ORGANIC MATRICES FOR THE DETECTION AND MONITORING OF ENVIRONMENTAL ANALYTES

M.Sc. Thesis By Tanishq Meena



BIOSCIENCES AND BIOMEDICAL ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY INDORE

MAY 2023

ORGANIC MATRICES FOR THE DETECTION AND MONITORING OF ENVIRONMENTAL ANALYTES

A THESIS

Submitted in partial fulfilment of the requirements for the award of the degree

of

Master of Science

By

Tanishq Meena



BIOSCIENCES AND BIOMEDICAL ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY INDORE

MAY 2023



INDIAN INSTITUTE OF TECHNOLOGY INDORE

CANDIDATE'S DECLARATION

I hereby certify that the work being presented in the thesis entitled ORGANIC MATRICES FOR THE DETECTION AND MONITORING OF ENVIRONMENTAL ANALYTES in the partial fulfilment of the requirements for the award of the degree of MASTER OF SCIENCE and submitted in the DEPARTMENT OF BIOSCIENCES AND BIOMEDICAL ENGINEERING, Indian Institute of Technology Indore, is an authentic record of my own work carried out during the time period from August 2021 to May 2023 under the supervision of Dr. Abhijeet Joshi, Associate Professor, Department of Biosciences and Biomedical Engineering.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other institute.

Jamely 5/2023

Tanishq Meena

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

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First and foremost, I extend sincere gratitude to **Dr. Abhijeet Joshi** for allowing me to be a part of and learn from his team. His unwavering support, both academic and personal, will always be cherished. I have grown tremendous lengths under his supervision.

I also want to acknowledge the efforts by fellow members of the TheraSens lab, namely **Dr. Sharan Rathnam, Mr. Tanmay Vyas, Mr. Badri Sahoo, Mr. Tileshwar Sahare, Ms. Palak Saket**, and **Mr. Yogesh**. Without their presence, learning would hardly have been as fun as it has been. All of their efforts in making me learn valuable hard and soft skills will always be prized. A great thing about everyone mentioned is that they do not discourage anyone from trying new things and learning. While encouraging others, they share their own insights and experiences whenever they can. I hope they continue to guide upcoming students as they have guided me.

I also thank my PSPC members, **Dr. Parimal Kar** and **Dr. Hem Chandra Jha** for their valuable inputs.

Extending my gratitude, I thank **Professor Suhas Joshi**, Director, IIT Indore for providing me the opportunity to learn from one of the most prestigious research institutions in India. I tip my hat to his efforts to make IIT Indore greater with each passing day. I thank **Professor Amit Kumar**, HOD BSBE, **Professor Prashant Kodgire**, DPGC Convenor, and **Dr. Parimal Kar**, Course Coordinator, BSBE, for their continuous valuable support to students not only during this project but also throughout the M.Sc. program.

In the same light, I express gratitude toward the **BSBE Faculty** and **BSBE Office** for every kind of support there can be. The inputs received from the faculty during evaluation sessions have only contributed to my growth as a student and as a person. Members of the BSBE Office have always been there to solve any problem that students may encounter, and I acknowledge their effort for the same.

I thank the members of Prof. Prashant Kodgire's **Molecular Immunology Lab**, specifically **Mr. Ankit Jaiswal** and **Ms. Surbhi Jaiswal** for going out of their way and sharing valuable insights about various topics throughout my time in the lab, whenever I sought them from them. They has no reason to, except their innate kindness, for which I will feel forever grateful. I thank other members of that lab for making my time there a bit easier.

Lastly, I thank my classmates and fellow lab mates, Ms. Isha Dhingra, Mr, Junaid Ahmed, Mr. Rishav Nag, Ms. Sushma Ahirwar, Mr. Nikhil Kumar, and Mr. Mohanchaitanya Reddy for all the laughs and happy memories I share with them, and for which they all have been a big reason. I learned teamwork and how to be more social from these guys, and I want to acknowledge them from the same. I thank them from never shying away from helping me or including me as a friend.

To sum up, I could not have asked for a better lab, better people, and better overall experience, and I would choose these people again if given a chance, in a heartbeat.

TM

This thesis is dedicated to everyone who was genuine in their approach toward me.

ABSTRACT

This work attempts to extend ongoing research in the domain of environmental monitoring. The first of the two objectives in this project has been carried forward keeping in mind the ever-growing problem of water pollution. One step toward water remediation is finding new ways to detect and/or remove pollutants from water, especially organic dyes. Many physical and chemical techniques have been employed for detecting dyes in water samples, the most recent of them being fluorescence-based sensors. This study focuses on checking the applicability of one such class of fluorescent biosensors, called carbon quantum dots, or CQDs, for this purpose. Spike and recovery experiments revealed the influence of different water matrices on the sensing ability of CQDs.

The next step after successfully detecting the analyte is its removal. For this purpose, a physical system consisting of alginate beads containing activated charcoal is used. Adsorption capacity of said system is tested to see the extent of adsorption of selected synthetic dyes carried out by it, under different parameters. Empirical data reveals that this system is an efficient method of ridding water from contaminants, specifically dyes.

LIST OF PUBLICATIONS

- Singh, Y., Meena, T., V S, Sharan Rathnam, Vyas, T., Joshi, A., Sonawane, A. Emerging applications of nanotechnology in human welfare, *Springer Nature*. 2022-
- Shanmuga, S. R., Deepak, T., Sahoo, B., Meena T., Singh, Y., and Joshi, A. Metallic nanocarriers for therapeutic peptides: Emerging solutions addressing the delivery challenges in brain ailments. *Journal of Experimental Pharmacology and Toxicology*. 2023

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ACRONYMS

S. No.	Acronym	Expansion (meaning)	
1.	CQD	Carbon Quantum Dot	
2.	HPLC	High Performance Liquid Chromatography	
3.	PA	Phthalic acid	
4.	TED	Triethylenediamine	
5.	TEM	Transmission Emission Microscopy	
6.	XRD	X – Ray Diffraction	
7.	FTIR	Fourier Transform Infrared Spectroscopy	
8.	EDX	Energy Dispersive X - Ray	
9.	UV – vis	Ultraviolet – visible regions of the	
		electromagnetic spectrum	
10.	OFS	Optic Fiber Spectrometer	
11.	LOD	Limit of Detection	
12.	LOQ	Limit of Quantification	
13.	MCT	Microcentrifuge tube	
14.	HPLC	High-Performance Liquid Chromatography	
15.	LC-MS	Liquid Chromatography – Mass Spectrometry	
16.	FITC	Fluorescein isothiocyanate	
17.	EPEC	Enteropathogenic E. coli	
18.	PMMA	Poly(methylmethacrylate)	
19.	NIR	Near Infrared	
20.	μΜ	Micromolar concentration of substance	
21.	Ru(bpy)	[Ru(bpy) ₃] ²⁺ Cl ⁻ ; Tris(bipyridine)ruthenium(II)	
		chloride	
22.	λ_{ex}	Excitation maximum; Peak wavelength of	
		excitation spectrum of a compound	

Chapter 1 Introduction

1.1 Overview

The term 'pollution' describes the presence of undesirable substances (called pollutants) in the environment. The presence of anything undesirable in the environment can be detrimental to the life living in that region. Usually, pollutants disrupt biological activities, for example, they can pose threat to the marine ecosystem by hampering the survival of fishes, or by inhibiting photosynthesis and eventual growth in aquatic plants. Upsetting of any number of species in any region can sometimes disturb the entire ecosystem; considering the interconnectedness of life everywhere, the harms posed to one life form are eventually harmful to life everywhere.

Pollution comes in various forms, characterized by the source of pollutant and the eventual target of the adverse effect. Major kinds of pollution are the pollution of air, water, and soil (**Fig. 1-1**).



Figure 1-1. Illustration showing the three major types of pollution, namely the pollution of air, water, and soil. *Image source: Water pollution with plastic bags in river Vector Image (vectorstock.com)*

Air pollution is majorly caused by the smoke and gases released by such sources as factories and industries, oil refineries, spontaneous and artificial forest fires, automobiles^[1]. On the other hand, water is polluted when unwanted substances are dumped in water bodies, big and small alike. A lack of necessary sanitation facilities is largely the reason for water pollution arising from domestic settings. Pollutants also arise from agricultural fields (owing to the excessive use of chemical fertilizers and pesticides), sewage, and spillage of oil and radioactive substances in oceans and rivers by factories, industries, and power plants^[2]. Similarly, many factors are implicated in causing soil pollution as well. Activities that cause soil pollution are both natural and man - made. Harmful chemicals, such as chlorine and perchlorate, can sometimes naturally get accumulated in the soil, leading to imbalances in the optimal soil composition. Artificial pollution - causing activities include excessive mining, foundry, and construction (contributes to the release and deposition of heavy metals), chemical waste dumping, uncontrolled release of fertilizers and pesticides, and accidental spills and leaks.

1.2 Adverse effects of pollution





Figure 1-2. Data showing deaths caused by various sources in 2015. Pollution ranked first in reasons for mortality. Image source: The Lancet, NPR

Any type of pollution, at any level and to any extent, comes with associated environmental problems. Pollution, in general, is the biggest environmental (and second overall) cause of mortality^[3], falling only second to severe diseases like AIDS and malaria. It was, at one point, the leading cause of mortality (**Fig. 1-2**).

Problems caused by pollution can be categorised as damage to the scenery and harm to the life in the affected region. Damage to the scenery simply means the aesthetic damage caused by the addition of undesirable pollutants and contaminants to the environment. Since humans are directly dependent on the proper functioning of the ecosystem, damage to the environment, although indirect, eventually adversely affects humans as well. The global economy requires approximately 100 billion tonnes of raw materials each year^[3]. By affecting the environment, pollution leads to increased demand of raw material which in turn leads to depletion of natural resources. This both negatively strains the environment as well as hinders economic growth.

Apart from the economic aspect, pollution comes with devastating health implications as well. All the agents associated with any kind of pollution are known to cause mild or serious health problems in humans and animals on which humans very dearly depend. Air pollution is considered the heaviest burden on the environment according to several sources, including the UN^[4]. It has been implicated with some fatal diseases, like heart disease and stroke, lung and related respiratory disorders, and cancer. As of 2019, over 99% people are living in regions where the strictest AQI guidelines (as set by WHO in 2021) were not being met (Fig. 1-3).



Figure 1-3. Illustration showing the annual mean levels of fine particulate matter PM_{2-5} . Each square represents a country. Darker squares show a higher concentration of PM_{2-5} particles. *Image source: Air Pollution Note – Data you need to know (unep.org)*

As essential a resource water is, it is still highly subjected to pollution (by means mentioned above). It alone caused the deaths of 1-8 million people in $2015^{[2]}$. Mortality rates are higher in third – world countries, where there is an overall unavailability to clean and drinkable water (**Fig. 1-4**). Some of the human health conditions associated with unsafe water consumption are giardia, typhoid, and cholera. On the environmental aspect, over – accumulation of nutrients in water can lead to eutrophication and the eventual choking of the water body (smaller bodies, like lakes, are more susceptible to this fate). Even in larger bodies, harmful chemicals, over long time periods, can lead to bio – accumulation. as the chemicals get accumulated in the tissues of the fishes. These fishes then get eaten by larger fishes, and those chemicals get transferred to the latter. In this way, increasingly greater amounts of chemicals end up as we go up the food chain. Another aspect is ocean acidification, which is problematic for coral life and shellfish.



Figure 1-4. Data showing deaths due to unsafe water consumption in 2019. Higher rates of mortality observed in low – income countries. Scale bar shows number of deaths per 100,000 people. *Image source:* <u>Clean Water - Our World in Data</u>

1.3 Organization of the thesis

This masters' project focuses specifically on the problem of water pollution. Keeping in mind the increasing ways in which water is getting polluted (and increasingly more inaccessible), the demand for water remediation methods are at an all – time high. This project attempts in this direction, developing and testing ways to detect and remove contaminants from water. In that light, the project holds two objectives, one for detection and the other for removal.

This thesis is organized in 3 overall chapters. The first chapter equips the reader with some background information regarding everything about pollution. With this knowledge, the reader can move to the more focused content in chapters 2 and 3- Chapter 2 deals with how sensors are used for our purpose of detecting certain kinds of analytes in water. Our work on carbon quantum dots is discussed. This chapter also tells how detection and monitoring studies are generally carried out. Chapter 3 builds up on the general objective of water pollution. It goes further to attempt ways to remediate water by ridding it from analytes that are detected in the previous chapter. Focused work using activated charcoal is explained in detail. The last chapter concludes the thesis and holds information about the possible directions this project can take.

The reader is advised to refer to the table of contents as well as the lists of figures and tables (all presented between pages xv and xix) for better navigation through the thesis.

1.4 Objectives

The project is divided into 2 objectives, both pertaining to water monitoring and remediation. The overall objective is "To check the application of different matrices in water remediation". Two sub – objectives within this broad one are as follows:

- 1. To check the applicability of CQDs in the detection of dyes in different water matrices
- To check the efficiency of charcoal infused alginate beads for the removal of dyes from water

Chapter 2 Dye Sensing using CQDs

2.1 Introduction

The issue of water pollution is ever – growing, and is, therefore, of unparallel concern, now more than ever (see chapter 1). Both natural and anthropogenic activities lead to the exploitation of potable water resources, making them scarcer. Anthropogenic activities that lead to water pollution include oil spills, leaching of pesticides and insecticides from agricultural fields into water sources, release of untreated wastewater from certain industries into local water bodies, among others causes (**Fig. 2-1**).



Figure 2-1. Various sources of water pollution

Industrial wastewater causes permanent damage to the ecosystem. Pollutants that make up most of the industrial wastewater are summarized in Table 2-1.

Industry	Major constituents	Contribution to water pollution	Reference(s)
	Dyes, salts, urea,		
Textile	H ₂ O ₂ , chlorinated	>14%	[5,6]
	compounds		
Agriculture	Pesticides, and	20/	[7]
	insecticides	3%	[/]
	Phenolic		
Petrochemical	compounds,	9%	[8]
	hydrocarbons,		
	Heavy metals		
Plastic and	(lead, mercury,	1%	[5]
rubber	cadmium)		
Painting	Volatile organic		
	compounds	10%	[9]
	(benzene, toluene)		

Table 2-1. Contribution of some industries to water pollution

2.1.1 Common water pollutants



Figure 2-2. Schematic diagram illustrating common water pollutants

Four major classes of water pollutants are as follows (**Fig. 2-2**). Microorganisms naturally make up a bunch of water. It is concerning when some problematic (disease - causing) microbes come to be present in water. Some of these are *Vibrio* species, *Legionella*, *Pfiesteria*, and *Naegleria*, among others^[10]. Their numbers in water is a function of prevailing environmental conditions, and the addition of certain chemicals can facilitate their excessive growth. Uncontrollable numbers of these organisms can lead to a number of waterborne diseases.

On the other end of the size spectrum, pollutants can also be macro. Anywhere from discarded plastic bottle and straws to oil spills. These pollutants generally interfere with the life forms in water bodies.

The other two classes of water pollutants are volatile organic compounds (VOCs) and inorganic chemicals. VOCs arise from sources like dry cleaning, paints, and cars exhausts. Common examples of VOCs include ethylene glycol, benzene, formaldehyde, toluene, xylene, and methylene chloride. VOCs have long been implicated in a variety of serious health problems, most common ones being respiratory problems (owing to their highly volatile nature) and skin and eye irritation. These are known carcinogens^[9]. Biggest inorganic offenders are heavy metals, such as arsenic, mercury, and copper, among other such metals. The hazards of these heavy metals have been thoroughly studied. Almost every heavy metal leads to life – threatening situations, from kidney complications and bone density issues to cancers^[11].

Another serious water pollutant is organic dyes. Dyes most commonly find their use in textile and other related industries. It is the untreated wastewater dumped uncontrollably by these industries in nearby water bodies that causes the problem (**Fig. 2-3**). It should also be noted that the textile industry not only contributes to water pollution, but also to air pollution^[12]. The hazards associated with dyes have been discussed in coming sections.

In this study, we will be working to detect organic dyes in different water samples. Further, we will test out the applicability of our detection method in different water sources.

2.1.2 Chemical dyes

Chemical dyes are pollutants that find their way in water bodies through the untreated industrial wastewater that is released into them. They even leach into and contaminate the groundwater, which then becomes unfit for domestic use, due to the adverse effects linked to it. These dyes are known to be mutagenic and carcinogenic, lead to organ failures, impairment of photosynthesis and inhibition of growth in plants, and accumulate in tissues of fish^[12]. **Figure 2-4** shows the general classification of organic dyes.



Figure 2-3. Colored effluent released from industries, particularly the textile industry. Image source: <u>https://wesustainabletextileforum.com/</u>

On top of that, these cause aesthetic damage to water bodies as prolonged release of coloured effluent from industries causes colour change in the water bodies in which they are released (Fig. 2-3)^[13]. Most synthetic dyes are known to be recalcitrant in the environment, and resistant to light, water, temperature, and biodegradation^[14].



Figure 2-4. Flowchart illustrating the classification of textile dyes

Dye-containing effluents released from industries have certain characteristics. These include elevated pH, increased chemical oxygen demand, bright colour, and elevated ecotoxicity^[15].

2.1.3 Classical methods of detecting dyes



Figure 2-5. Schematic diagram comparing the newer methods of analyte detection with the more classical methods

Keeping in mind the ecological and human health concerns associated with these harmful chemicals, the need for their detection in water becomes important. Since the earliest times, many methods have been put to work for the purpose of their detection. Some of the conventional methods are various chromatographic methods^[16], like thin-layer chromatography, HPLC, LC-MS, spectrophotometric methods^[17] like derivative spectrometry, kinetic spectrometry, sorption spectrometry, Raman spectrometry, and electrophoretic techniques like capillary electrophoresis^[18]. These conventional methods have some shortcomings. These include lengthy analysis time, high eluent usage (in the case of liquid chromatography), expensive, poor reproducibility, accuracy, and response time. On top of these points, these methods do not lead to point-of-care detection of analytes, which is the more sought – after approach of detection currently.

Some of the newer detection methods make the use of sensors for detecting dyes. Sensors are machines that can convert a physical quantity into a detectable signal with some coherent meaning. Based on the input (detectable quantity), sensors can be classified into various classes, including optical sensors, chemical sensors, and electrochemical sensors^[19]. Of recent, the optical sensors have been put to good use because of some advantages like high sensitivity and ease of use. These sensors include fiber optic sensors, colorimetric sensors, and fluorescence-based sensors. Of these, fluorescence-based sensors detect such physicochemical properties of analytes as fluorescence intensity, which changes according to a compatible change in concentration of that analyte. Fluorescent sensors are great for low-limit detection with decent sensitivity and selectivity^[20]. **Fig. 2-5** compares the features of conventional and recent detection methods

2.1.4 Fluorescent labels

Fluorescence as a phenomenon upon which sensors could be built has been explored quite extensively. Fluorophores with desirable properties can both be synthesized as well as found naturally. The choice of fluorophore can be made from a variety of them, including (a) small organic dyes, (b) chelated lanthanides, (c) biologically – available fluorophores (phycobiliproteins and GFP), (d) metal-ligand complexes like $[Ru(bpy)_3]^{2+}$, and (e) nanostructures, including $QDs^{[21]}$.

The physicochemical properties (which includes size and morphology, biocompatibility) of a fluorophore determines the potential for detection. When compared, organic dyes have few advantages over quantum dots (QDs). QDs are 0 – dimensional nanostructures (having each spatial dimension between 1 and 100 nm). What's interesting about them is that their properties are dependent on their mode of synthesis (which governs their size and shape) Fig. 2-6 illustrates how size determines the photoluminescence property of QDs. Smaller QDs generally emit shorter wavelengths (toward the blue of VIBGYOR), whereas larger QDs emit longer wavelengths. Considering the band gap theory of solids to explain the phenomenon, QDs observe an inverse relation between particle size and band gap. Band gap here refers to the energy gap present between the conduction and the valence bands (bands are energy levels that can host electrons, and from which electrons can jump to other levels of lower or higher energies). An increase in the QD size sees a decrease in the band gap (therefore emission of a photon with a longer wavelength.)





Figure 2-6. (a) Zero – dimensionality of QDs. (b) Quantum confinement effects in CQDs. Size-dependent variation in band gap and its correlation to emission wavelength

Fluorescent dyes and QDs differ variably in their optical properties. The absorbance of QDs gradually rises toward shorter wavelengths. Also, the emission wavelengths of QDs are tunable according to the excitation wavelength (and size). This property alone makes QDs a much more favourable candidate for detection studies (that use fluorescence) over organic dyes. Moreover, QDs show greater values for molar extinction coefficients as compared to organic dyes. This means that QDs would absorb more at a certain wavelength than dyes would, and more absorbance means more emission. QDs have been found to emit significantly both in the visible and NIR regions, whereas the NIR emission of dyes is limited (the dyes that do emit in NIR region are not very photostable). All these points turn the choice of fluorophore in the favour of QDs^[21].

One particular type of quantum dots, called carbon quantum dots, have been in the limelight for the last 15 years. These are preferred because of their desirable fluorescence properties. Their fluorescence emissions can be tuned according to the excitation wavelength^[22], and their sizes (carbon dots of various sizes can be synthesized by different methods). Nanoparticles other than carbon dots have been used for

detecting and removing industrial pollutants in water. Sharma et al.^[23] used a graphene/fluorescein system for detecting As^{3+} in drinking water, with a detection limit as low as 0.96 µg L⁻¹⁻ GQDs have been used for the detection of Hg²⁺, Pb²⁺, Fe³⁺, Ag²⁺, and Cu²⁺ with detection limits in the nanomolar range^[24]. Even carbon dots have been used for the detection of heavy metal ions. Yang et al. synthesized CQDs from citric acid and used them for the detection of Cu²⁺ and Hg^{2+[25]}. The principle of using CQDs for the detection of heavy metals is that heavy metals quench (decrease the fluorescence intensity) of CQDs, and therefore with increasing concentrations of heavy metals in water samples, the fluorescence of CQDs decreases. The use of CQDs in the detection of organic dyes has not been reported yet (**Table 2-2**).

Author(s)	Summary of Findings	Source(s)
Sharma et al. (2021)	Used a fluorescein/graphene system for	
	detecting As(III) ion in drinking water.	[23]
	Could detect as low as 0.96 μ g L ⁻¹⁻	
Revesz et al. (2022)	Used Graphene carbon dots for detecting	
	heavy metals. Attained detection limits in	[24]
	the nanomolar range	
Chaudhary et al. (2021)	Used CQDs from Phthalic acid and	
	Triethylenediamine to detect urea	[26]
	adulteration in milk	
Yang et al.	Synthesized carbon dots from citric acid	[07]
(2014)	and used them to detect Cu^{2+} and Hg^{2+}	[27]
Present study	Synthesis of CQDs from Phthalic acid	
	and Triethylenediamine to detect organic	-
	dyes in water matrices	

Table 2-2. Recently reported methods of detecting analytes in water

2.2 Quantum dot sensors

2.2.1 Carbon Quantum Dots

QDs behave as artificial clusters of semiconductive atoms having electrons with limited mobility due to their small size (a concept known as quantum confinement). QDs basically behave as individual atoms due to being so small. These molecules can absorb photons and re-emit them at longer wavelengths for a period, i.e., they are fluorescent. Semiconductor quantum dots, which were discovered before CQDs, were initially used for fluorescence-based sensing studies. However, semiconductor QDs are toxic and also cannot be used for long-term monitoring of a single molecule^[19]. CQDs are also an improvement over previously used organic dye fluorophores ,like FITC and Ru[(bpy)₃]²⁺, in that the former are more photostable, less toxic, have higher quantum yield, and are low-cost. Due to limitations like these, carbon-based QDs have been preferred over the past few decades in a variety of biomedical research areas.

Properties

Structurally, CQDs are quasispherical carbon-based nanoparticles with amorphous to crystalline carbon core. The CQDs are mostly made up of graphitic or sp² carbon, or combined sheets of graphene and graphene oxide^[28]. Detailed structure depends on the precursor(s) and the mode of synthesis used.

Optically, CQDs prepared from different precursors show different absorption spectra in different solvents, but the general range lies between the wavelengths 200 and 400 nm (the UV region)^[29]. CQDs have been shown to exhibit excellent fluorescence ability. The exact mechanism behind this property is not known, but the surface defects on CQDs have been linked to its^[30]. The photoluminescence and electronic properties of CQDs are highly tunable according to their size and the excitation wavelength, a property unique to CQDs (Fig. 10). Sizedependent excitation has to do with the phenomenon of quantum confinement, i.e., as the size of CQDs changes, the band gap between their conduction and valence bands changes. This variation in the energy gap leads to the release of photons of varying energy.

The quantum yield (efficiency of fluorescence) of CQDs can be further tuned by surface passivation (altering the functional groups on the surface of CQDs). In addition, the fluorescence emissions of CQDs are pH-dependent^[26,31–33]. Our CQDs, synthesized from phthalic acid and TED, show an increase in fluorescence as the pH of the solution is increased from 3-0 to 10.0.

Apart from the desirable optical properties mentioned above, CQDs are more biocompatible than their semiconductor counterparts and organic dye fluorophores, making them ideal for *in vitro* studies.

Due to the properties mentioned above, CQDs have been used in a variety of applications, including the sensing of metal ions in water^[30,34–36] and of other molecules, like dyes and other adulterants in food samples^[26,37], drug delivery^[38], *in vitro* and *in vivo* bioimaging^[39], and therapy^[40,41].

2.3 CHOICE OF DYES



Figure 2-7. Chemical structures of the 4 dyes that have been selected for this project

Four organic dyes have been selected for this project. These are methylene blue, methyl orange, methyl red, and bromocresol green (**Figure 2-7**). All of these are synthetic azo dyes (defined by having the azo, or the R–N=N–R', group). The bright colours of these aryl azo compounds are attributed to n-delocalization of electrons. On the one hand, methyl orange, methyl red, and bromocresol green are acidic (anionic), and are excellent pH indicators. On the other hand, methylene blue is a basic (cationic) thiazine dye, which is a redox indicator. All four are reported to be found in the effluent released from the textile industry.

The procedure for the sensing studies involves formation of calibration curves, followed by conduction of spike and recovery experiments. Spike and recovery study is done to test the effect a particular matrix (different water sources, in this case) has on the signal obtained from the analyte. This deviation is observed in comparison with the standard conditions.

2.4 MATERIALS AND METHODS

A. Materials

A1- For CQD synthesis: Phthalic acid (MW 166.14 g/mol) and Triethylenediamine (TED; MW 112-17 g/mol) were acquired from Sigma – Aldrich India[®] and used as received. Distilled water was used for the synthesis of the CQDs, and for preparing the working solutions.

A2- For dye detection: Methylene blue (318.85 g/mol), Methyl orange (MW 327.33 g/mol), Methyl Red (MW 269.3 g/mol), Bromocresol green (MW 698.01 g/mol). Distilled water was used for preparing the stock and working solutions.

B. Experimental setup for the preparation, and the monitoring studies. An 800 W microwave oven (Samsung, 230 V/50 Hz), a

UV – vis spectrometer (UV – 1900i, Shimadzu, Japan), an optical fiber spectrometer (OFS, Ocean Optics, USA) (Figure 2-8), Benchtop pH meter (Hanna Instruments[®])



Figure 2-8. The optical fiber spectrophotometer setup used for sensing experiments

C. Methodology

C1- Synthesis and characterization of CQDs. The CQDs were preparing by mixing phthalic acid and TED in a feed ratio of 1:1 (3 grams each) in 10 mL distilled water. The two solutes were dissolved thoroughly using a magnetic stirrer, following which the solution was microwaved at 800 W for 90 seconds. The solution changes from a transparent one to an orange one. The product was allowed to cool undisturbed at room temperature. After around 20 minutes, the solution was transferred in a plastic syringe (Dispovan[®]) and passed through a 0.22µ nylon syringe filter. Figure 2-9 shows a schematic explaining the procedure.



Figure 2-9. Schematic diagram showing the protocol employed for the synthesis of CQDs

The CQDs were characterized by transmission electron microscopy (TEM) for elemental analysis, morphology, and size (TEM CM200, Phillips India). X-Ray diffraction (XRD) was performed to determine the crystallinity of the material. By the means of lattice constant and crystalline phase, using a Rigaku Smart Lab X-Ray diffractometer with Cu X-rays ($\lambda = 1-540$ Å). The optical properties of the CQDs were analysed using fluorescence spectroscopy on an optic fiber spectrometer (Ocean Optics, USA) and a UV – vis spectrometer (UV – 1900i, Shimadzu). The chemical nature of the CQDs was determined using Fourier Transform Infrared Spectroscopy (FTIR) in the 4000 – 400 cm⁻¹ range. Lastly, Energy-dispersive X-Ray (EDX) analysis was done to determine the elemental configuration of water – soluble CQDs

C2- Dye sensing using CQDs. The pH response of CQDs was recorded by measuring the fluorescence of the dye dilutions at an λ_{ex} of 365 nm using an Optic Fiber Spectrometer. The selected pH range was 3-0 to 10.0. The obtained data was plotted as a curve of fluorescence intensity versus concentration of the dye **C3-** Spike and recovery experiments. The dilutions of the dyes were prepared in tap water (taken from the lab), and water taken from the Narmada and Kshipra rivers. From the data obtained, predicted concentration and percent recovery were calculated for the dyes and represented in the form of graphs. Parameters for characterizing the biosensor, such as linearity, sensitivity, resolution, and limits of detection and quantification were also calculated.

2-5 RESULTS AND DISCUSSION

2.4.1 Synthesis and characterization of CQDs. The process of microwave pyrolysis used (800 W, 90 s) of phthalic acid and TED (1:1) results in the production of desired CQDs. Phthalic acid was used a precursor because its polyaromatic structure is likely to give a long-wavelength fluorescence emission. TED was used as a surface passivant to enhance the CQD quantum yield.



Figure 2-10. Biophysical characterization of CQDs. (A-D) TEM images of CQDs. Spectroscopic characterization: Excitation and emission spectra of the synthesized CQDs. FTIR spectrum. EDX spectrum of CQDs. Fluorescence trend of CQDs at different pH

TEM was done for morphological characterization. Most of the CQDs were found to have sizes in the range of 5 ± 2 nm (Fig. 2-10A). UV-vis spectrophotometry gave the absorption spectrum with a maximum at a wavelength of 365 nm. The emission spectrum of CQDs was obtained using the OFS, giving maximum emission at 510 nm (Fig. 2-10B).

FTIR analysis was performed to identify the functional groups in the CQDs (**Fig. 2-10C**), with phthalic acid showing a broad peak at 3460 cm⁻¹, corresponding to the O–H stretch indicating the presence of carboxyl groups on phthalic acid. TED showed a peak at 3545 cm⁻¹, corresponding to the N–H stretch indicating the presence of amine groups on TED. The prepared CQDs showed peaks from 3380 to 3438 cm⁻¹, corresponding to the O–H and N–H stretches suggesting carboxyl and amine groups. The peaks also verify the amide (CONH) bond at 1560 cm⁻¹⁻ The EDX analysis was performed for elemental configuration of water-soluble CQDs (**Fig. 2-10D**).

2.4.2 Dye sensing using CQDs. The fluorescence response of CQDs at 510 nm exhibits a linear correlation when recorded with the OFS. The CQDs show an overall pH sensitivity where fluorescence intensity increases from pH 3-0 to pH 10.0 (Fig. 2-11). The dye solutions become more acidic as their concentration increases. Therefore, the results obtained are in accordance with the normal behaviour of CQDs observed in such conditions.



Figure 2-11. Overlay spectra (above) and scatter plot (below) showing the pH response of the synthesized CQDs. Fluorescence of the CQDs shown to be increasing with increasing pH

2.4.3 Parameters for characterizing the biosensor. Linearity, sensitivity, resolution, limit of detection, and limit of quantification were calculated from the calibration curves.. These were used to characterize the biosensor. The formulae for their calculation are given in Table 2-3- Linearity is a measure of the relative deviation of sensing behaviour from an ideal straight line. This measure can be used to show the relation between the measured physical quantity and the concentration of the analyte. Sensitivity indicates the extent of change in a sensor's output when the input (measured quantity) varies. Resolution is the smallest detectable incremental change in the input that can be detected in the

output. Limit of detection is the smallest concentration that gives a detectable signal, whereas the limit of quantification is the smallest concentration that can not only be reliably detected but also quantified with acceptable repeatability and accuracy.

Table 2-3. Calculation formulae used for calculating the analyticalparameters used for characterizing biosensors

Parameter	Calculation	
Linearity	Given by the R ² value of the calibration curve	
Sensitivity	Given by the slope of the calibration curve	
Resolution	Standard deviation of the lowest concentration	
	Sensitivity	
Limit of		
Detection	3-3 · Resolution	
Limit of		
Quantification	$2. \cdot LOD$	

2.4.4 Spike and recovery experiments

Spike and recovery data test the applicability of a sensor. We test the influence a particular medium (or matrix) has on the detection of the analyte and how. This is done by comparing the result obtained with the standard curve.



Figure 2-12. Methylene blue detection using CQDs. A) Calibration curve prepared taking ln fluorescence intensity versus concentration of dye $[\mu M]$. (B-D) Dye spiked with tap, Kshipra, and Narmada water, respectively.

Calibration shows a linear relationship between concentration of dye and fluorescence response of CQDs ($R^2 = 0.9961$). These CQDs show a 90% to 126% recovery when spiked with known concentration of methylene blue dye. When spiked with tap water, recovery falls in the range 92% (for 5 µM) to 99% (for 200 µM), with the maximum value being 126%. When spiked with Narmada water, desirable recovery is observed above 30 µM dye, having recovery value of 85% and above. In the case of Kshipra water, a trend like Narmada is seen, with concentrations above 30 µM show recovery above 85% (Fig. 2-12). The sensor is not found suitable for detecting concentrations below 25 µM.

B. Bromocresol green



Figure 2-13. Bromocresol green detection using CQDs. A) Calibration curve prepared taking ln fluorescence intensity versus concentration of dye $[\mu M]$. (B-D) Dye spiked with tap, Kshipra, and Narmada water, respectively

Calibration shows a linear increase of fluorescence response of CQDs with the concentration of dye ($R^2 = 0.9982$). Spiking with tap water shows excellent recovery of fluorescence signal, ranging from 93% to a maximum of 110%, when concentrations above 10 μ M are considered. When spiked with Narmada water, recovery usually lies between 80% and 99% (maximum being at 70 μ M). In the case of Kshipra water, recovery falls between 80% and 100%, with less recovery seen in concentrations below 15 μ M (Fig. 2-13). The sensor is not found suitable for detecting bromocresol green concentrations below 15 μ M.

C. Methyl orange



Figure 2-14. Methyl orange detection using CQDs. A) Calibration curve prepared taking ln fluorescence intensity versus concentration of dye $[\mu M]$. (B-D) Dye spiked with tap, Kshipra, and Narmada water, respectively

Calibration shows that the fluorescence response of CQDs increases linearly with the concentration of dye ($R^2 = 0.9981$). In tap water, we see an overprediction by the sensor, as predicted by the recovery values; all falling above 140%. Similar trends are observed in the other two matrices (**Fig. 2-14**).

D. Methyl red



Figure 2-15. Methyl red detection using CQDs. A) Calibration curve prepared taking ln fluorescence intensity versus concentration of dye $[\mu M]$. (B-D) Dye spiked with tap, Kshipra, and Narmada water, respectively

Fluorescence intensity of CQDs increase linearly with concentration of methyl red ($R^2 = 0.9868$). In tap water, recovery values obtained usually come around 100% to 130%. In Narmada water, the desired range of recovery from 80% to 120% is observed at concentrations between 30 µM and 150 µM. Similarly, in Kshipra water, this desired range of recovery is seen at concentrations above 10 µM. (Fig. 2-15). CQDs are not found to be applicable in the detection of Methyl red below 20 µM.

2.5 Conclusion of the first objective

The applicability of lab – synthesized carbon quantum dots as fluorescent sensors for the detection of selected organic dyes was tested in this objective. As expected, i.e., in accordance with literature, the fluorescence of CQDs showed a positive correlation with pH of the solution. Using this property, CQDs were used to detect one cationic and three anionic dyes. Increasing concentrations of these dyes changed the pH of the solution, and the fluorescence trend of the solution varied accordingly.

Spike and recovery experiments were carried out to test these sensors' detecting ability in three other water matrices, namely tap water, and water taken from the Narmada and Kshipra rivers (present locally in Madhya Pradesh). With what we found out; we can say that CQDs can be reliably used to detect these dyes beyond a certain concentration limit. This limit of detection depends on the dye. However, the general trend shows that CQDs fail to detect dyes below the lower limit of 15 μ M. Other methods would need to be employed for detection at lower concentrations. Ratiometric approach to the detection of these dyes could be tried out for better results.

Chapter 3 Dye Removal Using Charcoal and Alginate

3.1 INTRODUCTION

Building up on the original objective of using lab-synthesized PATED-CQD biosensors for detecting organic dyes, this objective focuses on their removal from different water matrices. The need for effective removal is only a natural extension of their detection. Purification is usually aimed at reducing the concentration of undesired substances (solutes, ions, suspended matter, and parasites) in water, and making it consumable according to pre-set standards.

3.1.1 Reported methods of dye purification

There are physical processes that have been employed for the water purification. These include natural purification processes like artificial groundwater recharge[42], simpler treatment methods like, sedimentation, filtration, and disinfection, chemical processes like coagulation and flocculation, and biological processes (Fig. 3-1).



Figure 3-1. Some of the commonly used separation (and purification) methods. *See text*

Among the chemical separation methods, coagulation and flocculation are the most widely used, and photocatalysis is the most recent. Both coagulation and flocculation are separate but connected processes, and used in tandem and in association with sedimentation to carry out the separation. In a mixture of charged particles (to be removed), coagulation serves to neutralize the charge on said particles, followed by the aggregation of these particles into larger blobs (to be called flocs). Larger flocs are easier to filter out. Photocatalysis is another purification process in which light is used to activate a catalyst to promote a chemical reaction. The mentioned catalyst is often a semiconductor (like TiO₂ or ZnO) which is capable of absorbing light in the UV - vis region. Upon absorbing light, the catalyst generates an exciton (electron – hole pair). Excitons then can participate in a variety of chemical reactions, like redox reactions, and radical formation. This methods has been extensively used in environmental remediation[43-47].

Biological methods of water purification rely on microorganisms to do the job, instead of chemicals. Microorganisms like nematodes and bacteria metabolically break down harmful chemical. Biological treatment usually goes through an aerobic and an anaerobic phase. The use of microorganisms is usually the second step in the overall purification process (rightly called 'secondary wastewater treatment'). The primary wastewater treatment is usually physical separation by dissolved air flotation and others similar processes (membrane separation and sedimentation)[48].

Physical processes majorly rely on different versions of sedimentation and filtration. Sedimentation allows the settling out of suspended particles in water over a period of time. This is followed by decantation of the more purified portion of water from the container. Filtration is the process where water (to be treated) is passed through a porous medium to catch impurities, allowing cleaner water to pass through. Screens, sand (and similar media), or membranes could be used for the purpose. Different materials used serve different applications. The use of membranes is observed in microfiltration and ultrafiltration. Other physical separation methods include reverse osmosis (RO) which involves filtering out of impurities from water using a semi – permeable membrane under high pressure.

Removal of pollutants by the means of their physical adsorption on suitable matrices is found to be the most ideal way. In this light, activated charcoal has been an excellent material of choice; Tran et al. reported the mechanism behind activated charcoal in removing the cationic dye methylene green[49]. Another water purification system was developed using activated charcoal and graphene nanoplatelets, with the latter complementing the former[50]. In this project, activated charcoal has been used after incorporating in hydrogels made from sodium alginate. Such a system was reported to be successful in remedying phenol contamination in drinking water[51], however a hybrid system like the one used in this project has not been reported for the purpose of dye removal from water yet.

3.1.2 Activated charcoal



Activated charcoal is an allotrope (Gr., 'alternate form') of carbon. It is commonly reported to be an excellent adsorbent for removing dissolved contaminants from water.

Mechanism of action

Activated charcoal is a material that is prepared to have a large surface area and high porosity (pretty much like a sponge). These properties make it an excellent adsorbent (adsorption, as opposed to absorption, occurs at the surface). Activated charcoal draws substances to its surface by the means of weak electrostatic forces. This causes their adsorption onto the charcoal. IT has been estimated that a gram of charcoal has surface area equivalent to the size of a football field (about $1,000 \text{ m}^2$) [49].

3.2 MATERIALS AND METHODS

A. Materials

Sodium alginate, obtained from Sigma-Aldrich[®], Calcium chloride (CaCl₂; MW 110.98 g/mol) obtained from EMPLURA[®], Activated charcoal powder, obtained from Loba Chemie Pvt. Ltd.

Instrumentation and miscellaneous

- UV vis spectrophotometer (UV 1900i, Shimadzu, Japan), magnetic stirrer (and beads), electrical balance
- Quartz cuvettes, beakers (30 mL, 50 mL), measuring cylinder (10 mL), plastic syringe (Dispovan[®])

B. Methodology

Preparation of alginate beads. 4% (w/v) sodium alginate was prepared by dissolving 800 mg sodium alginate powder in 20 mL distilled water. 2% (w/v) CaCl₂ powder was made by dissolving 600 mg in 30 mL distilled water. Sodium alginate solution was transferred into a plastic syringe and added to the CaCl₂ solution

drop by drop to allow the beads to form. The CaCl₂ solution must be stirred continuously at a moderate speed. The beads so formed were allowed to settle. The solution in the beaker was replaced with distilled water, and the beads stored in the refrigerator. **Fig. 3-2** depicts the protocol schematically.



Figure 3-2. Schematic diagram showing the steps to synthesize charcoal – containing alginate beads

- Encapsulation of activated charcoal into the alginate beads. 20 mL of 1 mg/mL charcoal suspension was prepared and sonicated. The charcoal suspension was added to sodium alginate solution and mixed magnetically. The rest of the procedure is the same as discussed above.
- **Preparing dilutions of dyes.** Necessary dilutions of the dyes were prepared for conducting the experiments.

Experimentation

 Purification of dyes using charcoal-infused alginate beads: The absorbance of each concentration of each of the dyes was measured and noted, using a UV – vis spectrometer (UV1900i, Shimadzu, Japan), 10 beads were put in each concentration of each dye. The beads were kept in the tubes for 30 minutes (for each tube). After 30 minutes, their absorbance was again recorded, and compared. The data was represented as a curve plotted with absorbance versus concentration of dye.

Adsorption studies:

Three parameters were tested out to check the adsorptive potential of charcoal beads that we synthesized. These are the effects of adsorbent dose, pH, and initial concentration of dye on adsorption. For the first parameter, a variable number of beads were placed in tubes containing a fixed initial concentration of dye (100 μ M). The contact time was taken to be 30 minutes. Absorbance of these solutions was measured before and after incubation, and absorbance values were compared. For studying the effect of pH, solutions of the four dyes having different pH were prepared. A fixed number of beads (15) were kept in each tube, containing the same initial concentration of each dye (100 μM). The tubes were kept undisturbed for 30 minutes. Initial and final absorbance values were compared and interpreted. For the last parameter, i.e., to study the effect of initial dye concentration on adsorption, different concentrations of each dye were prepared (5 μ M to 100 μ M). A fixed number of beads (15) were placed in each tube. Initial and final absorbance values were compared and interpreted accordingly. The contact time in this case was 30 minutes. All the experiments were repeated thrice, to obtain better data.

3.3 RESULTS AND DISCUSSION

 Dye removal using charcoal-containing alginate beads. Calibration curves were prepared for the 4 dyes to obtain the ideal behaviour of these dyes at different concentrations (Fig. 3-3). The charcoal-containing beads were kept in the MCTs containing different concentration of dyes for 30 minutes each, upon which the absorbance of each solution was measured and compared with the absorbance before adding the beads. The data obtained was plotted as absorbance versus concentration Powdered charcoal exhibits faster kinetics, and therefore a greater adsorptive capacity, due to increased surface area.



Figure 3-3. (A-D) Variation of absorbance of the four dyes with concentration. Curves plotted taking absorbance versus concentration (μM) of dye



Figure 3-4. Effect of adsorbent dose on adsorption of dyes

The first parameter to be tested out was the effect of adsorbent dose (Figure 3-4). It can be seen that increasing the adsorbent dose leads to a greater adsorption (portrayed in terms of 'percent reduction in concentration). This can be explained in terms of surface area and pores. As the amount of adsorbent increases, the active sites (the pores) in the adsorbent also increase. Higher adsorbent dosage may ultimately lead to saturation of the curve, explained by exhaustion of available active sites and, therefore, no further increase in adsorption rate.



B. Effect of initial concentration of dye

Figure 3-5. Effect of initial concentration of dye on adsorption of dyes

Figure 3-5 shows the adsorption trend as initial concentration of dye is varied. In the case of methylene blue and methyl orange, the overall adsorption capacity observed an increase and then a decrease in adsorption. This can be interpreted as the saturation of the curve (in the case of methyl orange) and a decreasing trend (in the case of methylene blue). On the other hand, methyl red and bromocresol green dyes showed an expected increase in adsorption rate as their concentration was increased. In these cases, the adsorption limit (which tells the saturation of the adsorbent by the adsorbate) was not observed. It can be deduced from the former two graphs that the activated charcoal – alginate system would work better in lower concentrations, as this range of concentrations is causing a supposed saturation of the adsorbent.



C. Effect of pH of solution

Figure 3-6. Effect of pH of dye on adsorption of dyes

The pH effect was observed in the 4.0 - 8.0 pH range. The relationship between adsorption and pH is given in **Fig. 3-6**. All 4 dyes show varied response. On the one hand, methylene blue saw a decreased adsorption by charcoal and alginate as the solution is made alkaline. Methyl red, on the other hand, showed an exactly opposite trend, with its adsorption facilitated by the alkalinity. Methyl orange and bromocresol green each observed a pH – optimum for maximum and minimum adsorption. pH 6.0 seems to be favourable for adsorption of bromocresol green, while giving the poorest adsorption response for methyl orange.

3.4 Conclusion of the second objective

Objective 2 saw the use of a system consisting powdered activated charcoal encapsulated inside calcium alginate beads. The adsorptive potential of both alginate and activated charcoal are both well – known and reported. The objective was planned in a way to test the same via experiments. A review of literature suggested that different parameters could be tested to see the effect of different conditions on the adsorption process. In that light, we tried to study the effects of three such parameters, described in the previous section. These are the effects of adsorbent dosage, pH, and initial dye concentration.

The data strongly suggests that the system is useful for the purpose of removing dyes from water samples. We believe that the current study could be applied to similar dyes to what we have used for this project.

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