



Faculty of Sciences
Department of Chemistry and Forensic Sciences

SYNTHESIS AND APPLICATION OF CARBON DOTS TOWARDS FLUORESCENCE DETECTION OF POLYCYCLIC AROMATIC HYDROCARBONS

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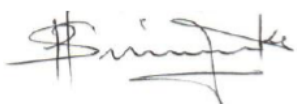
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ABSTRACT

Polycyclic aromatic hydrocarbons are organic pollutants that pose substantial health risks. Consequently, there has been a need for development of cost-effective and highly sensitive analytical techniques for monitoring these compounds. The study reports a rapid, facile, non-toxic and cheap strategy for synthesizing fluorescent CDs from monkey orange (*Strychnos cocculoides*) fruit for detecting and quantifying fluoranthene and anthracene in water samples. Synthesis of CDs involved hydrous pyrolysis (hydrothermal) of powdered monkey orange shells in a furnace at 200 °C for 3 h. Next followed purification steps that involved ultra-sonication, centrifugation, filtration and dialysis of the resultant solution. The synthesized CDs possessed high photoluminescence properties. They showed blue fluorescence when irradiated with Ultra-violet radiation at 365 nm. Characterization of the prepared CDs included extensive review of their optical properties, morphology and structure. Scanning electron microscopy and atomic force microscopy results showed uniform dispersion of the carbon dots with amorphous morphology and little signs of aggregation. The average particle size of the prepared CDs was calculated to be 3.6 nm. Fourier transform infra-red spectroscopy showed existence of hydroxyl, carbonyl, alkene, alkane, ester and ether functional groups. X-ray diffraction revealed the structure of CDs as amorphous with poor crystallinity. Raman spectroscopy results also showed the presence of turbostratic carbon. The synthesized CDs exhibited good solubility in water and excellent stability in aqueous, acidic and alkaline environments. Detection of anthracene and fluoranthene occurred through fluorescence enhancement whereby there is energy transfer between sensor and the specie. The figures of merit being the limit of detection and quantification for the target analytes were ranging from 1.4 µM to 2.0 µM and 4.3 µM to 6.1 µM respectively. Moreover, the method yielded percentage recoveries ranging from 95% to 102%.

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DEDICATION

This research project is dedicated to all the individuals who have played a part in my educational pursuit. Your unwavering support, belief, and inspiration has made this achievement possible. My solid support system, my loving family, whose endless encouragement and faith in me became the driving force of my academic journey. Your resolute faith gave me the strength to overcome challenges and pursue my dreams.

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LIST OF ABBREVIATIONS

AFM: Atomic force microscope

AN: Anthracene

CDs: Carbon Dots

DMF: Dimethylformamide

FL: Fluorescence

FLA: Fluoranthene

FTIR: Fourier-transform infrared

FQY: Fluorescence quantum yield

GC-FID: Gas chromatography flame ionization detection

GC-MS: Gas chromatography mass spectrometry

GQDs: Graphene Quantum dots

HPLC: High performance liquid chromatography

LOD: Limit of detection

LOQ: Limit of quantification

NAP: Naphthalene

NP-LC: Normal Phase Liquid Chromatography

PAHs: Polycyclic aromatic Hydrocarbons

PL: Photoluminescence

QDs: Quantum dots

RP-LC: Reverse phase liquid chromatography

SEC: Size exclusion liquid chromatography

SEM: Scanning electron microscope

UV-Vis: Ultraviolet visible

XRD: X-ray diffraction

CHAPTER 1

1.0 INTRODUCTION

1.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic pollutants characterized by their chemical structure which consists of multiple fused benzene rings that are primarily generated from an incomplete combustion of organic materials [1],[2]. They vary from two-ring naphthalene and naphthalene derivatives to sophisticated ring structures of up to 10 rings and they are mostly colorless, pale yellow or white solids [3],[4]. These compounds can be introduced into the environment via numerous processes including biomass combustion, industrial processes as well as vehicle emissions among others. Despite being synthesized chemically for various purposes, PAHs are primarily utilized as intermediates in the production of agricultural products, pharmaceuticals, lubricating materials, thermosetting plastics and other chemical products [1].

PAHs are labelled as emerging priority pollutants by the Environmental Protection Agency (EPA) because of their detrimental health effects [5],[6]. Certain PAHs are said to be having carcinogenic, mutagenic, teratogenic, and immunosuppressive properties [6],[7]. Because of their adverse effects, EPA has established regulatory guidelines and limits for PAHs in various environmental media with maximum permissible levels of contamination ranging from 0.2 to 200 parts per billion (ppb), depending on the specific PAH compound [8]. For instance, the general maximum contaminant levels of PAHs in water is 2 ppb [9] while benzo[a]pyrene, a widely recognized carcinogenic agent among PAHs, is set to a maximum contaminant level of 0.2 ppb in potable water [1],[10]. These potential hazard encouraged ongoing efforts to come up with efficient methods for detecting and identifying these compounds [2].

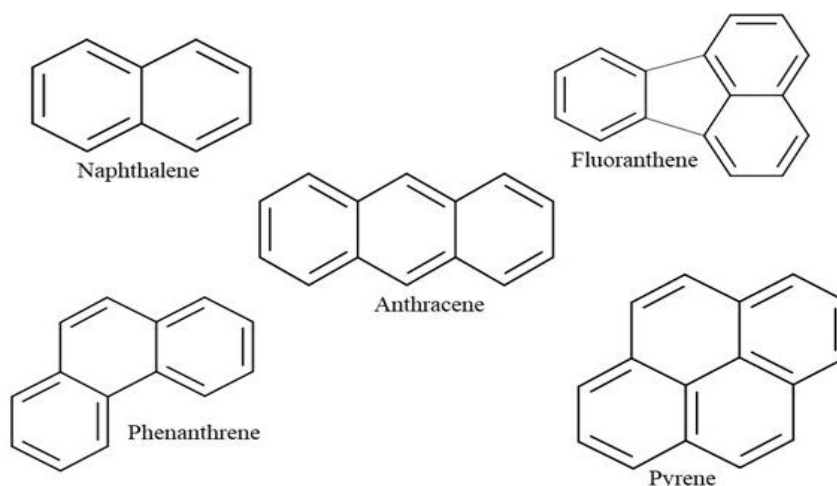


Figure 1: Chemical structures of some common polycyclic aromatic hydrocarbons

PAHs often occur at trace concentrations in the environment, hence their detection methods are aimed at improving sensitivity to accurately monitor them [11]. Researchers employed a variety of techniques to monitor and quantify PAHs, including chromatography [12]–[14], electrochemical methods [15], and immunoassays [16]. A study by Zuazagoitia and colleagues (2007) came up with a screening method for identifying PAHs in water by GC-FID [17]. Their reported LOD was $0.20 \mu\text{gL}^{-1}$, suggesting that the method can be well suited for preliminary screening. Huang *et al.*, (2013) documented a study involving determination of PAHs in water using HPLC coupled with fluorescence and diode array detection [18]. The findings indicated good recoveries and exceptional reproducibility. However, it is vital to note that chromatographic techniques are expensive, time consuming and labor intensive, especially where routine monitoring is required. They also require trained personnel and all that is not appropriate for fast and low-cost analysis [2],[7].

On the other hand, other studies report the use of immunoassays for detection of PAHs. For example, research by Sun *et al.*, (2018) established an approach involving a FL-linked immunosorbent assay, in which rhodamine B iso-thiocyanate served as the model fluorescent dye conjugated to the monoclonal antibody for the detection of phenanthrene [19]. Goryacheva and coworkers developed a FL polarization immunoassay for detecting PAHs, the study's limits of detection were reported to be 3.4 ng/mL , 0.9 ng/mL , and 1.1 ng/mL for anthracene, benzo[a]pyrene, and naphthalene respectively [20]. However, immunoassays are often characterized by low accuracy and are primarily suitable for preliminary screening tests

[21]. Their effectiveness is limited by their specificity for a particular analyte, which may pose challenges when dealing with the presence of multiple PAHs [22]. Regarding electrochemical techniques, it's important to note that environmental samples often have a various matrices including water, soil, and sediment. These matrices can contain a multitude of both organic and inorganic compounds. Unfortunately, this complexity can give rise to interference with the electrochemical response of PAHs, thereby diminishing the accuracy and reliability of measurements [23].

As an alternative to these techniques, researchers proposed the use of metal and graphene quantum dots (MQD, GQD) as PAHs' sensors [7],[24]–[26]. Yang *et al.*, (2010) documented work on the synthesis and application of cadmium telluride quantum dots prepared on titanium dioxide nanotubes for detecting PAHs through fluorescence resonance energy transfer [24]. Their research showed high sensitivity, as shown by a remarkably low limit of detection (15 pM) for Benzo[a]pyrene. In another study, Du and co-workers (2016) published the synthesis and application of iron oxide nanoparticles coated with gold nanoparticles for detecting 16 priority PAHs. They highlighted that their achieved limit of detection for their methodology (0.001 M) can be applicable in screening especially in highly contaminated areas [27]. Despite a lot of research being available, a significant drawback of metal quantum dots is their limited ability to disperse evenly in commonly used solvents, making their processing a challenge [28]. They have a higher likelihood of aggregation in some commonly used solvents, which makes them unsuitable for the design of nanosensors [29]. In addition, MQD typically contain heavy metals such as cadmium which can potentially leach from the sensor and lead to health and environmental concerns [30].

On another note, researchers discuss the utilization of graphene quantum for PAHs' detection. Nsibande and colleagues discuss the use of graphene quantum dots for sensing pyrene in water samples [7]. Their study involved the synthesis of two distinct types of GQDs probes (graphene oxide-GQDs and Carboxylate-GQDs). Notably, these probes demonstrated exceptional sensitivity, with limits of detection of 0.351 μM for GO-GQDs and 0.242 μM for CA-GQDs. Furthermore, they highlighted that GQDs are environmentally friendly and easy to synthesize [7]. However, their zero bandgap restrict their application on electronics, despite the presence of various strategies to widen the bandgap energy [31]. In contrast, this research presents the use of CDs, which are also nanoparticles, in the detection of PAHs.

1.2 Carbon dots

The identification of a novel type of carbon material in 2004 has developed a significant interest among scientists and researchers [32]. CDs have captured the attention of scientists for many years owing to the abundant availability of precursor materials and their remarkable mechanical, electrical, and optical properties [33]. They have acquired significant recognition not only for their biodegradable, water-soluble and chemically inert characteristics, but also for their facile surface functionalization [34]–[39]. This facilitated their use in wide range of applications including bio-imaging [40]. Ghadareh *et al.*, (2017) established a sensitive immunosensor consisting of CDs and an antibody [41]. This immunosensor was designed for the detection of CA125 a specific biomarker commonly associated with ovarian cancer. The study concluded that the immunosensor exhibits very excellent selectivity and sensitivity towards CA125 in cancer diagnosis. CDs have also been developed for anti-counterfeit applications [42]. Dinake *et al.*, (2022) synthesized CDs based invisible ink for currency anti-counterfeiting, the ink exhibited high photoluminescence and excellent chemical stability [32]. Wang and co-workers synthesized hollow CDs from bovine serum albumin for their application in drug delivery [43].

Even though CDs are deemed less toxic, a considerable number of research involving CDs used precursor materials that may involve toxic and costly chemicals that require complex synthetic routes [42]. Maleki *et al.*, (2019) conducted a study involving the preparation of nitrogen-doped CDs via pyrolysis of urea and citric acid in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the detection of hydroxy-PAHs [44]. Sun *et al.*, (2021) presented a method for sensing bezo[a]pyrene in water using CDs synthesized from 4-carboxyphenylboronic acid [45]. Moreover, Minervini *et al.*, (2022) reported work in which CDs were synthesized by glycothermal treatment of resorcinol (1,3-hydroxybenzene) using a strong base and acid catalysts to improve the CDs reaction yield [46]. These CDs were used to extensively investigate the mechanisms leading to PAHs' fluorescence. This study proposes the use of safer and cheaper alternatives for synthesizing CDs and applying them in detecting PAHs. The study intends to use bio-waste as it has shown promising results in effectively producing CDs, offering several benefits, including resource utilization, waste management, alleviation of resource scarcity, and reduction of environmental pollution [47],[48].

1.3 Problem statement

Environmental pollution is a major challenge for environmental regulators across the globe, as it poses risks to humans, aquatic life, and the overall ecosystem. The release of pollutants into the environment, largely resulting from human activities driven by industrial, agricultural and pharmaceutical advancements to mention a few, has unintended consequences. These exercises resulted to the generation of PAHs, a class of pollutants that are of significant concern to the world at large. These compounds hold significant environmental threats due to their potential adverse effects. Pollutants of this nature have been demonstrated to be mutagenic and carcinogenic, posing risks to humans and other organisms. Monitoring of PAHs is therefore very important. Current methods for monitoring PAHs, such as conventional chromatography, immunoassay and electrochemical techniques have notable drawbacks. Chromatography techniques are expensive and involve the use of many harmful chemicals. They also require trained personnel and have a lengthy sample preparation. On the other hand immunoassays can be less sensitive while electrochemical methods have challenges with complex matrices. All these may not be suitable for routine monitoring. In contrast, carbon dots offer a promising alternative for PAH sensing as they show great advancements. However, some of their synthesis methods still rely on toxic chemicals and complex procedures. Consequently, there is a need to develop cost-effective and simple analytical methodologies for detecting these pollutants. Therefore, these study aims to develop a simple, affordable analytical approach that incorporates the principles of green chemistry for PAH detection using CDs.

1.4 Significance

CDs are emerging as effective sensing probes for detecting PAHs in environmental samples. These methods can offer better approaches for monitoring PAH pollution, enabling better management of environmental contamination. In contrast, conventional methods for PAH analysis i.e. chromatography, electrochemical methods and immunoassays can be complex, time-consuming, less sensitive and laborious. Unlike conventional methods, CDs offer benefits in terms of simplicity, high sensitivity and cost-effectiveness for PAH detection. However, past research carried out on application of CDs in sensing PAHs has mostly utilized commercially available chemical precursors that are toxic and expensive for preparation of CDs. Therefore this work will utilize the facile synthesis of CDs from

abundant and ecofriendly precursors with tunable optical properties, enabling sensitive and selective detection of PAHs. This will promote sustainability and reducing the environmental harm

1.5 Aims

- The aim of the study is to synthesize CDs from monkey orange (*Strychnos cocculoides*) for detection of Polycyclic aromatic hydrocarbons.

1.5.1 Objectives

- To tailor the CDs for low level detection of PAHs by optimizing synthesis conditions
- To characterise the prepared CDs using techniques including AFM, SEM, XRD, UV-Vis, FTIR, Raman spectroscopy and FL spectrophotometry to better understand their structure, morphology, surface functional groups and optical properties so as to understand their interaction with the analytes.
- To use the developed CDs sensor for detection of PAHs in real water samples.

CHAPTER 2

2.0 LITRATURE REVIEW

2.1 Risks associated with exposure to polycyclic aromatic hydrocarbons

PAHs are a widely distributed group of chemically related compounds that persist in the environment and exhibit diverse structures with diverse toxicity [1]. They commonly occur in mixtures of two or more PAH compounds and they are harmful to the ecosystem and human health as they have adverse effects on living organisms [49],[50]. They can enter into the environment by numerous pathways and can be absorbed and accumulated by living organisms through the food chain [51]. According to Gao *et al.*, (2018), humans can come into contact with PAHs through ingestion, inhalation, and dermal contact [52]. In contrast, Fismes *et al.*, (2002) suggests that in plants, PAHs are absorbed from the soil via roots and translocated to other parts of the plant [53]. The rate of absorption is dependent on factors such as concentration, physicochemical state, water solubility, as well as the soil type [54]. The adverse impacts of PAHs exposure on an individual vary depending on numerous factors such as the route exposure, the duration and intensity of the exposure, as well as the toxicity levels of the PAHs [1],[55]. Other factors such as pre-existing health conditions and age can also have an impact on health outcomes [56].

Table 1: list of some of the EPA priority PAHs

Compound	Abbreviation	Ring number
Naphthalene	Na	2
Acenaphthene	Ac	3
Anthracene	An	3
Fluoranthene	Fla	4
benz[a]anthracene	BaA	4
benzo[k]fluoranthene	BkF	4
benzo[a]pyrene	BaP	5
dibenz[ah]anthracene	DBahA	5
benzo[b]fluoranthene	BbF	4

Studies revealed that being exposed to PAHs can result in development of cancer, genetic

mutations, as well as to weaken the immune system [57]. They can also cause adverse effects such as tumors, reproductive issues, developmental problems, liver and kidney damage as well as respiratory problems to mention a few [1],[58]. Research states that in workplaces, exposure to high levels of PAHs lead to workers experiencing symptoms like vomiting, eye irritation, diarrhea, nausea, and confusion [59]. Additionally, some PAHs e.g. naphthalene and benzo[a]pyrene cause skin irritation. Moreover, benzo[a]pyrene and anthracene are identified as skin sensitizers, meaning they have the ability to trigger allergic reactions [57],[60]. According Patel *et al.*, (2020), if naphthalene is inhaled or ingested in large amounts, it can cause the destruction of red blood cells [61]. A study by Brander *et al.*, (2010) concluded that inhabitants who lived close to the hazardous waste site of an abandoned creosote wood-treatment facility, an area highly concentrated with PAHs, showed prevalence of skin rashes compared to the occupants far from the site [49].

Another challenge is the ability of PAHs to form reactive metabolites when processed by the body. PAHs can be transformed into reactive intermediates like epoxides and dihydrodiols, which can covalently bind to cellular proteins and DNA leading to cellular damage, mutations, and cancer [25],[26]. Inhaling mixtures containing PAHs is linked to a higher risk of cancer, including skin, lung, bladder, and gastrointestinal cancer, among workers [64]. A report by Zhang *et al.*, (2013) indicated that in china, 1.6% of lung cancer cases can be attributed to the inhalation of PAHs [65]. In a study conducted by Adeyeye (2018), it has been found that animals exposed to high doses of certain PAHs for prolonged periods have developed lungs, stomach and skin cancers from inhalation, ingestion through food and dermal contact with benzo[a]pyrene being the most common PAHs to cause the cancer [1],[66]. Benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[ah]anthracene, and pyrene have been classified as possible carcinogens by the Environmental Protection Agency (EPA) [67].

Exposure to PAHs is commonly associated with immune suppression as the most frequently reported effect [68]. However, immune potentiation can also occur, where immune cells are stimulated to secrete more cytokines, leading to inflammation [69]. In some cases, this inflammation may actually promote tumor development or lead to hypersensitivity [1]. It has been reported that when ingested some PAHs may induce DNA adducts [70]. Meanwhile, a study carried out by Fardel (2010) has shown that exposure to PAHs can cause suppression

of the immune system in rodents [71]. Parvez *et al.*, (2019) suggests that this immune suppression may play a role in pathways by which PAHs cause cancer [72]. Research by Ewa *et al.*, (2017) and Vorderstrasse *et al.*, (2001) demonstrated that PAHs led to a decrease in the quantity of splenic B lymphocytes. Additionally, these substances inhibited antibody production and resulted in a decrease in the number of antigen-specific responder B cells [70],[73]. Additionally, exposure to PAHs is linked with the inhibition of T cell proliferation and a dose-dependent inhibition of the secretion of cytokines such as IL-2, IL-10, IL-17A, and IFN γ [69]. A study in Bangladesh analyzed the peripheral blood mononuclear cells from nearly 200 men and found an inverse correlation between PAH adducts and T memory cell phenotypes [74]. When PAH-DNA adduct levels were high, there was a high decline in the percentage of T cells [74].

Studies conducted on laboratory animals, such as mice, have shown that exposure to PAHs can negatively affect developing embryos [75]. Research confirms that benzo[a]anthracene, and naphthalene are embryo-toxic [76]. A study conducted by Stefansdottir *et al.*, (2023) suggests that consumption of high concentrations of benzo[a]pyrene during pregnancy is also being linked to malformations and decreased body weight in offspring of the mice [75]. These findings suggest that exposure to PAHs during gestation may pose a risk to the developing fetus and highlight the importance of minimizing exposure to these chemicals. A study conducted in Teplice, a district in northern Bohemia, has found a strong link between exposure to particles containing PAHs during early pregnancy arguing that they negatively affect fetal development [77]. Padula *et al.*, (2014) reported that being exposed to PAH pollution during pregnancy is associated with negative effects on the birth of a child, such as, premature delivery, decreased birth weight and congenital heart defects [78]. Research by Perera *et al.*, (2006) states that prenatal exposure to PAHs can lead to lower IQ scores at age three, as well as an increased risk of behavior problems at ages six and eight, and asthma [79]. Additionally, the umbilical cord blood of infants who have been exposed to PAH may show DNA damage, which has been associated with an increased risk of developing cancer [80].

2.2 Conventional methods used for analysis of polycyclic aromatic hydrocarbons

PAHs occur in environmental component (air, water, soil) at varying concentrations and forms, thereby necessitating the use of different analytical methods for each specific case

[50]. Consequently, several techniques are being developed and utilized for analysis of PAHs [22]. The techniques are classified into two: immunoassay and chromatography [21]. In addition, recent research has demonstrated promising progress in the use of nanomaterials for PAHs detection and analysis [81].

2.2.1 Immunoassays

Immunoassays are bioanalytical techniques that rely on the interaction between an antigen and an antibody to determine the quantity of the analyte present [82],[83]. It has been extensively used for detecting various analytes such as proteins, carbohydrates, and lipids [22],[83]. Over the years, significant breakthrough has been made in developing immunoassays for detecting PAHs in environmental components [22]. Several types of immunoassays are available, such as chemiluminescence, fluorescence and enzyme-linked immunoassays [84]–[86]. The key difference between these assays lies in the method of detection which can include ultraviolet (UV), fluorimetric, amperometric, or potentiometric detection [16]. These assays target PAHs based on their physical properties, such as their size and weight for instance, the BTEX RaPID assay is an enzyme immunoassay specifically designed to rapidly analyze small aliphatic PAHs as well as carcinogenic PAHs with more than four aromatic rings [87],[88]. Other assays are tailored to the type of sample being tested, e.g. Ensys PAH soil test kit from Strategic Diagnostic Inc. This particular kit is a qualitative and semi-quantitative assay that offers an estimated measurement of PAH concentrations in soil [88]. The Tigret Inc. Rapid Assay PAH test kit is utilized to analyze PAHs in water, offering qualitative, semi-quantitative, and quantitative results [16],[88],[89]. Similarly, the Envirogard kit from JSS Modern Water is designed to detect PAHs in water, specifically targeting the 16 PAH compounds outlined by EPA regulations [90],[91]. These kits are known for their speed and accuracy, making them valuable tools for environmental monitoring and management. However, it is important to note that immunoassays also have their limitations.

A study by Adeniji *et al.*, (2018) reported that immunoassays have the possibility of results biasness [21]. The study mentions that these methods have lower accuracy and precision for many aromatic compounds when compared to other standard methods. Therefore, they are generally used as presumptive test in the field rather than for precise analytical measurements.

Moreover, these assays are specifically designed for a particular analyte, which can pose challenges when multiple PAHs are present [84],[92]. Furthermore, certain analytes, such as Benzo[a]pyrene, have low immunogenicity and normally occur in mixtures of different PAHs [22]. These factors along with the small size of these analytes, makes their detection more difficult for immunoassays.

2.2.2 Chromatographic methods

2.2.2.1 Liquid Chromatographic methods

Liquid chromatographic (LC) methods are frequently employed for the analysis of PAHs [21]. There are several LC methods available, including normal phase liquid chromatography (NP-LC), size exclusion chromatography (SEC), reversed-phase liquid chromatography (RP-LC), and high-performance liquid chromatography (HPLC) [93]–[95]. HPLC methods are predominantly favored for their ability to effectively analyze thermally labile, semi-volatile, less volatile or non-volatile compounds. Adeniji *et al.*, (2018) Reported that HPLC coupled with fluorescence detection offers a highly sensitive approach for determining PAHs [21]. However, it may not offer sufficient selectivity when it comes to identifying individual PAHs within complex mixtures [96]. Wise *et al.*, (1993) states that RP-LC with fluorescence detection is a highly effective analytical method for separating PAH isomers [97]. This is particularly important for complex mixtures of PAHs found in environmental samples, which often contain many different isomeric structures that are challenging to separate, e.g. benzo[b]fluoranthene, and dibenz[a,c]anthracene [95].

According to a study by Sim *et al.*, (1998), it is difficult to accurately detect and quantify PAHs in mixtures, especially when their concentrations are very low and the interfering material is abundant [98]. However, study carried out by Fisher and co-workers proposes that SEC can be used to analyse PAHs mixed with other compounds [13]. This is because of their excellent ability to separate molecules based on their size. It makes it easier for the interfering compounds to be separated from the target analytes, making it easier to detect and quantify them accurately [13]. However, sample requires treatment involving liquid-liquid or solid-phase extraction prior to analysis to isolate analytes of interest [99]. Heaton *et al.*, (2012) Reports that the use of normal-phase systems in contemporary laboratories is environmentally harmful and costly, primarily due to the disposal of potentially toxic eluents [100]. Additionally, the utilization of alkanes and polar solvents (such as chloroform and

ethyl acetate) can lead to limited solubility of the analytes, posing further challenges [101].

2.2.2.2 Gas chromatographic methods

GC is one of the most commonly used method in the separation and detection of PAHs [21]. This technique commonly employs detectors e.g. flame ionization detector (FID) and mass spectrometry (MS) [14],[102]. The utilization of GC-FID is regarded as a valuable technique that can provide a high resolving power, particularly when capillary columns are employed. Extensive research is available on the application of this technique for quantifying PAHs in various environmental sample [14]. For example, an article published by world health organization (WHO) states that GC-FID is included in official methods used at the international level to analyze PAHs in both air and water [103]. One benefit of FID is that its response is directly related to the carbon content of the compounds. This means that even if a suitable reference standard is not available, it is still possible to measure and quantify compounds belonging to the same isomer group [14].

On the contrary Schenk *et al.*, (2016) reports that FID lacks the ability to distinguish between isomers, resulting in co-elution or overlapping peaks in the chromatogram [104]. The study further added that without further confirmation using additional techniques, it can be challenging to identify and quantify specific isomers using GC-FID alone. Furthermore GC-FID is a non-specific detector, meaning it does not provide specific information about individual compounds [105]. It measures the total concentration of all compounds that can be detected by the FID, including non-PAHs. This lack of selectivity can be a challenge when analysing complex samples containing a wide range of compounds, as it may be difficult to differentiate PAHs from other interfering substances [14]. The sensitivity of the FID is typically lower compared to more specific detectors such as MS [106]. PAHs mostly occur in low levels in environmental samples, for instance, a research conducted on the worldwide environmental dispersion of PAHs demonstrated that they can be detected at levels as low as 0.11 ng m^{-3} in air, 0.06 ng L^{-1} in water, and 21 ng g^{-1} in sediments, therefore FID may not be sensitive enough to accurately quantify them at low concentrations [81].

FID relies on the combustion of analytes, and some PAHs may undergo thermal degradation or decomposition at high temperatures leading to inaccurate quantification or loss of analyte [107]. Care must be taken to optimize the GC-FID conditions to minimize thermal

degradation. GC-FID is primarily suitable for the analysis of volatile compounds. However, some PAHs are non-volatile or have high molecular weights, making them less amenable to analysis using this technique [61]. These compounds may require sample preparation techniques such as extraction or derivatization to improve their volatility or detectability, which adds complexity to the analysis [96]. In summary, identification and quantification of PAHs using this detector can be affected by interferences from prevalent environmental contaminants such as phthalates, as well as unintended compounds co-extracted from carbon-based sources. Additionally, high column bleeding and inadequate separation of isomers, can also pose challenges. [108]. Therefore, it is crucial to perform thorough sample clean-up procedures to ensure precise quantification and identification of the analytes when utilizing this detector [109].

When analyzing for challenging sample matrices like oil or sediment, GC-MS is frequently employed to determine PAHs [110]. According to Poster *et al.*, (2006), The substantial reduction in interfering peaks in GC-MS chromatograms, as opposed to those obtained through GC-FID, established GC-MS as a better analytical technique in environmental PAH analysis [14]. A recent survey has provided a comprehensive review of advancements and uses of GC-MS in analyzing environmental samples. The focus is on the detection and determination of various persistent pollutants. GC allows for the separation of PAH compounds, while MS enables accurate identification of the PAHs present [110]. Therefore, the combination of these two methods is often employed to successfully separate PAHs in complex sample matrices to obtain accurate qualitative and quantitative results [111]. GC-MS offers numerous advantages when it comes to detecting PAHs, including better separation efficacy, quantitative accuracy, and the ability to work with small sampling volumes [112],[113]. As a result, researchers frequently utilize GC-MS to study the distribution of PAHs in the environment [111]. Nevertheless, the utilization of this technique necessitates the implementation of sample preparation techniques like liquid-liquid extraction and solid-phase extraction. These methods often require a substantial amount of solvent and sample material and can involve labor-intensive and time-consuming processes [114].

2.2.3 Nanomaterials in detecting polycyclic aromatic hydrocarbons

Recently, substantial growth has been made in material science, leading to the emergence of direct sensing techniques that provide numerous advantages compared to conventional

detection methods. These developments do not only address the limitations of traditional techniques, but also offer significant benefits e.g. portability, high sensitivity, and selectivity, making them highly desirable in numerous applications [81]. Novel materials such as metal quantum dots (MQDs) and graphene quantum dots (GQDs) are some great examples of these improvements [7],[115],[116]. While the current application of these sensing approaches is limited to certain environmental samples, their application is expected to expand in the near future [117].

MQDs are small, crystalline nanomaterials that possess excellent optical and electronic properties [81],[118]. These properties primarily arise from quantum confinement, where the behavior of electrons and holes within the MQDs is constrained due to their small size [25]. The effects of quantum confinement lead to distinct features such as tunable bandgaps, discrete energy levels, and improved charge carrier properties [119]. These unique properties make MQDs highly suitable for a wide range of applications such as optoelectronics and fluorescence sensing and they have consequently been utilized even in PAH detection [81]. MQDs possess a highly adaptable surface chemistry that enables modifications using different approaches. This versatility paved way for incorporating specific receptors that are designed for particular target analytes [5]. The interaction of MQDs' surfaces with PAHs can induce energy transfer to or from the MQDs, resulting in an increase or decrease of the fluorescence of the MQDs. This characteristic enables the detection of PAHs using MQDs [81]. MQDs may consist of various elements including Zinc Selenide (ZnSe), Zinc Sulfide (ZnS), Cadmium Telluride (CdTe), Cadmium Sulfide (CdS), Silver Sulfide (AgS) [120]. However, in the context of sensing PAHs, cadmium-based QDs have been predominantly reported in the literature. A study by Li *et al.*, (2009) analyzed and compared the performance of MQDs used as photoluminescence sensors for optical detection of pyrene and anthracene [115]. The study concluded that the relative FL intensities of CdTe QDs, SiO₂@CdTe, and CA[7]@SiO₂@CdTe QDs exhibited a linear increase corresponding to the increase in concentrations of anthracene and pyrene. Yang and colleagues showed the detection of Benzo[a]pyrene using CdTe QDs modified with titanium dioxide nanotubes (TiO₂) [24]. The detection of BaP was achieved by observing an increase in FL intensity, which was attributed to the occurrence of FL resonance energy transfer between the PAH molecules and the CdTe QDs.

Table 2: MQD and GQD sensors that have been developed for detection of PAHs and their LODs

QDs/GQDs	Modifier	Detection Technique	LOD	Detection Range (mol L ⁻¹)	References
GO-CdSeTeS/ZnS	L-cysteine	Fluorescence	2.26×10^{-9} μ M	$0.1-0.5 \times 10^{-6}$	[121]
GO-CdSeTe/ZnSe/ZnS	L-cysteine	Fluorescence	0.19 mg/L	$0.1-0.5 \times 10^{-6}$	[122]
CdTe	(TGA)/Cyclodextrins	Fluorescence	0.085 μ M	$5 \times 10^{-7}-7.5 \times 10^{-5}$	[123]
SiO ₂ @CdTe		Fluorescence	2.45×10^{-8} M	$0.1-50 \times 10^{-3}$	[81]
CA[7]@SiO ₂ @CdTe	Cyclodextrins	Fluorescence	2.94×10^{-8} M	$0.1-50 \times 10^{-3}$	[81]
CdTe (TiO ₂)	TiO ₂	Fluorescence	1.5×10^{-11} M	$4 \times 10^{-7}-4 \times 10^{-11}$	[24]

Even though MQDs show excellent potential as fluorescent probes in the detection of PAHs, this type of chemical sensor possess drawbacks [124]. Many quantum dots comprise of heavy metals e.g. cadmium, lead, and mercury. These metals can be potentially harmful to human health and the environment [30]. While efforts have been made to develop less toxic quantum dots, the toxicity issue remains a concern, especially in applications where the quantum dots may come into contact with biological systems or be released into the environment [119],[124],[125]. On the other hand, factors like heat, light, and moisture can cause MQDs to degrade over time, leading to a decrease in sensitivity, reliability, and overall sensing performance [126]. Moreover, the production of quantum dots, particularly those derived from rare or toxic materials, can be expensive, which hampers their widespread use in sensing applications, especially in large-scale settings [127].

In contrast, GQDs possess distinctive properties, including flexibility, photo stability significant pore volume, adjustable conductivity, bio-compatibility and easy surface modifications [128]. These characteristics make them highly well suited for the adsorption of organic pollutants, including PAHs [129]. Research carried out by Maluleke and co-workers

(2021) published the synthesis of graphene oxide (GO)-modified CuInS/ZnS QDs. These modified QDs are recognized for their notable adsorption properties with aromatic compounds, making them suitable for sensing PAHs [130]. The study's indicated that introducing varying concentrations of PAHs to the GO led to an increase in FL intensity. This enhancement was attributed to the π - π stacking interaction occurring between the chemical sensor and the PAHs. However vast numbers of researches reports that GQDs need modifications to improve their interaction with target molecules, particularly aromatic compounds [120]. Several studies have recently been conducted to explore strategies for enhancing the affinity of GQDs dots for these compounds e.g. turning of their bandgap. For example Tian and co-workers states that according to the required FL properties, it is possible to modify the bandgap of GQDs by introducing heteroatoms for doping [131]. However, this modification comes with the presence of defect states which are mostly observed when doping with non-metallic atoms such as fluorine, boron, and nitrogen [132]. Additionally, during the preparation process of GQDs, various defects can arise due to acidic processes involving chemical and isotopic impurities [132]. These defects impact both the size and absorption properties of GQDs, ultimately affecting their sensitivity. On another note, research has indicated that GQDs, particularly those derived from graphene with carboxyl group functionality, are generally non-biodegradable and may possess toxic properties, thereby restricting their use in significant quantities within living organisms [133]. Moreover, the expensive nature of graphene and similar materials hinders their application in cost-effective, large-scale manufacturing [134]. Consequently, researchers have focused on developing an alternative type of nanomaterial known as carbon dots, which offer simple synthesis process and versatile applications, without the associated complications [40].

2.3 Application of CDs in detection of polycyclic aromatic hydrocarbons

CDs are nanoparticles with a zero-dimensional quasi-spherical shape, and they possess numerous beneficial characteristics [47],[135]. These include strong photoluminescence properties, high stability, minimal toxicity, remarkable water solubility, favorable compatibility with biological systems, and easy surface modification properties as well as facile synthesis procedures [47],[136]. Recently, CDs have emerged as promising photoluminescence sensors for a wide range of applications including bio-imaging, biomedical and chemical sensing, optoelectronics, fertilizer production, separation processes, anti-counterfeit, drug delivery as well as serving as co-catalysts for environmental

remediation [136]. These CDs can be synthesized from chemicals and a variety of carbon containing sources, including bio-waste, such as orange peels, sorghum husk, watermelon, monkey orange and potato peels to mention a few [32],[137],[138]. Studies show that CDs have been successful in detection of a wide range of organic pollutants including PAHs. Extensive research has utilized the synthesis of CDs from chemicals for PAHs' detection and so far it has shown excellent results. For instance, a research study by Yang and coworkers used citric acid and urea to synthesis CDs for detecting benzo[a]pyrene in water [139]. This method exhibited remarkable sensitivity, with LOD and LOQ of 0.15 and 0.5 ng L⁻¹, respectively. Chen *et al.*, (2022) synthesised blue, cyan-blue and yellow CDs using m-phenylenediamine/o-phenylenediamine for the blue CDs, citric acid and ethylenediamine for the cyan blue CDs, o-phenylenediamine and L-glutamic acid for the yellow CDs. These CDs combined with four polyvinyl alcohol were used as the fluorescent probe for the detection of multiple PAHs using the inner filter effect [2]. The study concluded that the developed sensors were able to successfully quantify and distinguish between 16 PAHs including anthracene fluoranthene and naphthalene.

Due to the undesirable properties of chemical precursors used in the synthesis of CDs, researchers are now focused to using precursors that are less toxic and less costly such as bio-waste. For example, Bandi *et al.*, (2013) reported the preparation of onion waste derived CDs for multicolor imaging [140]. The use of CDs derived from tobacco was published by Miao *et al.*, (2018) for the detecting tetracycline [141] while Kumar and coworkers studied the fluorescence sensing of lead ions using CDs derived from Ocimum Sanctum [142]. Even though extensive research is available on the use of CDs synthesised from bio-waste for various applications, there has been limited studies where CDs synthesised from environmentally friendly precursors have been used to detect PAHs. In one of the few studies documented, Hashemzadeh and co-workers synthesised of CDs through the acid treatment of coconut shell biochar, which were then decorated with chitosan. These CDs were effectively used for both the detection and removal of anthracene from aqueous solutions [143]. Even though the study used an environmentally friendly starting material, the CDs were modified with toxic chemicals for their optimal utilisation. Therefore, this study continues the search for alternative less harmful ways that can be used for the synthesis of CDs and applying them on detecting PAHs.

CHAPTER 3

3.0 METHODOLOGY

3.1 Chemicals and Materials

The study used a range of chemicals, including potassium phosphate dibasic, potassium phosphate monobasic, naphthalene, fluoranthene, anthracene, and quinine sulfate, all of which were sourced from Sigma Aldrich. Methanol (98%) and dimethylformamide (DMF 98%) were procured from Honeywell. Rochelle Chemicals provided aluminum nitrate, lead nitrate, barium nitrate, cobalt nitrate, sodium nitrate, magnesium nitrate, copper nitrate, calcium nitrate, and silver nitrate. All chemicals were utilized in their as-received form. The unripe monkey orange (*Strychnos cocculoides*) or Mogorogorwane in native language was collected from Mogorosi village in the central district of Botswana and BIUST wilderness, only the shells were used for the study. Deionized water purified by Milli-Q system was used exclusively for the entire study. The water samples used were tap water from BIUST and water from Letsibogo dam (Selibe-Phikwe Botswana).

3.2 Synthesis of Carbon Dots

CDs were synthesized from monkey orange shells via hydrous pyrolysis (hydrothermal approach). The shells were washed with deionized water and air dried for two days. The shells were then ground into fine powder using mortar and pestle. The crushed monkey orange powder was mixed with deionized water at a ratio 1 g:10 mL, placed in a Teflon-lined autoclave and heated in a furnace at the optimized temperature and time of 200 °C and 3 h respectively. Thereafter, the autoclave was set to cool down naturally to ambient temperature overnight. After that, the obtained brown solution was agitated under ultrasonic vibration at 25 °C for 10 minutes and then centrifuged for 15 minutes at 7000 rpm. The resultant solution was then decanted and filtered through 0.22 µm membrane filter. Thereafter, the supernatant was dialyzed (retained molecular weight: 1000 Da) against deionised water in the dark for 48 h, and deionized water was changed once after the first 24 h. Ultimately, the desired CDs solution was obtained and kept in a refrigerator at 4 °C for future use.

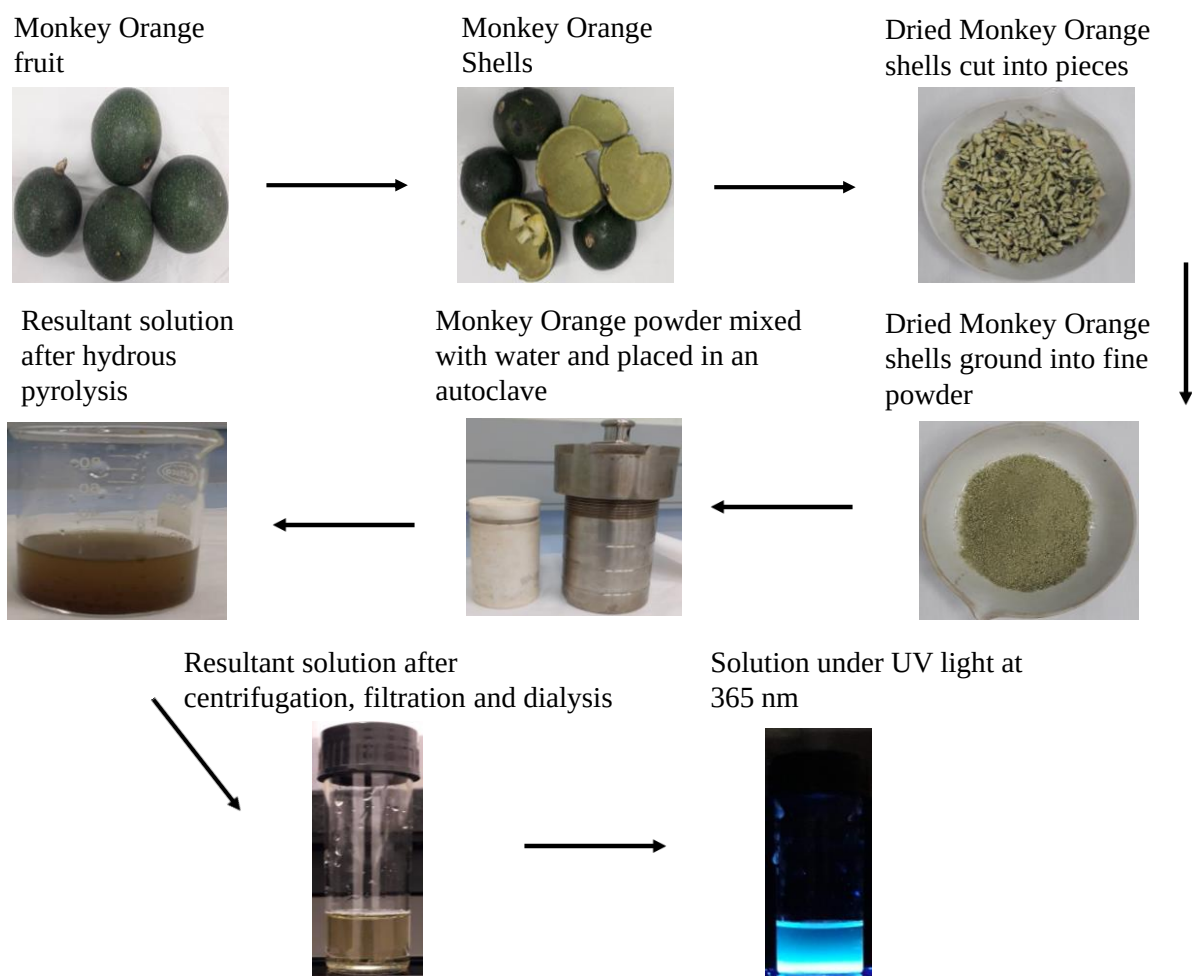


Figure 2: Schematic representation of hydrothermal synthesis of CDs.

3.3 Optimization of CDs synthesis conditions

The most effective synthesis conditions for temperature, pH and time, were experimentally established. Monkey orange powder was exposed to; varying carbonisation temperatures (100 °C to 300 °C) with increments of 50 °C, different exposure time (1 to 4.5 h) with increments of 30 minutes and varying pH conditions (5-8) with increments of 1 pH unit. This was followed by fluorescence intensity measurements of the product after the synthesis process as explained in the previous section 3.2. The conditions resulting in CDs producing the highest fluorescence intensity were considered the optimal synthesis conditions.

3.4 Instrumentation

3.4.1 Characterisation of CDs

Fluorescence measurements were performed on a Perkin Elmer LS 55 spectrofluorometer. UV-Vis results were recorded by Thermo Scientific Evolution 201 spectrophotometer. An atomic force microscopy (AFM), (Bruker AFM dimension edge in Scan Asyst Peak Force), was used to determine the size and distribution in the tapping mode. Scanning electron microscopy images were obtained with JSM-7100F microscope at 15kV. The functional group analysis was computed on Thermo Scientific Nicolet iS10 FTIR spectrometer operated at 32 scans and resolution of 0.50 cm^{-1} . The X-ray diffraction, data was obtained from a Bruker D8 Advance powder diffractometer, fitted with Lynx Eye XE energy dispersive strip detector, operating at 40 mA and 40 kv. Carbon structure was studied by Raman spectroscopy using Horiba Scientific equipment with; 532 nm edge laser as an excitation source, 600 grating and excitation wavelength 500 nm. Raman signals were acquired at 50% of the beam intensity with integration time of 0.64 ms resulting in a total acquisition time of 30 s.

All sonication procedures were made on a Sonic-Clean, Labotec ultrasonicator. Pyrolysis was done using carbolite AAF 1100 furnace. pH measurements were recorded on a thermo fisher scientific Omarion star pH meter. Centrifugation was done by Thermo Scientific, Heraeus Megafuge 40 Centrifuge. All mass measurements were taken on a Vibra HT analytical balance. The HPLC system was an Agilent series infinity 1260 equipped with a G1322A pump and in-line diode array UV detector using YMC-Pack ODS-AM column. The conditions for analysis were set in isocratic elution mode with mobile phase consisting of 100% methanol at a flow rate of 0.5 mL/min and column temperature of 30 °C. With all the analysis, the injection volume was set at 5 μL and a fixed wavelength of 274 nm.

3.5 Sample preparation for characterisation

FTIR, FL and UV-Vis measurements did not require any sample preparation. The resulting CD solution from section 3.2 was analysed as it is. For AFM analysis, the sample was prepared by placing a drop of the purified CDs solution on an AFM mica slide. The drop was then allowed to dry at room temperature in a desiccator for an hour. For analysis using XRD, Raman and SEM, the samples were prepared by washing the pellet from centrifugation with distilled water, drying it for 2 h then depositing a few drops of the resulting CDs solution on the dried pellet and allowing it to dry (section 3.2).

3.6 Determination of fluorescence quantum yield

The fluorescence quantum yield (FQY) of CDs was measured as relative fluorescence quantum yield and was determined following a procedure reported in literature [144],[145]. Owing to the similar emission range to CDs, quinine sulfate (0.1 M H₂SO₄ as solvent) was chosen as a reference standard, the FQY of CDs was calculated using the following equation adopted from [146],[147]:

$$\Phi_x = \Phi_{st} \left(\frac{Grad_x}{Grad_{st}} \right) \left(\frac{\eta_x^2}{\eta_{st}^2} \right)$$

Where Φ_x and Φ_{st} refers to FQY of the CDs and quinine sulfate respectively; $Grad_x$ and $Grad_{st}$ are gradients of the graph of integrated FL intensity against the absorbance for the CDs and Quinine sulfate respectively. η_x and η_{st} account for refractive index of CDs and quinine sulfate solution, respectively. To mitigate re-absorption effects, the absorption in the 10 mm fluorescence cuvette was maintained below 0.10 at both the excitation wavelengths of 340 nm and 310 nm [148].

3.7 Determination of band gap

Bandgap energy of CDs was determined experimentally using Uv-Vis spectroscopy and the Tauc plot method [149]. The synthesized CDs were characterized by UV-vis and the spectrum obtained was analyzed to achieve the bandgap energy.

3.8 Method validation

3.8.1 Specificity

Specificity study was experimentally determined by recording fluorescence intensities of CDs in the presence of metal ion interferences. The selected metal ions included (Al³⁺, Pb²⁺, Ba²⁺, Co²⁺, Na⁺, Mg²⁺, Cu²⁺, Ca²⁺, Ag⁺) at concentrations of 500.0 μ M

3.8.2 Sensitivity

To evaluate the viability of the quantification and the accuracy of the analytical outcomes, the figures of merits, i.e. limit of detection (LOD) and the limit of quantification (LOQ), were determined using a linear regression model at 95% confidence level. The calculation formulas for LOD and LOQ listed below were adopted from Devilal *et al.*, (2016) [150].

$$LOD = 3.3 * (\sigma/S)$$

$$\text{LOQ} = 10 * (\sigma/S)$$

Where σ represents the standard deviation of y intercepts of regression lines and S indicates the slope of the calibration curve

3.9 Stability tests

The stability of CDs was investigated under two experimental conditions being pH and sodium chloride concentration. The experiment aimed to evaluate how varying pH levels and salt content would affect the CDs' ability to maintain their FL intensity.

3.10 Quantitative Detection of polycyclic aromatic hydrocarbons

3.10.1 Preparation of solutions and samples

The CDs working solution was prepared by diluting 50 mL of CDs stock solution (section 3.2) in 100 mL volumetric flask, followed by 10 mL of pH 8 buffer solution (KH_2PO_4) and lastly diluting to the mark with distilled water. The resulting concentration of CDs working solution was 5000 ppm. PAHs stock solutions were prepared separately by weighing and dissolving the desired amount of each PAH of interest (Fla, An) in 98% dimethylformamide (DMF). Serial dilutions of the stock solutions were made using the same solvent (DMF). The water samples including tap water and Letsibogo dam water were filtered through a 0.22 μm membrane filter to remove suspended materials. The samples were spiked with a desired amount of the analytes of interest and subsequently analysed.

3.10.2 Quantitative detection of polycyclic aromatic hydrocarbons by the proposed model (CDs)

The prepared CDs were used as a fluorescent probe for quantitative determination of PAHs (An, Fla). The detection model involved adding 1 mL of CDs working solution to 3 mL of the prepared PAHs, followed by a one-minute interaction period. A standard curve was constructed using ten solutions with concentrations ranging from 1 to 10 μM for each PAH. The prepared solutions were then measured on a FL spectrometer. This model was applied to determine the concentration of An and Fla in Letsibogo and tap water samples.

3.10.3 Quantitative detection of polycyclic aromatic hydrocarbons by the Reference Model HPLC

Sample preparation involved filtering the samples through a 0.22 μM membrane filter. A standard curve was constructed using 5 solutions with concentration ranging from 2 to 10 μM (2 μM , 4 μM , 6 μM , 8 μM , 10 μM) for each PAH. The model was used as a reference for determination of concentrations of An and Fla in Letsibogo and tap water samples.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Characterization of the prepared CDs

Characterization of CDs employed several techniques to determine the structure, morphology, size, and carbon configurations of the CDs. These techniques included atomic force microscopy, X-ray diffraction, Scanning electron microscopy, and Raman spectroscopy. Additionally, Fourier transform Infrared spectroscopy was used to identify the distinct functional groups present on the surface of the as-prepared CDs. Moreover, absorption and fluorescence measurements were conducted utilizing UV-Vis spectroscopy and a Fluorescence spectroscopy, respectively.

4.1.1 Characterization by Atomic Force Microscopy

Size and topographic morphology of the CDs were investigated by the atomic force microscopy (AFM). Figure 3(a) shows that the prepared CDs are uniformly dispersed with amorphous morphology confirmed by the 3D image in Figure 3 (b), but some aggregation is observable. This findings are consistent with those of Sabet and coworkers [151]. Shamsipur *et al.*, (2018) suggest that the observed aggregations could possibly be as a result of AFM sample preparation [152]. The average particle size of the prepared CDs is calculated to be 3.69 ± 0.02689 nm.

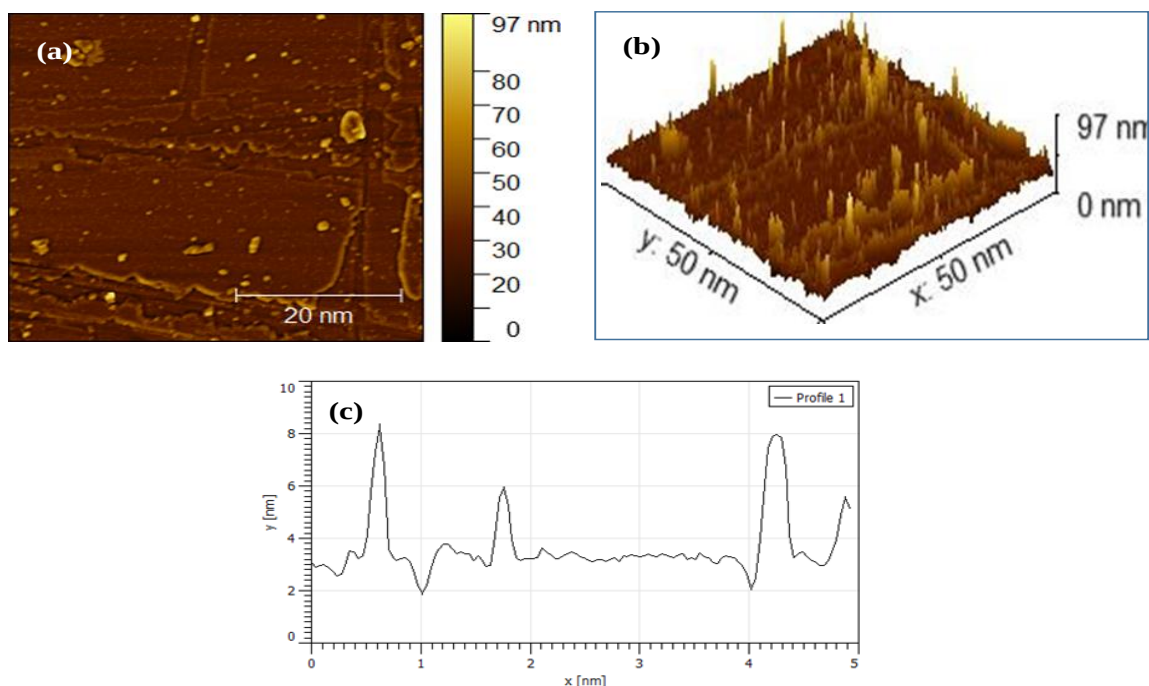


Figure 3: AFM characterisation of the CDs; (a) 2D image of the well dispersed CDs, (b) 3D image of the CDs showing amorphous structure, (c) the height distribution of the prepared CDs

4.1.2 Characterisation by scanning electron microscopy

The SEM image of the CDs is depicted by Figure 4. The results closely align with the AFM results as evident in Figure 4(a), where the CDs exhibit uniform dispersion with minimal aggregation. The particle size distribution from Figure 4(b) shows the average particle size of 3.6 nm.

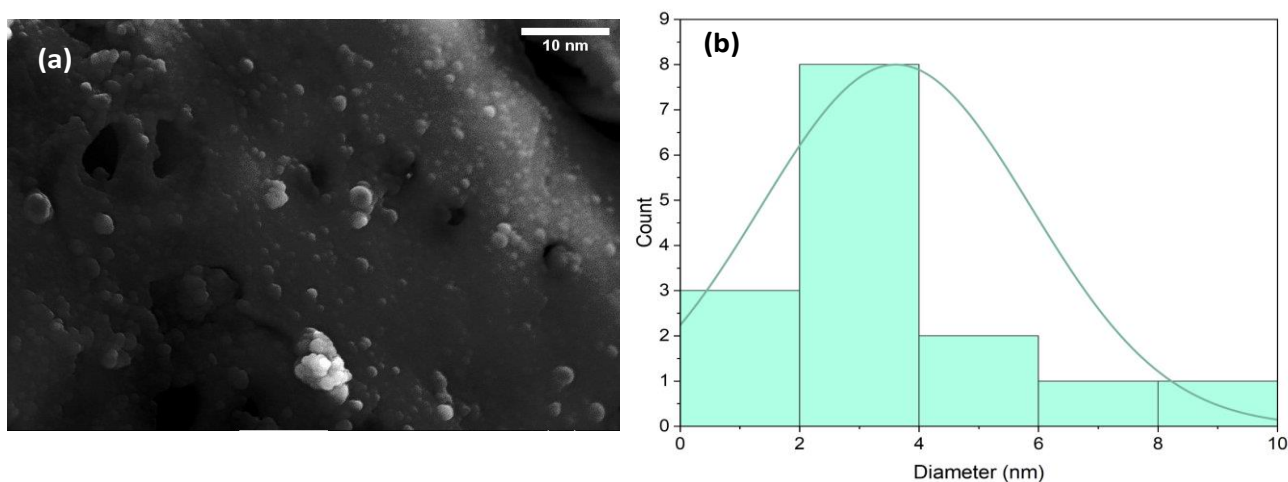


Figure 4: SEM characterisation of the CDs; (a) 2D image of the well dispersed CDs, (b)

Particle size distribution.

4.1.3 Characterization by X-ray diffraction

The XRD spectrum in Figure 5 (a) exhibits mostly a diffuse pattern with broad peaks which is a phenomenon well known for an amorphous material with little crystalline order [153]. The spectrum displays broad peaks at $2\theta = 15^\circ$, 21° , and 34° corresponding to the (110), (200) and (211) diffraction patterns of graphitic carbon respectively. The peaks 15° , 21° , and 34° corresponds to the interlayer spacing's of 5.90 Å, 5.98 Å and 4.56 Å respectively which according to Shamsipur *et al.*, (2018) indicates poor crystalline nature of CDs [152]. The peak observed at 21° in the XRD pattern could potentially be attributed to the distortion of the (110) planes resulting from the precursor, or it could arise from the presence of small circular structures formed during the reaction, which is consistent with the occurrence of similar peaks in XRD patterns of carbon materials [153]. The observed results explain the presence of turbostratic carbon structures which is supported by the Raman spectroscopy results [154]. Zhang *et al.*, (2010) and Mewada *et al.*, (2013) explained graphitic nature of the CDs with an inner layer spacing of $\sim 4.12\text{\AA}$ a result that aligns closely with our own findings [155],[156]. The XRD spectrum of the starting material in Figure 5 (b) shows the absence of a peak at $2\theta = 15^\circ$ when compared to the CDs spectrum. The introduction of a new peak in Figure 5 (a) might indicate a phase transition in the material. This could be changes in composition or the formation of a new crystalline phase.

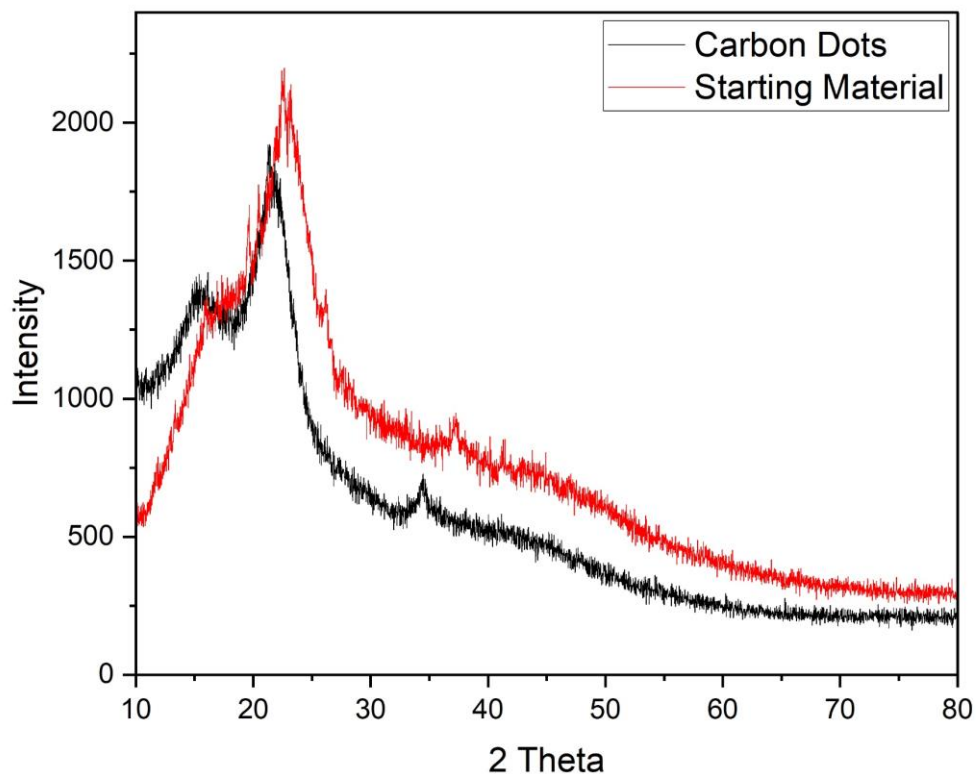


Figure 5: XRD spectra for both the CDs and the starting material.

4.1.4 Characterization by Raman Spectroscopy

The Raman spectrum of the prepared CDs shown in Figure 6 exhibits two peaks at 1324 cm^{-1} and 1591 cm^{-1} , corresponding to the Disorder (D) and Graphite (G) bands, respectively [157]. The D peak is attributed to the breathing modes of sp^2 carbon atoms in disordered or defective carbon materials, such as amorphous carbon, graphene edges, or carbon with sp^3 defects. The G band is associated with the in-plane vibrations of sp^2 carbon atoms in a crystalline carbon structure, such as graphene [158]. The intensity ratio of the D-band to G-band (I_D/I_G) has a value of approximately 0.57, confirming poor crystallinity of the prepared CDs [159]. These results corroborate the amorphous and poor crystalline nature of the CDs as determined using AFM and XRD techniques. Similar results were reported by Kumar *et al.*, (2013) with a slightly higher intensity ratio (I_D/I_G) being 0.59 [160].

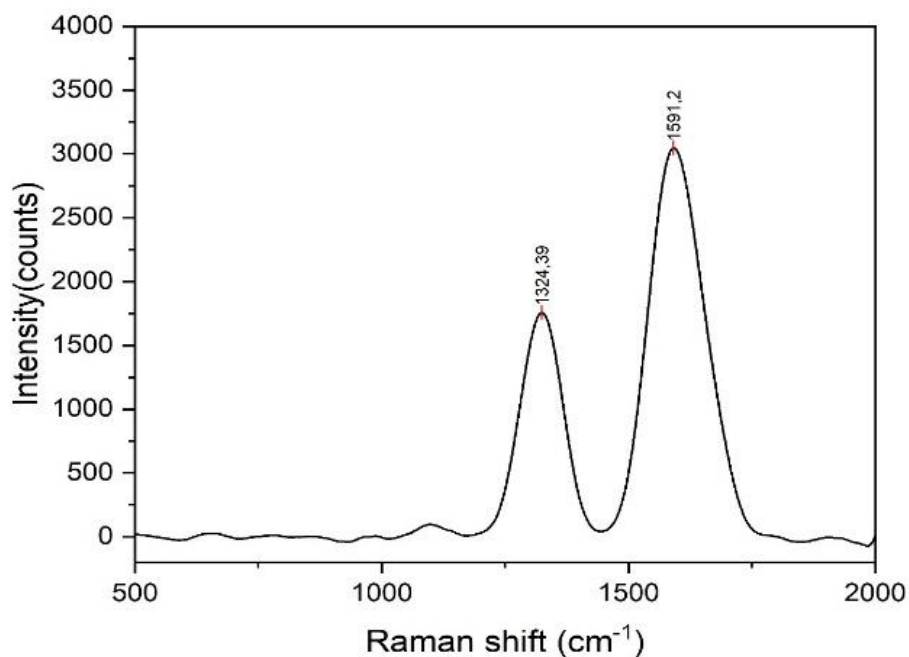


Figure 6: Raman spectrum of the as-prepared CDs

4.1.5 Characterization by FT-IR

The FTIR spectrum in of the CDs reveals characteristic peaks around 3400 cm^{-1} and 2912 cm^{-1} that are representative of hydroxyl (O–H) and an alkane group (C–H) respectively. The peak at 1710 cm^{-1} is a representative of the carbonyl group (C=O) while the ester group is represented by a peak at 1234 cm^{-1} . The peak at 1031 cm^{-1} shows the presence of the ether group (C–O–C) while an alkene group (C=C) is depicted by a peak at 1324 cm^{-1} . The corresponding spectrum of the starting material depicts peaks that closely resemble those observed in the spectra of the CDs. This similarity suggests that the carbonization process does not change the surface functional groups present in the starting material.

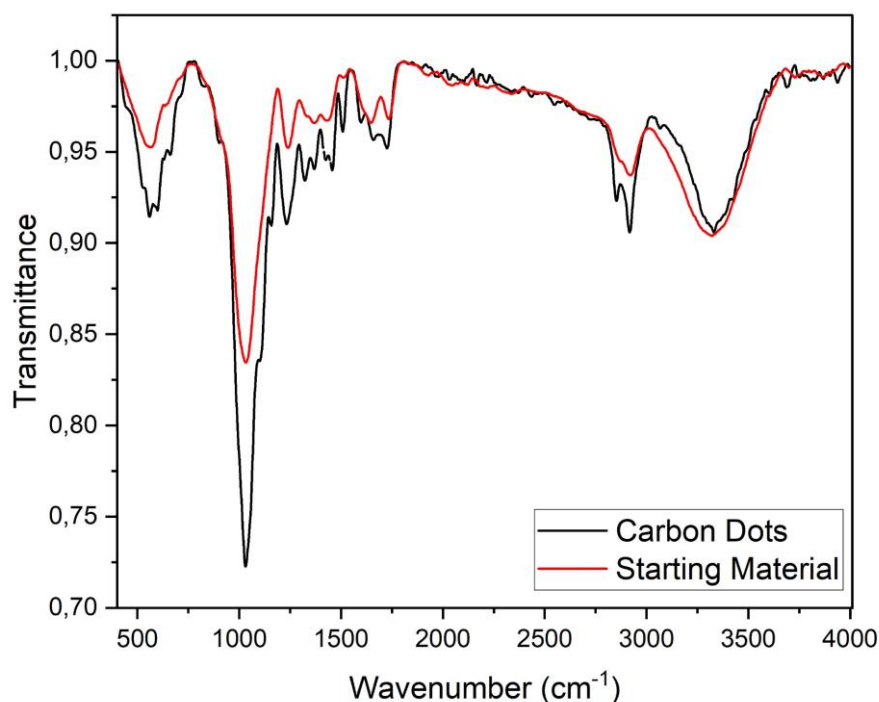


Figure 7: FTIR characterisation of the prepared CDs;

4.2 Optical Properties of the Synthesized CDs

Optical properties of the CDs were recorded by UV/Visible and fluorescence spectrometers, the obtained CDs show high water solubility and strong photoluminescence (Figure 8). Figure 8 (b) indicates the yellowish CDs aqueous solution under visible light while Figure 8 (a) shows a highly blue FL under UV irradiation at 365 nm. The bright blue PL of the CDs under UV light is strong enough to be seen with the naked eye.

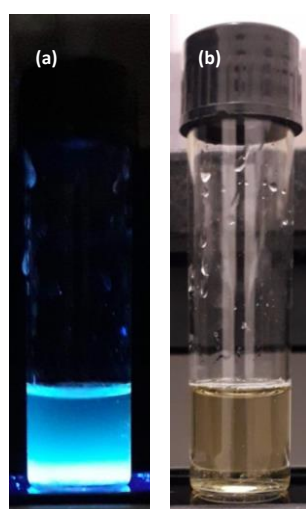


Figure 8: Images of CDs solution dispersed in water; (a) under UV light at 365 nm, (b) under visible light

4.2.1 Characterization by UV-Vis Spectroscopy

The absence of an absorption peak in the UV-Vis spectrum of the as-prepared CDs, as shown in Figure 9, can be attributed to the likelihood of a broad particle size distribution of the CDs which was discussed in section 4.1.2 (SEM). This broad spectrum suggests that there is no well-defined band edge in the UV-Vis region [138],[161],[162]. This observation is consistent with previous studies that synthesized CDs from biomass, yielding comparable spectra [32],[163].

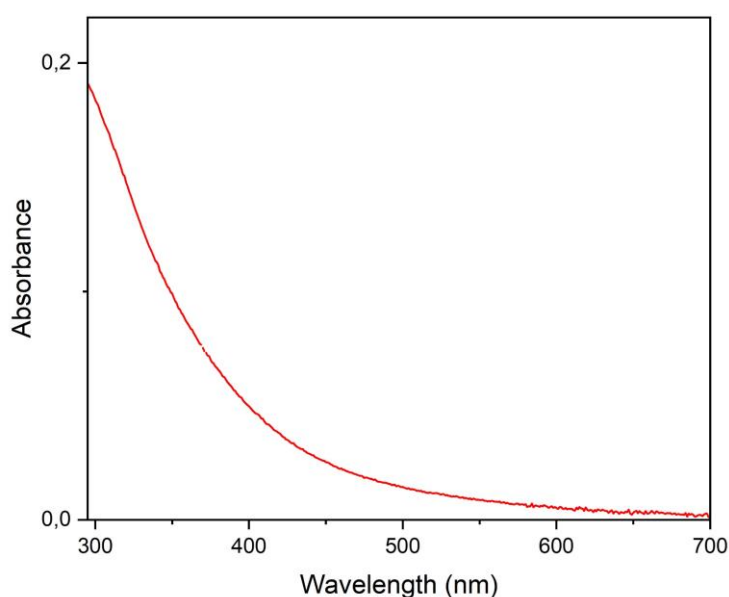


Figure 9: UV-Vis absorption spectrum of CDs

4.2.2 Characterisation by Fluorescence Spectroscopy

As depicted by Figure 10 (b) the FL emission spectrum of the CDs was recorded at excitation wavelengths 320–360 nm at intervals of 10 nm. When the excitation wavelength was changed from 320 to 340 nm, the FL intensity increased, whereas when the excitation wavelength increased from 340 to 360 nm, a gradual decrease in the FL intensity of the emission peak was observed. This reveals that the fluorescence exhibited by CDs is highly reliant on the excitation wavelength. The excitation dependent behavior can be attributed to defect states characterized by a wide energy distribution. These defects are found on either core of CDs or within oxygenated groups such as carbonyl, ether, and carboxylic acid [164]. The decrease in FL intensity could be due to re-absorption of emitted fluorescence [165]. The

optimum fluorescence intensity was observed at the excitation wavelength of 340 nm. Figure 10 (a) confirms 340 nm as the optimum excitation wavelength hence the mirror images of excitation and emission graphs. The spectrum in Figure 10 (b) depicts a red shift which may result due to changes in the CDs size, composition and surface chemistry [166]. A similar phenomenon was reported by Tesa (2021) who explains this behavior to the size-dependent shape and corresponding edge-state variations of CDs [167].

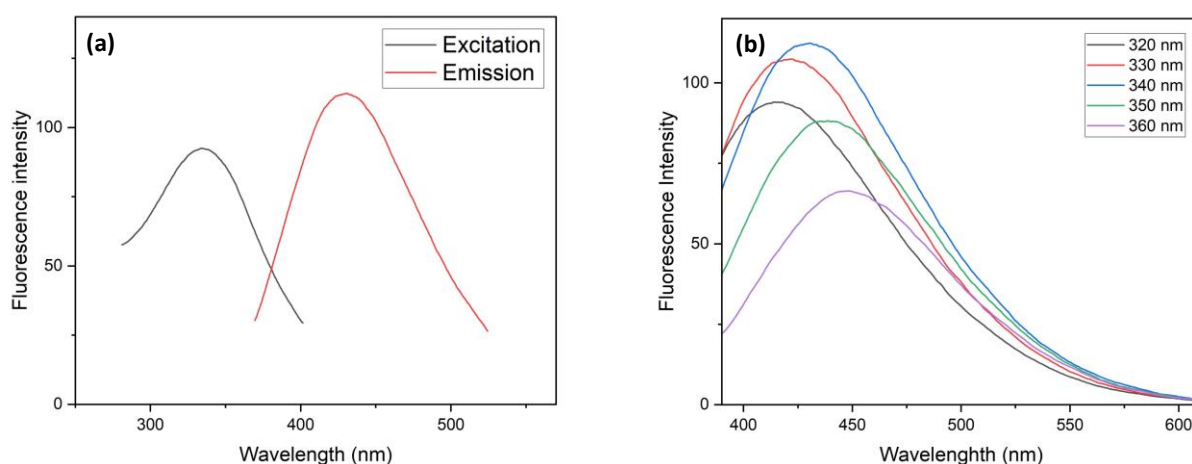


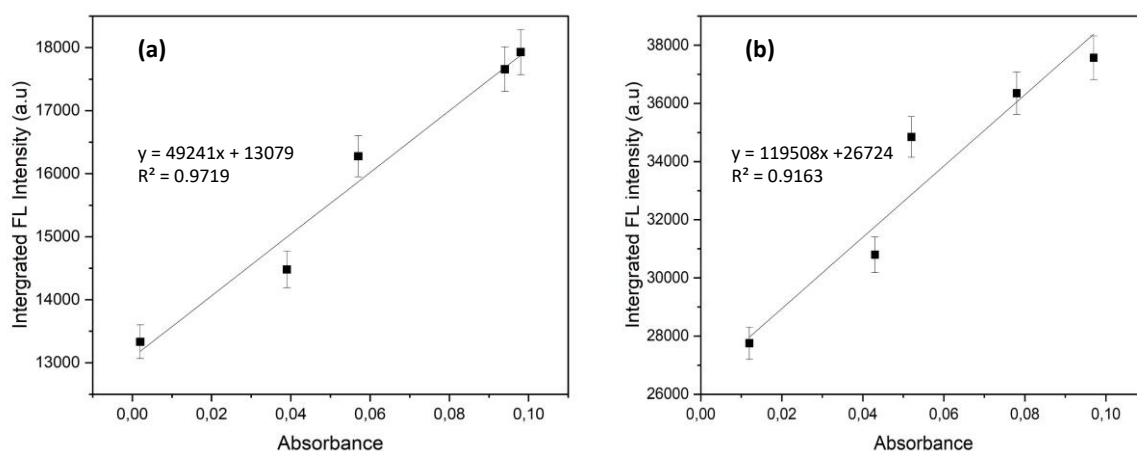
Figure 10: FL spectra of the as-prepared CDs (a) excitation and emission spectrum of the CDs, (b) emission spectra of the as-prepared CDs at different excitation wavelengths

4.2.3 Determination of the fluorescence quantum yield

The FQY of the prepared CDs was determined to be 19.4% which according to literature is good for CDs synthesized from biomass [168],[32]. Even though that is the case, Dinake *et al.*, (2022) suggests that CDs FQY can be further improved by surface passivation to introduce other functional groups that enhance CDs photoluminescent properties [32]. Quinine sulfate (54%, 0.1 M H_2SO_4) was used as a reference standard and linear plots were used to establish the FQY, with integrated fluorescence intensity plotted against absorbance for both the prepared CDs Figure 11 (a) and quinine sulfate standard Figure 11 (b). The gradients of the linear graphs in Figure 11 (a) and (b) were used in an equation stated in section 3.5 to calculate the FQY. The excitation wavelengths for the CDs and Quinine sulfate were maintained at 340 nm and 310 nm respectively. Table 3 show a compilation of some studies in which biomass was used for the synthesis of CDs with the FQY determined using quinine sulfate as reference standard. Notably, the FQY reported in these studies are comparatively lower than the one achieved in the current study.

Table 3: FQY of CDs from biomass starting material.

Biomass	FQY (%)	Reference
Pigeon egg yolk	16.34	[169]
Wheat Straw	9.2	[170]
Garlic	17.5	[171]
Pigeon egg white	17.48	[169]
Tobacco leaves	13.7	[168]
Monkey Orange	19.4	Developed method

**Figure 11:** Plots of absorbance vs integrated FL intensities for determination of FQY; (a) CDs, (b) Quinine sulfate

4.2.4 Determination of Bandgap energy

The Tauc plot method was used to determine the band gap energy of the prepared CDs based on the plot of absorbance versus wavelength. The calculated value was approximately 3.45 eV. This value aligns closely with the findings of Arroyave *et al.*, (2020) who reported a comparable band gap value of approximately 3.6 eV for their silver-coated CDs [172]. This value according to Yu *et al.*, (2016) is relatively large [37]. Bandgap energy is affected by the size of CDs, small particle size result to a higher energy bandgap [132],[173]. This infers that the CDs prepared in this study have small particle size. Additionally the as-prepared CDs can be considered as direct semiconductors with significant band gap that is well-suited for fluorescence measurements [174]. Moreover, the bandgap energy can also assume that the prepared CDs are typically more photo-stable.

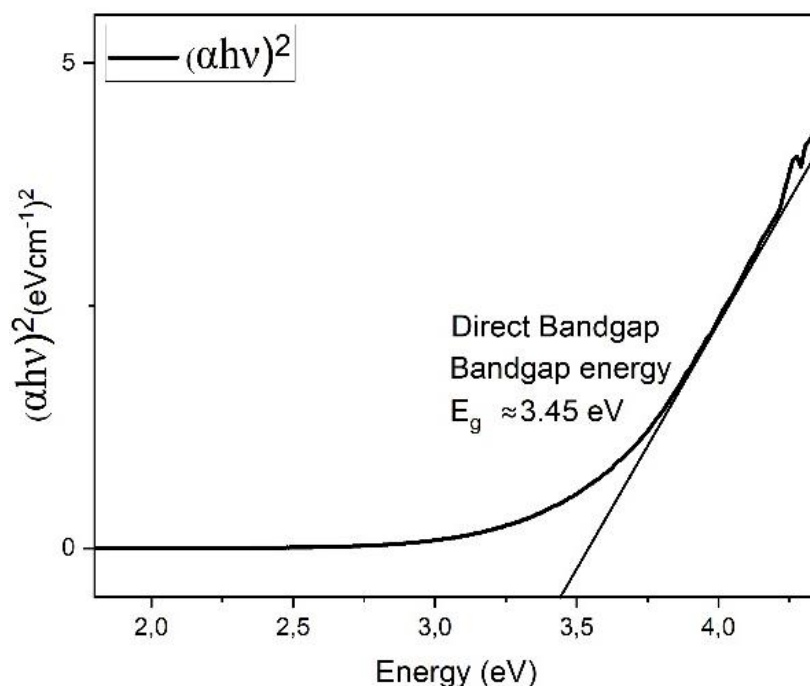


Figure 12: Spectrum of bandgap energy for the CDs

4.3 Optimization of CDs synthesis conditions

The sensitivity and selectivity of CDs in detecting PAHs is dependent on CDs optimum performance, therefore this section was focused to optimize CDs synthesis conditions. The objective was to optimize parameters including pH, synthesis temperature, and synthesis time so as to achieve the highest efficacy in detecting the PAHs compounds.

4.3.1 pH Optimization

As shown by Figure 13 (a), the FL of the CDs was found to exhibit a slight increase in intensity as the pH of the system was increased from 5 to 8. However, a subsequent increase in pH from 8 to 9 resulted in a decrease in FL intensity. Consequently pH 8.0 was determined to be optimal pH for the study. The result is in agreement with the pH dependence fluorescence behavior of CDs reported by Majoni and coworkers [174]. Although it can be inferred that the FL intensity of the CDs is influenced by the pH of the system, the observed changes are minimal, suggesting that the effect of pH on the fluorescence intensity is relatively minor.

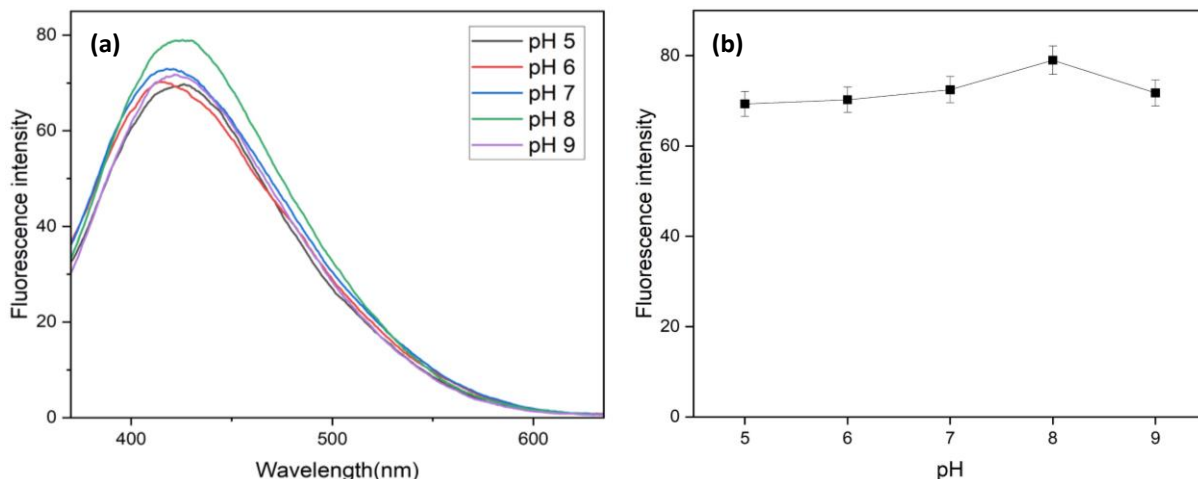


Figure 13: FL behaviour of CDs under different pH conditions; (a) FL intensity graph, (b) FL trend plot

4.3.2 Temperature Optimization

Temperature is known to affect the degree of carbonization of carbon core and the surface state therefore it is of vital importance to investigate the best synthesis temperature for the CDs. As depicted by Figure 14 (a) when temperature was increased from 100 °C to 200 °C, the FL intensity increased and there was a gradual decrease as the temperature increased from 200 °C to 300 °C. The maximum fluorescence intensity was achieved at 200 °C hence it was selected as the optimum synthesis temperature. Figure 14 (b) shows a simplified trend of the FL behavior of CDs as the temperature is changed.

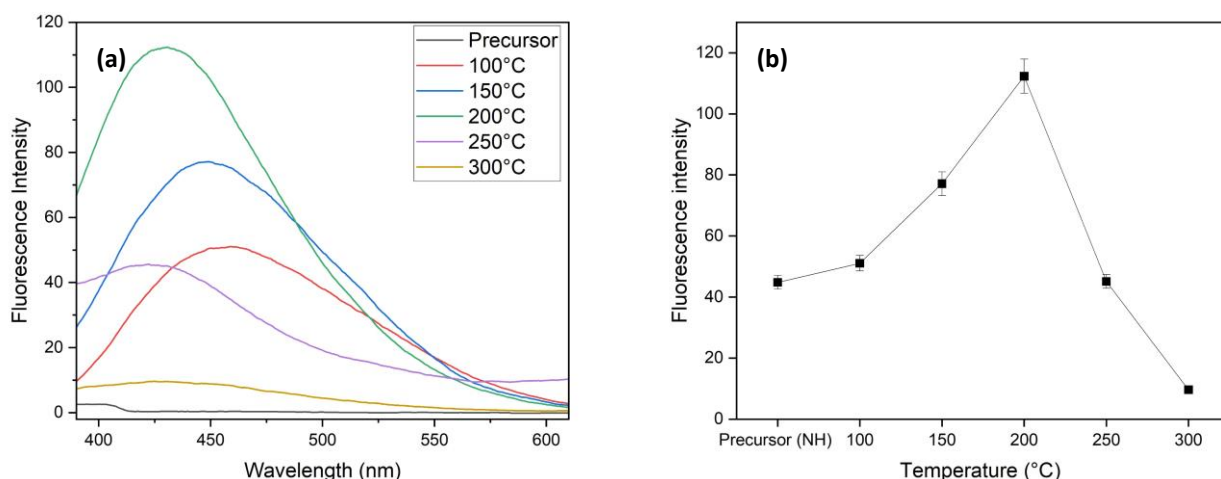


Figure 14: FL response of CDs under different synthesis temperatures; (a) FL intensity graph, (b) FL trend plot.

4.3.3 Synthesis Time Optimization

Figure 15 illustrates the impact of time on the fluorescence intensity of the system. Although the results are not showing a uniform trend, the graph shows a distinctive peak at an incubation time of 3 hours. Thus, 3 hours was conservatively chosen as the optimum synthesis time.

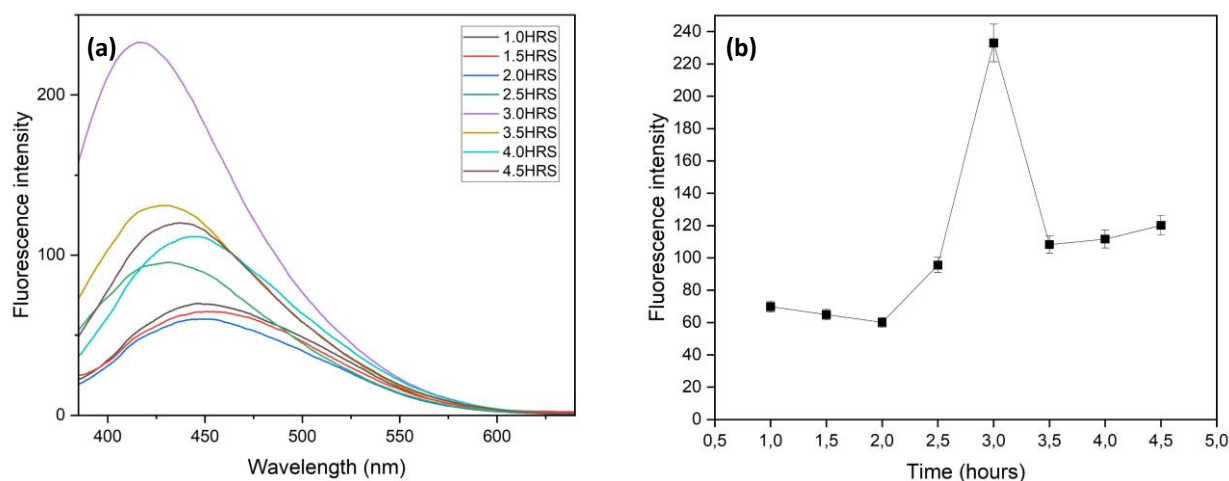


Figure 15: FL response of CDs under different synthesis time; (a) FL intensity graph, (b) FL trend plot.

4.4 Stability tests

To examine their stability, the as-prepared CDs were subjected to aqueous, acidic and basic conditions. One experiment examined the effects of varying concentrations of NaCl on the FL intensity of CDs. NaCl was selected to replicate the conditions resembling the environment in which the analytes are typically present. As shown in Figure 16 (a), the results indicated that the FL intensity of the CDs remained steady across the range of NaCl concentrations (0.2 to 1.0M). Even at high concentration of NaCl, the synthesized CDs proved to be very stable with no precipitate formation. Additionally, the study assessed the influence of pH from 5 to 9 on the FL intensity of CDs. Figure 16 (b) shows that there was minimal to no variation in the FL intensity except for a slight change at pH 8. These results demonstrated the good stability of CDs.

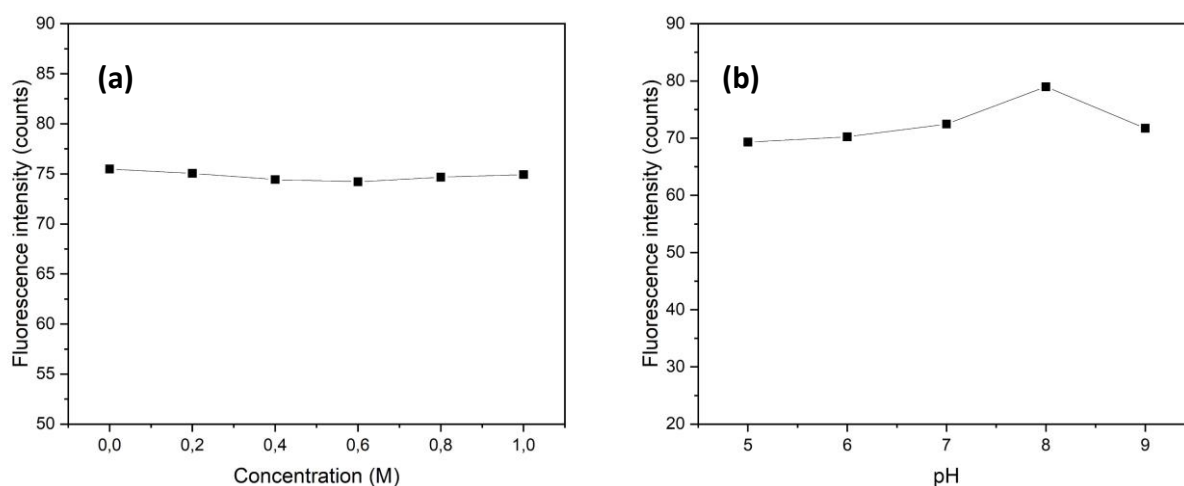


Figure 16: CDs' stability response; (a) different concentrations of NaCl, (b) varying pH environment of the reaction system

4.5 Fluorescence sensing

As shown by Figures 18 and 19 the detection of PAHs occurs through FL enhancement. This mechanism is described to be because of energy transfer between sensor and the target analyte. [5] Schematic illustration shown by Figure 17 summarises the sensing mechanism. This energy transfer may arise from various factors, including hydrogen bonding and pi-pi interactions [22]. The CDs were applied in detection of An and Fla as shown in Figures 18 and 19, the FL enhancement was observed upon addition of increasing concentrations of An and Fla. The FL enhancement was linear within 1-10 μ M range. The CDs showed better linearity in detecting Fla ($R^2 = 0.99583$) as compared to An ($R^2 = 0.99242$) however both results were satisfactory. For dissolving An and Fla, three solvents; DMF, acetonitrile and Dimethylsulfoxide (DMSO) were tested. The results indicated that DMF in its purest form has good solubility for the two PAHs. Consequently, it was chosen as the PAHs solvent.

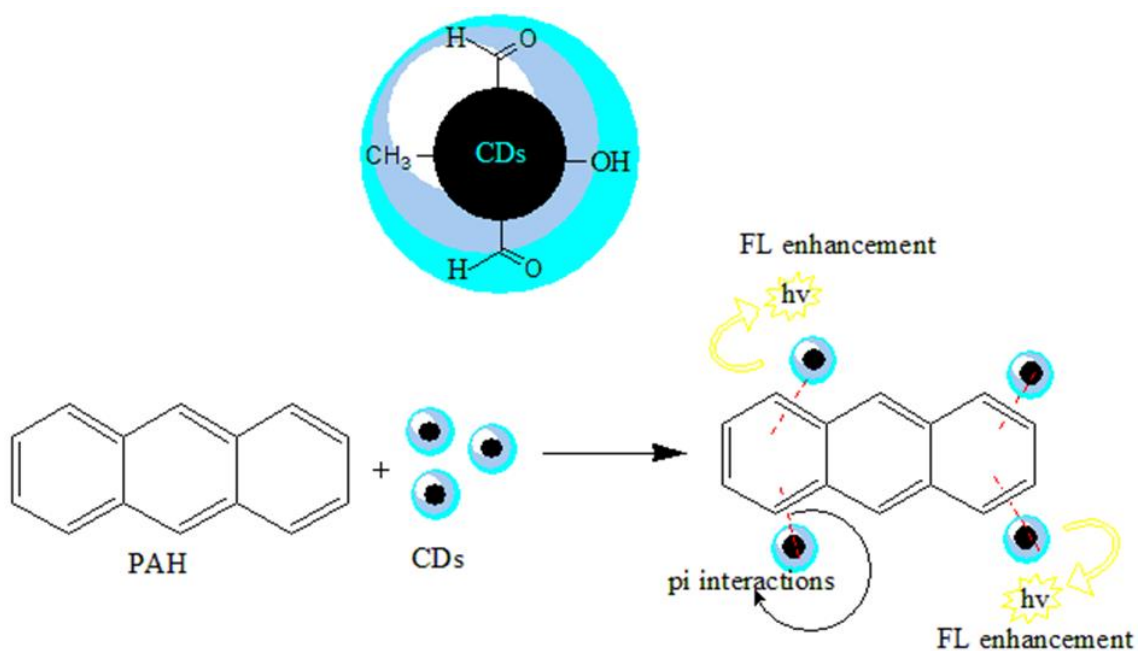


Figure 17: Schematic illustration of the interaction mechanism of CDs and PAHs resulting in FL enhancement

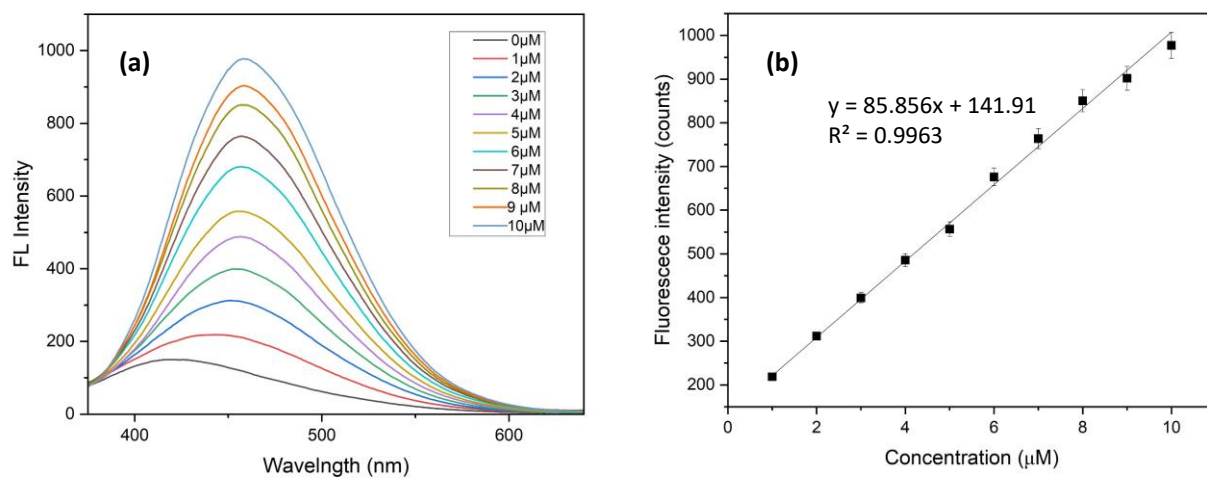


Figure 18: Fluorescence response of CDs and Fla; (a) spectrum of CDs with different concentrations of Fla, (b) corresponding calibration curve.

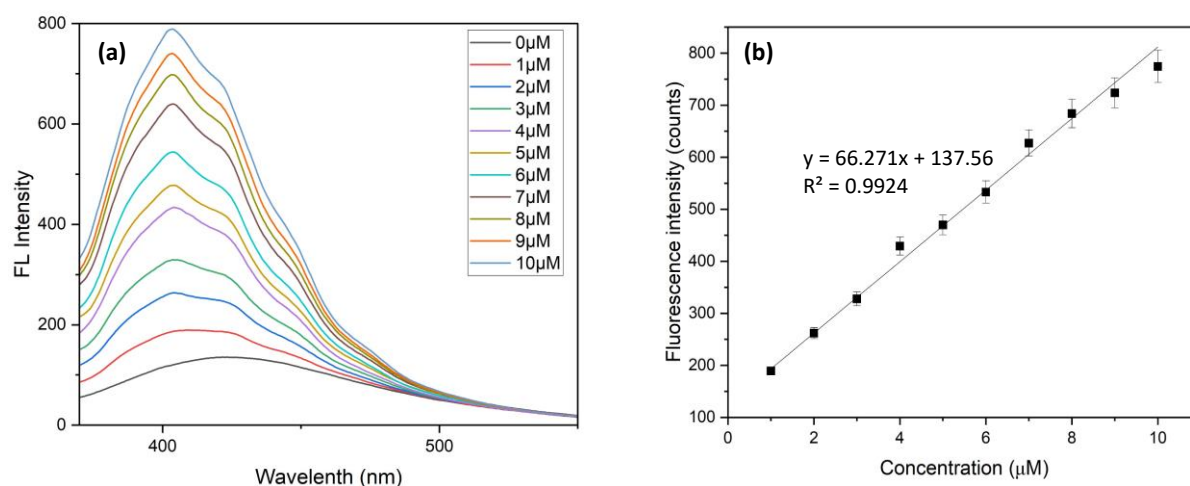


Figure 19: Fluorescence response of CDs and An; (a) spectrum of CDs with different concentrations of An (b) corresponding calibration curve

4.6 Quantitative detection of PAHs in water samples

In order to validate the reliability, sensitivity and applicability of the developed probe, the method was tested in real water samples, including tap water and water from Letsibogo dam. To fully utilize this method, HPLC was employed as a reference method. The reference method used a standard containing the two PAHs to determine the retention times for An and Fla. As shown by Figure 20, the results indicate that An and Fla elute at around 7.50 and 8.03, respectively. The chromatograms of the water samples shown by Figure 21 (a) and (b) impressively show that there was no An and Fla detected for the unspiked water samples as there are no peaks detected at retention times of 7.50 and 8.03 minutes. This result eliminated the possibility of false positives as there will be no additional concentrations of the two PAHs in the water samples upon spiking. The negative peaks observed in the chromatograms indicate that the molecules eluting from the column absorb less light than the mobile phase immediately before and after the peak. This phenomenon can occur when a relatively low detection wavelength is used and the mobile phase contains additives that significantly absorb light at these wavelengths [175]. Water samples being tap water and water from Letsibogo dam were spiked with concentrations of An and Fla with 7 μM for Fla and 8 μM for An. Using calibration curves for both proposed (CDs) and conventional method (HPLC), the determined concentrations for An in tap water was 8.13 while 7.19 was calculated for Fla using CDs. This results were better as compared to the HPLC results where 7.94 was found for Fla even though with An the calculated concentration was the same. As for Letsibogo dam water sample, CDs detected 7.64 An and 6.79 Fla compared to HPLC that determined

concentrations of 8.34 and 7.80 for An and Fla respectively. The recoveries of both An and Fla in the spiked samples for the proposed method were in the range of 95.31% –101.63%, while the reference conventional method recorded recoveries in the range of 101.63% – 111.42%. The summary of the results are tabulated by Table 4. In overall, the results for the proposed sensor proved to be better than the result from HPLC. These results demonstrated that the developed method had great potential for quantitative detection of An and Fla in real water samples.

Table 4: Quantification results of An and Fla in tap and Letsibogo water samples by developed method (CDs) and conventional method (HPLC)

		Developed method			HPLC method		
Sample	PAH	Added (μM)	Found (μM)	%Recovery	Added	Found	%Recovery
Tap water	An	8.00	8.13 ± 0.11	101.63	8.00	8.13 ± 0.05	101.63
	Fla	7.00	7.19 ± 0.07	102.71	7.00	7.94 ± 0.14	113.43
Letsibogo dam water	An	8.00	7.63 ± 0.20	95.31	8.00	8.34 ± 0.53	104.25
	Fla	7.00	6.79 ± 0.03	97.00	7.00	7.80 ± 0.15	111.42

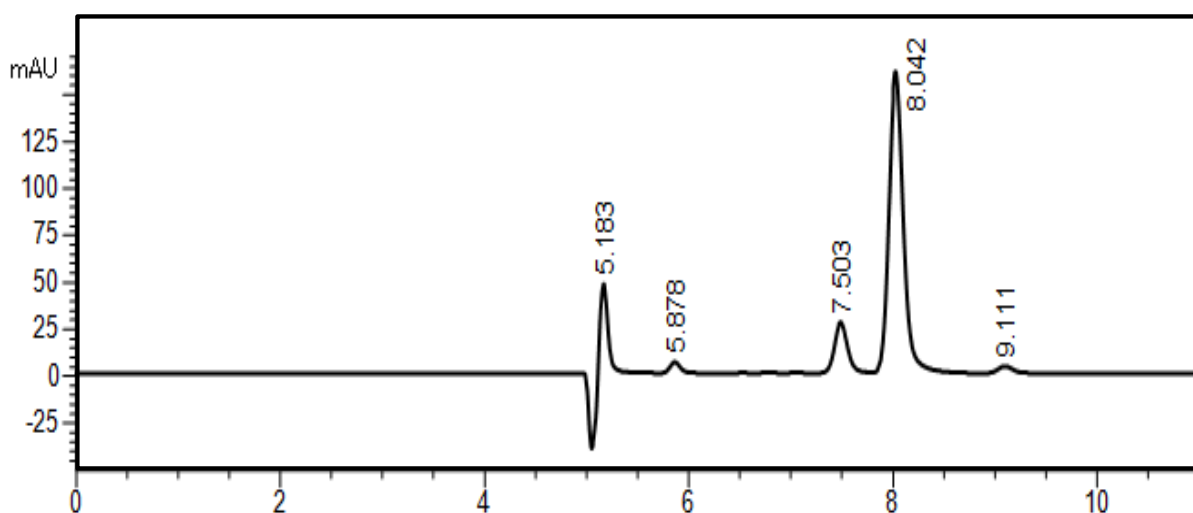


Figure 20: HPLC chromatogram of the standard containing An and Fla

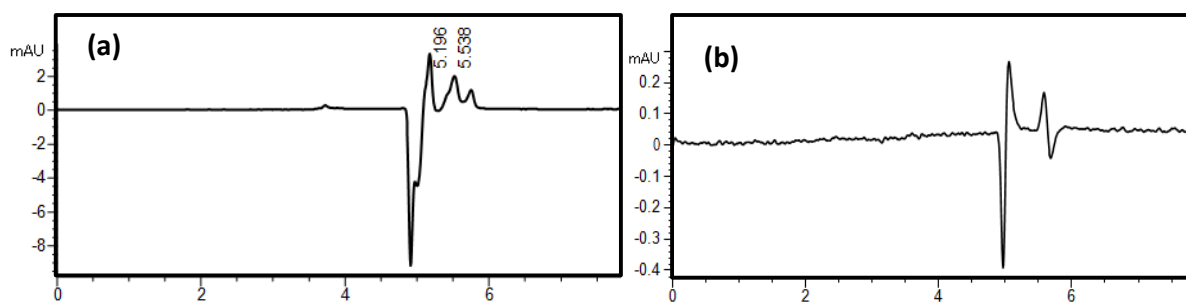


Figure 21: HPLC chromatogram for the unspiked water samples; (a) Letsibogo dam water (b) Tap water

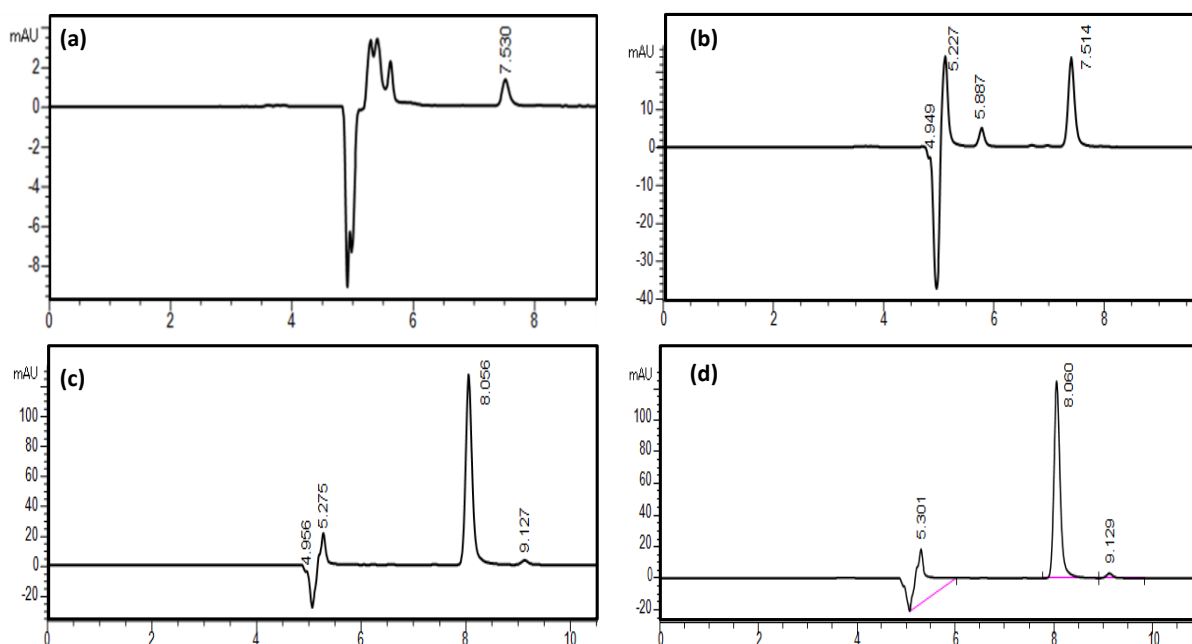


Figure 22: HPLC chromatograms for the spiked water samples; (a) An in tap water, (b) An in Letsibogo dam water (c) Fla in tap water (d) Fla in Letsibogo dam water

4.7 Method Validation

The developed method underwent validation in accordance with the guidelines set by the International Council for Harmonization (ICH) [176]. The validation process assessed the following parameters.

4.7.1 Sensitivity and Linearity

LOD and LOQ were obtained in respective PAHs using a linear regression model at 95% confidence level. $LOD = (3 \cdot \sigma) / S$, $LOQ = (10 \cdot \sigma) / S$, where σ represents the standard deviation of y intercepts of regression lines and S indicates the slope of the calibration curve. The developed method shows satisfactory sensitivity. Linearity was assessed by analysing the FL

intensity of 10 different concentrations for each PAH. Calibration curves were generated within the specified linearity ranges as illustrated in Figure 18 and 19 for the two PAHs. The robust linearity of the calibration curves was shown by a high coefficient of determination values. Linearity and figures of merit results are presented in Table 5.

Table 5: The coefficient of determination (R^2) of the calibration curve and results of the detection and quantification limits

PAH	R^2	Limit of Detection (μM)	Limit of Quantification (μM)
Fla	0.99583	1.39	4.23
An	0.99242	2.00	6.06

4.7.2 Accuracy

To validate the accuracy, a comparison was made with the results obtained from the reference method (HPLC). The assessment involved the analysis of two different concentrations of An and Fla. These concentrations were determined using the respective calibration curves, and the percentage recoveries ranged from 95% to 103%. A summary of these results is presented in Table 6.

Table 6: Percentage recoveries of PAHs in water samples

		Developed method			HPLC method		
Sample	PAH	Added (μM)	Found (μM)	%Recovery	Added (μM)	Found (μM)	%Recovery
Tap water	An	8.00 ± 0.80	8.13 ± 0.11	101.63	8.00 ± 0.80	8.13 ± 0.05	101.63
	Fla	7.00 ± 0.80	7.19 ± 0.07	102.71	7.00 ± 0.80	7.94 ± 0.14	113.43
Letsibog o dam water	An	8.00 ± 0.80	7.63 ± 0.20	95.31	8.00 ± 0.80	8.34 ± 0.53	104.25
	Fla	7.00 ± 0.80	6.79 ± 0.03	97.00	7.00 ± 0.80	7.80 ± 0.15	111.42

4.7.3 Precision

Precision was assessed by analyzing three replicates of concentrations for An and Fla, covering their respective linearity ranges. This evaluation included both intra-day precision (repeatability) and inter-day precision (intermediate precision). The %RSD was below 6% for all the PAHs in water samples, indicating a high level of repeatability and intermediate precision for the proposed method. The results of precision studies are presented by Table 7.

Table 7: Percentage relative standard deviation for intra-day (within one day) and inter-day precision (between days)

PAH	Sample	%RSD (intra-day)	%RSD (inter-day)
AN	Tap water	2.68	1.74
	Letsibogo	3.29	4.09
FLA	Tap water	5.21	3.00
	Letsibogo	1.07	4.16

4.7.4 Specificity

Different metal ions were each mixed with CDs to investigate their potential as interfering substances and the FL intensity of the CDs alone was also recorded. As shown in Figure 23, at a high concentration of 500 μ M, the interaction of CDs with different metal ion interferences are not showing significant differences in their fluorescence intensities, which suggests that the developed method is not affected by the investigated interferences. The analysis of variance was performed at $\alpha = 0.05$ and it showed that there is no significant difference between the mean values of the metal ion interferences.

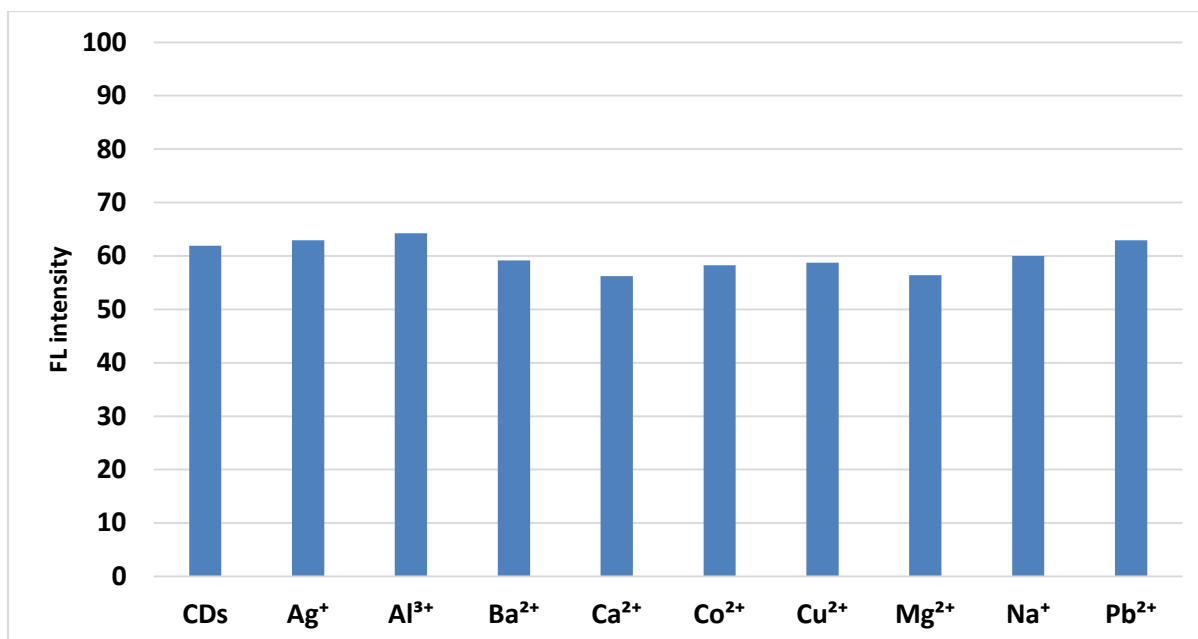


Figure 23: FL intensity of CDs interacted with different metal ions

4.7.5 Ruggedness

To evaluate the ruggedness, evaluations of intraday and inter-day precisions were conducted, as per table 7. The precision was notably good as shown by low percentage relative standard deviation (% RSD).

4.7.6 Robustness

Robustness was established from stability test as per section 4.3. The results indicate minimal changes to the CDs when pH and NaCl concentrations were altered. Moreover, the FL intensity remained relatively unaffected by these variations hence the method can be deemed robust.

4.7.7 Selectivity

Selectivity of the method was evaluated by studying the interaction of CDs with three specific PAHs, namely Fla, An, and Nap, each at 0.5 μ M. Figure 24 summarizes the experimental results. It shows that the fluorescence intensity was enhanced for Fla and An while it declined for Nap. This suggests that although all three analytes belong to the PAH class of compounds, the proposed method displayed preferential response towards Fla and An and demonstrated selectivity towards Fla and An and not towards Nap. Section 4.5 already

states that the mechanism of interaction between CDs and PAHs could be as a result of pi-pi interactions. Therefore this selectivity behavior could potentially be because An and Fla have larger and more extended pi-conjugated systems due to the additional benzene rings, compared to naphthalene. This extended pi-conjugation might lead to stronger interactions with CDs, resulting in more noticeable responses for the 2 PAHs.

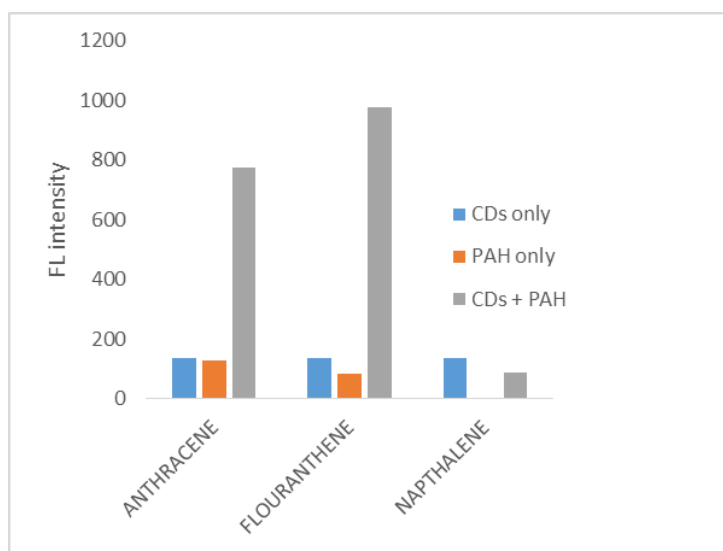


Figure 24: Interaction of CDs with different PAHs at concentration of 0.5 μM

CHAPTER 5

5.0 CONCLUSION

The ongoing release of PAHs into the environment by various processes stresses the need for their continuous monitoring. This is mainly important due to the potential adverse effects associated with these compounds. Consequently, it is important to come up with reliable analytical methods to consistently monitor PAHs in the environment. This study was aimed at developing a rapid cost effective technique which involved synthesising CDs from abundant monkey orange fruit and use their fluorescence properties for detecting PAHs in environmental samples. This aim was achieved by meeting the objectives as per section 1.5.1. The synthesised CDs possessed numerous advantages including high water solubility, high FQY (19.7%), excellent bandgap energy (3.45 eV), remarkable photoluminescence as well as small particle size (3.6 nm). They also displayed characteristic properties of CDs as reported in the literature, such as an amorphous structure with poor crystallinity indicative of turbostratic carbon. Additionally, they showed the presence of several functional groups, a common feature of CDs. The developed method was successfully optimized by systematically varying parameters such as temperature and time to achieve optimal sensing results. CDs were not only successfully synthesized from eco-friendly precursor materials, but also served as a reliable and sensitive probe for detection of An and Fla in real water samples. The results were better as compared to conventional HPLC method as evidently shown by better recoveries. Additionally the method showed low LOQ and LOD and high specificity. The methodology of the study reflect a reduction in harmful waste generation and the use of toxic reagents, contributing to a more sustainable approach to research. The success of CDs as probes for detection of PAHs opens opportunities for further research. Scientists may explore various biomass sources, synthesis techniques, and functionalization strategies to enhance CDs performance, potentially leading to even more advanced and tailored detection methods. These marks an important step towards sustainable and eco-friendly nanomaterial production.

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