as well as Parkes process of lead (Pb) metal derived from lead ores, having traces of metallic silver also yield small quantities of metallic silver as a side-product. The well-known cyanide process is the widely practiced metallurgical method for the bulk extraction of metallic silver using argentite ore as the starting material [2]. This process is generally marked with highly tedious multistep procedures to yield pure metallic silver.

The chemistry behind this cyanide process can be summarised as follows.

$Ag_2S+4NaCN \rightarrow 2Na[Ag(CN)_2]+Na_2S$

$2Na[Ag(CN)_2]+Zn \rightarrow Na_2[Zn(CN)_4]+\mathbf{2Ag}\downarrow$

The crushed and concentrated argentite (Ag_2S) ore is first treated with sodium cyanide, that results in the formation of sodium argento cyanide, which upon mixing with Zinc dust yields sodium tetra cyanozincate along with the precipitation of elemental silver, generally termed as spongy silver. This spongy silver is further treated with potassium nitrate to yield pure form of silver metal. The purity of the obtained silver is further ensured by conducting various electrolytic purification processes. Though cyanide process results quantitative extraction of metallic silver from the abundantly available argentite ore, utilization of excess concentration of toxic sodium cyanide as well as the associated tedious purification methods are considered as the major shortcomings of this method.

Hence, an innovative move for the bulk production/ extraction of shiny metallic silver from the precursor salt solution via an effortless strategy is highly solicited. To the best of our knowledge, this is the first report on the usage of carbon dots as a reducing platform for the effortless extraction of bulk metallic silver from its salt solution.

6.3.1 Experimental

(a) Extraction of metallic silver using table sugar derived carbon dots

About 5 mL of distilled water is made to boil in a glass vessel. To this hot water, 100 μL of 0.05 molar silver nitrate ($AgNO_3$) solution is added with constant stirring. The solution thus obtained is then cooled normally to room temperature. While cooling, the solution in the glass vessel turns reddish brown in colour after 15 minutes of standing (Figure 6.3 a). This reddish brown solution turns black after 3 hours (Figure 6.3 b). Interestingly, it was noticed that after 20 hours of storage, inner side of the glass bottle was seen covered with shiny mirror coating (Figure 6.3 c and d), indicating the deposition of metallic silver inside the glass vessel. Digital images of the solution displaying these sequential changes are provided in Figure 6.3. Hence, this method proposes an effortless strategy for the extraction of metallic silver using environmentally benign reducing agent, table sugar derived carbon dots.

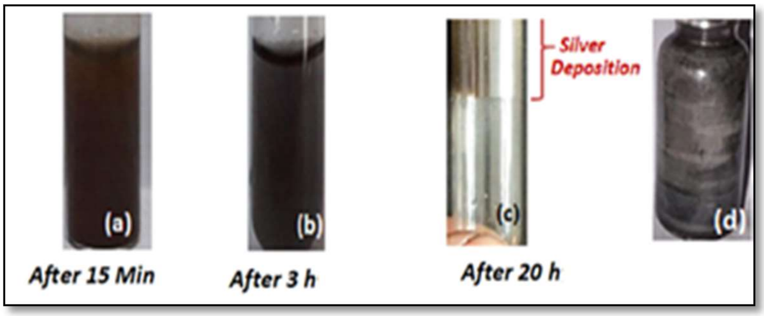
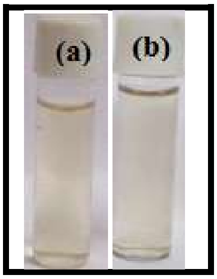


Figure 6.3: Digital images of silver salt solution after treating with TS-CDs at different time interval; 15 min (a), 3 h (b), 20 h (c) and glass vessel with silver coating (d).

(b) Confirming the participation of TS-CDs for silver extraction and excluding the possibility of other reagents in the reduction process

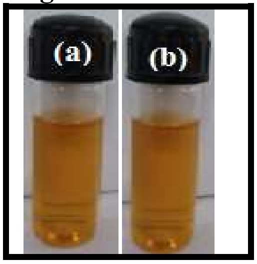
The vital role of TS-CDs for silver reduction, thereby resulting in metalic silver deposition is systematically verified by treating the precursosr salt solution ($AgNO_3$) with the possible intermediate moieties, starting from pure table sugar. For this, parallel experiments are conducted by altering the reagent, maintaining all the experimental conditions same. Results corresponding to each experiment in this regard are shown in the digital images (Figure 6.4) which is monitored after 3 days.

(i) **Normal table sugar solution**



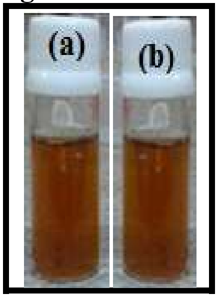
Images of test solution **(normal table sugar solution+ hot water+ $AgNO_3$ solution)** before (a) and after 72 hours (b)

(ii) **Caramelized table sugar solution**



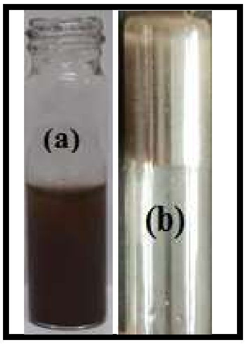
Images of test solution **(caramelized table sugar solution + hot water + Ag NO_3 solution)** before (a) and after 72 hours (b)

(iii) **Caramelized table sugar solution with ammonia**



Images of test solution **(caramelized table sugar with ammonia + hot water + Ag NO_3 solution)** before (a) and after 72 hours (b)

(iv) **Table sugar derived carbon dots**



Images of test solution **(caramelized table sugar solution with ammonia after microwave treatment; TS-CDs, as confirmed from the luminescence + hot water + Ag NO_3 solution**) before (a) and after 72 hours (b)

Figure 6.4: Digital images confirming the crucial role of TS-CDs for the reduction of Ag (I) to Ag (0); metallic silver deposition.

As Figure 6.4 illustrates, introduction of all other reagents into the hot aqueous solution of silver nitrate fails to bring the silver mirror formation/ metallic silver deposition on the glass vessel even after 72 hrs of observation. As Figure 6.4 (iii) indicates, even the addition of caramelized table sugar- ammonia mixture (precursor of TS-CDs) was not able to evoke the reduction process. Only after the formation of TS-CDs, which is verified through the appearance of photoluminescence, the aforesaid silver extraction/ silver reduction is noted under the prescribed experimental environment. Hence, all these observations confirm the crucial role of fluorescent TS-CDs enriched with oxygen functionalities in realising the reduction reaction.

6.3.2 Summary and Highlights

- Interestingly, present system was found to serve as an excellent reducing agent for the extraction of precious, lustrous metal, silver via an effortless process. Addition of minute amounts of TS-CDs into the hot aqueous solution of silver nitrate (precursor salt) results successive deposition of metallic silver inside the walls of glass vessel, confirming by the formation of shiny silver mirror coating after 20 hours of observation. Crucial role of TS-CDs in bringing the Ag (I) to Ag (0) reduction, thereby the silver deposition is systematically investigated and illustrated by various trial and error methods, which excludes the chance for participation of suspected possible intermediate in the reduction.

(II) Table Sugar Derived Carbon Dots for the Instantaneous Synthesis of Gold Nanoparticles

6.4 Introduction

Herein, the reducing ability of present table sugar derived carbon dots is exploited for the instantaneous preparation of gold nanoparticles by means of a benign as well as economic synthesis strategy. Carbon dot solution brings the easy reduction of Au (III) to Au (0) within few seconds, leading to the formation of gold nanoparticles (Au-NPs) of appreciable dimensions and extra-ordinary stability with the aid of trace quantities of bio-compatible stabilizing agent, starch. Thus obtained gold nanoparticle exhibits shelf life of more than 30 days, note-worthy to mention, when compared to previous attempts proceeding through chemically derived reducing agents and stringent experimental conditions. Excellent catalytic property of the prepared gold nanoparticles is illustrated through the hydrogenation of 4-nitrophenol to 4-aminophenol.

Though, literature unveils numerous efforts for fabricating gold nanoparticles using various green/naturally available materials (plant/leaf extracts, biological agents, micro-organisms, natural polymers), utilization of carbon dots to this effect is a rarely discussed area. It is worthy to mention that the precedented research attempts for carbon dot mediated gold nanoparticle formation involves the usage of harsh chemical conditions and prolonged synthesis routes. Most of such attempts demanded long reaction duration for the fabrication of the reagent carbon dots as well as Au (III) reduction (gold nanoparticle formation) [1-4]. Hence, in this context, the introduction of such an extremely benign reducing agent, which could result in instantaneous formation of gold nanoparticles with appreciable features and excellent catalytic activity, is highly note-worthy.

6.4.1 Experimental

(a) Instantaneous preparation of gold nanoparticles (AU-NPs)

About 10 mL of distilled water is subjected to heating at 60° C for 2 minutes. To this hot water, about 50μL of starch solution (0.3 wt%) is added as the capping/stabilizing agent with continuous stirring for preventing aggregation/agglomeration of nanoparticles. 25 μL of 0.05M precursor salt solution, Chloroauric acid ($HAuCl_4.3H_2O$), is added to the said solution with constant stirring. About 20 μL of the benign reducing agent, table sugar derived carbon dot solution is added slowly to the reaction mixture. Within 10 seconds, the solution turns wine red in colour, indicating the formation of gold nanoparticles (Au-NPs). Schematic representation summarizing the TS-CDs assisted gold nanoparticle synthesis is shown in Figure 6.5.

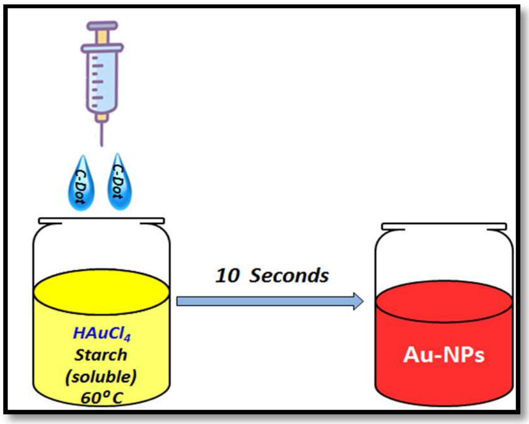


Figure 6.5: Schematic representation of TS-CDs assisted gold nanoparticle (AU-NPs) synthesis.

(b) Characterization of gold nanoparticles (AU-NPs)

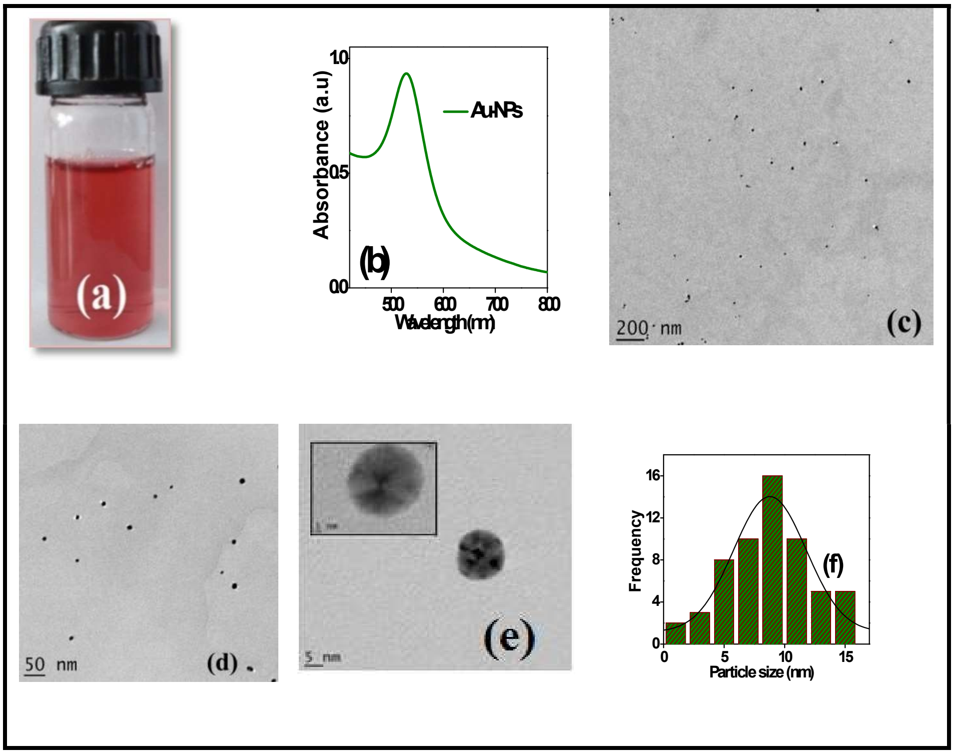


Figure 6.6 : Digital image of wine red coloured gold nano particle solution (a), UV-Visible absorbance curve indicating surface Plasmon resonance (b), HRTEM image of Au-NPs ; 200 nm (c), 50 nm (d) 5nm (e) (inset of Figure 6.6(e) contains HRTEM image of Au-NPs under 1 nm resolution), particle size distribution curve (f)

As-synthesised wine red solution (Figure 6.6 a) of nanogold is subjected to various characterization techniques for confirming the formation of gold nanoparticles. UV-Visible absorbance spectrum of the solution (Figure 6.6 b) displays the surface Plasmon absorption band at 527 nm, unique to gold nanoparticles, corroborating well with previous works in this regard. HRTEM images of the solution under different scales of resolutions are provided below in Figure 6.6 (c-e).

As evident from Figure 6.6 (e), hexagonal bi-pyramidal faceted gold nanoparticles is formed by the reducing action of table sugar derived carbon dot solution. The average particle size of gold nanoparticles is estimated as 8.3 nm, using the histogram plot of particle distribution (Image J software, version; 152-win-java8) (Figure 6.6 f).

(c) Investigating Catalytic Efficiency of Gold Nano Particles-

Reduction of 4-nitroPhenol (4-NP) to 4-amino Phenol (4-AP)

For ensuring the efficiency of prepared gold nanoparticles, a model catalytic reaction, hydrogenation of 4-nitro phenol (4-NP) to 4-Amino phenol (4-AP) is conducted. 100 µM solution of 4-NP is prepared as the stock solution for conducting the reaction. From this stock solution of 4-NP, about 1.7 mL is taken inside a 3 mL cuvette and is then mixed well with 1 mL of $NaBH_4$ solution in order to convert nitro phenol into nitrophenolate ion. For ensuring pseudo-first-order kinetics for the reaction, concentration of $NaBH_4$ was maintained 100 fold excess than that of 4-Nitrophenol solution. Addition of excess amounts of $NaBH_4$ turns the pale yellow coloured 4-NP solution in to bright yellow, confirming the formation of nitrophenolate ion. Corresponding spectral observation is provided below as Figure 6.7. Characteristic UV-Visible absorbance curve of nitrophenolate ion at 400 nm shows sequential decrease in intensity with the addition of 450µL of 1µM Au-NPs solution by maintaining Au-NPs: 4-NP concentration in the ratio 1:15, indicating 4-Nitrophenol reduction process. Introduction of aliquots of gold nanoparticles results decolourisation of bright yellow 4-NP solution (inset of Figure 6.7). Along with the gradual reduction of absorbance at 400 nm, sequential emergence of a new absorbance curve around 298 nm within few minutes of gold nano particle addition is evident in the Figure, corresponding to the absorbance of 4-Amino phenol (4-AP). This observation confirms the successful conversion of 4-NP to in to 4-AP with the addition of Au-NPs. Excellent catalytic activity of the prepared gold nanoparticles in affecting 4-NP reduction is thus assured.

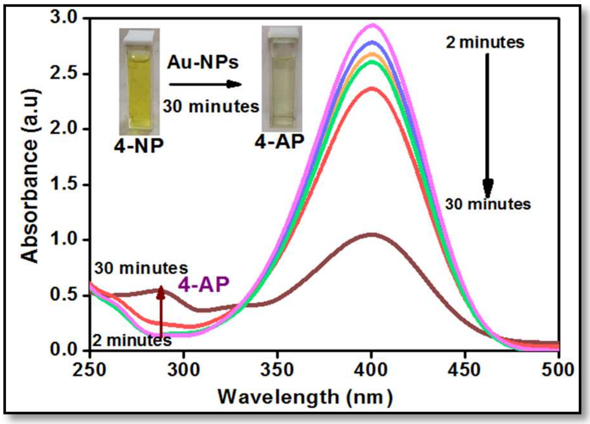


Figure 6.7: Demonstration of catalytic activity of the prepared gold nanoparticles: Catalytic hydrogenation of 4-Nitro phenol to 4-Amino Phenol using Au-NPs - (Reaction temperature: 27 °C)

6.4.2 Summary and Highlights

- The reducing action of the prepared TS-CDs is also exploited for the instantaneous preparation of efficient gold nanoparticles (Au-NPs) from Au (III). Owing to its excellent reducing ability, carbon dot solution brings gold nanoparticle formation in an almost instantaneous manner, within 10 seconds, highly appreciable compared to previous efforts in this direction. Formation of gold nanoparticle is further confirmed by various characterization analyses. Present system exhibits considerable surface Plasmon absorbance at 527 nm, unique to gold nano systems. Thus obtained gold nanoparticles possess average diameter size of 8.3 nm with hexagonal bi-pyramidal faceted morphology. Catalytic efficiency of the prepared gold nanoparticles is investigated by conducting hydrogenation of 4-Nitrophenol. Gold nanoparticles exhibit commendable catalytic activity in meagre amounts, under milder reaction condition.

☞

✓ Identification of TS-CDs as a green reducing agent through the effortless extraction of metallic silver from Ag (I) solution.

✓ Instantaneous formation of gold nanoparticles using highly reducible TS-CDs

✓ Achievement of gold nanoparticles with commendable catalytic activity

✓ Inherent bio-compatibility as well as extremely facile extraction strategy makes the present system as a promising green reducing agent.

> ✚ *In brief, successful extraction of metallic silver as well as instantaneous preparation of gold nanoparticles illustrate the excellent reducing ability of the present TS-CDs*

6.5 References

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Chapter 7

SUMMARY, HIGHLIGHTS AND FUTURE PERSPECTIVES OF THE WORK

- ***This chapter is devoted for summarizing the entire thesis. Herein, major highlights of our research findings and future scope of the prepared system are discussed briefly. As the material "carbon dot" is still remaining as a "mystic star in the nano-world", bright scope of scientific investigations is of course awaited.***

7.1 Summary of the Present Research Work

As is evident from previous chapters of the thesis, this was a genuine attempt for developing a novel strategy to carbon dots that ensures environmental compatibility, economic viability as well as effortless experimental procedure for designing bright luminescent carbon dots with promising features that could be exploited for a wide range of applications. For this, we have conducted a detailed literature survey regarding previous works on fluorescent carbon dot systems for significant applications in various fields, spending more time on numerous scientific reviews detailing their structure and properties. Owing to the increased thirst for environmental benign synthesis of any material, we focused our research on fabrication of promising carbon dot systems from abundantly available natural precursors. Interesting features of sugar prompted us to choose commercially available table sugar as the staring material for synthesising fluorescent carbon dots, which obeys one of the significant criteria of green chemistry i.e., safest raw material. Herein, we have utilized micro-wave energy for the successful formation of carbon particles of desired dimensions (below 10 nm) from table sugar-ammonia aqueous mixture within few minutes of irradiation. In this manner, the present research attempt can be viewed as a successful step for designing a benign, economic and easy formula to bright emitting carbon dots with promising features. Adequate characterisations confirm the formation of quasi-spherical carbon particles with average size of 3.8 nm with characteristic UV-Visible absorbance and fluorescence emission features. Spectroscopic studies confirm the presences of surplus

Oxygen bearing functionalities over the core of carbon dots. Carbon dots thus obtained are utilized for various applications including sensing of significant analytes, fabrication of efficient luminescent security markers, as well as a potent green alternative for chemical reducing agents.

The solution exhibited bright cyan emission with admirable photo-stability. The solution was also found to be highly bio-compatible in nature. Bright cyan emission of carbon dot solution was well exploited for the preparation of fluorescent markers with two different consistency; (i) luminescent ink and (ii) luminescent gel by mixing with naturally available binding agent, gum arabic/gum accacia, collected from the exterior bark of accacia tree. Luminescent ink was filled inside the cartilage of gel pen and successfully employed for patterning purposes. The prepared markers exhibit excellent applicability as it possesses strong adherence and patterning ability over various substrates, ranging from fabrics, plastics, glass plates and paper. Hence, these fluorescent carbon dot-gum accacia markers can serve as a promising anti-forgery/anti-counterfeiting tool for ensuring security to valuable files, documents and various branded commodities.

Apart from the fabrication of fluorescent markers, the system could serve as an excellent platform for quantitative monitoring of various significant analytes. The system offered naked eye monitoring of extremely lethal heavy metal ion, Pb^{2+} even at minute concentrations, i.e., 14 parts per billion, highly commendable when compared to the allowed concentration levels of the said ion in aqueous bodies. This novel observation was further scientifically corroborated well with the extra-ordinary affinity of Pb^{2+} ions with surface, carboxylate groups of carbon dot structure. Combination of carbon dot solution with sufficient quantities of xanthene dye, Rhodamine-6G, forms efficient FRET pair, which further serves as a promising sensing medium for highly toxic heavy metal, Hg^{2+} ions with appreciable detection limit in picomolar concentration (98 pM). Practical applicability of the system was also validated by conducting real sample Hg^{2+} sensing assays. Negligible error percentages associated with these experimental results confirm its adaptability as a common man's Hg^{2+}sensing device. Along with metal ions sensing, present carbon dot solution also offers quantitative monitoring of a set of nitro aromatic molecules with nitrobenzene parental frame work. The class of nitro aromatic molecule includes Nitro benzene, 4-Nitro aniline,4-Nitro phenol,4-Nitro toluene,4-Nitro benzaldehyde,4-Nitro benzoic acid and 4-chloro nitro benzene. The system enables highly selective and sensitive monitoring of all these analytes of similar structural origin with detection limit as low as femtomolar concentration. The reason for this aforementioned observation is explained well with the aid of widely accepted computational tools including global descriptors and donor-acceptor mapping. A plausible mechanistic route to the sensing action was also suggested based on the observations. Experimental reactivity order of all the nitro aromatic compounds was found to be in good agreement with the computational results(combined effect of global descriptors and the corresponding DAM plots). Apart from the luminescence based applications, these particles are also projected as a potent benign alternative for chemical reducing agents. Owing to the presence of abundantly available surface oxygen groups (hydroxyl, carboxyl, carbonyl etc.,) the sugar derived carbon dots enable successful extraction of metallic silver via extremely facile experimental strategy form metal salt solution ($AgNO_3$). The excellent reducing ability offered by sugar derived carbon dot solution was further extended to instantaneous synthesis of gold nanoparticles via benign and economic reaction route. The gold nanoparticles thus obtained exhibit commendable catalytic efficiency as evident by the successful conversion of 4-Nitrophenol to 4-Aminophenol.

7.2 Major Highlights of Present Work

- ✓ *Novel attempt for designing benign and economic synthesis strategy to fluorescent carbon dots form commercially available table sugar via well-known Maillard reaction.*
- ✓ *This is the first report discussing the utilization of naturally available gum accacia/gum arabic as a binding agent for making fluorescent gel and fluorescent ink.*
- ✓ *This is the first report on naked eye sensing of Pb^{2+} ions via simple aggregation of carbon dots.*
- ✓ *Quantification of Pb^{2+} ions using simple turbidimeter is achieved.*

- ✓ *This is the very first report on utilization of computational tools including global descriptors as well as donor-acceptor mapping for probing the sensing ability of carbon dot system.*

- ✓ *Present work discusses the first ever successful attempt for the extraction of metallic silver by means of an extremely facile strategy\y from metal salt solution, utilizing the strong reducing ability of carbon dots. This extraction procedure was found to be highly economic, benign and commendably effortless.*

- ✓ *Small concentrations of carbon dots are utilized for instantaneous preparation of gold nanoparticles with excellen catalytic ability through an economic procedure using trace amounts of benign capping agent (starch solution).*

- ✓ *The prepared fluorescent markers (PL-Gel and PL-Ink) exhibit commendable adherence over a wide range of substrates including cotton thread, glass plate, plastic sheet and paper.*

7.3 Sugar Derived Fluorescent Carbon Dots – Scope and Future outlook

- **Bio-Markers/bio-imaging tools:** Owing to its excellent bio-compatibility as well as commendable photo-stability, they can be further utilized for fabricating promising imaging tools for tracing the functioning of various body cells/tissue.

- **Drug Delivery applications:** As carbon dot structures were reported to be extremely sensitive towards structural modifications including heteroatom doping/surface functionalisation etc,.fine tuning of its structural frame works will mould them as promising carriers for various drug molecules to the target sites. Extreme biocompatible nature possessed by the carbon dots ensures safe delivery of drugs within living body cells without any harmful side effects.

- **Pesticides/insecticide sensor:** As the fluorescence intensity of the carbon dot solution was found to be quenched by the addition of some external agents including heavy metal ion and nitro aromatic molecules, the class of introduced analytes can be extended to large organic structures too, comprising of significant insecticides/pesticides.

- **Extending the optical sensing feature towards other analytes by fine tuning the structure of carbon dots:** Structural modifications by means of surface passivation/functionalisation/ hetero atom doping will tailor the features of TS-CDs for desired applications. By tuning the surface functional groups, luminescence behaviour of the carbon dot solution gets altered and may thereby result numerous possible quenching interactions with a wide spectrum of external agents including ions (cations and anions), organic molecules, bio-molecules, reaction parameters including pH, viscosity, temperature etc.,

- **Great scope for scientific investigations on structure as well as photoluminescence mechanism of carbon dots:** Despite the innumerable research efforts in the field of carbon dots, the unavailability of a widely-accepted structural format as well as unanimous fluorescence mechanism leaves this area as a hot spot for intense /meticulous research and investigations. Thus, carbon dot research is still in a developing phase. This will open many doors to research efforts including alterations in synthesis strategy such as tuning of reaction time, temperature, pH, solvents, energy source and concentration of reaction mixture. Systematic analysis of various characterization results of each product obtained after aforesaid alterations in the synthesis procedure will definitely shed light on the structural aspects as well as the underlying fluorescence features/mechanism of the system.

- ➢ *Some studies were conducted in this regard by altering the adopted synthesis procedure of table sugar derived cyan emitting carbon dots. For this, we have conducted the carbon dot synthesis by changing some reaction parameters such as pH, pyrolyzing temperature of sugar, time of irradiation as well asthe synthesis strategy. Corresponding results are summarized in table 7.1.*

Reaction Conditions	**Digital image of solution under 360 nm excitation in UV chamber**	
Present Work		
Microwave irradiation of sugar slurry and dilute ammonia solution at 120 ^{0}C for 3 minutes (pH = 5.6)		**Intense cyan Emission**
Same reaction conditions, except **the change in pH to 4.5 with the addition of 0.1N HCl**		**Intense white Emission**
Same reaction conditions, except **the change in pH to 1.3 with the addition of 1N HCl**		**Intense yellow-orange emission**
Except the pyrolysis conditions, all other parameters kept as same ***(pyrolysis temperature- 150 ^{0}C &irradiation time- 5 minute)***		**Intense yellow emission**

- Altering the pyrolysis temperature of sugar from 150ºC to 180ºC and microwave irradiation mode (7minutes at 150ºC).

Feeble green emission

- Keeping all the reaction parameters same but changing the reaction method from microwave irradiation in to hydrothermal route:

Hydrothermal : 190⁰ C for 5 hrs in a muffle furnace

Feeble yellow emission

- Keeping all the reaction parameters same but changing the reaction method from microwave irradiation in to hydrothermal route:

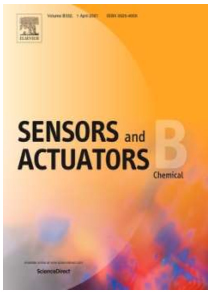
Hydrothermal : 220⁰ C for 6 hrs in a muffle furnace

Intense orange emission

Table 7.1: Observations of various reaction schemes adopted for synthesising carbon dot solutions by altering reaction parameters/ synthesis strategy.

- Deep probing in to the optical features of the solutions is believed to bring some scientific conclusions regarding the fluorescence mechanism, as well as the structural features of the sugar derived carbon dot system. Many research efforts in literature end up with some vague statements regarding the interpretation of exact structure and fluorescence mechanism of carbon dot systems. We strongly believe that meticulous analysis of characterization results of these samples will definitely pave way to significant findings regarding the structure and fluorescence feature of sugar derived carbon dots. Studies in this direction are in progress.

LIST OF PUBLICATIONS

1		*Table sugar derived carbon dots- a naked eye sensor for toxic* Pb^{2+} *ions*, **V.A. Ansi**, N.K. Renuka, ***Sens. Actuators, B., 264 (2018) 67-75***(Elsevier) DOI: *10.1016/j.snb.2018.02.167*
2		*Exfoliated graphitic carbon dots: Application in heavy metal ion sensing,***V.A Ansi**, N.K Renuka, ***j. Lumin., 205 (2019) 467-474***(Elsevier) DOI:*10.1016/j.jlumin.2018.09.061*
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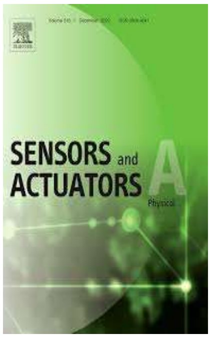
4		*Fluorescent carbon nanodots as efficient nitro aromatic sensor analysis based on computational perspectives*, **V.A. Ansi** , K.R. Vijisha, K. Muraleedharan, N.K Renuka**, *j. Sens. Actuator .A Phys.,302 (2020) 111817*** (Elsevier) DOI:*10.1016/j.sna.2019.111817*

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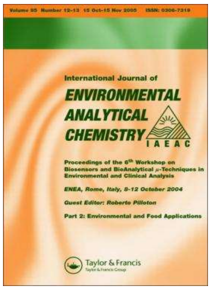
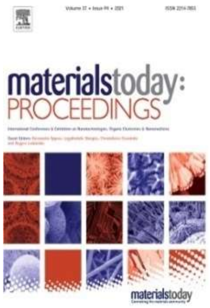
8		*Acetic acid derived carbon dots as efficient pH and bio-molecule sensor*, **Ansi V.A**, Ritu G, Thasleena panakkal, Aji A Anappara and N.K Renuka, ***int j environ an ch., 2019.10.1080/03067319.2019.1669581*** (Taylor and Francis) DOI: *10.1080/03067319.2019.1669581*

9		*Sucrose derived luminescent carbon dots as a promising bio-medical agent***, V.A. Ansi**, N.K. Renuka***, Mat.Today: Proceedings., 18 (2019) 1724–1728***(Elsevier) DOI:*10.1016/j.matpr.2019.05.269*
10		*An elegant and handy selective sensor for ppt level determination of mercury ions* , M. Anju, T. Divya, M. P. Nikhila, T. V. Arsha Kusumam, A. K. Akhila, **V. A. Ansi** and N. K. Renuka, ***RSC Adv., 6 (2016) 109506-109513*** (RSc)

LIST OF PRESENTATIONS

- Oral presentation at International conference on energy environment and advanced materials for sustainable future, ICEEAMSF-***2017*** at Kongu Engineering College, Erode [ISBN No 978-81-933005-2-7]
- Oral presentation at International conference on advanced materials for technological applications,ICAM-18 ***(2018)*** held at PSGR Krishnammal college for women, peelamedu, Coimbatore
- Oral presentation at National seminar Frontiers in chemical sciences (FCS-2020) Advances in electrochemistry and materials science held at Calicut university, Thenjipalam 29-31 january ***2020***