

Carbon Dots as a Fluorescent Sensor for Dopamine Detection

A PROJECT REPORT

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CANDIDATE'S DECLARATION

We, Archi Gupta, Roll No. 23/MSCPHY/76 and Suyash Mishra, Roll No. 23/MSCPHY/79 students of M.Sc. Physics hereby declares that the project Dissertation titled "Carbon Dots as a Fluorescent Sensor for Dopamine Detection" which is submitted by us to the Department of Applied Physics, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: June 9, 2025

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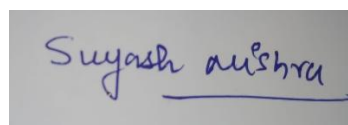
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LIST OF SYMBOLS AND ABBREVIATIONS

CDs	Carbon Dots
DA	Dopamine
PL	Photoluminescence
UP	Ultrapure Water
QY	Quantum Yield
AA	Ascorbic Acid
BSA	Bis-(trimethylsilyl)acetamide
Fruc	Fructose
UA	Uric acid
Hb	Haemoglobin
Glu	Glucose
Th	Thiourea
L-cys	L-cysteine
Gl	Glucotiaon
Vit B12	Vitamin B12
PMT	Photomultiplier tube
XRD	X-ray diffraction
UV-VIS	Ultraviolet – Visible
FT-IR	Fourier Transform Infrared

HR-TEM	High Resolution Transmission Electron Microscopy
IFE	Inner Filter Effect
SQ	Static Quenching
B-H plot	Benesi Hildebrand Plot
S-V plot	Stern-Volmer Plot
LOD	Limit of Detection

ABSTRACT

This study explores the hydrothermal synthesis of carbon dots (CDs) from green tea, along with a thorough analysis to evaluate their size and crystallinity. The average diameter of the CDs is 4.02 ± 0.76 nm. The CDs demonstrate a distinct absorbance peak at 279 nm, revealing excitation-dependent photoluminescence (PL). The rising concentration of dopamine (DA) correlates with a reduction in the PL intensity of the CDs, thereby illustrating the efficacy of quenching. The CD-based sensor exhibits exceptional sensitivity and selectivity, evidenced by a linear Stern-Volmer (S-V) response spanning from 0.0 to 79 μM , with a detection limit established at 3.71 μM . The suggested mechanism for the decrease in photoluminescence intensity involves the inner filter effect and static quenching, which arise from the formation of complexes between CDs and DA.

CHAPTER -1

INTRODUCTION

1.1 Nanotechnology

Human history moving from the Stone Age to modern times proves that the insatiable thirst drives progress. Stone Age history was defined by discovering and utilizing fire, which started burning it and changing human life. An essential requirement for survival coupled with the creative source developed through fire. Another epoch similar to that defining moment of fire is before our eyes now-the Quantum period.

Quantum physics provides an unparalleled possibility to improve material characteristics and technological innovation. Thus, the advance can determine the next course of human civilization. While exploring further into the scientific boundaries, we need to consider the consequences our endeavors would have on the environment. Earth is our home as well as the habitat of innumerable other organisms. We owe ourselves to maintain this planet's ecological balance while seeking technological progress.

The goal is to seek ways to adopt quantum technologies to make sustainable solutions at no cost to the environment's health within the development trajectory. It gives way to exploring both a challenge of innovation and conservancy in laying grounds for fruitful, harmonious ends in equal measure for man and the planet. Nanotechnology is the process of developing and fabricating functional items at the nanoscale. We call the science that underlies nanotechnology nanoscience.

The word "nanotechnology" first used by Professor Norio Taniguchi in 1974, is currently used to refer to a subfield of technology that focuses on structures smaller than 100 nanometers and primarily involves the control of individual atoms and molecules [1]. The revolutionary speech that Dr. Richard P. Feynman gave in 1959, titled "There is plenty of room at the bottom" [2], opened up a new universe of prospects in almost every branch of research. Nanotechnology,

sometimes known as nanoparticle technology, is a term often used to refer to the study and development of nanoparticles in various fields, including medicine, electronics, and agriculture [3]. The tremendous progress in nanotechnology over the last several decades has inspired researchers to develop dependable and practical techniques for producing nanomaterials that vary in size from one nanometer to one hundred nanometers [4]. The peculiar optical, electrical, physicochemical, and magnetic characteristics of nanoparticles are the source of the rising interest in these materials [5]. These properties are markedly different from those of their bulk counterparts. There is a vast variety of forms that carbon may take, including graphite, fullerenes, and diamond. Carbon is the element found in the most incredible abundance on Earth. Within nanotechnology, a new category of carbon nanomaterials, which includes graphene, carbon nanofibers, fullerenes, CDs, and nanodiamonds, has become a focal point of significant attention.

The interdisciplinary nature of nanotechnology fuels innovation, offering many applications in medicine, energy efficiency, and environmental solutions. This reflects the ability to change materials at the nanoscale to solve complex problems in many challenging areas. Nanoscale particles are different from macroscale particles in four ways: At the nanoscale, gravity forces are negligible, but electromagnetic forces dominate.

At the nanoscale, quantum physics is superior to classical physics in explaining motion and energy. The surface-to-volume ratio is higher at the nanoscale, and Random (Brownian) molecular motion becomes significant.

Nanomaterials become highly significant for the complete advancement of human beings.

Scientists use nanomaterials to develop diagnostic and management instruments for diseases that are pandemic worldwide. In 2019, nanomaterials were applied to diagnose and manage COVID-19, a deadly disease affecting many people worldwide. Many applications of nanomaterials are used in the construction and architecture industries. In the year 2020, 800 building products that apply nanotechnology were developed. Nanoparticles are tiny particles of size between 1 to 100 nanometers and can be manipulated at atomic and molecular levels. Its

small size provides some specific physical, chemical, and biological properties different from those in bulk materials. Engineered for specific applications, Nanoparticles have been used in medicine, electronics, optics, and material research. With a composition that includes organic and inorganic metals, polymers, and ceramics, they exhibit higher reactivity, strength, and conductivity [6,7].

1.2 Literature review

1.2.1 Quantum Dots

An investigation in semiconductors has assumed, quite literally, new dimensions. Their values are two, one and zero. Electrons in newly developed devices can be confined to planes, lines, or mathematical points quantum dots.[8]

One of the inventions in nanotechnology comprises nano-sized particles known as QDs. Quantum dots are fluorescent semiconductor nanoparticles consisting of a core material included within a shell made from another semiconductor material, with a diameter ranging from 2 to 10 nm. QDs are semiconductor particles at the nano-meter scale that exhibit distinctive optical and electronic characteristics. QDs are sufficiently small for quantum mechanical effects to prevail. The term "artificial atoms" is used due to their discrete energy levels, which are similar to those of individual atoms, in contrast to bulk materials that exhibit continuous energy bands. Their characteristics render them exceptionally adaptable for uses in bioimaging, photovoltaics, and optoelectronics.[9,10]

1.2.2 Quantum Confinement

The energy of the quantum dot is determined to be $\frac{h^2}{8md^2}$ (where d represents the length of the confinement), rather than $\frac{h^2}{8ma^2}$ (with a denoting the confinement radius), as stated in [11].

Mesoscopic semiconductor systems exhibit a spectrum of behaviors that span from classical to quantum mechanical phenomena. After completing his PhD in solid-state physics at Syracuse University in 1983, Reed became a member of Texas Instruments. His team achieved a

significant milestone by creating the first lithographically defined quantum dots in 1987. Since 1990, he has been a professor of electrical engineering at Yale University. The solution is found within the realm of quantum mechanics, guided by the foundational concepts established by Heisenberg's uncertainty principle. A particle, such as an electron, cannot possess a position with absolute precision while simultaneously having an arbitrary precision in momentum at any specific moment. As the confinement of an electron becomes more stringent, the uncertainty in its momentum increases correspondingly. It extends to exhibit a broader spectrum of momentum, resulting in an increased average energy. Should the electron be restricted to an infinitely narrow layer, its energy would correspondingly become infinite. The discrete energy levels of electrons within a semiconductor are fundamentally constrained by temperature and material's intrinsic properties. The influence of uncertainty principle becomes paramount when electrons are restricted to a very thin layer. Under the assumption that the electrons lack the necessary energy to escape confinement, they behave as if they exist in a purely two-dimensional state. This expression is definitive. Electrons are confined to a two-dimensional plane, restricting their movement in third dimension. In the quantum wire, particles exhibit freedom in a single dimension, while in a quantum dot, particles are confined with no freedom in any dimension. The length scale for a free conduction electron in typical semiconductors is approximately 100 angstroms (Å). An angstrom measures 10^{-10} meters, roughly corresponding to the radius of a hydrogen atom. An electron within a cubic semiconductor structure, measuring 100 angstroms per side, is essentially restricted to a singular point [8].

1.2.3 Carbon Dots (CDs)

CDs were first synthesized in 2004 as a byproduct of the purification of single-walled carbon nanotubes. These nanomaterials have outstanding and highly tunable fluorescence properties that make them suitable for many applications in biomedicine, optronics, sensing, and catalysis. CDs have excellent photostability, nanoscale dimensions, highly tunable PL, biocompatibility, electrochemiluminescence, and excellent multi-photon excitation (up-conversion) properties.

CDs have low toxicity and stability, making them easily functionalized by biomolecules; thus, they can serve as efficient vehicles for drug delivery and biological imaging. On the other hand, significant applications of CDs have been reported in sensors, optronics, and electrochemical luminescence. The synthesis of carbon quantum dots typically involves the surface modification of carbon nanoparticles using organic or polymeric reagents. Most synthesis techniques depend on carbonizing a carbon precursor, which frequently leads to inadequate control over those properties while producing CDs with varying optical properties. Various natural sources, including fruit juice, melon skin, pomelo, food, grass, and other plant leaves, are being utilized to form CDs. Using as a precursor in the hydrothermal carbonization process leads to the synthesis of CDs. Carbon is a black solid traditionally known to be insoluble in water and poorly fluorescent. The attention toward carbon-based quantum dots with good solubility and strong luminescence was extensive. Due to such a phenomenon, they have become known as carbon nano-lights. Significant development of carbon-based systems over the last several years in their synthesis, properties, and applications is an undeniable reality. Photoluminescent carbon-based quantum dots are superior to traditional semiconductor quantum dots and organic dyes, and they have high (aqueous) solubility, robust chemical inertness, facile modification, and high resistance to photobleaching. The excellent biological properties of carbon-based quantum dots, such as low toxicity and good biocompatibility, entrust them with potential applications in bioimaging, biosensors and biomolecule/drug delivery. The outstanding electronic properties of carbon-based quantum dots as electron donors and acceptors, causing chemiluminescence and electrochemical luminescence, bestow broad potentials in optronics, catalysis and sensors[12,13].

1.2.4 Applications of CDs

1. Biomedical and clinical applications: -

a. Imaging through quantum dots: -

Different imaging modalities are performed with quantum dots, encompassing in vivo cells and connective tissue imaging of tumours, along with diagnostic imaging applications. This utilization of quantum dots stands out as one of the most prevalent.[14,15,16].

b. Intracellular imaging: -

In many instances, tissues are quite thick, making penetration through the probe significantly challenging. Consequently, quantum dots are ideal for non-interfering tissue imaging. QDs find application in immunological studies. Experimental evidence demonstrates that the reticuloendothelial system swiftly absorbs the quantum dots. Imaging of lymphatic system and cardiovascular system is achieved using quantum dots. The paramagnetic quantum dots were synthesised and conjugated with Cyclic RGD peptide for targeting human endothelial cells; this agent was employed for tumour investigation. Bioconjugate quantum dots are utilized to label proteins for the purpose of minimally invasive mapping of living cells [14,15,16].

c. Tissue imaging: -

Tissue imaging resents challenges, as various types of sensors and biomarkers within tissues are often difficult to identify and recognise using fluorescence methods. To address this issue, quantum dots are considered beneficial for imaging purposes. Their narrow emission peak facilitates the identification of additional sensors or indicators on a reduced cell surface area. Antibodies linked with quantum dots serve a purpose in tissue imaging [16].

d. Tumour imaging: -

Initially, tumour imaging was performed using fluorescence microscopy; however, it is limited in spatial resolution due to low intensity. Recently, quantum dots have been utilised for tumour imaging due to their high magnitude along with bright luminescence. This advancement is achieved through improved retention and permeation, allowing for the sustained presence of quantum dots in the metastatic site over extended durations[17].

e. Pharmaceutical Application: -

The pharmaceutical domain primarily encompasses both diagnosis and drug delivery mechanisms. Regarding diagnosis, there is a preference for magnetic resonance imaging with metal ions. Recently, quantum dots have attracted interest due to their optical properties, have high photostability, reduced dimensions, and biocompatible features, rendering them appropriate for use in biological systems and the pharmaceutical sector.

2. Drug delivery through quantum dots: -

Aqueous solubility is essential for quantum dots in biological applications. The enhancement of water solubility in quantum dots can be accomplished by conjugating a mercaptan group to them. Following conjugation, there is an increase in the size of the quantum dots, which impedes their clearance by the kidneys and their uptake by the reticuloendothelial system. This can alter the physical characteristics of quantum dots would entail extending their half-life and preventing acidic degradation[18,19].

3. Detection of metastatic cancer cells: -

Metastatic cancer involves the movement of tumour cells from the original organ to other organs via the bloodstream. It disseminates throughout the body and possesses the potential to trigger multiple forms of cancer. In the past, detection systems relied on radiation treatments involving magnetic resonance, which were both expensive and posed risks to patients. Isolating and detecting circulating tumour cells, particularly when they are present in low concentrations, poses significant challenges. In this context, antibody-conjugated quantum dots are utilised as they facilitate real-time imaging. Yali Wang and colleagues conducted a study focused on the detection of micro trap states in lung cancer through the magnetised nanoparticles and QDs arrays[20].

4. Drug release study through QDs: -

The study on drug release conducted with HPLC-MS presents certain limitations, as it assesses the quantities of free and encapsulated drugs independently. The conjugate based on quantum dots and FRET effectively tackles this issue [19]. This method relies on the principles of

fluorescence emission spectra from quantum dots and the excitation spectra of fluorescence receptors, demonstrating the quenching of fluorescence. Improvement of the fluorescence properties of donor molecules and the fluorescence characteristics of acceptor molecules[21].

5. QDs used for gene delivery: -

Gene therapy is a novel strategy for addressing biological diseases and disorders. Among the genetic vectors, plasmid DNA, mRNA, and siRNA are employed for gene silencing. Previously, gene delivery efficiency was significantly hindered by inadequate cellular uptake. However, enhancing quantum dots with various materials has been shown to raise their uptake by cells [21].

6. Neuroscience study through QDs: -

Quantum dots facilitate the exploration of neuronal structures. The study concerning brain delivery highlights a significant limitation: the selective permeability of biological molecules through the blood-brain barrier, which relies on their small size and lipophilicity [22]. Existing literature indicates that carbon dots can readily traverse the blood-brain barrier. Quantum dots offer extended fluorescence and optical properties that are advantageous for brain imaging and drug delivery within the brain. An innovative technique in a recent study has resulted in the creation of biocompatible water-soluble quantum dot micelles that maintain optical properties and improve uptake in hippocampal neurons[15,23].

1.3 Green Tea: -

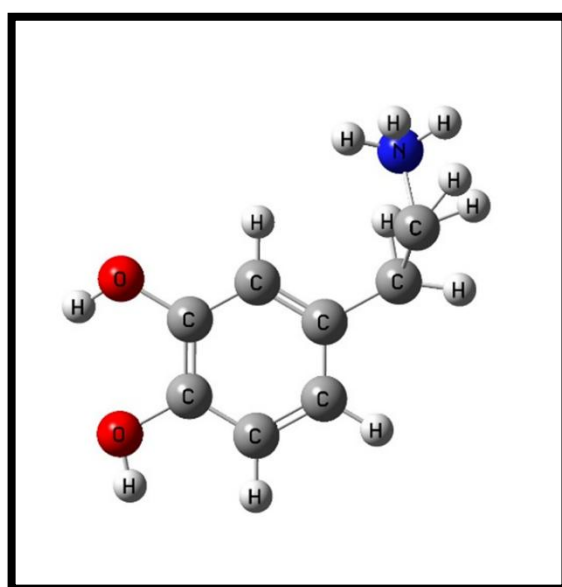
Tea is the world's second most consumed drink, generating around 7.02 billion metric tons of fresh leaves yearly [24]. After use, spent tea leaves (STL) become waste rich in cellulose (5.6–19.3%), hemicellulose (5.2–20.4%), lignin (11.9–26.8%), and bioactive like polyphenols (6.1–19.96%),[25,26] caffeine, and amino acids. Unlike paddy straw, these bioactive make STL more persistent in the environment [24,27].

About 70–80% of tea waste is in landfills or burned [28] which releases methane, VOCs, and phenolic compounds, polluting air, soil, and water.[27] Burning adds particulate matter to the air, while landfill STL leaches phytotoxic tannins, harming microbes and taking up arable land [29].

1.4 Dopamine (DA): -

DA functions as a hormone and an important neurotransmitter within the neurological system. It also substantially influences human conduct because of its role as a hormone in the circulatory system. Within the brain, dopamine is responsible for two crucial activities: it regulates the heart rate, blood pressure, renal function, and vascular activity; it also influences a variety of cognitive processes, including excitement, happiness, pleasure, sleep, learning, and accuracy; and it regulates mental states, including sadness. This may result in symptoms such as a lack of motivation, recurrent learning experiences, variations in mood, exhaustion, emotions of despair and disillusionment, and abnormalities in sleep patterns [30]. Consequently, a shortage of dopamine might appear in these symptoms. Alterations in the number of neurotransmitters are indicative of an extensive range of ailments, including cancer, neurological diseases such as Alzheimer's and Parkinson's, schizophrenia, epilepsy, euphoria, and memory loss, as well as euphoria. For this reason, the development of a DA-level monitoring approach that is sensitive, selective, user-friendly, and accurate is of the utmost importance for the diagnosis and treatment of mental diseases. Methods such as spectrophotometry, electrochemistry, enzymatic methods, and techniques based on chromatography have been described as effective for diagnosing diabetes. Consequently, several studies have been carried out to locate a method that is both exact and cost-effective. Electrochemical and optical procedures are the ones that are used the most often for neurotransmitter detection [31]. This is due to the various properties that each employ. Through the use of a straightforward, economical, and environmentally friendly

hydrothermal process, we were able to produce CDs from Assam tea in the current investigation. Not only does the use of a natural precursor guarantee sustainability, but it also removes the need for using harsh chemicals and complex surface passivation methods. In their manufactured state, the CDs demonstrated remarkable PL capabilities and were used as optical probes to detect DA sensitively and selectively. The synthesized CDs are viable for DA sensing because of their good PL behaviour, high water solubility, biocompatibility, and low toxicity. These characteristics set them apart from other candidates. This study proposes a method that is both environmentally friendly and cost-effective for the fabrication of carbon-based fluorescent nanoprobe, which have the potential to be used in biosensing and clinical diagnostics processes.



Scheme 1. Structure of DA.

CHAPTER -2

CDs AND IT'S SYNTHESIS APPROACH

2.1 Quantum Dots

Man-made nanoscale crystals known as QDs have unique optical and electrical characteristics. When exposed to UV light, they may transport electrons and release a variety of colored lights. These synthetic semiconductor nanoparticles offer a plethora of possible uses, such as solar cells, composites, displays, illumination, fluorescent biological labelling, and medical imaging. Types of quantum dots, the quantum confinement effect arises because of the dividing of the energy levels in QDs, leading to an increase in semiconductor energy gap as the nanocrystal size decreases. Figure 2a illustrates the various types of quantum dots through a pictorial representation. QDs can be categorized into three main types:

- (a) according to their composition or structure
- (b) based on their size
- (c) depending on the material utilized for their preparation.

a. According to structure, QDs are mainly classified as

1. Core-Type Quantum Dots
2. Core-Shell Quantum Dots
3. Alloyed Quantum Dots.

b. According to size, QDs are classified as

They are divided into two types i.e. larger QDs and smaller QDs.

Larger QDs (LQDs): - LQDs have a diameter of 5–6 nm and emit shorter frequency with orange or red colored emission. For example: Photo voltaic and LED.

Smaller QDs (SQDs): - They have a diameter of approximately 2–3 nm and emit shorter wavelengths with blue or green colored emission. Upon addition of ligand, carrier molecule and polymer coating; its size can be increased. These QDs are used in imaging, biological

applications and biosensors.

c. According to the material used: -

QDs are classified as Quantum dots and are divided into two types:

- i. Semiconductor quantum dots
- ii. carbon-based quantum dots

i. Semiconductor QDs: -

Semiconductor quantum dots were first come across in 1981. Semiconductor QDs are three-dimensional nanoparticles at the nanometre scale, composed of inorganic materials such as heavy metals and non-heavy materials. These entities exhibit a spherical morphology, characterized by a crystalline structure and dimensions of less than 6nm. The properties include size-dependent photoluminescence, excitation-independent photoluminescence, and a long lifespan. These substances exhibit amphiphilic characteristics, possessing an extensive surface area along with excellent photostability, semiconductor properties, and colloidal behaviour. Their benefits in terms of functionalization are more pronounced. Semiconductor quantum dots, commonly referred to as quantum dots, are synthesized using a bottom-up approach.

ii. Carbon dots (CDs): -

Much attention has been focused on the most recent member of this family, CDs, which are generally less than 10 nm [32]. CDs are a fascinating field of research that falls under the umbrella of carbon nanomaterials. Because of their unique properties, which include a high degree of biocompatibility, a low level of toxicity, and favourable solubility, they are an attractive alternative to the typical semiconductor quantum dots [33]. It is widely accepted that CDs are carbon-based materials with a quasi-zero dimension, and fluorescence is the defining characteristic of these materials. In 2004, fluorescent carbon nanoparticles were published for the first time. This was the result of an unexpected finding that occurred while purifying single-walled carbon nanotubes [34] . Researchers have developed a special interest in CD fluorescent

and photoluminescent features [35]. This interest is primarily because CDs have applications in biomedicine, catalysis, sensing, cell imaging, fluorescence staining, and diagnostic examination. The hydrothermal carbonisation method is popular because of its uncomplicated simplicity, economic benefits, and environmentally benign properties. CDs generated from waste biomass have lately been shown to possess many potentials, including their availability, affordability, non-toxicity, and biocompatibility [36]. The hydrothermal approach does not necessitate strong acids or post-synthesis surface passivation operations [37]. This particular method is advantageous when natural sources are employed as starting materials. Techniques of biosynthesis that make use of natural resources, such as sugarcane [38], wheat straw [39], coriander [40], and other natural resources, have been more popular in recent years. In comparison to more traditional methods of synthesis, these methodologies make use of ingredients that are readily accessible and provide more economical options. The cores of CDs are generally amorphous or nanocrystalline, and most of their constituents are carbon atoms that have been hybridised with hydrogen sp^2 [41].

2.2 Synthesis Approach

Different strategies are used to synthesize nanomaterials, each with the aim of achieving particular characteristics and uses. Common methods for synthesizing nanomaterials include the following.

1.Top-Down approach: -

Using this technique, the bulk is split into smaller pieces, which are further broken into nanomaterials.

Integrated circuit manufacturing serves as an illustration of this methodology. The division of macrocrystalline structures forms nanocrystalline structures.

a. Mechanicanosynthetic Methods: -

Mechanical techniques provide the most cost-effective approaches for the large-scale production of nanomaterials. Ball milling stands out as one of the simplest ways available. Ball milling generates nanomaterials through mechanical attrition, where kinetic energy from a grinding medium is imparted to a material that is being reduced [42].

b. Lithographic Methods: -

Widely recognized methods are energy-intensive and demand costly equipment and facilities. However, they are top-down techniques that primarily produce micron-sized features. Lithography has been used to produce printed circuits and computer boards for several decades [42].

c. Laser Ablation: -

The interaction of a laser beam with a solid or liquid surface results in the ejection of materials from that surface. At lower levels of flux, particularly, the heat generated from the interaction between the laser beam and the surface often leads to the sublimation or evaporation of the material present on it. However, typically, the material at higher levels of flux experiences a transformation into plasma. Typically, "laser ablation" denotes the process of removing material through the application of a pulsed laser. A continuous wave laser beam may also be employed for material ablation, given that the laser intensity is sufficiently high [43].

2. Bottom-Up approach: -

Nanomaterials are created by assembling the tiniest units, or atoms. It is employed in constructing fundamental materials, such as the assembly of atoms and molecules.

Sol-gel, hydrothermal, physical, and chemical vapor deposition are a few of the techniques of Bottom-Up approach.

a. Chemical Vapour Deposition: -

Chemical vapour deposition is a gas-phase process wherein reactive constituents interact over a catalyst or pre-templated surface to yield nanostructured materials. The cost-effective production of carbon nanotubes is achieved through chemical vapour deposition [42] .

b. Sol-Gel: -

The sol-gel process typically employs the use of metal alkoxides or organometallic, inorganic salts as the precursors. In the process, the precursor" 's series of hydrolysis and polycondensation reaction form a colloidal suspension or a sol. The sol-gel process is described as the conversion of a system from a liquid "sol" (mostly colloidal) into a solid "gel" phase. Then, the gel was dried and calcinated at different temperatures in order to retrieve the metal oxide nano-powder. Sol-gel method has the unique option of tuning the target products' shape, morphology, and textural properties. In comparing it with the high-temperature technique, a range of significant advantages enable the formation of the metastable system, which is very hard and impossible for most temperature techniques: having enhanced purity and compositional homogenizing of the product with a moderate temper[42].

b. Hydrothermal Method: -

Hydrothermal synthesis generally takes place within a pressurised apparatus known as an autoclave, employing an aqueous solution to facilitate the reaction . The temperature within the autoclave can surpass the boiling point of water, thereby attaining the pressure of vapour saturation. Hydrothermal synthesis is a widely utilised technique for the preparation of metal oxide nanoparticles, accomplished through the hydrothermal treatment of peptised precipitates obtained from a metal precursor in an aqueous environment. The hydrothermal method has demonstrated efficacy in controlling grain size, particle morphology, crystalline phase, and

surface chemistry through the regulation of solution composition, reaction temperature, pressure, and solvent characteristics[42].

2.2.1 Synthesis Methods for CDs

CDs can be created using various techniques, each with unique benefits and drawbacks. The size, shape, intended qualities of the nanoparticles, and the intended use all influence the synthesis technique selection. The following are some typical methods for creating nanoparticles:

Two approaches prepare methods for preparation of carbon quantum dots Carbon quantum dots. The first is the top-to-bottom approach, which includes converting larger carbon components into smaller ones. The other is the bottom-up approach, where the smaller carbon compounds are merged to produce the larger compounds.

Following methods are used for the preparation of carbon quantum dots-

- a. Arc discharge technique
- b. Laser Ablation technique (discussed earlier)
- c. Microwave ablation
- d. Electrochemical oxidation
- e. Hydrothermal technique/solvothermal treatment. (previously discussed)

a. Arc Discharge Technique: -

This approach utilizes a hierarchical strategy. This approach entails transforming carbonaceous materials, particularly single-walled carbon nanotubes, into smaller compounds that exhibit

size-dependent characteristics. The main carbon precursor decomposes in a sealed reactor's anodic electrode, leading to high-energy plasma formation. The arrangement of carbon atoms is altered. Consequently, when incorporated into a cathode, the carbon dots produced through this method exhibit excellent solubility in aqueous environments [21].

c. Microwave Ablation: -

CDs are synthesized from carbon-based precursor molecules. The precursors utilized in this study include carboxylic acid, citric acid, and carbohydrate-based compounds, specifically glucose and fructose. This method employs microwave radiation in conjunction with water-solubilizing surfactants to enhance the carbonization of various precursors. PEG surfactants enhance the emission properties of quantum dots and facilitate surface passivation. The incorporation of inorganic ions improves carbonization and elevates quantum yield. As reported in the literature, fluorescent carbon dots have been synthesized from diverse sugars and PEG through microwave methods. This method synthesizes oxygen-rich CDs and carbon-based electrocatalysts. Nasser Arsalani et al. synthesized gelatin-based CDs, which were passivated with polyethylene glycol using a microwave-assisted method. The carbon dots were designed for the delivery of methotrexate. Quantum dots produced via this technique are utilized in bio-imaging and cellular staining applications[21,44,45].

d. Electrochemical oxidation: -

This method employs a top-down approach and operates under mild conditions. This technique utilizes graphene, carbon nanotubes, and nanofibers as bulk carbon materials. The electrolysis reaction takes place in both acidic and basic environments. Various post-treatments are employed to enhance the solubility and fluorescence efficiency of the synthesized CDs. The synthesis of multi-walled carbon nanotubes involves the rolling of graphene layers on carbon paper, subsequently followed by chemical vapour deposition. For instance - fluorescent carbon quantum dots were synthesized using this approach from multi-walled carbon nanotubes in the presence of tetrabutylammonium ion in acetonitrile,

yielding CDs with a size of 0.5 nm. Their emissive range spans from 400 to 500 nm, exhibiting a quantum efficacy of 6.5%. CDs synthesized through electrochemical methods demonstrate effectiveness in electronic applications, catalysis, and biological imaging. Ganesan Muthusankar and colleagues synthesized nitrogen-doped carbon dots via an electrochemical process combined with copper oxide for the selective detection of NSAIDs in berries [21,44,46].

CHAPTER - 3

HYDROTHERMAL SYNTHESIS OF CDs

3.1 Hydrothermal method

The hydrothermal technique uses water as a solvent in a closed system at a certain temperature and pressure to complete the reaction, simulating the formation of crystals during the mineralization process in the sample. Properties of water, such as vapor pressure, density, viscosity, surface tension, and ionic product, will all alter significantly in hydrothermal conditions. It can clearly lower system reaction temperatures and produce highly crystalline products with small size distributions, excellent purity, and little aggregation [47,48,49].

3.2 Synthesis process

3.2.1 Green Synthesis: -

The transformation of potentially abundant biomass into materials of technical significance has emerged as a recent area of interest. Experts are currently seeking next-generation materials that are environmentally sustainable and cost-effective. A bottom-up technique outperforms a top-down strategy, as it results in fewer surface defects on CDs and achieves a higher production yield. The bottom-up technique also includes the synthesis of CDs through hydrothermal treatment [50].

Biomass waste, described from its green, affordable, abundant, easily accessible, and carbon-rich nature, introduces a promising option as a precursor for creating CDs. The management and

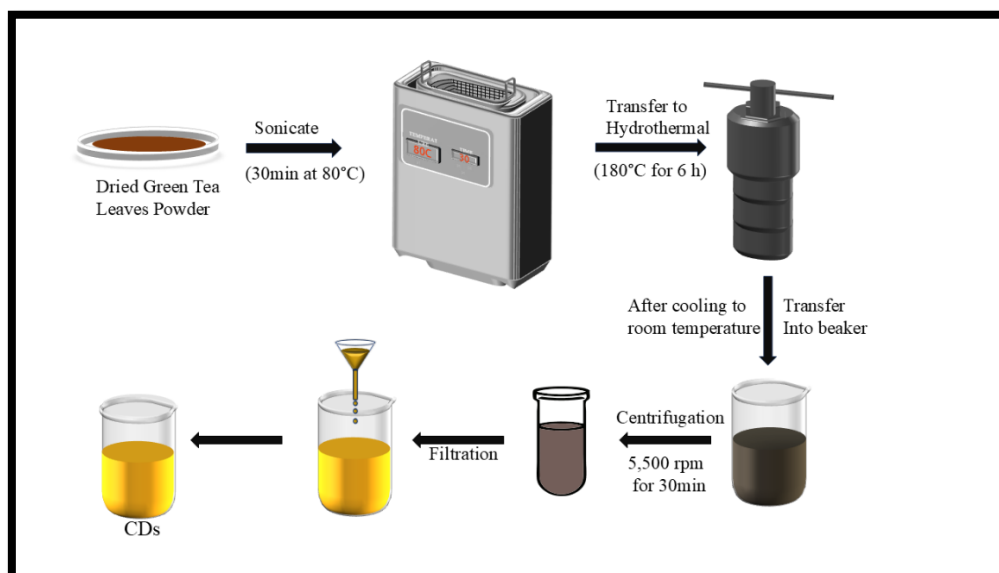
renewal of biomass waste presents an important confrontation in today's world. Effective use of biomass waste not only helps in reusable obstacles but also gives a low- budget source for CDs synthesis. This plan considered using biomass waste as a low-budget and sustainable precursor.

3.3 Materials

Organic handcrafted green tea was collected from the Charaideo District in Assam, India, as a precursor for creating CDs. This tea was then stored in arid conditions at ambient temperature. (C₈H₁₁NO₂), which was the chemical formula of dopamine. The Dopamine (C₈H₁₂ClNO₂), which was the salt form of dopamine, was acquired from CDH chemicals. The analytes Ascorbic acid (AA), Urea, Fructose (Fruc), Uric acid (UA), Haemoglobin (Hb), Bis-(trimethylsilyl) acetamide (BSA), Glucose (Glu), Glucotiaon (Gl), Thiourea (Th), L-cysteine (L-cys), Vitamin B12 (Vit B12) were present in lab. Ultrapure (UP) water was used to prepare solutions for testing purposes.

3.4 Sample preparation

Using an ultrasonic processor, 0.5 g of green tea powder was mixed in 30 mL of UP water, and the mixture was then ultrasonically treated for 30 minutes at 80 °C. For six hours, the mixture was heated to 180 °C in an autoclave chamber coated with Teflon. To get rid of the sediment, the mixture was put into the centrifuge tube and swirled for 30 minutes at 5500 rpm once it had cooled to room temperature. A filter paper was used to filter the supernatant. The practicality and viability of our study were confirmed by the use of the filtered liquid, which was the result of an inexpensive and simple synthesis method, for further characterization with little modification, as reported in [51] as shown in Scheme 2.



Scheme 2. Schematic representation of the synthesis process of CDs

3.5 Characterization Technique

Several characterization techniques are employed to analyze CDs, elucidating their structural, optical, and chemical properties. Using a Thermo Fisher Technai 200 device, high-resolution transmission electron microscopy (HR-TEM). X-ray diffraction (XRD) studies produced high-resolution diffraction patterns using a Rigaku K-alpha system that helped to identify crystalline phases and evaluate lattice parameters precisely. UV-vis spectroscopy experiments were conducted to analyze absorption spectra utilising a dual-beam UV/VIS/NIR spectrometer (Lambda 750, Perkin Elmer, USA). A quartz cuvette with an optical path length of 10 mm was utilized for the samples. The Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon) was employed to obtain PL and PL-excitation measurements, set up with a PMT, a 450W Xenon lamp, and flash lamps. The Fourier Transform Infrared (FT-IR) analysis was conducted using the Nicolet iS50 Tri-detector system, enhanced with a gold flex spectrometer and gold optics, providing precision and exceptional resolution of 0.09 cm^{-1} . The integrated Diamond ATR module enhances the ability to analyse the $400\text{--}4000\text{ cm}^{-1}$ range, provides insight into molecular vibrations, and facilitates identifying significant functional groups in the CDs

CHAPTER - 4

RESULTS AND DISCUSSION

4.1 Structural and morphological characteristics of CDs

While the amorphous nature of the CDs implies that they are sp^3 hybridized [31], the strong peak detected at 23.9 degrees in the X-ray diffraction pattern of the CDs corresponds to the (002) plane, which has a graphitic character. This is seen in Fig. 1.

The vibrations related to hydroxyl and amine functional groups situated on the surface of the CDs are represented by the FT-IR peak identified at 3318 cm^{-1} . The elongation of O-H and N-H hydrogen bonds causes these vibrations [52,53]. The hydrophilic properties of the CDs, which are essential for their absorption in water, have been validated by the peaks that have been seen. Both the optical characteristics and the stability of carbon dots are improved due to the presence of these functional groups, which shows that their effective passivation does so [52,53]. The stretching vibrations of the nitrile ($C\equiv N$) bond give rise to the peak at 2088 cm^{-1} [54,55]. The observations of a second peak at 1635 cm^{-1} may indicate the existence of one or more functional groups. $C=O$ stretching, which is connected with carboxyl or carbonyl groups; $C=C$ stretching, which is related to the structure of graphitic carbon; and perhaps $C=N$ stretching or N-H bending vibrations are three examples of these types of vibrations [54]. A partial graphitization of the carbon core is shown by the peaks that were found, which show presence of oxygen-containing functional groups on surface of CDs. As demonstrated in Fig. 2, HR-TEM analysis was used to investigate the synthesized CDs, as shown in Fig. 3a. The last peak seen at 1016 cm^{-1} is suggestive of C-O stretching vibrations present in alcohols, ethers, or esters [55]. Put sample solution on a grid made of copper, and then let it dry at room temperature for a while. According to the data shown in Figure 3b, the average dimensions of the disseminated CDs were determined to be 4.02 ± 0.76 nanometers. It has been revealed that the spherical particles exhibit a substantial degree of polydispersity.

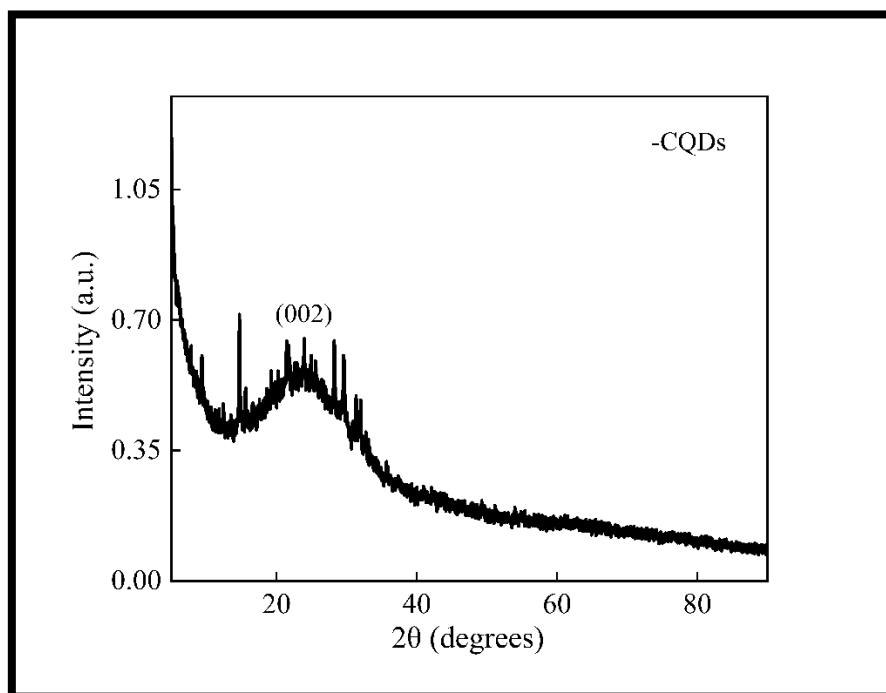


Fig. 1. XRD pattern of the obtained CDs deposited on the thin film

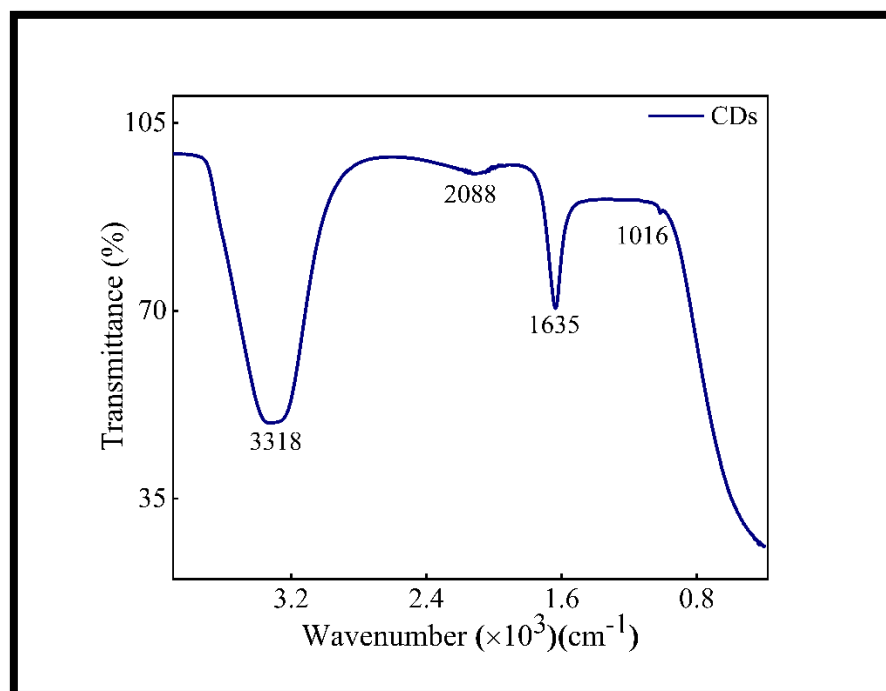


Fig. 2. FTIR spectrum of synthesized CDs dispersed in water.

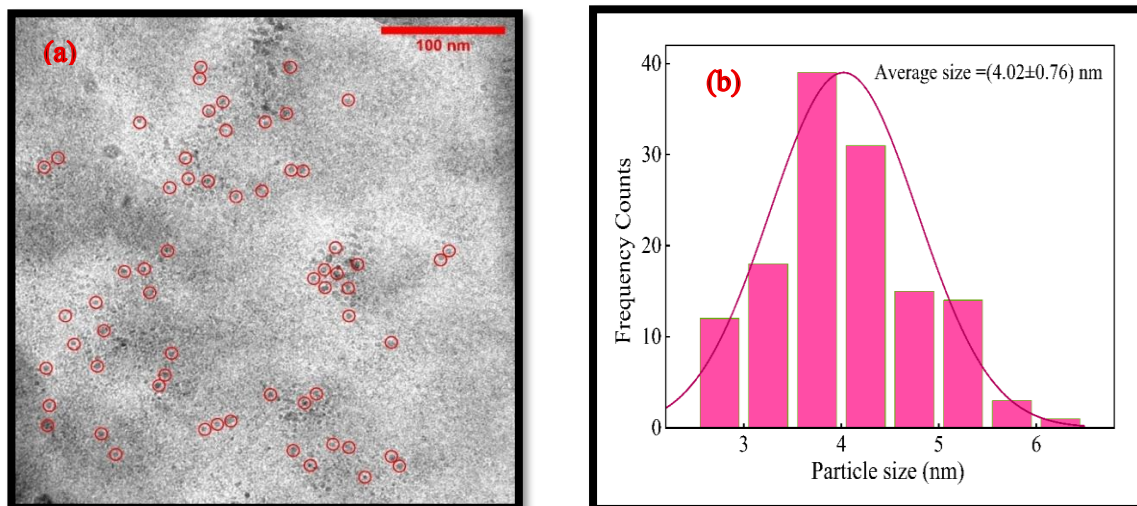


Fig. 3. HR-TEM images of obtained CDs (a) with particle size distribution (b) optical properties of the synthesized CDs

According to the second derivative of the UV-vis spectra, the most noticeable peak of CDs is recorded at 279 nm [56], which is obtained from green tea leaves. This peak is shown in Figure 4. The peak indicates an electronic transition between π and π^* , which is associated with aromatic systems. This suggests a conjugated π -electron system due to the presence of C=C bonds with sp^2 hybridized carbon domains in the CDs, which implies a partial graphitic carbon core [57,58,59,60]. In addition, consistent UV-vis spectra have been recorded from various precursors, which is evidence that aromatic carbon structures have been preserved [60,61]. The Tauc method involves plotting $(\alpha h\nu)^n$ against photon energy ($h\nu$). The absorption coefficient, denoted as α alpha, is determined by calculating the absorbance (A) using the formula $\alpha = 2.303 \times \frac{A}{d}$ (where d represents the path length of the cuvette). The value of n is determined by the characteristics of electronic transition, with $n=2$ for direct allowed transitions and $n = \frac{1}{2}$ for an indirect allowed

transition [62,63]. There is a linear zone close to the absorption edge, as shown by the indirect Tauc plot, which is represented by the equation $((\alpha h\nu)^{1/2} \text{ vs. } h\nu)$ [64]. The extrapolation of this linear section to the energy axis $(\alpha h\nu)^{1/2} = 0$ results in an optical bandgap energy of about 3.8 eV, as seen in Fig.6. The presence of an indirect bandgap indicates that electronic transitions need a shift in momentum that is aided by phonons and is influenced by surface states and defects in the CDs.

As shown in Fig. 7a, the CDs have a strong photoluminescence (PL) with an emission wavelength of 435 nm and an excitation wavelength of 340 nm. Additionally, Fig. 7b displays the normalized (PL). Quantum confinement, the development of aromatic compounds, and surface traps are some of the factors that may affect their characteristics. The recombination of excitons is the driving force behind the observed emissions [65,66]. When stimulated at 340 nm, the PL emission at 435 nm implies that the carbon core and surface functional groups contribute to the PL. The emission wavelength serves as a measure of the degree of conjugation [67,68]. The interaction between the core and surface states makes it possible to alter characteristics, which are impacted by the amount of surface passivation and the existence of PL [68,69]. In addition, Quinine sulphate, which has a QY of 54%, was used as a reference to determine that the QY of GQDs is 3.2% by applying the formula provided by Equation (1).

$$QY_{\text{sam}} = QY_{\text{ref}} \times \left(\frac{PL_{\text{sam}}}{PL_{\text{ref}}} \right) \times \left(\frac{Abs_{\text{ref}}}{Abs_{\text{sam}}} \right) \times \left(\frac{\eta_{\text{sam}}}{\eta_{\text{ref}}} \right)^2 \text{-----(1)}$$

The quantum yield of quinine sulphate is denoted by the symbol QY_{ref} , which serves as a reference, while the symbol QY_{sam} represents the quantum yield associated with CDs. While the reference and CDs are responsible for light absorption, which is represented by the symbol Abs , integrated areas of the PL spectra are represented by the symbols PL_{sam} and PL_{ref} . In addition, the symbol η represents refractive index of UP water and sulphuric acid, which are used as the solvents for CDs and reference standard.

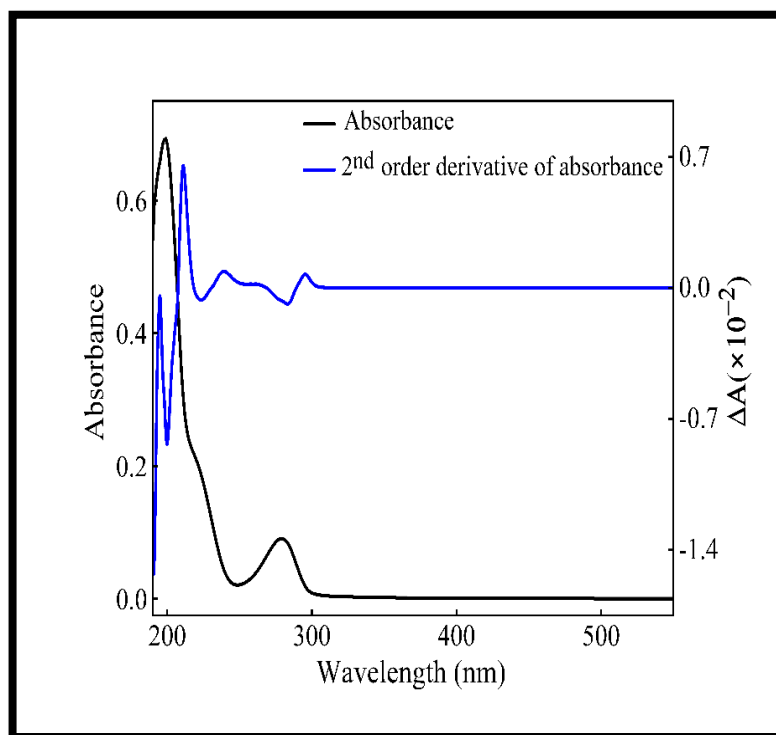


Fig. 4. Absorbance spectrum of synthesized CDs with its 2nd derivative

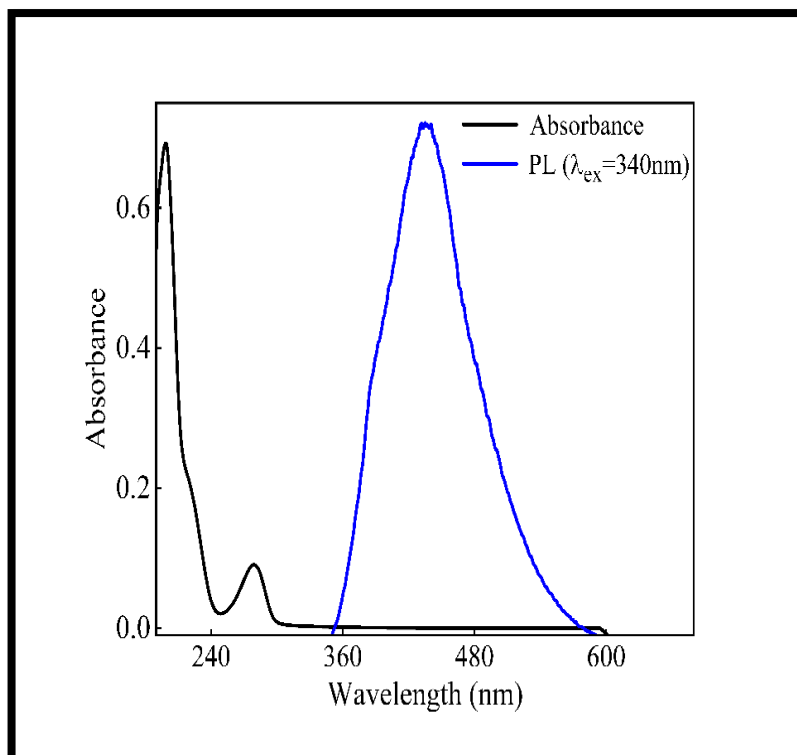


Fig. 5: Overlapped absorbance and PL spectra with excitation wavelength 340 nm of CDs

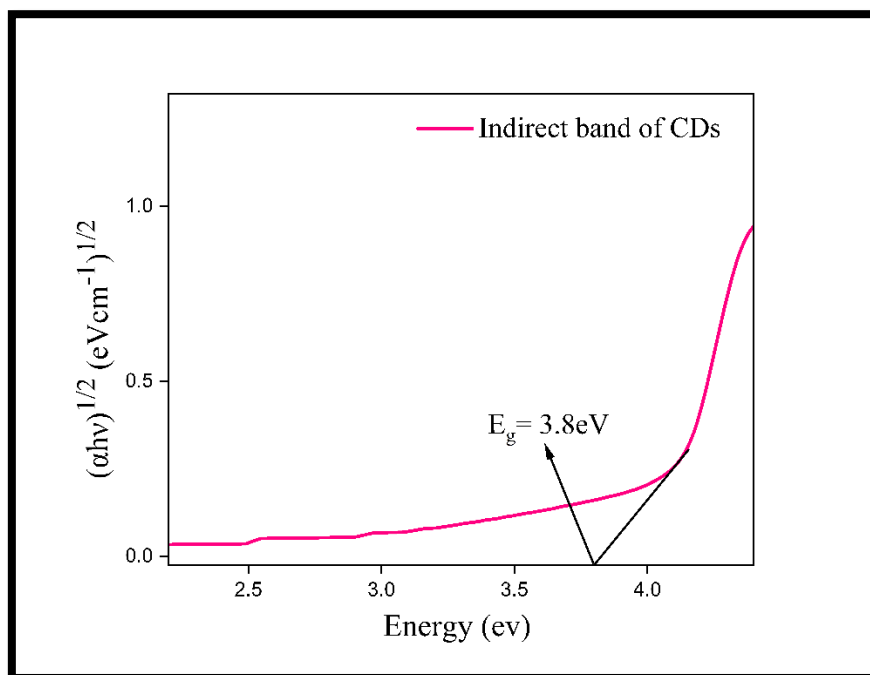


Fig. 6. Tauc plot by UV-vis spectrum of CDs which is shown in the figure.

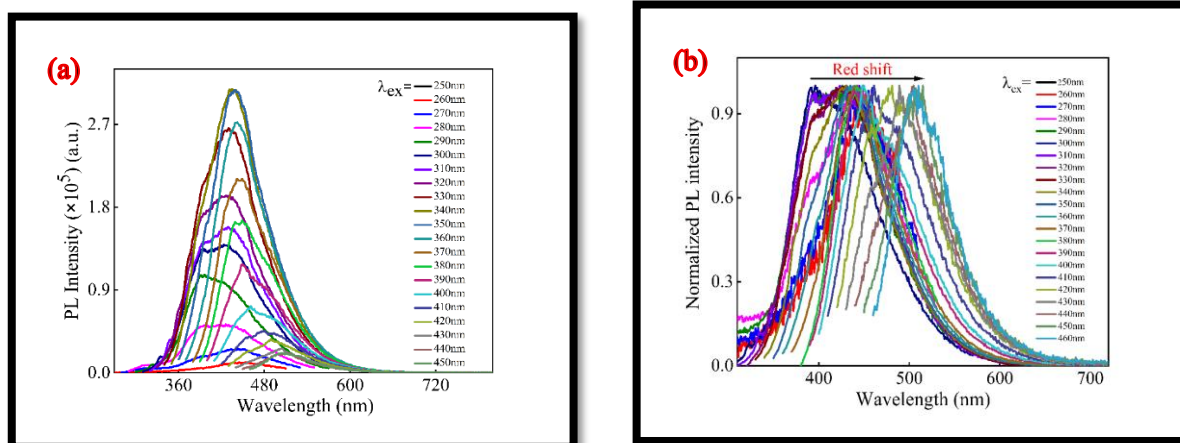


Fig. 7. PL spectra of CDs at the different excitations ranging from 250 - 450 nm (a) and normalized PL spectra at different excitations of CDs (b).

4.3. DA sensing

4.3.1 Effect of DA on the absorbance and PL of CDs

Fig. 8a presents the relationship between concentration and absorbance, highlighting an increase at 279 nm, with saturation observed at $\geq 79 \mu\text{M}$ of DA concentrations. The detailed formation of CD-DA complexes demonstrates static quenching mechanisms related to the ground state, evidenced by a consistent fluorescence lifetime corresponding to the complex [70]. The linear Benesi-Hildebrand (BH) plot ($R^2=0.96$) confirms a 1:1 binding stoichiometry, yielding a binding constant (K_b) of $2.05 \times 10^4 \text{ M}^{-1}$, consistent with moderate-affinity CD-based sensors [71,72]. In Fig. 8b, the modified spectra suggest interactions on the surface mediated by hydroxyl groups, while the catechol groups of DAs interact with the oxygen functionalities of CDs through hydrogen bonding and π - π stacking [73,74].

Adding DA significantly diminished the fluorescence intensity of the generated CD dots when subjected to identical experimental conditions. In light of this phenomenon, we propose that a cost-effective fluorescent probe for determining Dopamine could be developed through fluorescence quenching [31]. Dynamic and static quenching represent two prevalent techniques for photoluminescence quenching. The quencher and the excited-state fluorescent molecule interact through diffusion in dynamic quenching. The static quenching mechanism involves the interaction between the quencher and the ground-state fluorescent molecule, forming a complex. To clarify the quenching mechanism, the S-V equation presented in Eq 2 can be utilized:

$$\frac{F_0}{F} = k_{sv}[Q] + 1 \text{ -----(2)}$$

In this equation, k_{sv} represents the S-V quenching constant, calculated as $k_{sv}=7.9 \times 10^4 \text{ M}^{-1}$. The variable $[Q]$ denotes the DA concentration, while F_0 and F indicate the PL intensities of the CDs at 435 nm in the presence and absence of DA, respectively. Fig. 9b demonstrates a linear relationship between $\frac{F_0}{F}$ and DA concentration across the range of 0.1–79 μM , with an R^2 value of 0.96 [75]. Fig.9b demonstrates the progressive quenching of the PL of CDs with the addition of

different concentrations of DA. Additionally, Fig. 9a demonstrated a significant linear correlation between PL quenching and DA concentration within the 0–79 μM . The estimated detection limit (LoD) for DA, calculated using the formula $3\sigma/K$, is 3.71 μM [76]. In this context, σ represents the standard deviation derived from the PL spectra of blank QDs, while K denotes the corresponding slope.

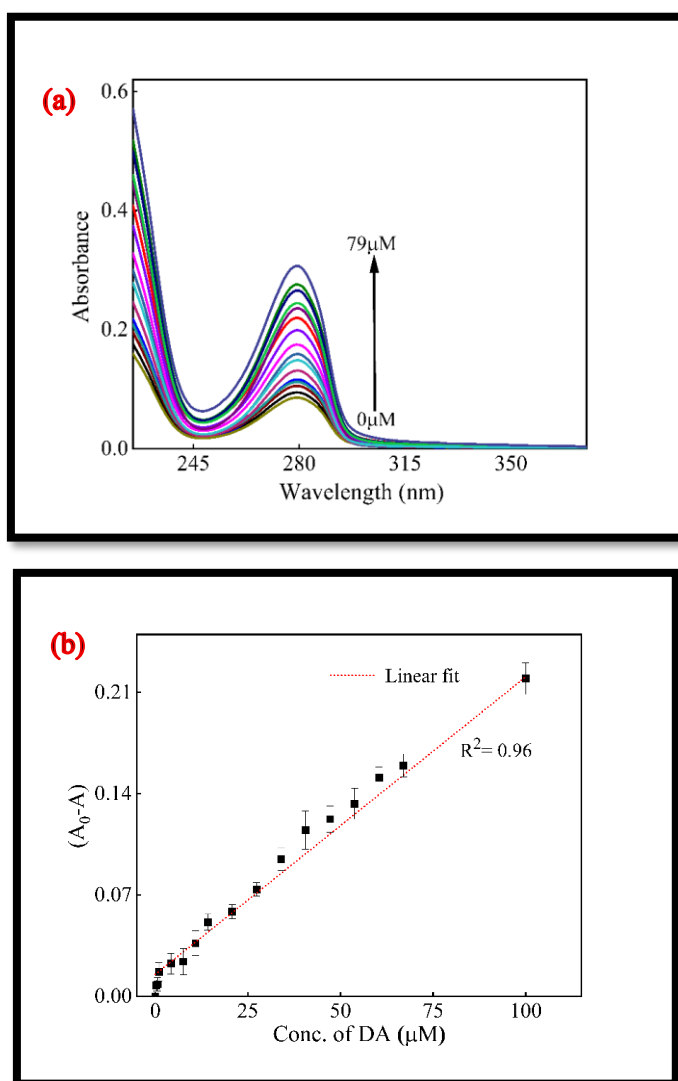


Fig. 8. Absorbance spectra of CDs in the absence and presence of various (0.00 - 79 μM) concentrations of DA (a) and the corresponding B-H plot (b).

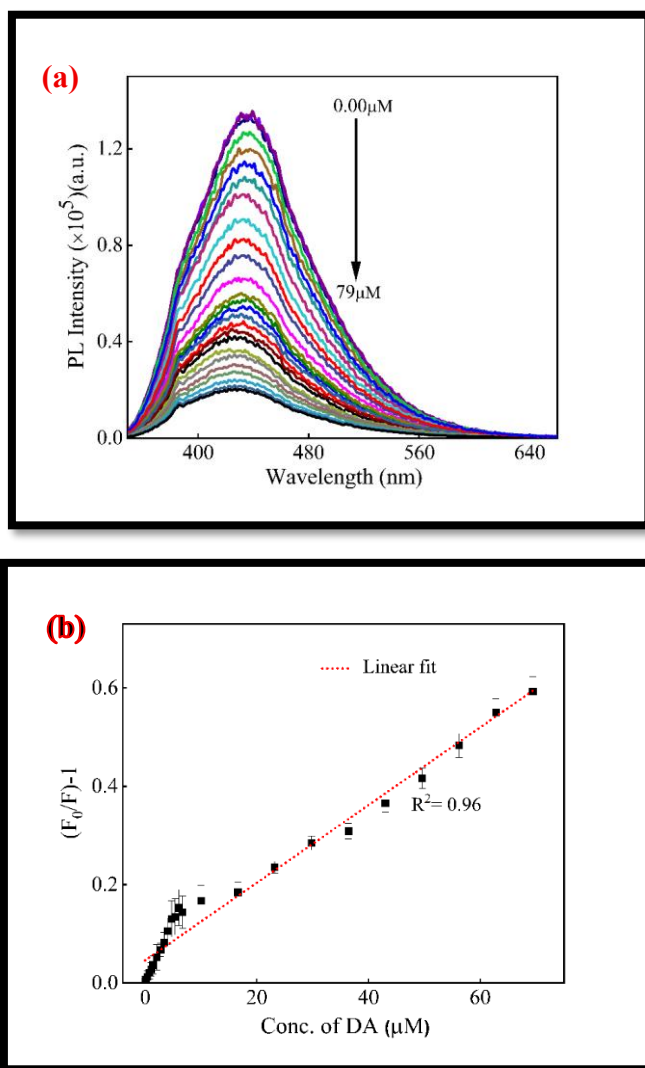


Fig. 9. PL spectra of CDs in the absence and presence of various (0.00 -79 μM) concentration of DA with 340 nm excitation wavelength (a) and the corresponding linear S-V plot (b).

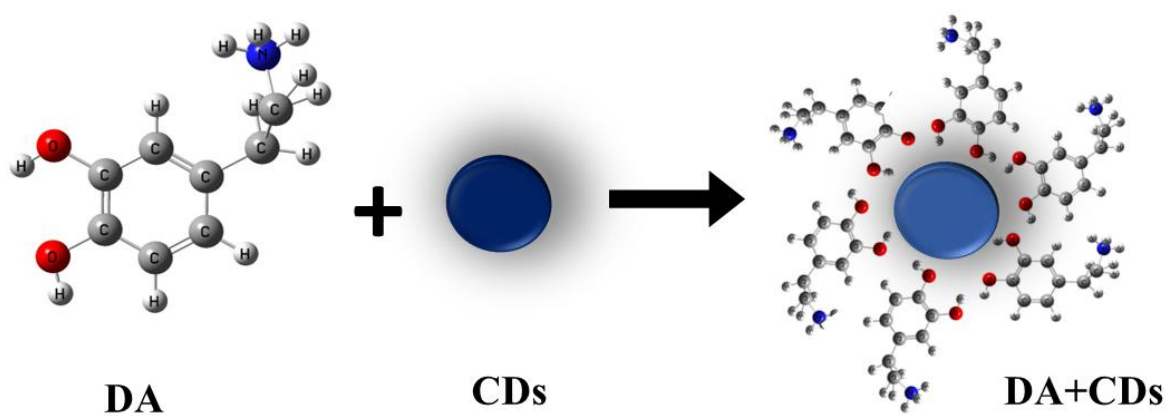


Fig. 10. Complex formation by the interaction of CDs with DA.

4.3.2 Selectivity and Sensitivity

Researchers evaluated the impact of chemicals and biomolecules widely found in human blood on the measurement of dopamine to determine the selectivity of the proposed method for dopamine sensing. CDs were added to the solutions of biomolecules that were analyzed, which comprised the analytes AA, Urea, Fruc, UA, Hb, BSA, Glu, Gl, Th, L-cys, and Vitamin B12, as well as inorganic ions such as Ca^{2+} , Mg^{2+} , Hg^{3+} , Al^{3+} , and Fe^{2+} (Fig. 11a). The fact that these proteins and ions caused minor changes in CDs PL is readily apparent. Additionally, DA, which was present in conjunction with the interferents that were mentioned before, was used in the comparison testing. According to the results 11b, these experiments were carried out at room temperature to establish that the compounds in question had a negligible impact on DA, which may be quantified [73].

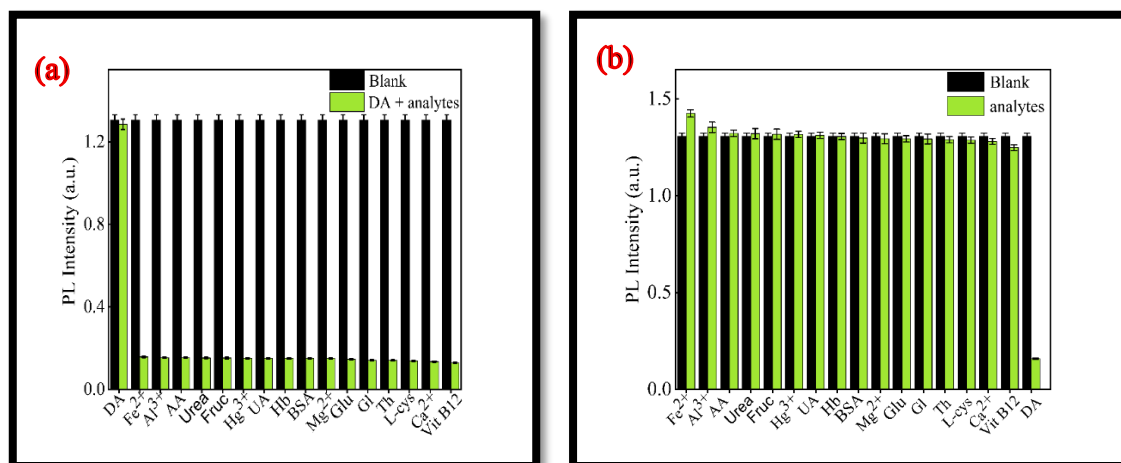


Fig. 11. The bar graph of PL intensity quenching of CDs with fixed concentration (79 μM) of different analytes (a) and with a fixed concentration of DA (79 μM) along with different analytes (79 μM) representing the anti-interference effect (b). The green bar shows the change in PL intensity in the presence of various analytes, whereas the black bars of (a) and (b) represent the PL intensity in the absence of analytes.

4.3.3 Proposed Mechanism of CDs

Uncertainty persists on the mechanism of CDs. The luminescence of CDs is affected by a wide variety of parameters, one of which is the recombination of electron-hole pairs that result from

photogeneration on surface of CDs. The excitation of electrons in radiative traps is the end consequence of this process, which is influenced by several factors, including the quantum confinement effect [68]. CDs are influenced by the effectiveness of electron and hole recombination, which is accomplished by interactions between molecules or ions and the surface groups of CDs [77].

Displays the overlap that exists between the CD excitation spectra and the DA absorbance spectra. A significant overlap exists between the peak of DA and the excitation of DA. Because DA absorbs some excitation light owing to spectral overlap, the IFE is principally responsible for governing the PL quenching of DA by CDs. Through this particular form, the DA is directly responsible for absorbing light. As a consequence of this, the fluorescence intensity is reduced as a result of the effective excitation of CD [78,79,80]. Evidence from reports and observations from the literature suggests that static quenching plays a role in the process. The formation of non-fluorescent ground-state complexes between DA and CDs results in static quenching, reducing the intensity of the fluorescence detected [80,81]. It has been claimed that complex formation may cause IFE and static quenching in CD-based DA sensors [63]. This phenomenon has been seen in Fig. 12. The sensitivity and selectivity of CD-based PL sensors for DA detection are improved due to this dual process. During ten months, the researchers monitored the PL intensity at 435 nm to evaluate the CDs' level of stability. The extraordinary consistency of CDs is shown by the fact that there is no change in intensity, as seen in Fig. 13b. In addition, researchers investigated the several ways in which pH affected the PL properties of CDs. Through a wide pH range extending from 2.0 to 12.0, the following data reveals that the PL intensity remains unchanged almost the whole time. This suggests that CDs are very stable and have a remarkable tolerance to pH fluctuations in solution, as seen in Fig. 13a.

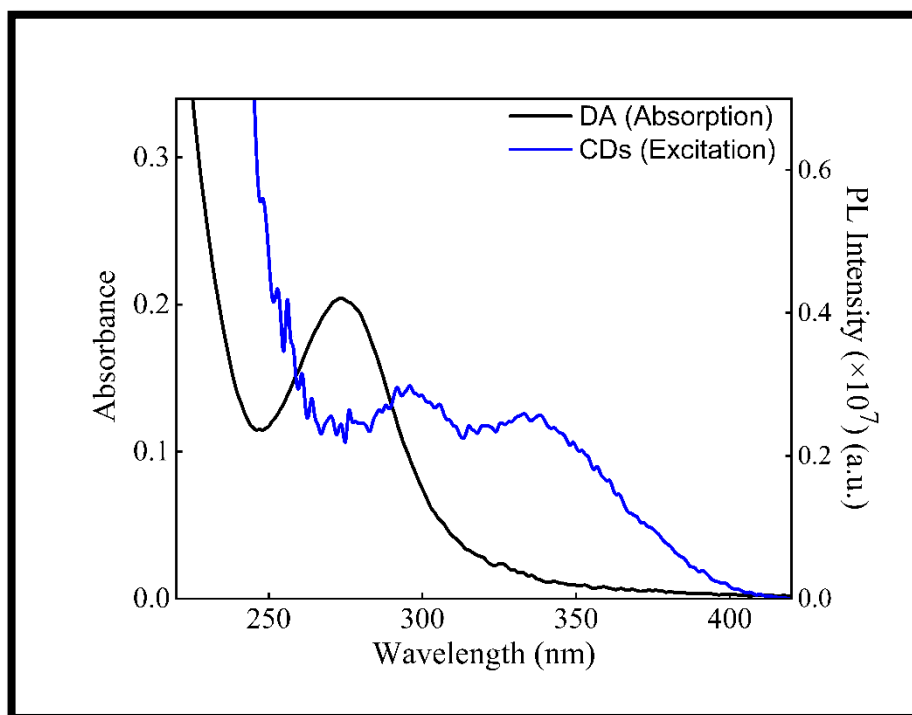


Fig. 12. Spectral overlap of the absorption spectrum of DA and the PL excitation spectra of CDs.

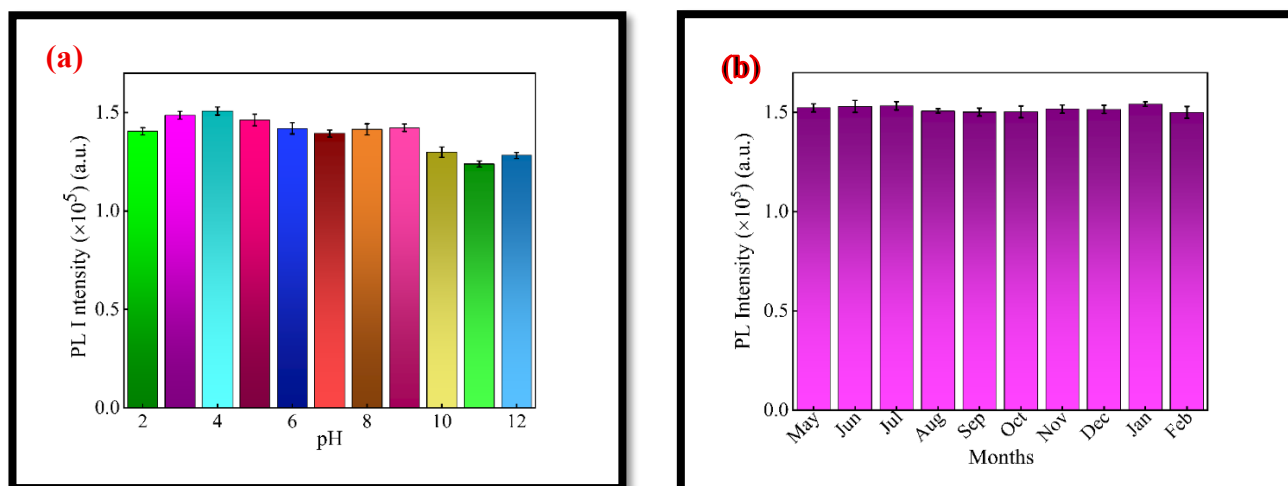


Fig. 13. PL spectra at excitation wavelength of 435 nm of CDs as a function of different pH (2-12) (a) and PL spectra of CDs at 435nm monitored in the repeated interval up to 10 months, representing the stability of CDs (b).

CHAPTER -5

CONCLUSIONS

5.1 Conclusions

In order to generate CDs with an average diameter of 4.02 ± 0.76 nm, which were produced from green tea, the hydrothermal synthesis technique was used. If the CDs are irradiated at a wavelength lower than 365 nm, they exhibit a remarkable blue photoluminescence (PL), reaching its highest intensity in the 435 nm band when stimulated at 340 nm. Over a wide range of DA concentrations, ranging from 0.0 to 79 μM , the probe/sensor exhibited a linear response, exhibiting remarkable sensitivity and selectivity. Furthermore, it had an amazing detection limit of 3.71 μM . The PL of CDs drastically dropped after establishing a non-fluorescent ground-state complex. This was owing to the interaction between the donor and the acceptor, which strongly shows that static quenching occurred, with the internal field effect acting as the mechanism responsible for the quenching. When detecting DA, the CDs showed impressive selectivity and sensitivity.

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APPENDIX

A1 – PLAGIARISM REPORT

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