



Faculty of Engineering

**Synthesis of Willow Leaves and Apricot Leaves-based Carbon Quantum
Dots Fluorescent Probes and their Applications in Biosensing**

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Synthesis of Willow Leaves and Apricot Leaves-based Carbon Quantum Dots
Fluorescent Probes and their Applications in Biosensing

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Metal cations and biomolecules are essential for a wide range of physiological processes. The unique nanostructure of carbon quantum dots (CQDs) gives them excellent fluorescence properties and biocompatibility as fluorescent probes, which have great potential for applications in biosensing. However, current research on CQDs as fluorescent probes faces three major challenges: the use of unsafe and toxic chemical raw materials, low quantum yield, and low accuracy. A technique was devised to synthesize CQDs fluorescent probes from non-toxic willow leaves. An experiment was conducted for 12 h at a pH of 7.4 and a temperature of 200°C. Based on the ability of Cu^{2+} ions to form a complex with CQDs and pyrophosphate (PPi), an "off-on-off" fluorescent probe was developed. This probe enables the selective detection of pyrophosphatase (PPase) with a CQDs dosage of 50 μL , a PPi concentration of 5.0×10^{-4} mol/L, and a response time of 40 min. The results indicated that the linearity of PPase was satisfactory within the range of 0 to 0.8 U/mL, represented by the linear equation $y = -656.83x + 710.43$, with a linear correlation coefficient of $R^2 = 0.9968$. This method is expected to be applicable to the detection of human serum samples. The use of willow leaves gives the CQDs a quantum yield of 12.1% only. Therefore, a novel phosphorus-doped biomass CQDs (P-BCQDs) fluorescent probe was designed. The addition of sodium pyrophosphate gives the quantum yield as high as 25.4%. The P-BCQDs exhibit a response time of 2 minutes for the detection of Hg^{2+} at a pH of 7, with concentrations of Hg^{2+} ranging from 0 to 20 $\mu\text{mol/L}$. A linear relationship between the F_0/F of the P-BCQDs and Hg^{2+} concentration was established, with a detection limit of 9 nmol/L. Notably, the fluorescence of the P-BCQDs- Hg^{2+} system gradually increased with the concentration of glutathione (GSH) ranging from 0 to 10 $\mu\text{mol/L}$. This method is expected to enable the simultaneous detection of intracellular Hg^{2+} and GSH. Finally, to enhance the quantum yield

and to address the issue of low detection accuracy associated with a single fluorescent probe, a novel ratiometric fluorescent probe was developed by incorporating gold nanoclusters (AuNCs). CQDs with a quantum yield of 27.6% were synthesized using non-toxic, chemically rich apricot leaves as raw materials. These CQDs were then combined with GSH-conjugated AuNCs, which were synthesized through the GSH reduction of chloroauric acid to, form an AuNCs/CQDs ratio fluorescent probe. The quantitative detection of Cu(II) was accomplished through visual analysis of colour change and the ratio of emission peak intensities. During the reaction, the probe changed colour from pink to purple to blue at varying concentrations of Cu(II). The linear range of the AuNCs/CQDs ratiometric fluorescent probe for Cu(II) was 0-120 $\mu\text{mol/L}$, and the limit of detection was 0.65 $\mu\text{mol/L}$. This method is intended for the visual detection of intracellular Cu(II).

Keywords: Willow leaves and apricot leaves, carbon quantum dots, fluorescent probe, pyrophosphatase, mercury ions, glutathione, Cu(II)

Sintesis Daun Willow dan Daun Apricot berasaskan Karbon Titik Kuantum Probe Pendarfluor dan Aplikasinya dalam Biosensing

ABSTRAK

Kation logam dan biomolekul adalah penting untuk pelbagai proses fisiologi. Struktur nano titik kuantum karbon (CQDs) yang unik memberikan ia sifat pendarfluor yang sangat baik dan biokompatibiliti sebagai probe pendarfluor, yang mempunyai potensi besar untuk aplikasi dalam biosensing. Walau bagaimanapun, penyelidikan semasa mengenai CQD sebagai probe pendarfluor mempunyai tiga cabaran utama: penggunaan bahan mentah kimia yang tidak selamat dan toksik, hasil kuantum yang rendah dan ketepatan yang rendah. Satu teknik telah dicipta untuk mensintesis probe pendarfluor CQD daripada daun willow yang tidak toksik. Ujikaji telah dijalankan selama 12 jam pada pH 7.4 dan suhu 200°C. Berdasarkan keupayaan ion Cu^{2+} untuk membentuk kompleks dengan CQD dan pirofosfat (PPi), probe pendarfluor "off-on-off" telah dibangunkan. Probe ini membolehkan pengesanan terpilih pirofosfatase (PPase) pada dos CQDs 50 μL , kepekatan PPi 5.0×10^{-4} mol/L, dan masa tindak balas 40 min. Keputusan menunjukkan bahawa kelinearan PPase adalah memuaskan dalam julat 0 hingga 0.8 U/mL, diwakili oleh persamaan linear $y = -656.83x + 710.43$, dengan pekali korelasi linear $R^2 = 0.9968$. Kaedah ini dijangka boleh digunakan untuk pengesanan sampel serum manusia. Penggunaan daun willow menghasilkan kuantum CQDs hanya 12.1%. Oleh itu, probe pendarfluor biojisim berfosforus (P-BCQDs) telah direka bentuk. Penambahan natrium pirofosfat memberikan hasil kuantum setinggi 25.4%. Masa tindakbalas P-BCQDs adalah selama 2 minit untuk pengesanan Hg^{2+} pada pH 7, dengan kepekatan Hg^{2+} antara 0 hingga 20 $\mu\text{mol/L}$. Hubungan linear di antara the F_0/F of the P-BCQDs dan Hg^{2+} kepekatan telah diperolehi dengan had pengesanan 9 nmol/L. Sistem pendarfluor P-BCQDs- Hg^{2+} meningkat secara beransur-ansur

dengan kepekatan GSH antara 0 hingga 10 $\mu\text{mol/L}$. Kaedah ini dijangka membolehkan pengesanan serentak Hg^{2+} dan GSH intraselular. Akhir sekali, untuk meningkatkan hasil kuantum dan menangani isu ketepatan pengesanan rendah yang dikaitkan dengan probe pendarfluor tunggal, probe pendarfluor ratiometrik novel telah dibangunkan dengan menggabungkan nanocluster emas (AuNCs). CQDs dengan hasil kuantum sebanyak 27.6% telah disintesis menggunakan daun aprikot. CQDs ini kemudiannya digabungkan dengan AuNCs konjugasi GSH, yang disintesis melalui pengurangan GSH asid kloroaurik untuk membentuk probe pendarfluor bernisbah AuNCs/CQDs. Pengesanan Cu(II) dicapai melalui analisis visual perubahan warna dan nisbah keamatan puncak pelepasan. Semasa tindak balas, probe bertukar warna daripada merah jambu kepada ungu dan biru pada kepekatan Cu(II) yang berbeza-beza. Julat linear probe pendarfluor ratiometrik AuNCs/CQDs untuk Cu(II) ialah 0-120 $\mu\text{mol/L}$, dan had pengesanan ialah 0.65 $\mu\text{mol/L}$. Kaedah ini bertujuan untuk pengesanan visual intrasel Cu(II).

Kata kunci: Daun willow dan daun aprikot, kuantum karbon biojisim, probe pendarfluor, pirofosfatase, ion merkuri, glutathione, Cu(II)

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LIST OF ABBREVIATIONS

A	Absorbance
AD	Alzheimer's Disease
AIE	Aggregation-Induced Emission
Au@AgNPs	Ag-coated Gold Nanoparticles
BCQDs	Biomass Carbon Quantum Dots
BCQDs-AL	BCQDs based on Apricot Leaves
BCQDs-WL	BCQDs based on Willow Leaves
BSANPs@AgNCs	Bovine Serum Albumin Nanoparticles-stabilized Ag Nanoclusters
CQDs	Carbon Quantum Dots
CQDs@Cu-IIP	Carbon Quantum Dots and Cu Ions Imprinting Polymers
DQE	Dynamic Quenching Effects
EPA	U.S. Environmental Protection Agency
F	Fluorescence Peak
FL	Fluorescent Light
FRET	Fluorescence Resonance Energy Transfer
FTIR	Fourier Transform InfraRed
GSH	Glutathione

HOMO	The Highest Occupied Molecular Orbital
IFE	Internal Filter Effects
kiwi@AuNCs	Kiwi juice stabilized Gold Nanoclusters
LUMO	The Lowest Unoccupied Molecular Orbital
MFE	Meta-induced Fluorescence Enhancement
N	Nitrogen
N-CDs/AgNCs	N-doped Carbon Dots/Ag Nanoclusters
NSCDs	Nitrogen and Sulfur co-doped Carbon Dots
P-AuNPs	Gold Nanoparticles from Padina Australis
P-BCQDs	Phosphorus-doped Biomass CQDs
P-BCQDs-WL	P-BCQDs based on Willow Leaves
PD	Parkinson's Disease
PET	Photoinduced Electron Transfer
Pi	Inorganic Phosphate
PPase	Pyrophosphatase
PPi	Inorganic Pyrophosphate
QY	Quantum Yield
RTP	Room-Temperature Phosphorescence
S	Sulfur

s	Standard reference
SPR	Surface Plasmon Resonance
SQDs	Sulfur Quantum Dots
SQE	Static Quenching Effects
TEM	Transmission Electron Microscopy
u	The Substance to be Measured
UV-vis	UltraViolet-visible
XPS	X-Ray Photoelectron Spectroscopy
XRD	X-Ray Diffraction

CHAPTER 1

INTRODUCTION

1.1 Research Background

Several diseases derived from heavy metal ions and endogenous active substances in organisms (enzymes, proteins, etc.) have emerged as a significant issue in worldwide public health (Dai et al., 2021). For example, mercury can cause damage to the lungs, kidneys, nervous system, digestive system and immune system, and excessive exposure can even be fatal (Low et al., 2024). Copper is an essential trace element for living organisms. Adults contain 100-200 mg of copper, it binds to enzymes and helps with blood clotting, hormone maturation, and cellular processing for energy (He et al., 2024). But too much copper can kill cells. Common hydrolase pyrophosphatase (PPase) is essential for bone production, glucose metabolism, and DNA synthesis (Zhang et al., 2023). In addition, PPase can serve as a promising potential biomarker for certain clinical diseases. Reduced glutathione (GSH) is capable of anti-oxidation, toxin clearance, and other critical physiological functions. An elevated amount of it suggests the onset of conditions like hepatitis, pneumonia, or potentially cancer (Timson et al., 2024). Therefore, it is crucial to identify the concentrations of heavy metal ions and biologically active compounds for the diagnosis and treatment of associated illnesses.

Table 1.1: Limit Values for Metal ions in Daily Drinking Water

No.	Metal ions	China (mg/L)	United States of America (mg/L)	European Union (mg/L)	References
1.	Arsenic	0.01	0.01	0.01	Thakkar et al. (2021)
2.	Cadmium	0.005	0.005	0.01	Kolaprath et al. (2023)
3.	Chromium	0.05	0.1	0.05	Omer et al. (2019)
4.	Lead	0.01	0.015	0.01	Rawat et al. (2023)
5.	Mercury	0.001	0.002	0.001	Karami et al. (2020)
6.	Selenium	0.01	0.05	0.01	Thakur et al. (2021)
7.	Aluminum	0.2	0.05-0.2	0.2	Vyas et al. (2023)
8.	Iron	0.3	0.3	0.3	Chatzimitakos et al. (2017)
9.	Manganese	0.1	0.05	0.05	Mohaghehpour et al. (2022)
10.	Copper	1.0	1.3	2.0	Rooj et al. (2018)
11.	Zinc	1.0	5	-	Khan et al. (2020)

Fluorescence analysis, which is highly selective, sensitive, and inexpensive, has been widely used for quantitative testing in the field of environmental analysis and disease diagnosis (Chopra et al., 2022). According to the relevant national standards, the contents of various metal ions in daily drinking water (Table 1.1) have certain requirements (Yan et al., 2022; AbhijnaKrishna et al., 2022). Most of the ions and small molecules are nonfluorescent and cannot be analysed by direct fluorescence analysis but need to be indirectly measured by external fluorescent probes. Therefore, it is essential to create a specific and targeted fluorescence analysis method to precisely measure the concentrations of ions and small molecules (Table 1.2).

Table 1.2: Detection of PPase, Hg²⁺, Cu(II), and GSH by Fluorescence Analysis

No.	Target	Probe	Real Sample	References
1.	PPase	Poly(acrylic acid)-Ca ²⁺ (Ce ³⁺)	human serum	Fan et al. (2024)
2.		CQDs@Cu-MOF nanotubes	human serum	Zhang et al. (2023)
3.	Hg ²⁺	Nitrogen-doped carbon dots	lake water	Nelson et al. (2024)
4.		Gold nanocluster/CQDs	cosmetics and wastewater	Chen et al. (2024)
5.	Cu(II)	A julolidine-coupled azine-based reversible chromo-fluorogenic	real environmental	Maiti et al. (2024)
6.		Zinc nitride quantum dots	river, canal, and tap water	Singh et al. (2024)
7.	GSH	Silver-riboflavin complex and nitrogen-doped carbon dot nanoprobe	human serum	Alhazzani et al. (2024)
8.		Sulfur-nitrogen co-doped carbon dots	HeLa cells	Liu et al. (2023)

In recent years, fluorescent nanoparticles have garnered significant interest within the area of imaging analysis and biosensing, and have been greatly developed. However, traditional fluorescent dyes and semiconductor quantum dots usually have several disadvantages, such as easy photobleaching and high cytotoxicity, which limit their practical application. To overcome these shortcomings of traditional fluorescent probes, Xu et al. (2004) discovered for the first time a new carbon-based zero-dimensional nanoluminescent material with a particle size between 1~10 nm, namely carbon quantum dots (CQDs), (Sun et al., 2006) (Figure 1.1).

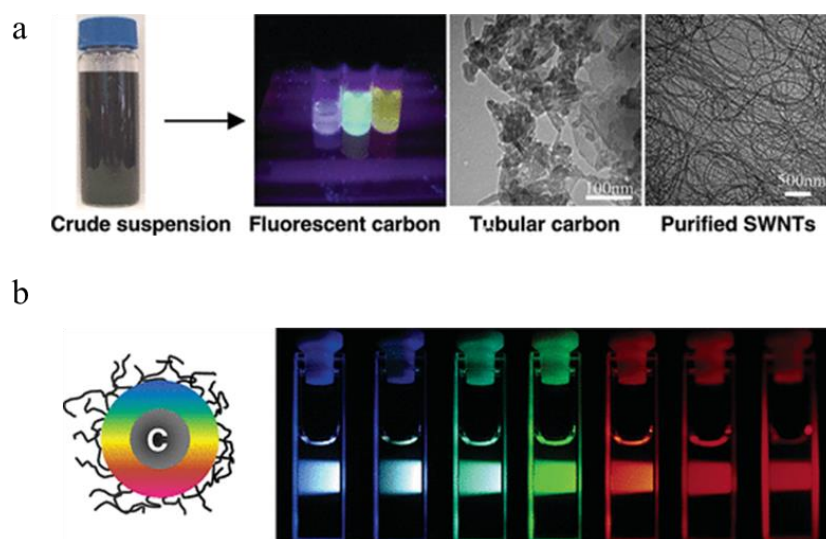


Figure 1.1: (a) the First Discovery (Xu et al., 2004) and (b) the First Name (Sun et al., 2006) of CQDs

Compared with traditional fluorescent probes, CQDs have the advantages of adjustable optical properties (Li et al., 2019) good biocompatibility, and high photostability (Liu et al., 2019), so they are recognized as excellent substitutes for traditional fluorescent probes in the fields of biosensing (Yu et al., 2023; Pourmadadi et al., 2023) (Figure 1.2) and biomedicine (Naik et al., 2022; Wareing et al., 2021).

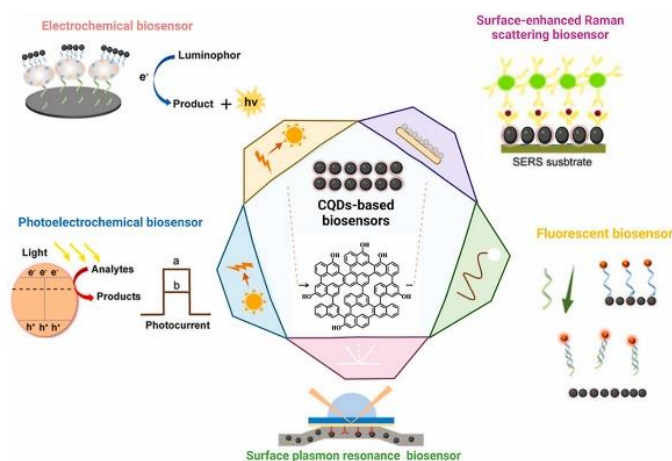


Figure 1.2: CQDs-based Biosensors (Pourmadadi et al., 2023)

However, there are many challenges in commercial production. For example, the use of chemicals as precursors can cause environmental pollution and is harmful to humans; low quantum yield affects the performance of fluorescent probes; low detection accuracy cannot be used for the detection of actual samples. As shown in Figure 1.3, this is one of the key reasons why CQDs received considerable research attention over the last 10 years.

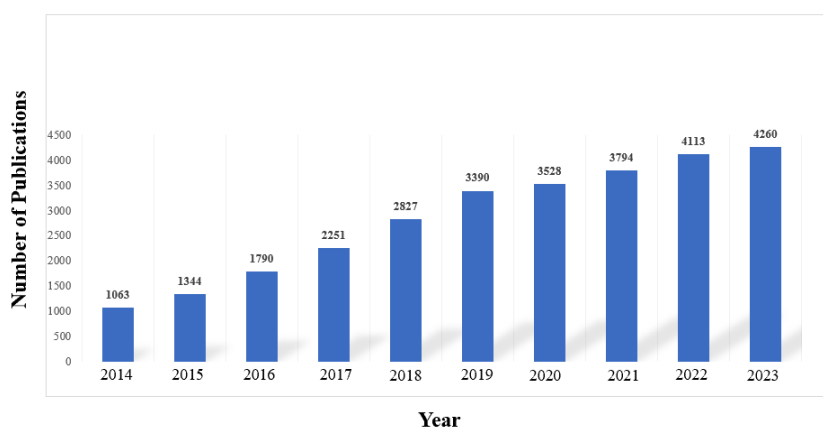


Figure 1.3: Number of Publications with the Title "CQDs" Retrieved from the Web of Science during 2014-2023

1.2 Problem Statement

The synthesis of CQDs commonly involves the use of organic sources as raw materials (Kong et al., 2024). The selection of organic compounds is due to the occurring functional groups in the organic sources, such as carboxyl, hydroxyl, and aldehyde functional groups which offer more possibilities and active sites for the chemical reactions to happen. However, many organic compounds have varying degrees of toxicity which are harmful to the environment and human health. For example, ethylenediamine (Mirlou-Miavagh et al., 2024), o-phenylenediamine (Hu et al., 2024), acetone (D'Souza et al., 2024), toluene (Astafiev et al., 2019) may cause cancer and other serious diseases with long-term

exposure. Furthermore, they can cause burns to human skin, eyes and respiratory tract during the preparation (Thakur et al., 2023). Therefore, there is a need to find non-toxic raw materials that are suitable for replacing the aforementioned organic chemicals in synthesizing the CQDs.

Many defect sites are usually present on the surface of the CQDs (Kumar et al., 2024). These defects are due to factors such as incomplete reactions during synthesis (Zhang et al., 2024), introduction of impurities (Javeria et al., 2024), or uneven distribution of functional groups on the surface (Hess et al., 2017). Defect sites can become complexation centres for electrons and holes, leading to non-radiative complexation of the excited state electrons and holes before they return to the ground state, thus reducing the quantum yield. As a result, the quantum yield of most CQDs is generally low, less than 10% (Xu et al., 2023), which seriously limits their applications in the field of fluorescence sensing and bioimaging. Therefore, after analysing reaction conditions and improving purification as well as separation techniques, further exploration on how to incorporate other elements into the nanostructure of CQDs on time in achieving efficient synthesis of the CQDs with high quantum yield is a key focus in the development process of CQDs as the literature is lacking such detailed information, to date.

Most of the fluorescent probes constructed based on CQDs use single-emission fluorescence intensity as the output signal (Chen et al., 2023; Li et al., 2024), which is susceptible to interference from fluorescence intensity from instrumental parameters (Hu et al., 2024) and photobleaching (Chen et al., 2024); thus, the accuracy of detection needs to be improved. On the other hand, ratio metric fluorescent probes can realize the detection of the substance to be measured by the ratio of two or more fluorescent signals. The

fluorescence intensity ratio of the ratio metric fluorescent probe is a linear correlation between the measured substance concentration during the detection process, resulting in effective mitigation of environmental factors. The accuracy of detection is significantly improved by adding instrumental noise to the detection results (Thakur et al., 2024). In order to eliminate background interference caused by a single fluorescent probe, it is crucial to select the appropriate fluorescence strategies when detecting real samples. This is crucial for enhancing the sensitivity and accuracy of the assay and even for achieving visual detection.

1.3 Hypotheses

Leaves are a potential raw material to synthesize CQDs because they are rich in chemical compounds, are safe for humans, and are environmentally friendly. The synthesis of CQDs fluorescent probes using leaves as precursors can effectively reduce environmental pollution and play a role in protecting experimental safety.

The quantum yield of biomass CQDs could be increased by performing elemental doping, such as doping with phosphorus. Furthermore, the quantum yield differs when using willow and apricot leaves due to differences in their chemical composition.

A ratiometric fluorescent probe can be designed using the fluorescence energy resonance transfer effect by introducing gold nanoclusters in the CQD system. Ratiometric fluorescent probes are capable of effectively eliminating background interference and improving detection accuracy when compared to single fluorescent probes.

1.4 Objectives

This study aims to synthesize novel leaves-based CQDs fluorescence probes with high quantum yield and accuracy for detecting PPase, Hg^{2+} , GSH, and Cu(II) in actual

samples. The study aims to achieve the following specific objectives. The study aims to achieve the following specific objectives.

i. To devise a technique for synthesizing the CQDs using non-toxic and environmentally friendly material such as willow leaves as a single fluorescent probe for selectively detecting PPase.

ii. To synthesize P-doped biomass CQDs with high quantum yield and use them as a single fluorescent probe for selective and sensitive detection of Hg^{2+} and GSH.

iii. To design AuNCs/CQDs from AuNCs along with non-toxic and environmentally friendly material CQDs with greater detection accuracy as a ratiometric fluorescent probe for selectively, sensitively, accurately, and visually detecting Cu(II).

1.5 Scope of the Research

i. The scope of this study mainly focuses on the fluorescent probes based on CQDs, which were synthesized by a hydrothermal method, based on willow leaves and apricot leaves. The research focuses on investigating how fluorescence intensity can be affected by reaction time, hydrothermal temperature, and reaction pH.

ii. Sodium pyrophosphate was used as a doping reagent. Chloroauric acid was used to prepare gold nanoclusters. Copper chloride, PPase, mercuric chloride, and GSH were employed for the preparation of standard solutions for the targets. Tap water and lake water were used as actual samples.

iii. Morphology, size distribution, surface functional groups, crystal structure, elemental composition, optical properties, and carbonization degree of the obtained CQDs fluorescent probes were investigated using Transmission Electron Microscopy (TEM),

Fourier Transform Infrared Spectroscopy (FTIR), x-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), UV-vis spectroscopy, fluorescence spectrophotometer, and Raman spectroscopy.

iv. Willow leaves and apricot leaves based-CQDs fluorescent probes were used for the detection of PPase, Hg^{2+} , GSH, and Cu(II), and the effects of CQDs dosage, response time, detection pH and concentration of detectors on the fluorescence intensity were studied.

v. The accuracy of willow leaves and apricot leaves based-CQDs fluorescent probes was checked by the spiked recovery method.

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CHAPTER 2

LITERATURE REVIEW

Metal cations and biomolecules play significant roles in maintaining normal human activities and resisting diseases (Hast et al., 2023). Hg^{2+} is highly toxic to the human body and can damage the human kidney (Abdeldayem et al., 2022), nervous system (Suhelmi et al., 2020), and cardiovascular system (Lopes-Araújo et al., 2023), and even at very low concentrations, it can accumulate in the body and thus cause harm. According to a survey, the average monthly water consumption of Chinese households is approximately 10 tons, and according to national regulations, the mercury content in residential water should not exceed 0.001 mg/L. Hence, it is crucial to monitor Hg^{2+} in the environment and the human body. Cu(II) is abundant in nature and is used in all aspects of human life. However, excessive amounts of Cu(II) can cause serious environmental pollution (Cui et al., 2023) and have toxic effects on living organisms (Wang et al., 2023; Zhang et al., 2024). For these reasons, the Cu(II) limits are 15.7-23.6 $\mu\text{mol/L}$ in human blood and 20 $\mu\text{mol/L}$ in drinking water according to EPA. In fact, monitoring Cu(II) levels can be an important marker for environmental pollution and biomedical diagnosis. As a water-soluble hydrolase, PPase plays a crucial role in the energy cycle by hydrolyzing PPi, releasing a significant amount of energy to fuel biosynthesis reactions (Gao et al., 2023b). The expression of PPase has also been linked to clinical conditions like gastric, ovarian, and breast cancers (Zhang et al., 2023). Researchers are still striving to create an easy, effective, and extremely sensitive method for detecting PPase, which would be beneficial for diagnosing and preventing diseases. GSH is a ubiquitous tripeptide present in nearly every cell of the body. It is an important intracellular regulator with antioxidant, free radical scavenging, and immune-enhancing properties.

Therefore, the rapid and accurate detection of them is highly important. Thus, many analytical methods, including potentiodynamic voltammetry (Shen et al., 2023), inductively coupled plasma-mass spectrometry (ICP-MS) (Van Acker et al., 2023), atomic absorption spectroscopy (AAS) (Drosaki et al., 2022) and atomic emission spectroscopy (AES) (Ronda et al., 2022), have been documented in the literature for identifying Cu(II). However, the use of large instruments for these techniques is not conducive to swift on-site detection and is costly (Wang et al., 2021).

Fluorescence analysis has received extensive attention in the fields of sensing and imaging analysis and has been greatly developed (Yue et al., 2022). However, traditional fluorescent dyes and semiconductor quantum dots usually suffer from disadvantages, such as easy photobleaching and high cytotoxicity, which limit their practical application. Compared with traditional fluorescent probes, CQDs have the advantages of tunable optical properties (Wang et al., 2020), good biocompatibility, and high photostability (Guan et al., 2023) and are recognized as excellent substitutes for traditional fluorescent probes.

In 2004, CQDs were initially identified during the synthesis of single-walled carbon nanotubes and electrophoretic purification by Xu et al., Two years later, Sun et al., first applied the surface passivation technique to the preparation of CQDs, which greatly improved the structure and fluorescence properties of their surface. Since then, research based on CQDs has developed rapidly in biosensing (Azargoshasb et al., 2024; Xu et al., 2022), imaging (Wang et al., 2024), and medical fields (Das et al., 2024; Li et al., 2024; Sharma et al., 2024).

2.1 Raw Materials of CQDs

As shown in Table 2.1, CQDs were often prepared by organic compounds rich in functional groups. However, many organic compounds possess varying degrees of toxicity, which is detrimental to the environment and human health. For instance, ethylenediamine (Mirlou-Miavagh et al., 2024), o-phenylenediamine (Hu et al., 2024), acetone (D'Souza et al., 2024), and toluene (Astafiev et al., 2019) may cause serious diseases such as cancer with long-term exposure. Moreover, they can burn human skin, eyes, and respiratory tract during the preparation process (Thakur et al., 2023). Hence, there is a necessity to find non-toxic raw materials suitable for replacing the aforementioned organic chemicals in the synthesis of CQDs.

Table 2.1: Raw Materials of Organic Compounds for the Synthesis of CQDs.

No.	Raw Materials	References
1.	NaOH, Acetone	Hou et al. (2015)
2.	Citric acid, Ammonia	Wang et al. (2016)
3.	p-Phenylenediamine, Diethylene triamine Pentaacetic acid, and Gd ³⁺ ions	Gao et al. (2017)
4.	1,4-Phenylene diisocyanate	Han et al. (2018)
5.	Toluene	Astafiev et al. (2019)
6.	1,6-Hexanediamine dihydrochloride	Ding et al. (2020)
7.	4-Chlorophenol	Wang et al. (2020)
8.	Citric acid, Urea	Vercelli et al. (2021)
9.	Methylene violet	Zhu et al. (2023)
10.	4,6-Diaminoresorcinol dihydrochloride	Katakami et al. (2023)
11.	Ethylenediamine	Mirlou-Miavagh et al. (2024)
12.	o-Phenylenediamine	Hu et al. (2024)
13.	Tartaric acid and 4-Aminosalicylic acid	Hu et al. (2024)

CQDs synthesized from biomass open a green path to create new nanomaterials from non-toxic natural resources. Biomass is any material of plant or animal origin that can be used as a carbon source for biomass CQDs (Goswami et al., 2024). Biomass has a variety of sources, both from waste and from non-waste renewable products. As shown in Table 2.2, various types of biomass have been used as initial carbon sources to synthesize biomass CQDs, such as tangerine juice (Aslan et al., 2021), marine polysaccharides (Torres et al., 2023), and chicken egg white (Cardoso-Ávila et al., 2024).

Table 2.2: Development Map of Biomass CQDs between 2015-2024

No.	Raw Materials	References
1.	Orange pericarp	Du et al. (2015)
2.	Lotus root	Gu et al. (2016)
3.	Peanut shells	Ma et al. (2017)
4.	Broccoli	Arumugam et al. (2018)
5.	Potato starch	Qiang et al. (2019)
6.	Eggshell Membrane	Ye et al. (2020)
7.	Tangerine juice	Aslan et al. (2021)
8.	Garlic peels	Jeevika et al. (2022)
9.	Marine polysaccharides	Torres et al. (2023)
10.	Chicken egg white	Cardoso-Ávila et al. (2024)

Waste biomass, such as leaves or food waste, is the most environmentally friendly and not only contributes to waste management but is also rich in chemicals (Wareing et al., 2021). In recent years, more and more people have been using leaves as a biomass carbon source to prepare biomass CQDs. As shown in Figure 2.1, biomass CQDs prepared from aegle marmelos leaves for detecting Fe^{3+} with high selectivity by Rani et al (2024). The linear range of Fe^{3+} was 0-20 μM with a detection limit of 0.18 μM . But in China, compared to aegle marmelos leaves, aloe vera leaves have the advantage of being suitable for all

seasons. Wang et al (2024) prepared biomass CQDs based on Aloe vera leaves and combined them with GSH-encapsulated copper nanoclusters to construct ratiometric fluorescent probes for detecting allylcarbamate. The linear range of allylcarbamate was 0.5-200 μM with a detection limit of 0.15 μM .

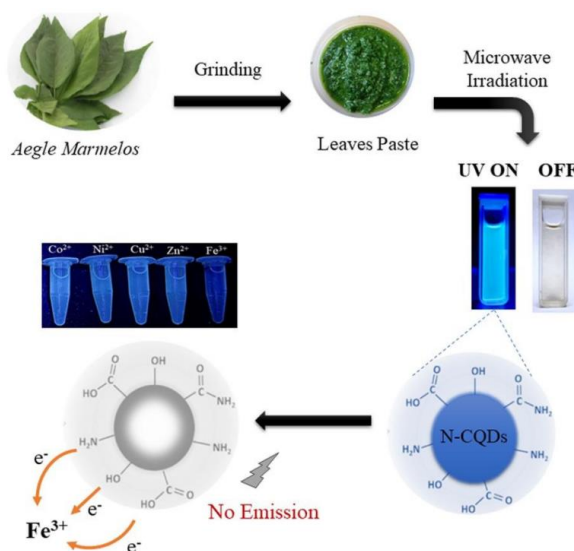


Figure 2.1: Biomass CQDs Prepared from Aegle Marmelos Leaves for Detecting Fe^{3+} (Rani et al., 2024)

As shown in Table 2.3, various types of leaves have been used as carbon sources to prepare biomass CQDs, due to their rich chemical composition. Willow leaves and apricot leaves are also rich in chemicals. Willow leaves contain mainly salicylic acid and salicin (Chauvet et al., 1987). Apricot leaves usually contain a variety of chemical components such as flavonoids, tannins, and volatile oils (Bielecki et al., 1977). These chemicals, not only can act as raw materials for biomass CQDs, but also are beneficial to improve the fluorescence properties of biomass CQDs, such as improving quantum yield (John et al., 2021) and optical stability (Dua et al. 2023). Furthermore, willow trees are found throughout China (Yan et al., 2023) and apricot trees are planted in more than 60 countries around the world (Prgomet et

al., 2020). Therefore, using willow leaves and apricot leaves as raw materials to synthesise biomass CQDs.

Table 2.3: Synthesis of Biomass CQDs Using Leaves as a Biomass Carbon Source

No.	Carbon Source	References
1.	Purslane leaves	Amer et al. (2021)
2.	Trachelospermum jasminoides leaves	Kumari et al. (2022)
3.	Eucalyptus tree leaves	Saleem et al. (2022)
4.	Maple tree leaves	Chellasamy et al. (2022)
5.	Birch leaves	Tang et al. (2023)
6.	Peach leaves	Ren et al. (2023)
7.	Dried date palm leaves	Saleem et al. (2023)
8.	Ginkgo biloba L. leaves	Fei et al. (2023)
9.	Aloe leaves	Wang et al. (2024)
10.	Aegle marmelos leaves	Rani et al. (2024)

2.2 Properties of Biomass CQDs

Typically, biomass CQDs consist of a sp² or sp³ hybrid carbon core (Britto et al., 2024), and their surface functional groups commonly include hydroxyl, carboxyl, and epoxy groups, which make them highly water soluble (Pan et al., 2024). However, the varying crystallinity and intricate microstructure of biomass CQDs result in diverse properties. The basic characteristics of biomass CQDs include the following four areas.

2.2.1 UV-visible Absorption Properties

Biomass CQDs synthesized by different methods have different chemical structures, which usually exhibit strong absorption properties in the UV region and extend to the wavelength range of visible light. This is caused by the π - π^* transition of the C=C bond and the n - π^* transition of the C=O and C=N bonds (Yang et al., 2023).

The UV-visible absorption characteristic peaks of the CQDs are mainly influenced by the species of surface groups, the size of the π - π -conjugate structure, and the O/N content ratio in the carbon core (Yuan et al., 2017; Qu et al., 2016). The UV-Vis absorption spectra of CQDs prepared by different process conditions may be different. For example, compared with the absorption peak at 270 nm of CQDs prepared by the electrochemical method (Zhou et al., 2007), the absorption peak of CQDs prepared by the laser ablation method is red-shifted to 280 nm (Yang et al., 2012), and the width of the half-peak is narrower. The surface functionalization also affects the UV-Vis absorption. For instance, the absorption spectra of the amine-functionalized CQDs are significantly red-shifted and appear in the region of 330~600 nm in Figure 2.2 (Li et al., 2018).

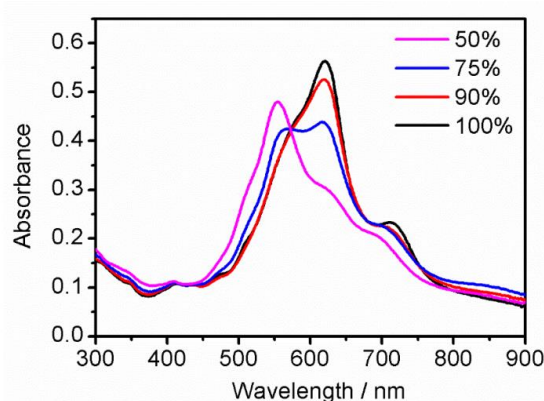


Figure 2.2: Absorption Spectra of CQDs in Mixed Solutions of DMSO and H₂O
(Li et al., 2018)

2.2.2 Fluorescence Properties

The fluorescence properties are among the most remarkable properties of CQDs. By utilizing different fluorescent substances like cadmium or lead in traditional quantum dots,

rare earth nanomaterials, and organic small molecule dyes, CQDs exhibit superior light stability, quality, increased fluorescence QY, reduced toxicity, improved biocompatibility, abundant low-cost resources, and extensive applications. The luminescence of CQDs consists of both photoluminescence (Figure 2.3) and upconversion photoluminescence (Figure 2.4). The first type of luminescence is referred to as Stokes luminescence, while the second type is known as anti-Stokes luminescence. The former occurs when the emitted wavelength is greater than the excitation wavelength, whereas anti-Stokes luminescence occurs when the excitation wavelength is greater than the emitted wavelength. Currently, recognized fluorescence mechanisms include quantum domain effects, surface-state luminescence, and molecular-state luminescence. The mechanism of upconversion luminescence usually occurs when the electrons of CQDs absorb multiple photons simultaneously, leading to their excitation to higher vibrational levels. Whereas photoluminescence is the opposite.

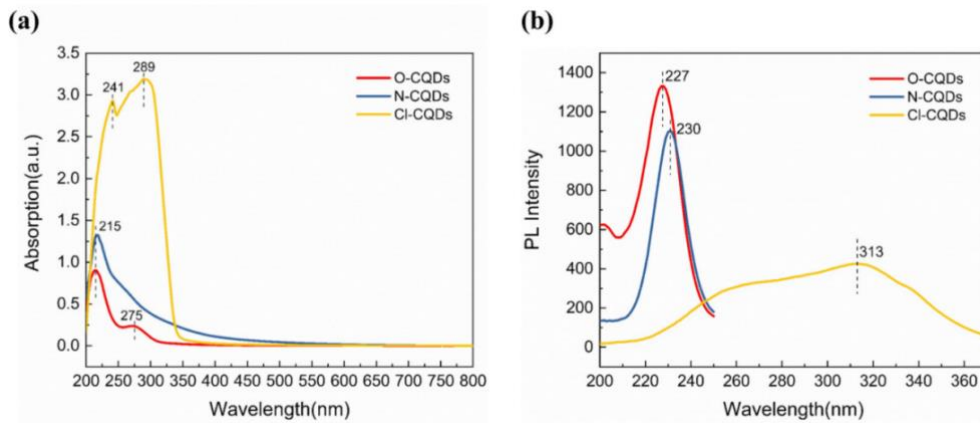


Figure 2.3: (a) UV-Vis Absorption Spectra and (b) PL Spectra of O-CQDs, N-CQDs, and Cl-CQDs (Liu et al., 2023)

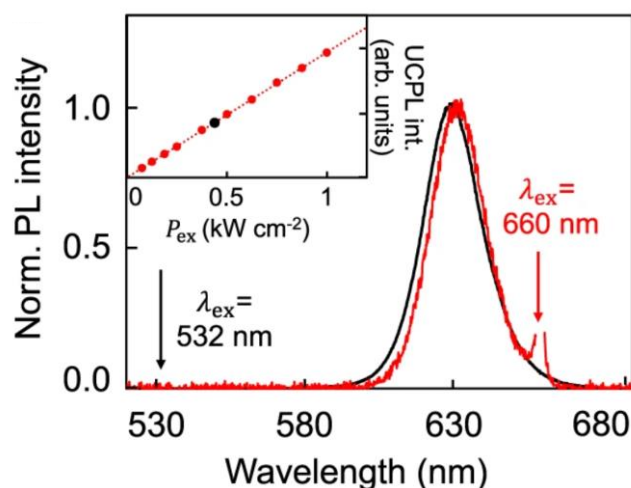


Figure 2.4: Normalized Upconversion Photoluminescence (Red) and Downconversion Photoluminescence (Black) Spectra Excited at 660 and 532 nm, respectively (Ye et al., 2021)

Typically, CQDs emit blue or green fluorescence, which restricts their biomedical applications. Lately, numerous research studies have created CQDs that produce red or near-infrared fluorescence through modifications in the source materials (La Ferla et al., 2023) and reaction parameters (Benner et al., 2023). Wang et al., (2024) successfully prepared CQDs with red emission at 607 nm under 514 nm excitation via the solvothermal method in N,N-dimethylformamide with citric acid and 1,8-diaminaphthalene as precursors (Figure 2.5). Strong red-emitting CQDs were prepared by controlling the content of methylene violet for hypochlorite detection and bioimaging (Figure 2.6). In addition, red CQDs can also be prepared by surface modification through doping (Figure 2.7) or material mixing during synthesis (Benner et al., 2023).

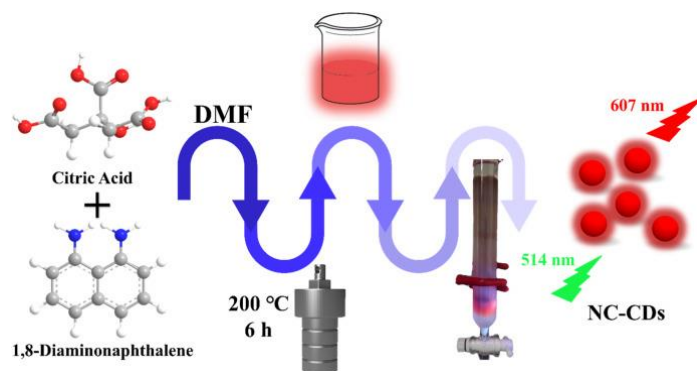


Figure 2.5: Schematic Diagram Illustrating the Synthesis of NC-CDs (Wang et al., 2024)

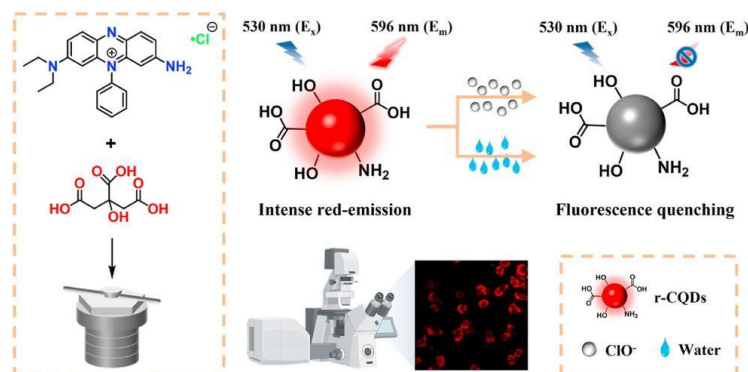


Figure 2.6: Facile Synthesis of Efficient r-CQDs as a Multifunctional Platform for Biosensing and Bioimaging (Zhu et al., 2023)

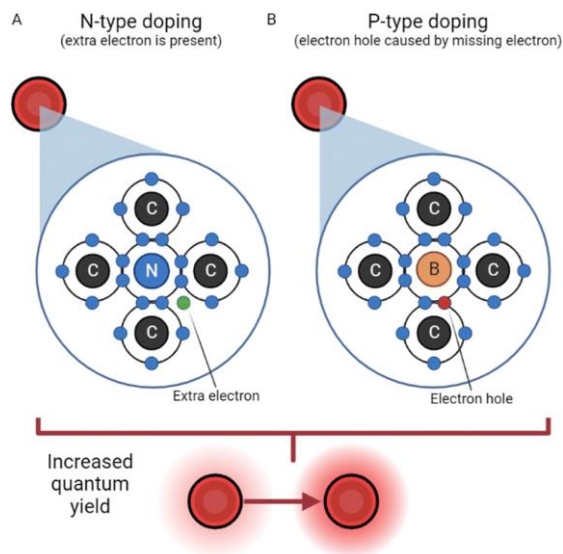


Figure 2.7: Mechanism of the Preparation of Red Carbon Dots by N or P Doping (Benner et al., 2023)

On the other hand, because high-colour-purity CQDs have relatively large advantages in bioimaging and photoelectric devices, Scientists have been researching CQDs that have limited emission ranges (Xian et al., 2022; Sun et al., 2023). Previous studies have reported that the full width at half-maximum (FWHM) of some CQDs ranges from 20-40 nm (Liu et al., 2020; Yuan et al., 2018; Yuan et al., 2020). Yuan et al., (2018) synthesized a series of multicolour luminous CQDs with an FWHM of 30 nm, which did not depend on the excitation wavelength (Figure 2.8). The synthesized CQDs have high crystallinity and a unique rigid triangular structure, and a large number of hydroxyl groups on their surface can cause weak electron-photon interactions, which results in high colour purity. Recently, Liu et al., (2020) prepared CQDs with a FWHM of 20 nm and a QY of 59%. The experimental results showed that the CQDs consist of a carbon core and an outer layer modified by polymer chains (Figure 2.9). The results showed that the QY is increased and the FWHM is decreased by the N-doped heterocycle and the extensive conjugate system.

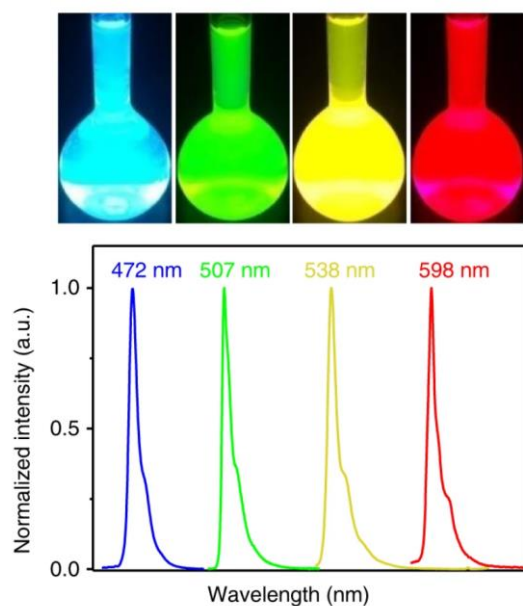


Figure 2.8: PL Spectra of CQDs (Yuan et al., 2018)

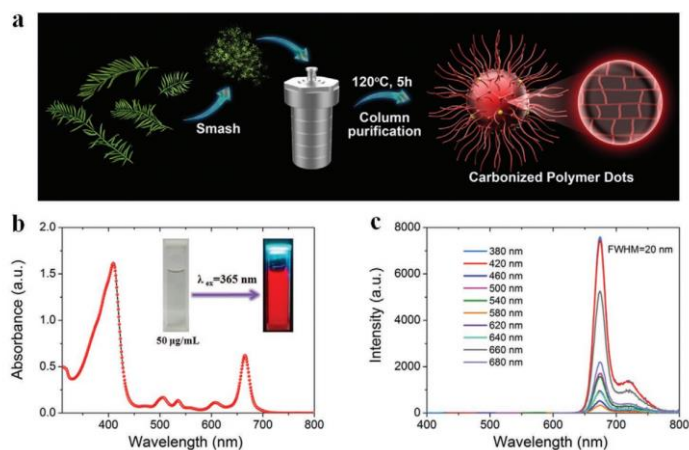


Figure 2.9: Synthesis (a), UV (b), and PL (c) Spectra of Deep Red Emissive CQDs (Liu et al., 2020)

Moreover, most CQDs have an excitation wavelength dependence, which is the phenomenon in which the fluorescence emission wavelength redshifts as the excitation

wavelength increases. This is possible because of the multiple fluorescence emission centres of CQDs and the wide distribution of different energy levels. Therefore, CQDs can change the wavelength of their fluorescence emission directly by changing the excitation wavelength without changing their chemical structure or size, which greatly facilitates their application in multicolour fluorescence biological imaging.

2.2.3 Phosphorous Light Properties

Room-temperature phosphorescence (RTP) is a unique property of CQDs. RTP is generated by two main mechanisms: (i) the shift from the excited singlet state (S1) to the triplet state and (ii) the emission from the lowest excited triplet state (T1) to the ground state. Additionally, heightened spin-orbit coupling can aid in exciton transitions from singlet to triplet states to enhance RTP generation. Moreover, inhibiting the nonradiative transition of the triplet exciton also plays a crucial role in RTP production (Liu et al., 2024; Li et al., 2024). RTP materials have been widely used in bioimaging, biosensing, and optical devices. Conventional RTP materials typically consist of inorganic or metal complexes, but their drawbacks such as high toxicity and cost significantly restrict their potential uses. In recent years, researchers have developed and synthesized CQDs with a high fluorescence QY and long phosphorescent afterglows (Tao et al., 2018; Li et al., 2019). It has been shown that C=O and C=N bonds promote RTP production with strong spin coupling between them, which can lead to a low singlet-triplet splitting energy. Moreover, N, P, and halogen heteroatoms can also enhance the C=O bond and C=N, the $n-\pi^*$ transition in the bond, and the transition from the singlet to the triplet state (Jiang et al., 2020). N-doped CQDs with ultralong phosphorescent afterglow were synthesized by a one-step hydrothermal method by Xia et al. (2020). In addition, they adjusted the carbon content by varying the reaction time

in order to manage the phosphorescent glow and emission wavelength of the created CQDs, resulting in a range of CQDs with varying RTPs (Figure 2.10). Further experimental and theoretical analyses confirmed that the red phosphorescence of the produced CQDs originates from the molecular state emission centre found in the polymer or carbon hybrid structure, as well as the presence of nitrite functional groups. Nevertheless, its cross-linked supramolecular structure can efficiently restrict the nonradiative conversion of triplet excitons and enhance the generation of RTP. Currently, the preparation and application of CQDs with RTP are still in the early stages of research. In the future, the fluorescence quantum yield, phosphor afterglow lifetime, and stability of CQDs should be improved by studying the reaction raw materials, pretreatment methods, and reaction conditions.

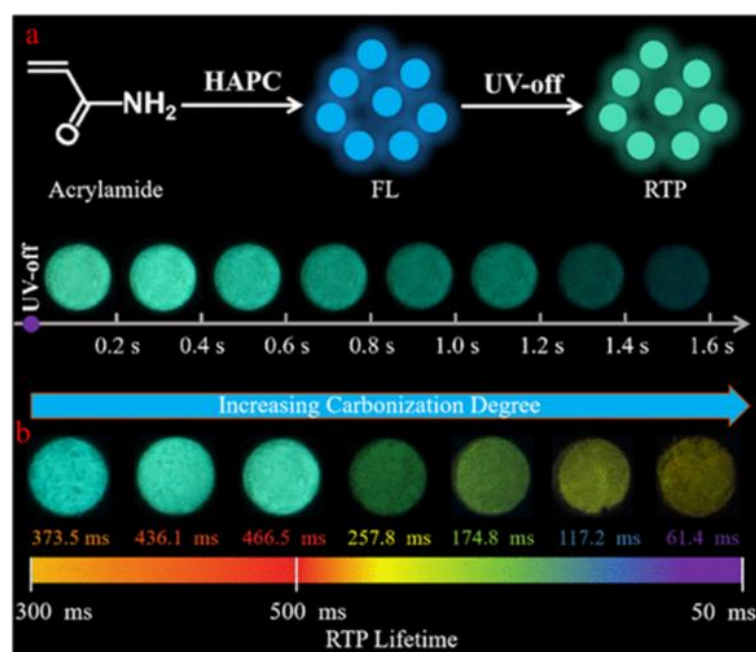


Figure 2.10: (a) A Diagram of how AN-CPDs are Made Using an HAPC method and their FL and RTP Emission Characteristics when in a Solid Form. (b) The RTP Emission of the AN-CPD Powder Produced at 200°C, taken at Various Time Intervals after the UV Light is Turned Off (Xia et al., 2020)

2.2.4 Chiral Properties

Chiral properties play an important role in chiral drug recognition, chiral molecular biology, and chiral chemistry (Rezaei et al., 2024; Zhao et al., 2024). Recently, an increasing number of researchers have developed and prepared chiral CQDs (Figure 2.11). Among the two approaches for synthesizing CQDs, bottom-up approaches are generally easier for synthesizing chiral CQDs. When the precursor material itself is a chiral molecule, no other chiral ligands are introduced, and direct chiral CQDs can be synthesized.

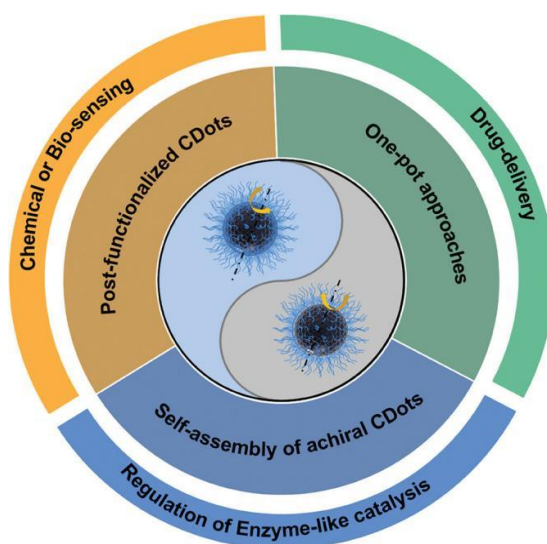


Figure 2.11: Synthetic Strategies for Accessing CQDs and their Chiral Effects on Various Applications (Zhao et al., 2024)

Chiral CQDs were created by heating a mixture of fructose and chiral cysteine molecules in a sodium hydroxide solution at normal pressure (Wang et al., 2024). In addition, this recently developed technique can be broadened by substituting the fructose starting material with ascorbic acid, sucrose, or lactose. Wang et al. (2022) created L-/D-Trp-CDs by combining citric acid and L-/D-tryptophan. The L-Trp-CDs were employed to control

laccase activity, resulting in an 87.26% inhibition rate, which was notably higher than that achieved with D-Trp-CDs (57.84%) in identical reaction settings. The key factor influencing enzyme activity in this system is the chiral functional groups rather than the carbon nuclei.

2.3 Preparation of Biomass CQDs

Various biomass CQDs have been synthesized from natural products (i.e., orange juice, coffee grounds, silk, green tea, eggs, eggshell film, soybean milk, flour, bananas, sweet peppers, honey, coriander leaves, garlic, bergamot, aloe, lemon juice, pear juice, hair, etc.) or waste materials (i.e., waste paper, paper ash, petroleum coke, etc.) as precursors (Lou et al., 2021; Durrani et al., 2023). As shown in Figure 2.12, two primary synthesis techniques commonly employed are known as the "top-down" and "bottom-up" methods (Shen et al., 2012; Mao et al., 2021). The "top-down" approach involves utilizing physical or chemical techniques such as chemical oxidation and electrochemical synthesis to transform large sp² carbon structures into smaller CQDs. While these techniques are straightforward, user-friendly, and efficient in terms of time, they are only applicable to materials containing extensive sp² carbon structures, like graphene, carbon fibre, and carbon black. Although this method cannot accurately control the size and shape distribution of the product, it is precise because of the diversity of sizes and shapes, and it also provides an opportunity for the development of CQDs with adjustable emission wavelengths. The "bottom-up" approach involves utilizing small molecules as precursors for carbon sources to prepare CQDs by hydrothermal methods and microwave-assisted methods. This method enables organic molecules to form sp² carbon domains through intermolecular coupling during carbonization to form CQDs whose size and morphology can be controlled. Although this method has the disadvantage of being time-consuming and complex, the synthesized CQDs are relatively uniform in size and controllable in morphology, and the requirement for carbon

sources is relatively low. All the discussed synthetic approaches with corresponding advantages and disadvantages are presented in Table 2.4.

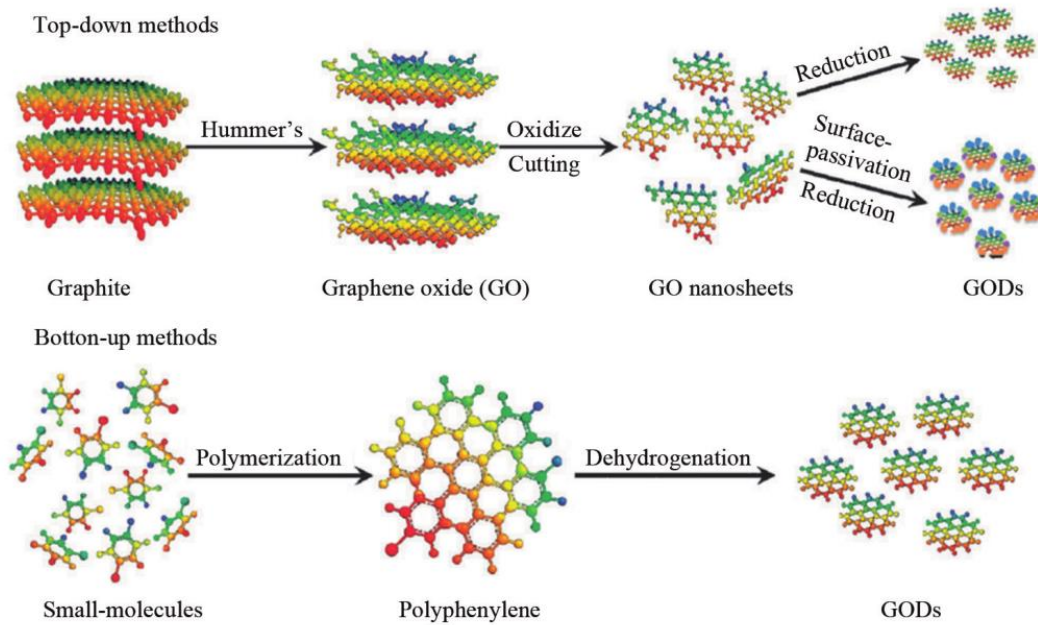


Figure 2.12: Diagram Illustrating both Top-Down and Bottom-Up Methodologies for the Synthesis of CQDs (Shen et al., 2012)

Table 2.4: Summary of the Advantages and Disadvantages of Biomass CQDs Synthesis Methods (Šafranko et al., 2021)

Method	Advantages (Pros)	Disadvantages (Cons)
“Top-Down” Approach		
Arc discharge	- Simple procedures	- Difficult to control particle size
Laser ablation	- Cheap raw material	- Expensive equipment
Chemical oxidation	- Mass production	- Long processing time
Electrochemical oxidation		
“Bottom-Up” Approach		
Hydrothermal/solvothermal treatment	- Good control of particle size and morphology	- Often include complex procedure
Carbonization	- Low-cost	- Possible aggregation
Microwave-assisted synthesis	- Facile synthesis and surface functionalization	
Thermal decomposition and pyrolysis		

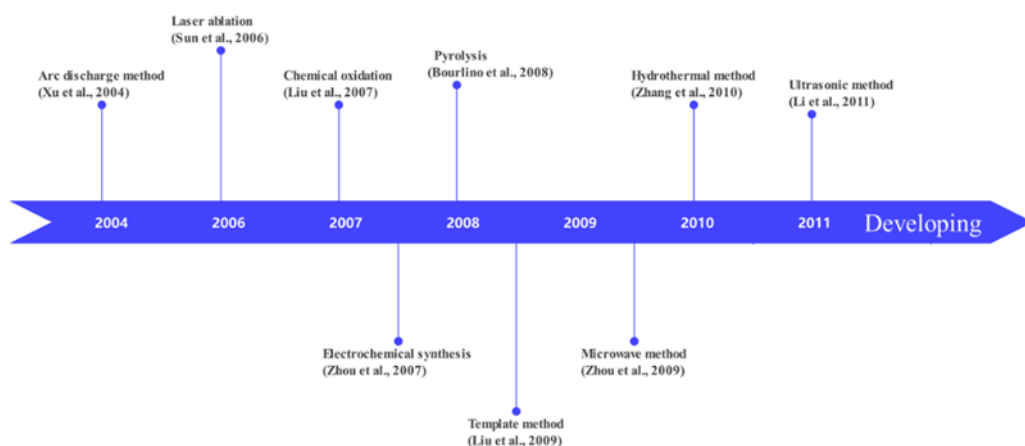


Figure 2.13: History of CQDs Synthesis Methods

As shown in Figure 2.13, there are approximately nine common methods for synthesizing CQDs. This chapter will mainly introduce the following three types.

2.3.1 Chemical Oxidation

Table 2.5: Overview of Biomass CQDs Produced from Various Natural Sources using Chemical Oxidation Methods

Carbon Source	Oxidizing Agent	Application Field	References
Anthracite coal	H ₂ O ₂	Pollutant control	Hu et al. (2016)
Anthracite coal	O ₃	Biosensor	Liu et al. (2018)
Palm shell powder	CF ₃ COOH	Biosensor	Soni et al. (2018)
Green tea leaves	H ₂ SO ₄	Biosensor	Hu et al., (2020)
Muskmelon fruit	H ₃ PO ₄	Biosensor	Kailasa et al. (2019)
Waste tea residue	HNO ₃	Biosensor	Gunjal et al. (2019)

Chemical oxidation is a method for obtaining CQDs by oxidizing carbon source precursors, such as H_2O_2 and $\text{HNO}_3/\text{H}_2\text{SO}_4$, as shown in Table 2.5. This technique does not need specialized equipment for large-scale production. Additionally, the oxidation process generates numerous oxygen-containing functional groups (like OH and COOH) on the CQDs' surface, giving them strong hydrophilic properties and chemical reactivity. Xu et al. (2014) oxidized green and yellow fluorescent CQDs using sucrose as a carbon source by H_3PO_4 at a relatively low temperature (60°C). The prepared CQDs have good dispersion, narrow particle size distribution, excellent stability over a wide pH range (2.0-13.0), high ionic strength and UV irradiation and show great application potential in biological imaging (Figure 2.14). Bao et al. (2015) synthesized CQDs with adjustable emission wavelengths by oxidizing carbon fibres with HNO_3 and ultrafiltration. By controlling the reaction time, temperature, and HNO_3 concentration and adjusting the size and surface oxidation degree of the CQDs, CQDs with different fluorescent colours were obtained. This research introduces a novel approach for creating CQDs with adjustable emission wavelengths based on two aspects of the experiment and mechanism (Figure 2.15).

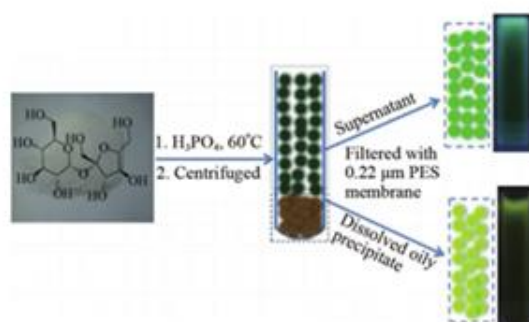


Figure 2.14: Diagram showing the Process of Creating Dual-Colour CQDs by Phosphoric Acid Oxidation of Sucrose (Xu et al., 2014)

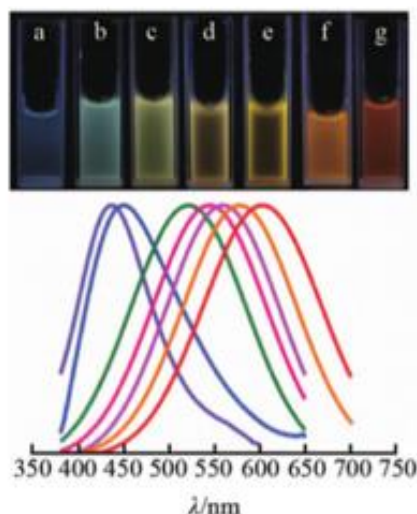


Figure 2.15: Optical Images and Fluorescence Emission Spectra of Multicolour CQDs (Bao et al., 2015)

Kumari et al. (2018) prepared green fluorescent CQDs with high stability and strong fluorescence intensity by an ultrasonic-assisted chemical oxidation method using waste polyolefin cracking residue as a raw material without any chemical modification. CQDs have good selectivity and sensitivity for Cu^{2+} detection in actual water samples. Simultaneously, the CQDs also have favourable cell penetration and minimal toxicity, making them suitable for efficient bioimaging of triple-negative breast cancer cells.

In short, chemical oxidation is a method of obtaining CQDs by oxidizing carbon source precursors. The advantage of this method is that the raw material is inexpensive, but it requires expensive equipment.

2.3.2 Hydrothermal Method

The hydrothermal method (Figure 2.16) refers to the preparation of products by high-temperature polymerization and carbonization of precursor molecules. The reaction is easy to control and causes little environmental pollution (Nazibudin et al., 2023). It is a direct and effective method for material preparation (Xian et al., 2022). BCQDs derived from various carbon sources prepared using hydrothermal methods and their properties are presented in Table 2.6. The QY of most biomass CQDs without functionalization is generally less than 10%, which seriously limits their applications in biosensing and bioimaging.

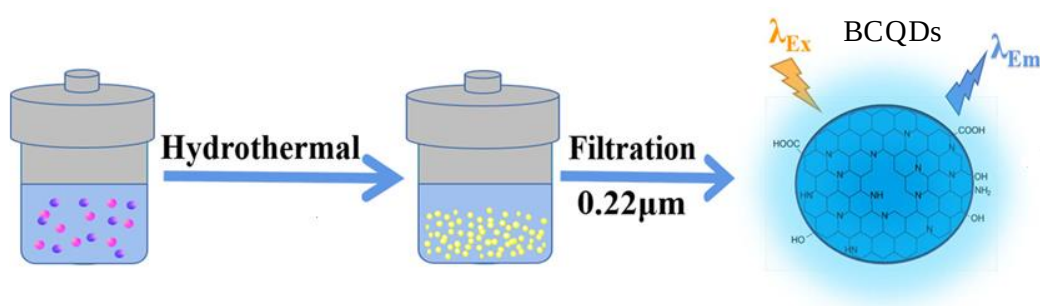


Figure 2.16: Schematic Diagram of BCQDs Prepared by a Hydrothermal Method

Table 2.6: BCQDs Prepared by Hydrothermal Methods with Different Biomass Carbon Sources and their Properties

No.	Biomass Carbon Source	Reaction Conditions	QY	Application	References
1.	Saccharum officinarum juice	120°C	5.67%	Cell imaging and Ion detection	Mehta et al. (2014a)
2.	Willow Bark	200°C	6%	Photocatalysis	Qin et al. (2013)

Table 2.7 continued

No.	Biomass Carbon Source	Reaction Conditions	QY	Application	References
3.	Potatoes	170°C	6.14%	Cell imaging	Mehta et al. (2014b)
4.	Wheat straw	250°C	9.2%	Cell imaging	Yuan et al. (2015)
5.	Cornflour	180°C	7.7%	Cell imaging and Ion detection	Wei et al. (2014)
6.	Fish scales	200°C	9%	Ion detection	Zhang et al. (2018)
7.	Groundnuts	180°C	7.8%	Ion detection	Roshni et al. (2019)
8.	Ocimum sanctum	180°C	9.3%	Cell imaging and Ion detection	Kumar et al. (2017)
9.	Sweet potato peels	200°C	8.9%	Fluorescence sensing	Liu et al. (2019)
10.	Fish scale collagen peptides	200°C	9.29%	Fluorescence sensing	Xu et al. (2023)

Zhang et al. (2010) produced fluorescent CQDs for the first time using a hydrothermal technique. Zhao et al. (2015) synthesized blue fluorescent nitrogen-sulfur codoped CQDs by a hydrothermal reaction using garlic as a carbon source precursor. In contrast to certain conventional fluorescent dyes, CQDs demonstrate outstanding optical characteristics, good chemical and photochemical stability, and excellent biocompatibility,

so they are ideal visual probes for imaging both in vivo and in vitro. Furthermore, the CQDs also exhibit effective scavenging of free radicals, showing their multifunctional applications in biological imaging and antioxidation.

Ding et al. (2017) synthesized red fluorescent CQDs in high yield (28%) by a hydrothermal method using lemon juice and ethanol as the raw materials. The typical size of the red fluorescent CQDs was measured to be 4.6 nm and the emission wavelength at 631 nm was independent of the excitation wavelength. At the same time, the CQDs have low cytotoxicity and good photostability. Given their environmentally friendly synthesis methods and good optical properties, CQDs show great potential for use in bioimaging and light-emitting diodes.

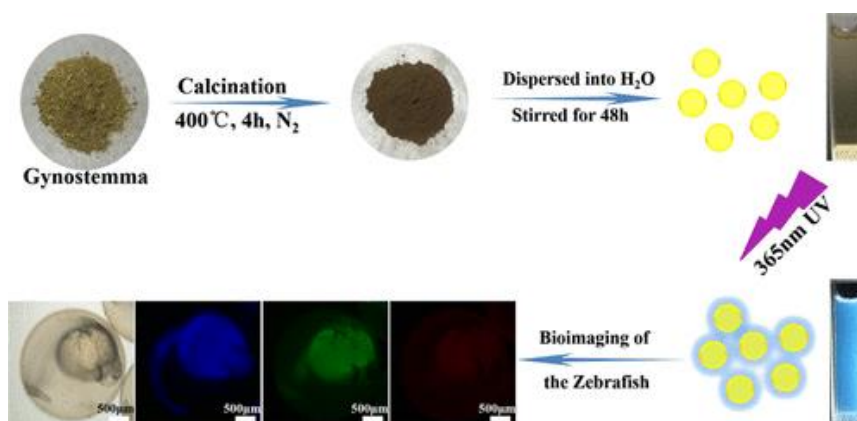


Figure 2.17: Synthesis of CQDs from Gynostemma for Use in Bioimaging (Wei et al., 2019)

Wei et al. (2019) used natural gynostemma pentaphyllum as a carbon source precursor, synthesized blue fluorescent CQDs with an average particle size of approximately 2.5 nm and good dispersion by a high-temperature hydrothermal method, and evaluated the biotoxicity of the CQDs using zebrafish embryos (Figure 2.17). The findings indicated that

around 90% of zebrafish embryos successfully hatched and survived when exposed to CQDs, with less than 10% showing deformities. This suggests that the CQDs demonstrated favourable biocompatibility. In addition, the CQDs promoted the mRNA expression of related genes in zebrafish, encoded more antioxidant proteins, and had both imaging and therapeutic effects. Studies have shown that fluorescent CQDs are potential candidates for the biological imaging and treatment of diseases caused by excessive oxidative damage, such as cancer, ageing, and other ageing-related diseases.

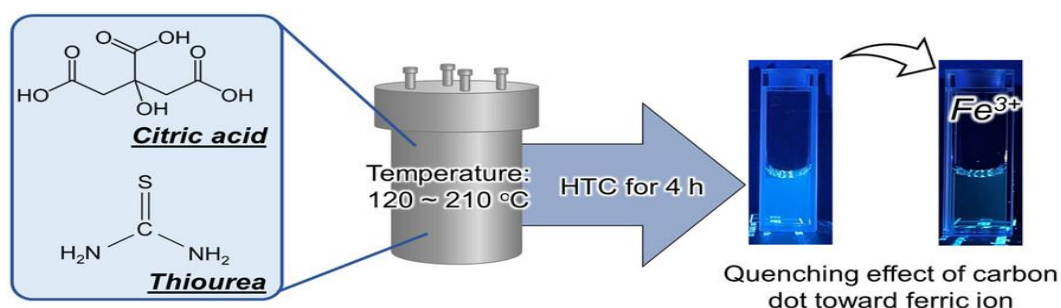


Figure 2.18: Examination of CQDs Produced at Varying Hydrothermal Carbonization Temperatures and their Abilities in Detecting Fe^{3+} (Ahn et al., 2024)

Han et al. (2024) prepared N-doped CQDs (N-CQDs) from orange peels through a hydrothermal process, resulting in a QY of 35.37%. In another interesting work, CQDs were fabricated by hydrothermal carbonization at 120 to 210°C (Ahn et al., 2024). Temperature changes have a significant impact on specific recognition, as the chemical structure and optical properties of the resulting CQDs can be distinguished. Both the heteroatom-doped core structure and the functionalized surface state show changes related to the mentioned temperature changes. CQDs synthesized at 180°C contain properly balanced doped heteroatoms and surface functional groups and further show a quenching effect of up to 17% for Fe^{3+} (Figure 2.18).

Overall, the hydrothermal method refers to the preparation of products by high-temperature polymerization and carbonization of precursor molecules. The advantage of this method is good control of the particle size and morphology, but it includes complex procedures.

2.3.3 Microwave-assisted Method

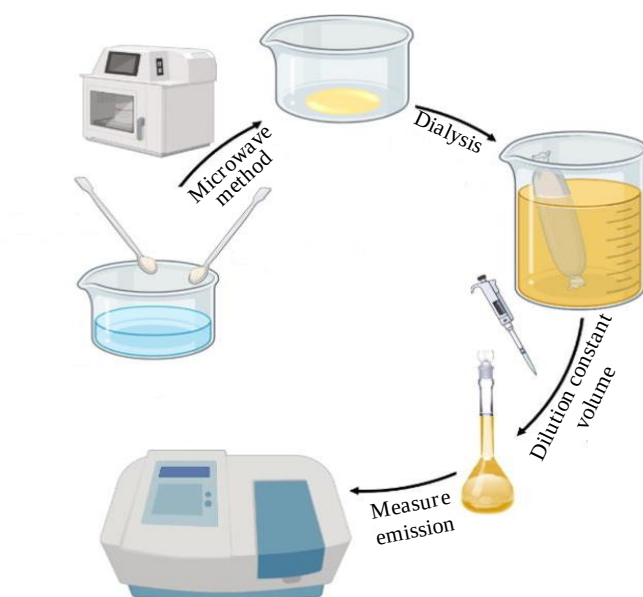


Figure 2.19: Schematic Diagram of BCQDs Prepared by a Hydrothermal Method

Microwave-assisted synthesis (Figure 2.19) is an efficient and time-saving method. It can significantly increase the yield and quantum yield of CQDs (Xu et al., 2023). Ichthyotic collagen peptides were utilized as precursors to synthesize CQDs via hydrothermal (CQDs-HT) and microwave (CQDs-MW) techniques. The researchers compared the optical characteristics of the CQDs produced using two different methods and analyzed how the heating process affected the structure of the CQDs. Both types of CQDs emit blue light and show emission properties that depend on the excitation. Moreover,

microwave heating is rapid and uniform, which is more favourable for N self-doping. While the N doping in CQDs-HT is minimal, there is a high proportion of pyrrole N present. In contrast to the microwave reaction vessel's limited sealing, the traditional hydrothermal method's controlled high-temperature sealing environment facilitates thorough dehydration and condensation of the precursors, resulting in the formation of smaller CQDs. Thus, compared with CQDs-MW, CQDs-HT exhibited a greater QY (9.29% vs. 4.86%) (Figure 2.20).

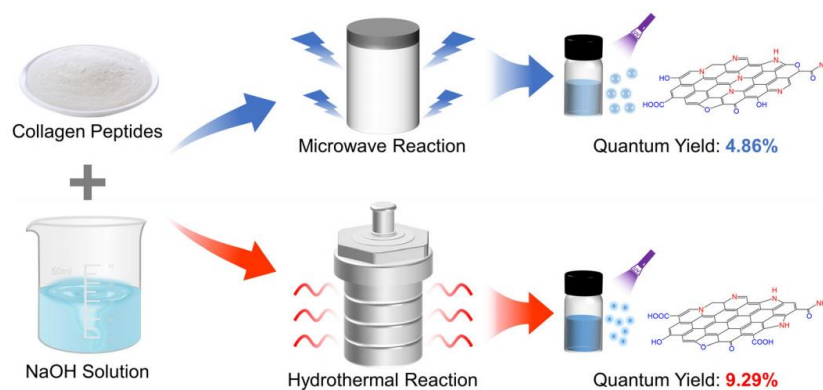


Figure 2.20: Diagram Illustrating the Variances in the Production of CQDs through Traditional Hydrothermal Methods and Microwave Techniques (Xu et al., 2023)

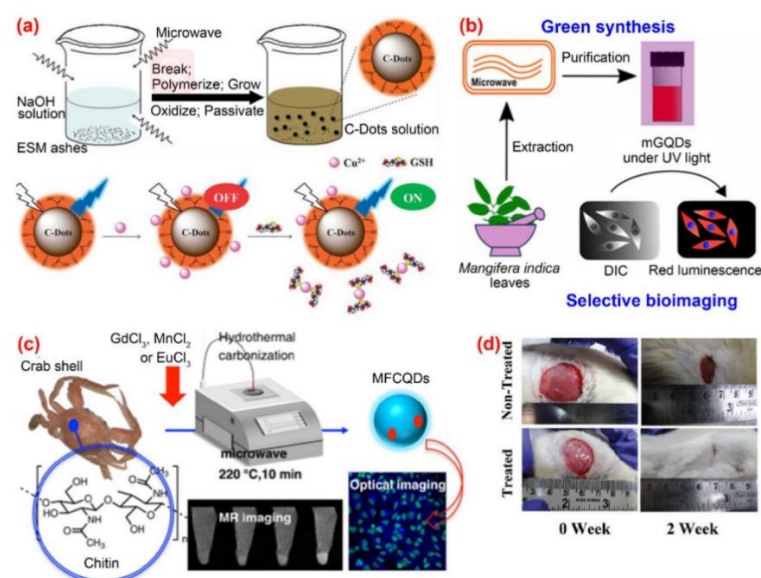


Figure 2.21: The Production of Biomass CQDs using Microwave-Assisted Methods. (a) Eggshell Membranes (Kumawat et al., 2017); (b) Mango Leaves (Yao et al., 2017); (c) Crab Shell (Bankoti et al., 2017); (d) Testing the Effectiveness of C-dots Made from Onion Peel in Promoting Wound Healing (Bankoti, et al., 2017)

Figure 2.21 illustrates the process of producing biomass CQDs using microwave-assisted methods. Liu et al. (2015) synthesized green fluorescent CQDs by a microwave-assisted method using glucose and sodium polyacrylate as carbon sources. CQDs have good biocompatibility and excellent photostability. Furthermore, the authors used folic acid-modified green CQDs to specifically recognize folate receptors on the surface of tumour cells, which can not only target tumour cells but also distinguish tumour cells from normal cells. Rodriguez-Padron et al. (2018) synthesized blue fluorescent CQDs with a particle size of 17.5 nm by a microwave-assisted method using lignocellulose residue in wood pulp as the raw material. This study showed the application prospects of these materials for the conversion of wastes into useful fluorescent nanomaterials.

Recently, Liu et al. (2019) synthesized blue fluorescent CQDs using pear juice as a carbon source and ethylenediamine as a nitrogen source. Nitrogen doping introduced by

ethylenediamine reduces the band gap energy between the LUMO and HOMO and improves the quantum yield and fluorescence properties of the CQDs. This carbon quantum dot has good biocompatibility and fluorescence stability, can be used for highly sensitive detection of the ovarian cancer tumour marker CA-125, and has the potential for medical diagnostic applications. Wang et al. (2019) synthesized polychromatic fluorescent CQDs using fructose as a carbon source and NH_4HCO_3 as a nitrogen source. The appearance of multiple energy levels and a larger sp^2 domain are the main reasons for the redshift with increasing concentration. Based on this characteristic, the CQDs were successfully used as fluorescent ink and biological imaging agents. This research introduces a novel technique for creating CQDs with polychromatic emission.

Although the rapid preparation of biomass CQDs can be achieved by microwave methods, the obtained CQDs usually have uneven particle size distributions.

2.4 Luminescence Mechanism of CQDs

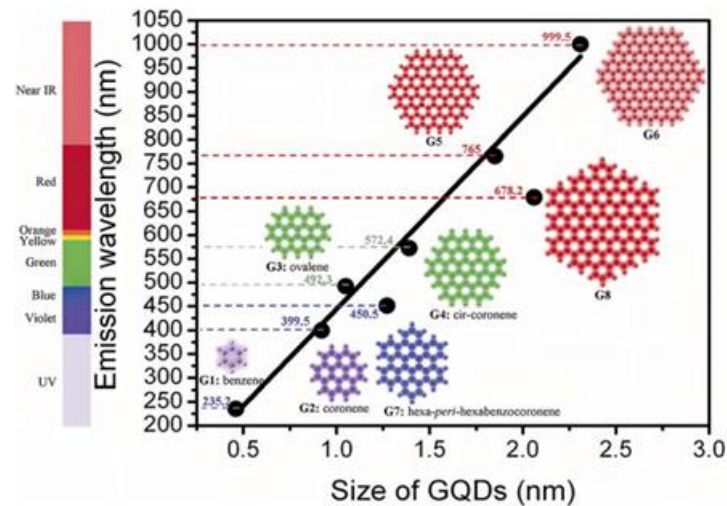


Figure 2.22: The Correlation between the Size of Particles and the Wavelength of Luminescence in Graphene CQDs (Wang et al., 2023)

The luminescence mechanism of CQDs is closely related to their preparation process. CQDs prepared from different raw materials, different preparation methods, and different pretreatment methods usually have different components and structures, which can lead to different optical properties (Figure 2.22). Currently, the most recognized carbon quantum dot fluorescence mechanism involves a quantum domain effect, surface-state luminescence, and molecular-state luminescence.

2.4.1 Quantum-restricted Domain Effect

CQDs typically have a size smaller than 10 nm, so some researchers speculate that similar to the luminescence mechanism of quantum dots, the luminescence performance of CQDs is also related to the quantum confinement effect (Yuan et al., 2018; Kozak et al., 2016). When semiconductor crystals are nano leveled, the grain boundaries have a significant effect on the electron distribution within the crystal, which leads to the occurrence of energy relaxation dynamics related to the band gap and size (Yan et al., 2013), which is the quantum limit effect.

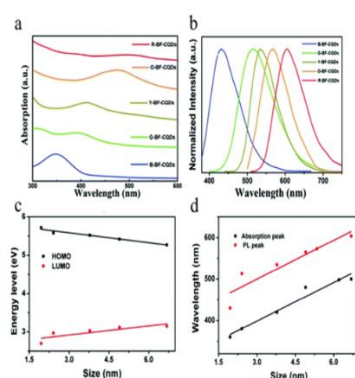


Figure 2.23: (a and b) The Absorption and PL Spectra of CQDs of Different Sizes, (c) HOMO and LUMO Energies for CQDs of Different Sizes, (d) Absorption and PL Spectra of CQDs of Different Sizes (Yuan et al., 2017)

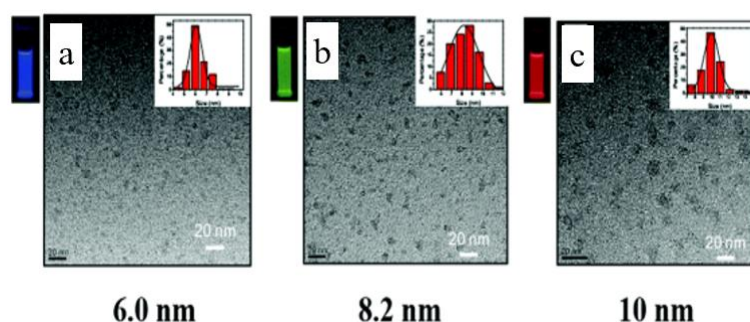


Figure 2.24: TEM Images of CQDs of Different Sizes (Jiang et al., 2015)

Yuan et al. (2017) synthesized a series of CQDs emitting different wavelengths of fluorescence (blue, green, yellow, orange, and red light, using citrate and diaminobanaphthalene) by changing the reaction conditions. Unlike most previously reported CQDs, these CQDs have no excitation wavelength dependence, high surface passivation, high crystallinity, and high fluorescence quantum yield (75%). By analysing their spectra, their UV-visible absorption wavelengths were 350 nm, 390 nm, 415 nm, 480 nm, and 500 nm, which coincide with the corresponding maximum fluorescence excitation wavelengths (Figure 2.23), indicating that their fluorescence emission comes from the bandgap transition. Moreover, these carbon quantum materials were found to have different particle sizes by transmission electron microscopy. With increasing particle size, the fluorescence emission redshifted, which revealed that the fluorescence of the CQDs came from the quantum domain effect. Jiang et al., (2015) prepared three CQDs with particle sizes of 6 nm, 8 nm, and 10 nm (Figure 2.24), emitting blue, green, and red fluorescence, respectively, which is consistent with the characteristics of the quantum domain effect. Li et al. (2010) and Peng et al. (2012) synthesized CQDs with size dependence and quantum

domain effects by electrochemical oxidation and chemical oxidation, respectively (Figure 2.25).

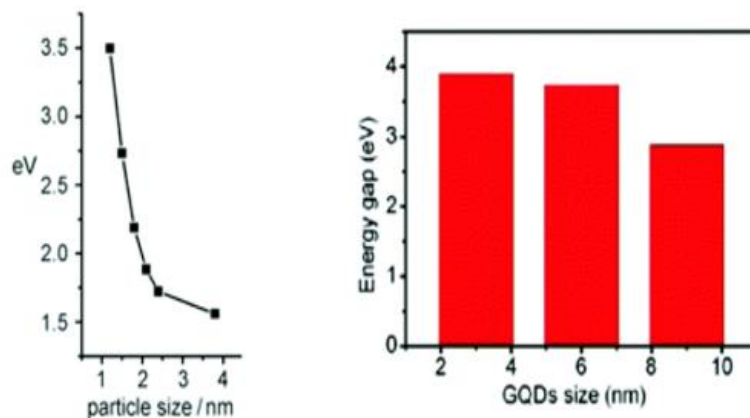


Figure 2.25: Relationship between the CQDs Size and Band Gap (Li et al., 2010; Peng et al., 2012)

2.4.2 The Surface-state Luminescence

The surface state (surface oxidation degree and surface functional groups) is closely related to the optical properties of the material. The process of chemical decomposition of carbon-based materials or the polymerization/carbonization of small molecule precursors is usually an essential step for the preparation of CQDs. This highly activated process (surface oxidation and local carbonization) makes the chemical structure of the CQDs surface very diverse and complex, including sp^2 hybridized carbon, sp^3 hybridized carbon, surface traps, and various functional groups (Gharat et al., 2019; Shen et al., 2019). Many studies have shown that the fluorescence intensity of CQDs is related to the degree of oxidation on the surface and that the intensity of the fluorescence emitted during surface oxidation increases. This is because the greater the degree of oxidation of the CQDs surface is, the more traps there are on the surface, and the more excitons will promote exciton recombination to

produce radiation (Bao et al., 2011). Ding et al. (2016) prepared CQDs by a one-pot water heat method using urea and p-phenylenediamine and subsequently purified a series of CQDs emitting blue, green, yellow, and red fluorescence. Transmission electron microscopy images of the CQDs show that the average particle size of the CQDs that emit different colours of fluorescence is 2.6 nm (Figure 2.26), which indicates that the fluorescence of the prepared CQDs does not come from the quantum domain effect. In addition, by analysing the FT-IR and XPS of the CQDs, it was found that the wavelength at which fluorescence is emitted will redshift as the number of hydroxyl groups and the oxygen content of the carbon quantum dot surface increase. This is because when the oxygen content on the surface of the CQDs rises, the difference in energy levels between the highest occupied molecular orbital and the lowest unoccupied molecular orbital decreases.

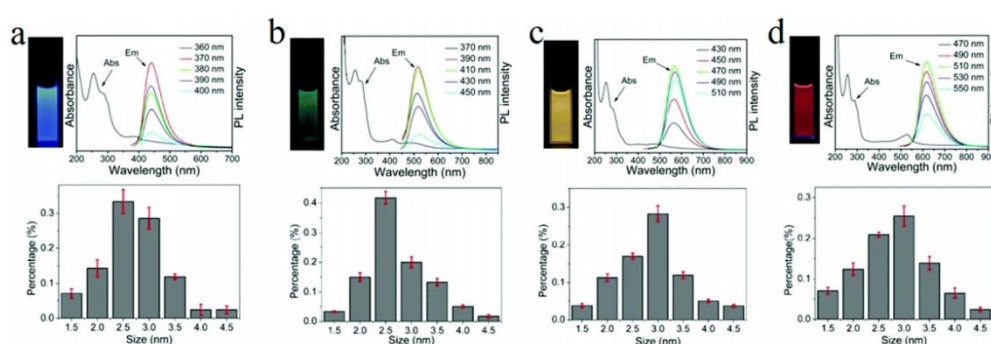


Figure 2.26: The Absorption, PL Spectra, and Size Distribution of Blue, Yellow, and Red Emission CQDs, respectively (Ding et al., 2016)

Zhang et al. (2017) prepared CQDs from citrate amine and ethylenediaminetetraacetic acid by a solvothermal method and synthesized a series of CQDs emitting blue, green, white, yellow, orange, and red fluorescence by changing the ratio of citrate amine to ethylenediaminetetraacetic acid. By analysing the TEM electron microscope

map, FTIR spectrogram, and XPS energy spectrum of the resulting CQDs, it was found that they had the same particle size and oxygen content. However, they differ in their amide group content, and their emission fluorescence redshifts as the concentration of the amide groups rises. Because the increase in the N to functional group content narrowed the energy gap and redshifted the emission fluorescence.

2.4.3 Molecular Luminescence

Many studies have shown that in the process of bottom-up synthesis, CQDs are modified on the surface so that they emit fluorescence (Benner et al., 2023). Reactive functional groups (e.g., carboxyl and amino groups) present in the small molecule precursor structure can react with each other, undergo further condensation, polymerization, and carbonization, and eventually form CQDs. This highly reactive process readily produces some fluorescent small molecule byproducts, which are modified on the carbon quantum dot surface. Xiong et al. (2018) prepared three CQDs through a hydrothermal process involving citric acid and ethylenediamine, citrate and hexamethylenetetramine, and citric acid and triethanolamine. By comparing the ultraviolet-visible absorption spectrum, steady-state fluorescence emission spectrum, fluorescence lifetime, XPS energy spectrum, and FTIR spectrum of the three CQDs synthesized with acid (a pyridine family fluorescent dye), the following conclusions were drawn: blue fluorescent CQDs synthesized from ethylenediamine and red fluorescence from surface-modified acid molecules. There are also experiments showing that some small fluorescent organic molecules with high fluorescence quantum yields are produced during the synthesis of CQDs and are dispersed in solution. Therefore, accurate purification steps to eliminate interference from some fluorescent small molecules are essential for the preparation of CQDs.

2.5 The Fluorescence Detection Mechanism of CQDs

CQDs are becoming more popular in fluorescent sensors due to their minimal toxicity, strong photostability, and impressive biocompatibility. In addition, their small size, large specific surface area, and abundant surface functional groups make CQDs highly reactive, making them suitable for application as fluorescent probes in fluorescent sensors. Changes in the surrounding environment, such as changes in temperature, ionic strength, and solvent conditions, may all affect the optical properties of CQDs. The principle of a fluorescence sensor based on CQDs is that the recognition components on the CQDs interact with the target analyte, causing a change in its fluorescence properties, and this change has a quantitative linear relationship with the concentration of the target analyte. According to the different fluorescence signals, fluorescence sensors based on CQDs can be divided into four types: quenching type, enhanced type, ratiometric type, and wavelength displacement type.

2.5.1 Quenched Type

As shown in Figure 2.27, in theory, the mechanisms of quenched fluorescent sensors are mainly divided into static quenching effect (SQE), dynamic quenching effect (DQE), light-induced electron transfer (photoinduced electron transfer, PET), fluorescence resonance energy transfer (FRET) and internal filter effect (inner filter effect, IFE). Here, we introduce them one by one.

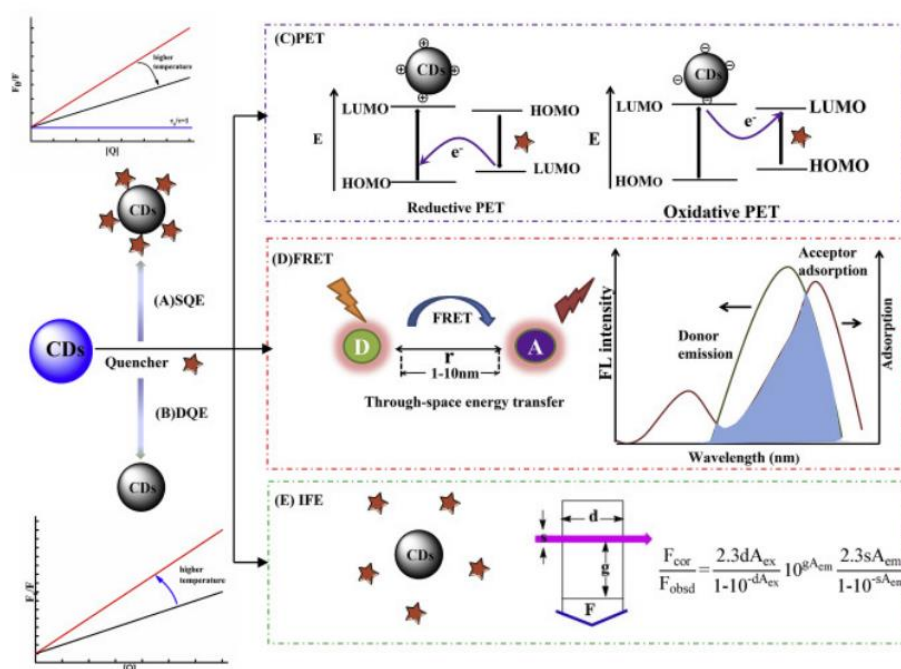


Figure 2.27: Quenching Mechanisms of Sensors based on CQDs (Molaei et al., 2020)

i. Static quenching effect

The static quenching effect is the process of CQDs reacting with a quencher to generate a nonfluorescent ground state complex. When the static quenching effect occurs, (1) the fluorescence life of the CQDs does not change significantly; (2) the UV-visible absorption spectrum of the non-CQDs is displaced during the reaction process; and (3) the temperature increase decreases the stability of the ground state complex, thus inhibiting the static quenching process and resulting in the gradual recovery and enhancement of the fluorescence intensity. The chemical interaction between the CQDs and the quencher is one of the essential conditions for the static quenching effect, so the surface functionalization of the CQDs favours the generation of the static quenching effect (Hijazi et al., 2023). N-CQDs were synthesized by the protection/deprotection of an amine group and an amidation reaction

on the surface of the CQDs, and the catechol content was detected by a static quenching effect (Yang et al., 2021). CQDs were synthesized by a simple hydrothermal process using mustard seeds as the carbon source (Zhang et al., 2022). With the formation of a nonfluorescent ground state complex between the CQDs and Fe^{3+} , Fe^{3+} detection is achieved by an overfluorescence quenching mechanism (Figure 2.28).

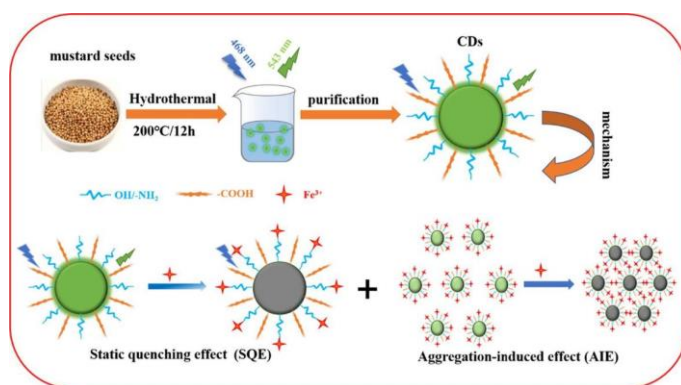


Figure 2.28: Schematic Diagram of the Preparation of CQDs and the Mechanism of Fluorescence Quenching to Fe^{3+} (Zhang et al., 2022)

Guo et al. (2021) prepared sulfur-doped CQDs (S-CQDs) using 3-aminobenzene and detected azithromycin (AZM) by a static quenching effect. An analysis of the zeta potential map (Figure 2.29A) revealed that the zeta potential of the CQD-AZM mixture was intermediate between that of the CQDs and AZM, indicating that strong electrostatic interactions occurred between them, resulting in the formation of the carbon quantum dot-AZM complex. After detection, the average fluorescence lifetime of the CQDs was 4.69 ns, which was between 4.64 ns and 4.86 ns (Figure 2.29B). This showed that the fluorescence duration of the CQDs remains the same even after AZM is added, which is consistent with the process of the static quenching effect.

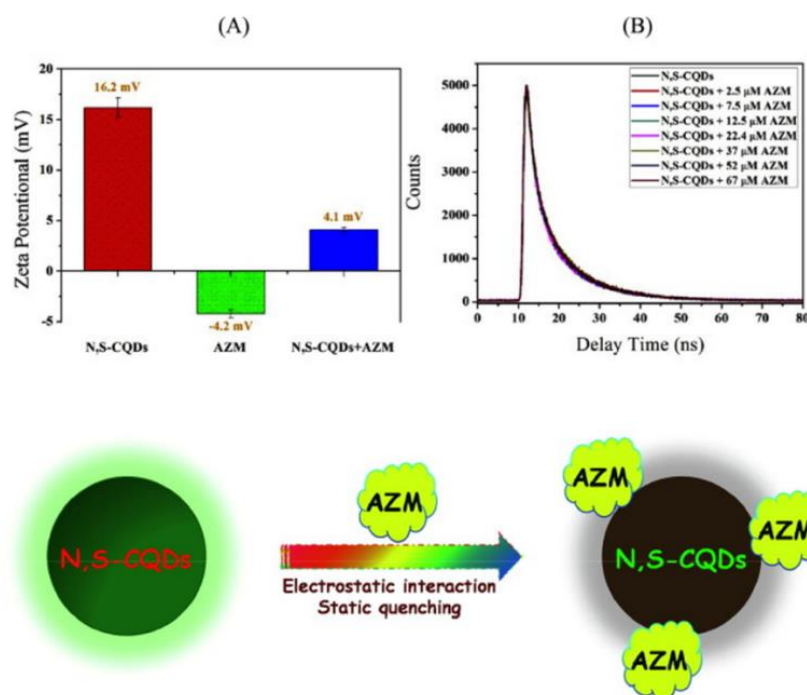


Figure 2.29: (A) Zeta Potentials of S-CQDs, AZM and the Mixture of S-CQDs and AZM. (B) Fluorescence Decay Curves of S-CQDs in the Absence and Presence of Different Concentrations of AZM (Guo et al., 2021).

ii. Dynamic quenching effect

The DQE is the process of electron transfer or energy transfer between the excited CQDs and the quencher and the collision back to the ground state. Compared with the SQE, the DQE has several different characteristics: (1) the fluorescence life of the CQDs will change significantly; (2) because dynamic quenching does not generate new matter, the UV-visible absorption spectrum of the carbon quantum dot will be maintained and unchanged; (3) the quencher will not react with the carbon quantum dot; and (4) an increase in temperature will promote the dynamic quenching effect (Zu et al., 2017).

Wang et al. (2016) synthesized CQDs by the hydroheat method using boronic acid and glucose and detected the content of iron ions by the dynamic quenching effect between

them and iron ions. The results showed that the concentration of iron ions is linear in the range of 0-16 $\mu\text{mol/L}$ and conforms to the Stern-Volmer equation described above. Moreover, the fluorescence lifetime of the CQDs changes, indicating that this detection mechanism has a dynamic quenching effect. Wei et al. (2021) synthesized red fluorescent CQDs (RCQDs) from sodium citrate and formamide, which were successfully used for detecting Cu^{2+} . Cu^{2+} can quench the fluorescence of R-CQDs, and further experiments have shown that the quenching mechanism combines both static and dynamic quenching effects.

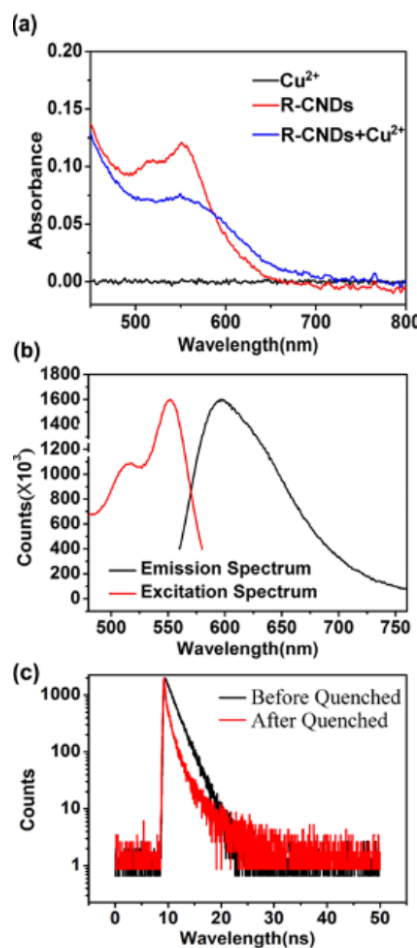


Figure 2.30: UV-vis Absorption Spectra of R-CQDs, a Water Solution of Cu^{2+} and R-CQDs+ Cu^{2+} ; (b) Emission Spectrum and Excitation Spectrum; (c) Fluorescence Decay Curves of the R-CQDs and R-CQDs- Cu^{2+} (Wei et al., 2021)

Figure 2.30a reveals that the UV-visible absorption peak of the R-CQDs changed significantly (which is consistent with the characteristics of the static quenching effect. They also detected a change in the fluorescence lifetime of CQDs before Cu^{2+} were added. In Figure 2.30c, the fluorescence lifetime of the R-CQDs can be fitted to an exponential decay equation with an average fluorescence lifetime of 1.3 ns. When Cu^{2+} are added, the fluorescence lifetime of the R-CQDs can be fitted to a double exponential decay equation, and the average fluorescence lifetime is 1.49 ns. This result indicates that the quenching mechanism is a dynamic quenching process. Considering the above results, it can be inferred that the mechanism of CQDs is a combination of static and dynamic quenching effects.

iii. Photoinduced electron transfer

PET is associated with the electron transfer process between excited CQDs (electron donors/acceptors) and quenchers (electron donors/acceptors). Quenchers can form complexes with CQDs and can excite CQDs' doctoral state electrons back to the ground state through nonradiative emission. According to the different types of CQDs, photoinduced electron transfer can be divided into prototype photoinduced electron transfer and oxidative photoinduced electron transfer. As the acceptor of electrons, driven by the energy gap between the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO), CQDs can accept electrons from the donor quencher by photoinduced electron transfer. In contrast, during oxidative photoinduced electron transfer, the excited CQDs are transferred to the quencher. The oxidized photoinduced electron transfer is driven by the energy gap between the LUMO of the carbon quantum dot and the HOMO of the quencher (Fan et al., 2017). In the photoinduced electron transfer quenching

FRET is a process in which the excited-state energy of a carbon quantum dot used as an energy donor is directly transferred to nonradiative energy transfer with dipole-dipole coupling as a quencher of the energy receptor. This process does not produce photons, and the fluorescence emission spectrum of the CQDs overlaps with the UV-visible absorption spectrum of the quencher. Moreover, for FRET to occur, the carbon quantum dot and the quencher need to be within 1-10 nm (Ma et al., 2024).

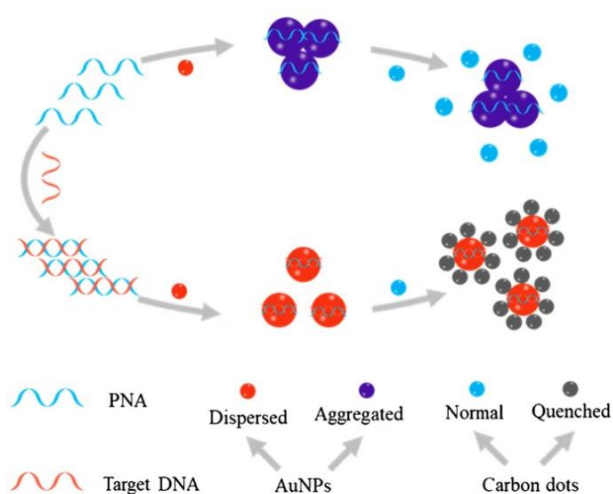


Figure 2.32: Schematic Diagram of the DNA Detection Strategy Using the CQDs/AuNPs System (Ma et al., 2024)

FRET-based fluorescent sensors have benefits including excellent sensitivity and minimal detection thresholds. Gao et al. (2020) designed a fluorescent sensor for detecting DNA based on FRET between CQDs and gold nanoparticles (AuNPs) (Figure 2.32). In the absence of DNA, peptide nucleic acid can cause AuNPs to come together, leading to the inhibition of the FRET interaction between CQDs and AuNPs and resulting in the recovery of the system's fluorescence intensity. However, when DNA is present in the system, the peptide nucleic acid can specifically recognize the DNA and form complexes with it, leaving

the gold nanoparticles in a dispersed state. This allows the CQDs to effectively undergo FRET with the gold nanoparticles, resulting in fluorescence quenching and the detection of the DNA content. The fluorescent sensor has high sensitivity and a low detection limit (0.21 nmol/L).

v. Inner filter effect

The conditions generated by the IFE include the following: (1) the UV-visible absorption spectrum of the quencher effectively overlaps with the fluorescence emission spectrum of the CQDs, and (2) the distance between the CQDs and the quencher is greater than 10 nm. Compared with other fluorescence quenching mechanisms, including FRET or PET, the IFE process has several characteristics: (1) the IFE-based sensor does not require the covalent combination of CQDs and the quencher, and the position and fluorescence lifetime of the absorption peak of the CQDs do not change; (2) there is no electron transfer or energy transfer; (3) CQDs do not require the modification of surface functional groups; and (4) the distance between the CQDs and the quencher is greater than 10 nm. All these features make the IFE-based fluorescence sensor relatively easier to construct and more feasible. There are two modes of IFE systems. One is composed of three components: a fluorescent luminescent material, a fluorescent absorber, and an analyte. The other is made up of two components, a fluorescent luminescent material, and an analyte, which is also a fluorescent absorber. The second mode greatly simplifies the operation of the experiment relative to the first mode.

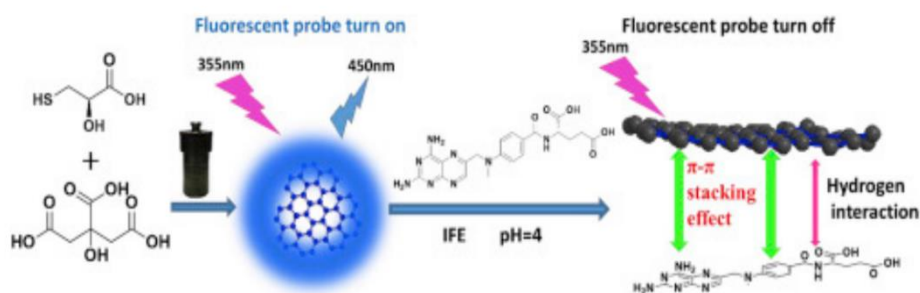


Figure 2.33: Schematic Diagram of a Sensitive Fluorescence Sensor Constructed based on the Inner Filter Effect of S-CQDs (Zhao et al., 2019)

Zhao et al. (2019) prepared and synthesized novel S-CQDs as a fluorescent probe to detect methopurine (Figure 2.33). The -NH₂, -COOH, and -OH groups on the surface of these CQDs can participate in hydrogen bonding interactions with methopurine. Furthermore, the aromatic heterocycle of these CQDs can be targeted to recognize methionine by π - π stacking interactions occurring between the pteridine ring and the benzene ring of methionine. Furthermore, studies of the HOMO and LUMO levels of the system and the fluorescence lifetime confirmed that the mechanism of the process is the IFE.

Sidhu et al. (2019) constructed a fluorescence sensor based on the IFE between floral gold nanoparticles and CQDs and successfully detected aflatoxin B1 in large bean paste. The surface is a distant sub resonance peak of floral gold nanoparticles that overlaps well with the fluorescence emission peak of CQDs, so it was selected as a fluorescence quencher. In the presence of floral gold nanoparticles, the fluorescence of CQDs is quenched, while their fluorescence lifetime is not changed. Therefore, the quenching mechanism of this fluorescence sensor is the IFE.

2.5.2 Enhancement Type

Enhanced fluorescent sensors are CQDs showing weak fluorescence or no emission fluorescence in the absence of an analyte. When the analyte is added, the fluorescence of the CQDs is enhanced. There are two modes of fluorescence-enhanced response: indirect fluorescence-enhanced response. First, the fluorescence of CQDs is quenched by nonanalytes. When the analyte is added, the fluorescence of the CQDs is restored and indirectly enhanced. The other is a direct fluorescence-enhanced type, in which the analyte acts directly with the CQDs to enhance its fluorescence. Aggregation-induced emission (AIE) (Ding et al., 2023) and meta-induced fluorescence enhancement (MFE) (Han et al., 2021) are the two main direct enhanced fluorescence sensors. In the AIE mechanism, the groups on the surface of CQDs can coordinate with the analyte to produce aggregation, thus inhibiting the enhancement of fluorescence from the nonradiative decay process. The MFE mechanism typically relies on the surface plasmon resonance (SPR) phenomenon exhibited by metal nanostructures (such as gold nanoparticles and silver nanoparticles), which increases the local electric magnetic field strength and leads to the enhancement of the surrounding CQDs fluorescence.

Phosphorus/nitrogen-doped CQDs were prepared and successfully used for the detection of formaldehyde by Qu et al. (2020). The results showed that when formaldehyde is added, formaldehyde can react with CQDs, inducing the aggregation phenomenon of CQDs and effectively limiting the vibration and rotation process of CQDs, thus reducing the nonradiative attenuation process and leading to fluorescence enhancement.

2.5.3 Ratiometric Type

Most fluorescence sensors use fluorescence quenching at a single wavelength or fluorescence enhancement as the output signal for quantitative analysis, which causes the fluorescence signal to be susceptible to many analyte-independent factors, such as fluctuations in the excitation light source, probe concentration, light scattering of the sample matrix and fluctuations in the solution environment. A ratiometric-type fluorescent sensor detects an analyte by recording changes in the ratio of fluorescence intensity at two wavelengths, which greatly reduces the impact of changes in the external factors described above (Fang et al., 2024). Therefore, ratiometric fluorescent sensors have more reliable and accurate experimental results than those based on a single wavelength. There are two modes: the reference mode and the dynamic mode. In the reference mode, after the addition of the target analyte, a fluorescence channel without signal change can serve as the reference, while another fluorescence channel with increased, weakened, or displaced changes in the fluorescence signal can act as the output signal. In dynamic mode, there are two dynamic fluorescence channels, and after the addition of the analyte, the fluorescence signal changes at both wavelengths, possibly through the same or opposite changes (Dai et al., 2023).

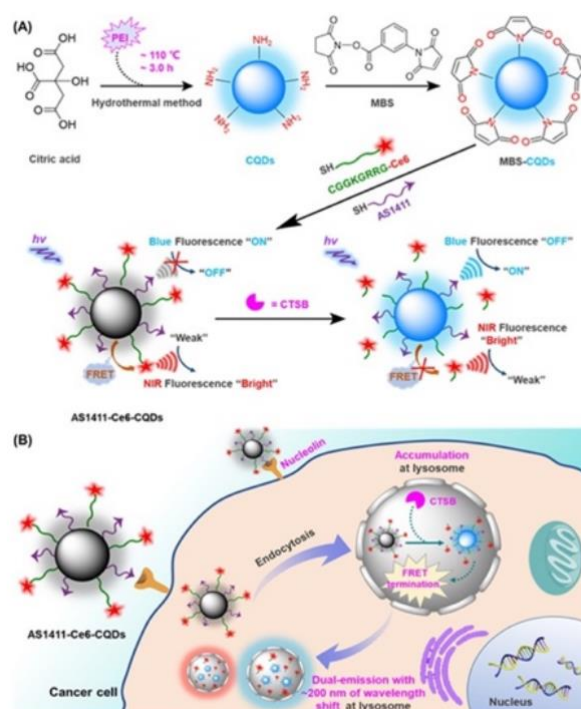


Figure 2.34: (A) Illustration of the Procedure for the Assembly of the Fluorescent Probe (AS1411-CQDs-Ce6). (B) Illustration of the Mechanism of Action of the Fluorescent Probe (AS1411-CQDs-Ce6) for the Fluorescent Imaging of Endogenous CTSB in Living Cancer Cells (Shen et al., 2021)

Among the ratiometric fluorescent responses, most biometric fluorescent sensors are based on the FRET mechanism (Mao et al., 2021). Shen et al. (2021) modified CQDs with a near-infrared molecular dye (Pep-Ce 6) and a nucleolin-targeting aptamer (AS1411) to establish a proportional fluorescent probe (AS1411-CQDs-Ce 6) targeting the malignant tumour marker cathepsin B (CTSB) (Figure 2.34). The FRET process occurs between the CQDs and Ce6 in the system, the fluorescence of the carbon CQDs is quenched at 450 nm, and the fluorescence of Ce6 at 650 nm is enhanced. Through nucleolin-mediated endocytosis, CQDs can selectively enter cancer cells with CTSB overexpressed in the lysosomes of cancer cells, cleaving Pep-Ce 6 to separate the Ce 6 dye from the CQDs, thereby inhibiting the FRET process between them and causing fluorescence to change from red to blue within

cancer cells. The amount of CTSB was determined by the ratio of red to blue fluorescence intensities. The results showed that the fluorescent probe has high selectivity and high sensitivity (detection limit of 0.096 ng mL^{-1}) and has promising applications in the clinical diagnosis of malignant tumours in the future.

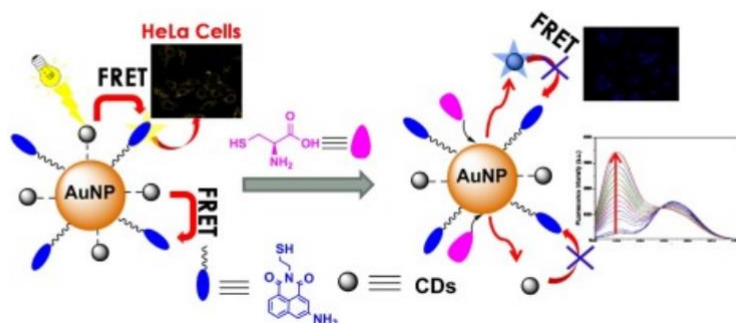


Figure 2.35: Schematic Diagram of the Procedure of the FRET Paired Ratiometric Fluorescence Probe based on CQDs and AuNPs for Cysteine Detection (Sidhu et al., 2019)

Sidhu et al. (2019) constructed a ratiometric fluorescent sensor based on the FRET interaction between CQDs and naphthalene (L1)-modified AuNPs for determining the cysteine (Cys) content (Figure 2.35). Upon excitation at 360 nm, the same fluorescence emission as that of naphthalene dimethimide was observed because of the FRET mechanism between the CQDs and naphthalene dimethimide. When Cys is added, the fluorescence emission intensity at two different wavelengths changes. This is because the sulfhydryl group in Cys strongly adsorbs the gold nanoparticles, replacing the CQDs on the surface of the AuNPs, thus inhibiting the FRET interaction between the CQDs and naphthalene dimethimide. The sensor was effectively utilized to detect human HeLa cells and Cys in serum by comparing the fluorescence emission intensity at two different wavelengths.

2.5.4 The Wavelength Displacement Type

A shift in the fluorescence emission wavelength or Stokes shift can also be used as an output signal for design and fluorescence sensors, and the fluorescence emission wavelength displacement or Stokes displacement is linearly correlated with the concentration of the analyte in the fitted range. Yang et al., (2019) synthesized piperazine-modified CQDs that were successfully used for the detection of oxytetracycline by FRET (Figure 2.36). P-CQDs can undergo FRET between them and oxytetracycline through hydrogen bonding and electrostatic interactions, leading to the displacement of the strongest emitting fluorescence of P-CQDs. The results showed that the shift in the fluorescence emission wavelength scales linearly with the concentration of oxytetracycline. Moreover, the visual detection of oxytetracycline can be achieved based on fluorescence colour changes.

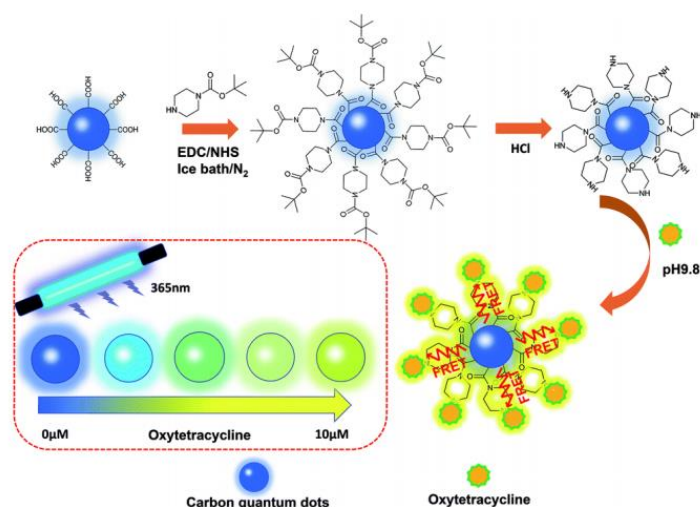


Figure 2.36: The Process of Creating P-CQDs and Detection Mechanism for OTC (Yang et al., 2019)

2.6 Application of Biomass CQDs

Biomass CQDs have the advantages of tunable optical properties (Wang et al., 2020), good biocompatibility, and high photostability (Guan et al., 2023) and are extensively applied in biosensing, imaging, and medical fields (Wareing et al., 2021; Xu et al., 2022).

2.6.1 Array Sensing

The surface of CQDs contains numerous active functional groups, which can be altered by modifying the raw materials, synthesis techniques, or reaction parameters. These functional groups give CQDs unique nonspecific interactions with a variety of target substances: the same kind of CQDs and different targets will produce different fluorescence response signals. Different CQDs and the same target to be tested will also produce different fluorescence response signals, which can be distinguished by principal component analysis or linear discriminant analysis, thus identifying different targets to be tested. Therefore, CQDs have become an excellent candidate material for the receptor unit of array sensors. Xu et al. (2017) synthesized four different CQDs in 1 min using four natural amino acids (leucine, isoleucine, glycine, and threonine) as raw materials and used these four CQDs as sensing units to construct a label-free sensor array for pattern recognition of proteins. When CQDs of the same type interact with 8 different proteins, they will elicit varying fluorescence reactions. Similarly, when a single protein interacts with four different types of CQDs, the resulting fluorescence responses will also differ.

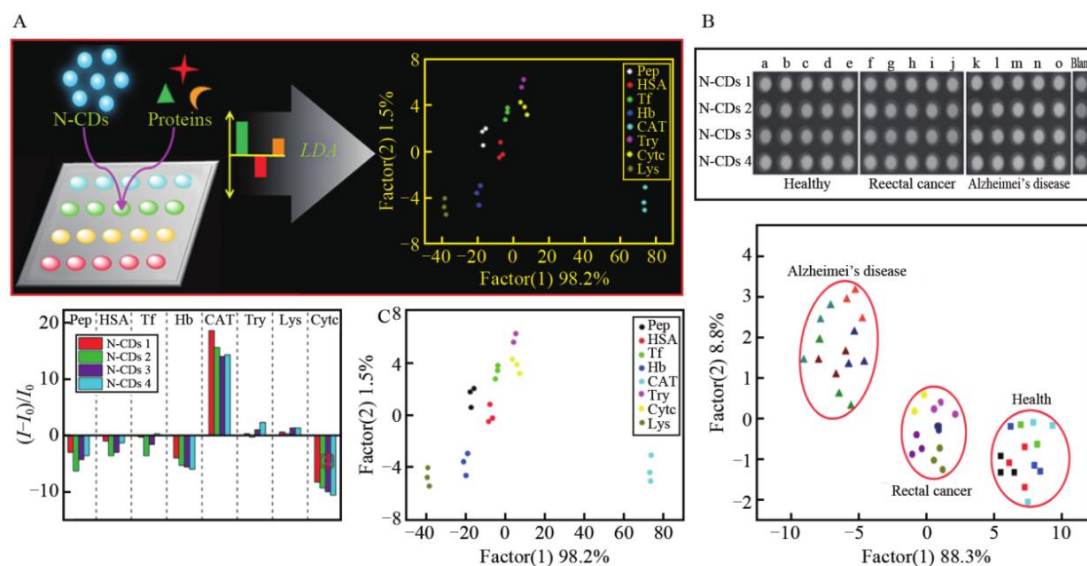


Figure 2.37: Schematic Diagram of the CQDs based on the Sensor Array for Protein Identification (A); Discrimination Results of the Patients and Healthy Individuals Obtained by the CQDs based on the Sensor Array (B) (Xu et al., 2017)

Based on these cross-response signals, eight proteins with varying isoelectric points and molecular weights can be accurately recognized. When compared to conventional protein recognition sensor arrays, array sensors offer three clear benefits: (1) Four types of CQDs can be utilized as inductive receptors without the need for additional surface modification. The process of preparing them is efficient, convenient, and requires minimal time and effort; and (2) in complex environments such as urine, eight proteins can also be effectively distinguished. (3) The serum of individuals with rectal cancer, patients with Alzheimer's disease, and healthy individuals can be accurately distinguished, revealing the broad potential of clinical auxiliary diagnosis (Figure 2.38).

2.6.2 Paper-based Sensing Analysis

Due to the advantages of simplicity, speed, and visibility to the naked eye, paper-based sensors based on CQDs have great application prospects in rapid visual detection applications in the environmental, pharmaceutical, clinical, and food fields. Zhou et al. (2016) reported a test strip for dose-sensitive visual detection of As(III) based on blue-green fluorescent CQDs and red fluorescent cadmium telluride quantum dots. Blue-green, fluorescent CQDs were used as stable internal standards, while GSH and dithiothreitol-modified cadmium telluride quantum dots were used as signal probes for the sensitive response of As(III). When As(III) was added to a mixed solution of blue-green fluorescent CQDs and red fluorescent cadmium telluride quantum dots, as the As(III) concentration increased, the fluorescence of the solution gradually changed from red to cyan, and the detection limit was 1.7 ppb ($\mu\text{g/kg}$). When a mixed solution of blue-green fluorescent CQDs and red fluorescent cadmium telluride quantum dots were printed on filter paper, it was surprising that with increasing As(III) concentration, the fluorescence of the test strip changed from pink to orange, khaki, then to light yellow, yellowish-green, and finally to cyan, which could be distinguished from As(III) at concentrations as low as 5 ppb. This study revealed a new practical application field of the test strip (Figure 2.39A). In addition, Liu et al. (2017) reported a visual test strip based on red and blue fluorescent CQDs to achieve semiquantitative visual detection of Cu^{2+} . The residual p-phenylenediamine on the surface of the red CQDs effectively binds to Cu^{2+} to produce strong absorption in the 400~600 nm range, which overlaps with the fluorescence emission range of blue fluorescent CQDs. This coordination allows the blue CQDs to adsorb to the surface of the red CQDs. Due to spectral energy transfer, the blue fluorescence is quenched, while the red fluorescence is unaffected. Based on this idea, a test strip was designed and prepared, the visual detection

of Cu^{2+} from blue to orange was achieved, and the detection limit was 25 nmol/L (Figure 2.39B).

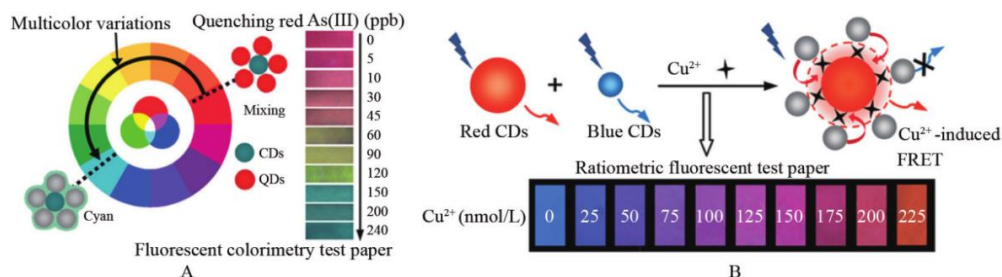


Figure 2.38: Schematic Illustration of the CQD-based Test Paper for the Detection of (A) As(III) and (B) Cu^{2+} (Zhou et al., 2016; Liu et al., 2017)

2.6.3 Biological Imaging

In contrast to conventional organic fluorescent dyes and semiconductor quantum dots, CQDs exhibit consistent optical characteristics, and superior biocompatibility, and are straightforward to synthesize and modify (Li et al., 2020). It is widely used in biosensors, biological imaging, nanodrug loading, and other fields. Notably, compared with most green or yellow fluorescent CQDs, red fluorescent CQDs sense interference in biological imaging and have unique advantages in the fields of cell and in vivo imaging (Liu et al., 2020). Recently, the development of high-performance near-infrared fluorescent CQDs as contrast agents for cell imaging and in vivo imaging has become a research hotspot. Sun et al. (2016) prepared efficient red fluorescent CQDs with a QY of 22.9% for nuclear staining and photothermal therapy. The results showed that the CQDs exhibit near-infrared fluorescence emission, low cytotoxicity, high photostability, and high photothermal conversion efficiency. More importantly, the CQDs are mainly enriched in RNA-rich nuclei in cells, and nuclear imaging can be achieved in both single-photon and two-photon modes (Figure 2.40A).

Furthermore, the CQDs also exhibit a high level of efficiency in converting light into heat (43.9%), showing a good effect on the photothermal therapy of tumours. This study provides not only a simple and convenient method for the preparation of efficient near-infrared fluorescence emission CQDs but also a new method for their application in the biomedical field. Li et al. (2019) synthesized a red fluorescent carbon quantum dot in vivo optical imaging contrast agent emitted in the near-infrared II region using watermelon juice as a raw material and applied it to imaging in mice.

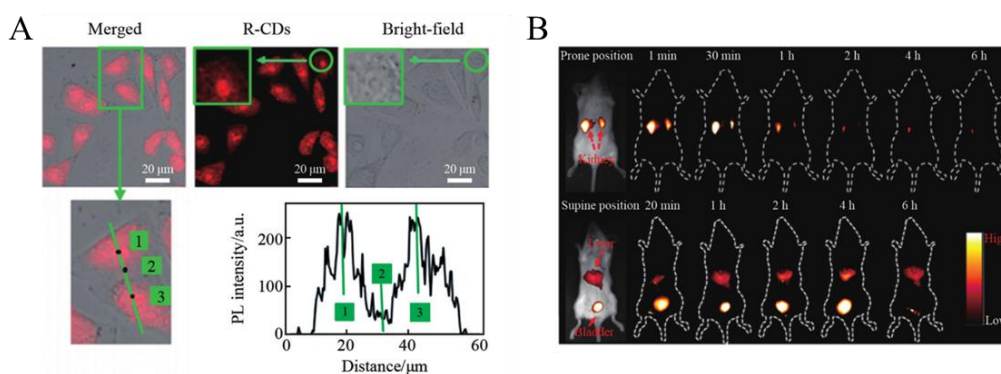


Figure 2.39: CQDs Based on Bioimaging: (A) Red Emissive CQDs as Nucleolus Imaging and Photothermal Cancer Therapy Agents (Sun et al., 2016); (B) CQDs with NIR-II Emission for In Vivo Optical Imaging and Photothermal Therapy (Li et al., 2019)

The findings indicate that when stimulated by an 808 nm laser, the CQDs emit a red fluorescence peak ranging from 900 to 1200 nm, which can be used for optical imaging of the near-infrared II region in mice (Figure 2.40B). Approximately 65% of the CQDs were found to be eliminated through mouse urine within a span of 6 hours, suggesting that the kidneys can efficiently clear the CQDs from the system. Furthermore, the CQDs exhibit a significant photothermal efficiency of 30.6% when exposed to 808 nm light, making them promising materials for cancer therapy.

2.7 Enhancing Quantum Yield of Biomass CQDs

Compared with traditional fluorescent dyes, the quantum yield of CQDs from biomass is generally less than 10%, which severely limits their application prospects in bioimaging and fluorescence sensing. It is found that heteroatom doping of CQDs not only enriches the surface fluorescence active sites of CQDs and improves their fluorescence quantum yields, but also realizes the functionalized regulation of their fluorescence properties and effectively broadens their application scope, which is the main means for the preparation of CQDs with a high fluorescence QY in biomass at present.

Nitrogen is the most common doping element for CQDs (Prakash et al., 2023). Due to the high electronegativity of nitrogen atoms, nitrogen doping not only improves the fluorescence QY of CQDs, but also realizes the modulation of the fluorescence emission peak position of CQDs (Zhang et al., 2023).

In general, the preparation of heteroatom-doped biomass CQDs can be realized in the following two ways: (1) directly selecting heteroatom-rich biomass materials as the initial carbon source; (2) additionally adding heteroatom-rich organic small molecules or polymers rich in heteroatoms.

Figure 2.37 illustrates that direct preparation of heteroatom-doped CQDs using biomass materials containing many hetero-elements reduces the number of reaction steps and is currently a popular method to enhance the QY of biomass CQDs. As Zinnia is rich in carbon, nitrogen, and oxygen, recently Huang et al. (2018) prepared N-CQDs for Fe^{3+} ion detection by microwave treatment of Zinnia. The QY of the prepared N-CQDs is as high as 27%, which is better than most biomass CQDs. In addition, nitrogen-rich Jinhua Buddha's hand (Yu et al., 2015), roseheart radish (Liu et al., 2017) and Chinese cabbage (Niu et al.,

2015) can also be prepared by hydrothermal reaction to obtain biomass N-CQDs with excellent fluorescence characteristics, with quantum yields up to 50.78%, 13.6% and 37.5%, respectively.

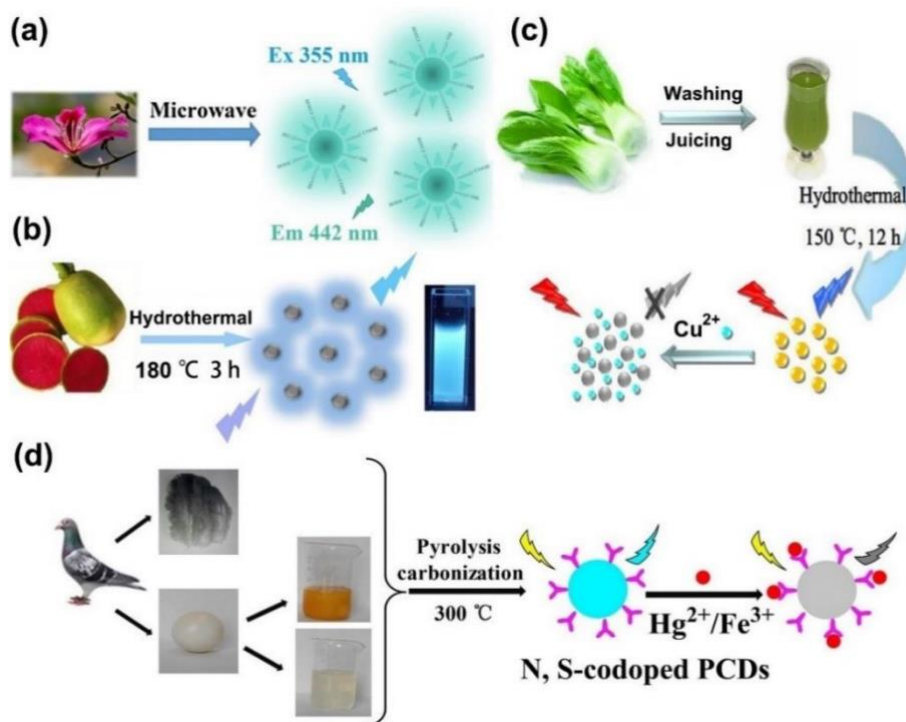


Figure 2.40: Preparation of Heteroatom Doped Carbon Dots Using (a) Zinnia, (b) Cabbage, (c) Rose Heart Radish and (d) Pigeonpea as Carbon Sources (Huang et al., 2018; Liu et al., 2017; Niu et al., 2015; Ye et al., 2017)

Doubly doped CQDs can be prepared using biomass materials enriched with a variety of heterogeneous elements. Ye et al. (2017) obtained high quantum yields of N/S double-doped CQDs from pigeon feathers, eggshells, yolks and faeces as precursors of biomass carbon source, with quantum yields of 24.87% (feathers), 17.48% (eggshells), 16.34% (yolks) and 33.50% (faeces), respectively. The prepared CQDs showed high sensitivity and selective fluorescence bursts for Hg^{2+} and Fe^{3+} , with detection limits of 10.3 nM and 60.9 nM, respectively. In addition to this, Zhao et al. (2015) have reported the preparation of N/S

double-doped CQDs by hydrothermal reaction using goose feathers and garlic, with final fluorescence quantum yields as high as 17.1% and 17.5%, respectively.

Although the direct preparation of CQDs from heterogeneous element-rich biomass materials is a relatively simple process, it can effectively improve the quantum yield of biomass CQDs and broaden their application prospects. However, this method requires a high level of biomass carbon source, and few biomass carbon sources are known to be available. By adding additional heteroatomic dopants, the limitations of the above method in the selection of carbon source can be solved, and the type and content of doping elements can be adjusted according to the demand, which is the focus of this thesis.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research Materials

In this study, willow leaves and apricot leaves were picked from the Qilu Institute of Technology campus. Quinine sulfate, Cysteine(Cys), Aspartic acid (Asp), Histidine (His), Glutamine (Gln), Lysine (Lys), Alanine (Ala), Threonine (Thr), Leucine (Leu), Glutathione (GSH), Arginine (Arg) were purchased from Shanghai Yuanye Biotechnology Co. Chloroauric acid, sodium pyrophosphate and other chemical compounds employed in this study in Table 3.1 were procured from Shanghai McLean Biochemical Technology Co. Ultrapure water (18.2 mΩ.cm) was obtained from a water purification system (Milli-Q, Merck, Germany) for use throughout the study.

Tap water samples were obtained from the faucet. After opening the tap and letting the water flow for a period of time, it was collected in a clean container. Its water quality was characterized as colourless and transparent, with no obvious odour and a pH value between 6.5 and 8.5. Used after filtration with 0.45 μm and 0.22 μm aqueous phase filter membranes respectively. Lake water samples were usually collected at various locations around the lake, using clean containers 2 m below the surface to avoid contamination. The pH was generally between neutral and weakly alkaline, and the appearance is usually clearer, light blue or light green, with no obvious pungent odour, and a faint watery or earthy taste. Used after filtration with 0.45 μm and 0.22 μm aqueous phase filter membranes respectively.

Table 3.1: The Chemical Reagents Used in the Study

No.	Chemical Reagents	Grade
1.	Pyrophosphatase	Biological Reagent
2.	Copper Chloride	Analytical Reagent
3.	Sodium Pyrophosphate	Analytical Reagent
4.	Mercuric Chloride	Analytical Reagent
5.	Glutathione	Biological reagent
6.	Chloroauric Acid	Analytical Reagent
7.	Quinine sulfate	Analytical Reagent
8.	Cysteine	Biological Reagent
9.	Aspartic acid	Biological Reagent
10.	Histidine	Biological Reagent
11.	Glutamine	Biological Reagent
12.	Lysine	Biological Reagent
13.	Alanine	Biological Reagent
14.	Threonine	Biological Reagent
15.	Leucine	Biological Reagent
16.	Arginine	Biological Reagent
17.	Potassium Bromide	Spectrographic Purity
18.	Ethanol	Analytical Reagent
19.	Trypsin	Biological Reagent
20.	Bovine Serum protein	Biological Reagent
21.	Serum immunoglobulin	Biological Reagent
22.	Streptavidin	Biological Reagent
23.	Cobalt nitrate	Analytical Reagent
24.	Ferric chloride	Analytical Reagent

Table 3.1 continued

No.	Chemical Reagents	Grade
25.	Nickel sulfate	Analytical Reagent
26.	Calcium chloride	Analytical Reagent
27.	Magnesium sulfate	Analytical Reagent
28.	Cadmium sulfate	Analytical Reagent
29.	Mercuric chloride	Analytical Reagent
30.	Lead nitrate	Analytical Reagent
31.	Silver nitrate	Analytical Reagent
32.	Potassium chloride	Analytical Reagent
33.	Sodium chloride	Analytical Reagent
34.	Sodium fluoride	Analytical Reagent
35.	Potassium iodide	Analytical Reagent
36.	Sodium sulfide	Analytical Reagent
37.	Sodium nitrite	Analytical Reagent
38.	Sodium nitrate	Analytical Reagent
39.	Sodium phosphate	Analytical Reagent
40.	Phosphate buffered solution	Analytical Reagent
41.	Sodium sulfite	Analytical Reagen
42.	Sodium carbonate	Analytical Reagen

3.2 Characterization Techniques

For this research, Transmission electron microscopy (TEM), Fourier transform infrared spectrometer (FTIR), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), Ultraviolet-visible spectroscopy (UV-vis), Fluorescence spectrophotometer, and Laser confocal Raman spectroscopy seven instruments were used to the morphology and

size, chemical functional group, elemental composition, crystal structure, optical property, and carbonation degree of CQDs, respectively.

3.2.1 Morphology and Size

The fine structure of the sample, which was less than 0.2 μm and could not be clearly observed under an optical microscope, was observed using a transmission electron microscope (TEM) (JEM-2100, JEOL, Japan) (Santwana et al., 2022). TEM is mainly used to characterize the particle size of BCQDs and gold nanoparticles. This technique was primarily employed in this study to assess the potential of BCQDs, gold nanoparticles, and fluorescent probes.

According to previous studies, TEM can be used to directly observe the morphology and structure of nanoparticles at extremely high magnification, especially for measuring nanoparticle size, as performed by Jiao et al. (2022), which is intuitive and very convenient. Appendix B shows the TEM that was used for this study. The solutions tested (CQDs, AuNCs, and AuNCs/CQDs) at a concentration of 0.2 mg/mL were prepared and sonicated for 10 min to fully disperse the sample. Ten microliters of the above sample solution were dropped onto an ultrathin copper mesh and allowed to dry naturally. The morphology and size distribution of the samples were characterized using TEM with an accelerating voltage of 200 kV.

3.2.2 Chemical Functional Groups

Infrared spectroscopy involves analysing the absorption spectrum created when molecules of a compound absorb infrared light of a wavelength and vibrate. By examining the frequency of the absorption band, researchers can deduce the presence of specific groups

or bonds in the molecule. Changes in this frequency can also indicate the presence of neighbouring groups or bonds, ultimately leading to the determination of the molecule's chemical structure through spectral analysis. Mixtures and compounds were quantitatively analysed by changes in the intensity of characteristic absorption bands. Therefore, the structural information of the BCQDs and gold nanoparticles was mainly characterized by FT-IR. The functional groups in the samples were analysed using FT-IR.

In a previous study, FT-IR was used to characterize the chemical structure of the nanoparticles, as described by Sunkar and Bhukya (2022). Appendix B shows the FT-IR spectrum (Nicolet is5, Thermo Fisher, United States) used for this study. First, aqueous solutions of CQDs, AuNCs, and AuNCs/CQDs were freeze-dried and processed to powder. Then an appropriate amount of powder was taken and mixed with KBr crystals by thorough grinding. The infrared absorption test was carried out by the KBr pressing method with a resolution of 4 cm^{-1} . The test range was $400\sim 4000\text{ cm}^{-1}$.

3.2.3 Elemental Composition

X-ray photoelectron spectroscopy (XPS) (Escalab 250, Thermo Fisher, United States) is a technique for analysing the chemical properties of the surface of substances. XPS measures the elemental composition, empirical formulas, and chemical and electronic states of elements in materials.

XPS was used to characterize the surface chemical properties of the nanoparticles as described by Sivakumari et al. (2023). Appendix B shows the XPS used for this study. The content and bonding state of each element in the samples were determined using XPS with a monochromatic Al-K α radiation source ($h\nu=1458.6\text{ eV}$). The samples were processed to a

size suitable for the sample stage. The position of the observation sample was determined by determining the height of the sample stage and the focusing height. The contents of C, O, Au, N, etc., were determined according to the test requirements.

3.2.4 Crystal Structure

X-ray diffraction (XRD) (Ultima IV, Rigaku, Japan) is a research technique utilized to gather data on the composition of a substance. By examining the diffraction pattern of the material using XRD, researchers can determine the structure or morphology of the atoms or molecules within the material. Appendix B shows the XRD patterns of the samples used in this study.

In this study, XRD was mainly used to characterize the crystal structure of the CQDs, AuNCs, and AuNCs/CQDs. After the solution was dried to form a solid on the machine, the samples were scanned and detected at 10°C/min in the 2θ range from 25 to 90°.

Based on previous studies, XRD can be used not only to characterize the crystallinity of nanoparticles but also to measure the crystallinity without destroying the sample, and the measurement results are reliable, as reported by Jain (2022).

3.2.5 Optical property

i. UV absorption spectra are molecular spectra that result from the movement of valence electrons. By utilizing UV-vis spectroscopy, the composition, content, and structure of a substance could be analyzed, determined, and inferred based on the absorption of UV-vis light by the molecules or ions present by scientists. Consequently, UV-vis analysis

was mainly used to determine the maximum excitation wavelength of fluorescent probes. The absorption curves were measured by an ultraviolet-visible absorption spectrometer.

In a previous study, UV-vis spectroscopy was used to determine the characteristic wavelengths of nanomaterials, as described by Jiao et al. (2022). A UV-vis instrument (GENESYS 50, Thermo Fisher, United States), as shown in Appendix B, was used in this study to measure the characteristic wavelength range of 190-800 nm. The biomass CQDs probe solution was taken and diluted with deionized water to the appropriate concentration and the UV-visible absorption spectra were measured. The UV-visible absorption spectrum of quinine sulfate was measured simultaneously with the quantum yield measurement and the two are compared.

ii. A fluorescence spectrophotometer was a device utilized to examine the fluorescence spectrum given off by liquid-phase fluorescent markers. A range of physical characteristics such as the excitation spectrum, emission spectrum, fluorescence intensity, quantum yield, fluorescence lifetime, and fluorescence polarization were yielded by this analysis. Insights into the bonding and structure of molecules from different perspectives were also provided. By utilizing these physical characteristics, general quantitative analysis and structural alterations of molecules in different settings could be deduced. Consequently, the connection between molecular structure and function was aided in elucidating.

In this research, excitation and emission information about fluorescent probes was mainly obtained by the instrument and it was determined whether there was an energy resonance transfer effect between biomass CQDs and gold nanoclusters. The different samples were analysed using a fluorescence spectrometer with an Xe lamp as the excitation light source. Based on a previous study, a fluorescence spectrophotometer was used to

determine the excitation wavelength, emission wavelength, and fluorescence intensity of the nanoparticles of the nanomaterials, as described by Jiao et al. (2022). The fluorescence spectrophotometer (F-2700, HITACHI, Japan) was used in this study.

The biomass CQDs probe solution was taken and diluted with deionized water to 0.1 mg/mL. The excitation slit was adjusted to a width of 3 nm, while the emission-receiving slit was set to 6 nm. Fluorescence spectra were then recorded with various excitation wavelengths ranging from 320 to 800 nm. Appendix B shows the fluorescence spectrophotometer (F-2700, HITACHI, Japan) that was used in this study.

3.2.6 Carbonation Degree

Laser confocal Raman spectroscopy can provide many important information about the structure of materials. For example, molecular structure and composition, steric regularity, crystallization and fate, molecular interactions, and the structure of surfaces and interfaces. In this research, the instrument was mainly used to examine structural changes in the transition from biomass carbon sources to CQDs. The structural disorder of a carbon material is usually characterized by the ratio of the intensity of the structurally disordered D peak to that of the graphite-crystallized G peak, R ($R=I_D/I_G$). The smaller the value of R , the more graphitized the carbon material is. For biomass CQDs, the smaller the R -value, the higher the degree of carbonization and the higher the quantum yield (Kumar et al., 2024).

Appendix B shows the Raman spectrometer (InVia Reflex, Renishaw, UK) that was used in this study to analyze the Raman spectra of CQDs in biomass from different carbon sources. 2 mg of biomass CQDs powder was taken on a clean metal sheet and uniform pressure was applied with a press to form a flat sheet. The instrument parameters were set

as follows: the laser wavelength was 633 nm, the laser power was 50 mW, the spectral resolution was $2\text{--}3\text{ cm}^{-1}$, the objective lens magnification was 50 $\times$, the grating lines were 1800 lines/mm, the integration time was 30s, the cumulative number of passes was 3, the spectral range was $200\text{--}3500\text{cm}^{-1}$, and the number of scans was 10.

3.3 Research Variables

The fluorescence properties of CQDs are primarily influenced by quantum size effects, surface defect states, bandgap transitions, and surface passivation (Kang et al., 2020; Yan et al., 2019). It has been revealed that the luminescence behaviour of CQDs is governed by the combination of two or more of the four factors rather than any one of them alone. Since alteration of the synthesis conditions is the primary means of regulating the properties of CQDs, the impact of some of the major synthesis conditions on the properties of CQDs was therefore discussed in the following.

3.3.1 The Impact of Raw Materials

The functional groups on the surface of CQDs prepared by hydrothermal method with different raw materials are different. The functional groups on the surface of CQDs greatly affect the fluorescence characteristics of CQDs (Han et al., 2024). Furthermore, the interaction force with the target is different, so the functional groups on the surface of CQDs also affect the type of target.

3.3.2 The Effect of Reaction Temperature

The synthesis of fluorescence CQDs with leaves involves a carbonization process. Since carbonization is an endothermic process (Pecchi et al., 2020), temperature is a key factor in the synthesis of CQDs. Zhu et al. (2013) suggested that the optimum synthesis

temperatures were different when different plant leaves (e.g., lotus leaves, pine needles, the oriental plane leaves and palm leaves) were used to synthesize CQDs. The synthesis temperature should not be too low or too high. A low synthesis temperature may prevent the complete carbonization of the leaves to CQDs. However, at a very high temperature, the leaves will be over-oxidized, and the surface structure of CQDs will be destroyed, thus causing deterioration in the optical performance of the CQDs (Deme et al., 2023). So in this study, the temperature range for synthesis was 170-240°C.

3.3.3 The Effect of Reaction Time

The effect of reaction time on the optical properties of CQDs is quite similar to that of reaction temperature (Nammahachak et al., 2022). A long reaction time will lead to the destruction of the surface structure of CQDs due to over-carbonization. A short reaction time will cause insufficient carbonization of the carbon source, and hence result in CQDs with weak fluorescence emission. It should be noted that the effect of reaction time on the optical properties of CQDs is temperature-dependent. The effect of reaction time is meaningful only when the reaction is carried out under a proper temperature. If the reaction temperature is not high enough, no useful end-product will be obtained even when ultra-long reaction time is adopted (Kang et al., 2020). In addition, the reaction time has a significant effect on the fluorescence QY of CQDs synthesized by hydrothermal method (Kumar et al., 2024). The QY of CQDs first increased and then decreased with the increase in reaction time. Therefore the range of reaction time in this study was 5-15 h.

3.3.4 The Effect of pH Value

The effect of solution pH on the fluorescence emission intensity of CQDs obtained by hydrothermal method using different raw materials is different (Lei et al., 2019). In this study, due to the different chemical compositions of willow leaves and apricot leaves, the CQDs contain different kinds of carboxyl groups, hydroxyl groups and other functional groups on their surfaces. Thus, it causes protonation and deprotonation under different pH conditions. Therefore, the pH range in this study was 6.2-8.6.

3.3.5 The Effect of Heteroatom Doping

The introduction of heteroatoms into the framework of carbon atoms can effectively modulate the electrical properties, and internal and surface chemistry of CQDs (John et al., 2021). Since phosphorus atoms have five electrons in their outermost orbitals and have similar electronegativity to carbon, phosphorus atoms were doped into CQDs in this study. In addition, the doping of phosphorus atoms can modulate the energy state density of CQDs, thus enhancing the fluorescence emission intensity of CQDs (Kalaiyarasan et al., 2020).

3.4 Research Process

The research methodology of this study was mainly focused on experimental work. The experimental works included both the preparation of fluorescent probes based on biomass CQDs and in vitro experiments. The experimental work for this study was implemented to accomplish both the aim and objectives of the study. The first objective was that a technique for synthesizing the CQDs from willow leaves was devised and the CQDs were used as a single fluorescent probe for the selective detection of PPase. Based on objective 1, The second objective was that P-doped biomass CQDs with high quantum yield

were synthesized from willow leaves and were used as a single fluorescent probe for the selective and sensitive detection of Hg^{2+} and GSH. The third objective was that AuNCs/CQDs were fabricated from AuNCs and low-cost biomass CQDs with higher quantum yield from apricot leaves and were used as a ratiometric fluorescent probe for the selective, sensitive, accurate, and visual detection of Cu(II). Figure 3.1 shows the research methodology flowchart. All the variables with a range of three objectives were summarized in Table 3.2.

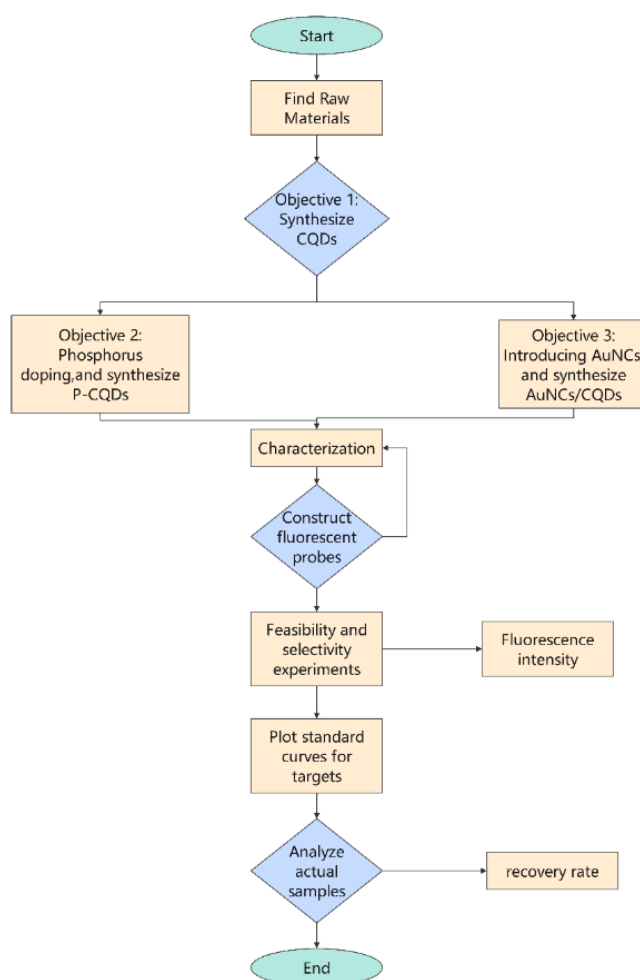


Figure 3.1: Research Methodology Flow Chart

Table 3.2: The Variables with Range of Study in Synthesis and Detection of CQDs

Objectives	Conditions	Variables	Variable Range
Objective 1	Synthesis	Reaction Temperature	180°C, 190°C, 200°C, 210°C, and 220°C
		Reaction Time	10 h, 11 h, 12 h, 13 h, and 14 h.
		Reaction pH	6.2, 6.6, 7.0, 7.4, 7.8, 8.2, and 8.6
	Detection	CQDs dosage	30 µL, 40 µL, 50 µL, 60 µL and 70 µL
		Response time	10min, 20min, 30min, 40 min, 50min, and 60min.
		PPI concentration	1.0×10 ⁻⁴ mol/L, 2.0×10 ⁻⁴ mol/L, 3×10 ⁻⁴ mol/L, 4×10 ⁻⁴ mol/L, 5×10 ⁻⁴ mol/L, and 5.5×10 ⁻⁴ mol/L
Objective 2	Synthesis	Reaction	3:1, 2.5:1, 2:1, 1.5:1, 1:1, 1:1.5, 1:2.5, 1:3
		Reaction Temperature	170°C, 180°C, 190°C, 200°C, 210°C, 220°C, 230°C, and 240°C
		Reaction Time	5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, and 12 h
	Detection	Response time	15 s, 30 s, 45 s, 60 s, 75 s, 90 s, 105 s, and 120 s
		pH	3, 4, 5, 6, 7, 8, 9, 10, and 11
Objective 2	Synthesis	Reaction Temperature	180°C, 190°C, 200°C, 210°C, 220°C, and 230°C
		Reaction Time	8 h, 9 h, 10 h, 11 h, 12 h, 13 h, 14 h, and 15 h
	Detection	Volume ratio	1:1, 1.5:1, 2:1, and 3:1
		Response time	1min, 2 min, 3 min, 4 min, 5 min, 6 min, 10 min,
		pH	6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, and 8.2

3.4.1 Detection of PPase

The methodology flow chart for objective I is shown in Figure 3.2.

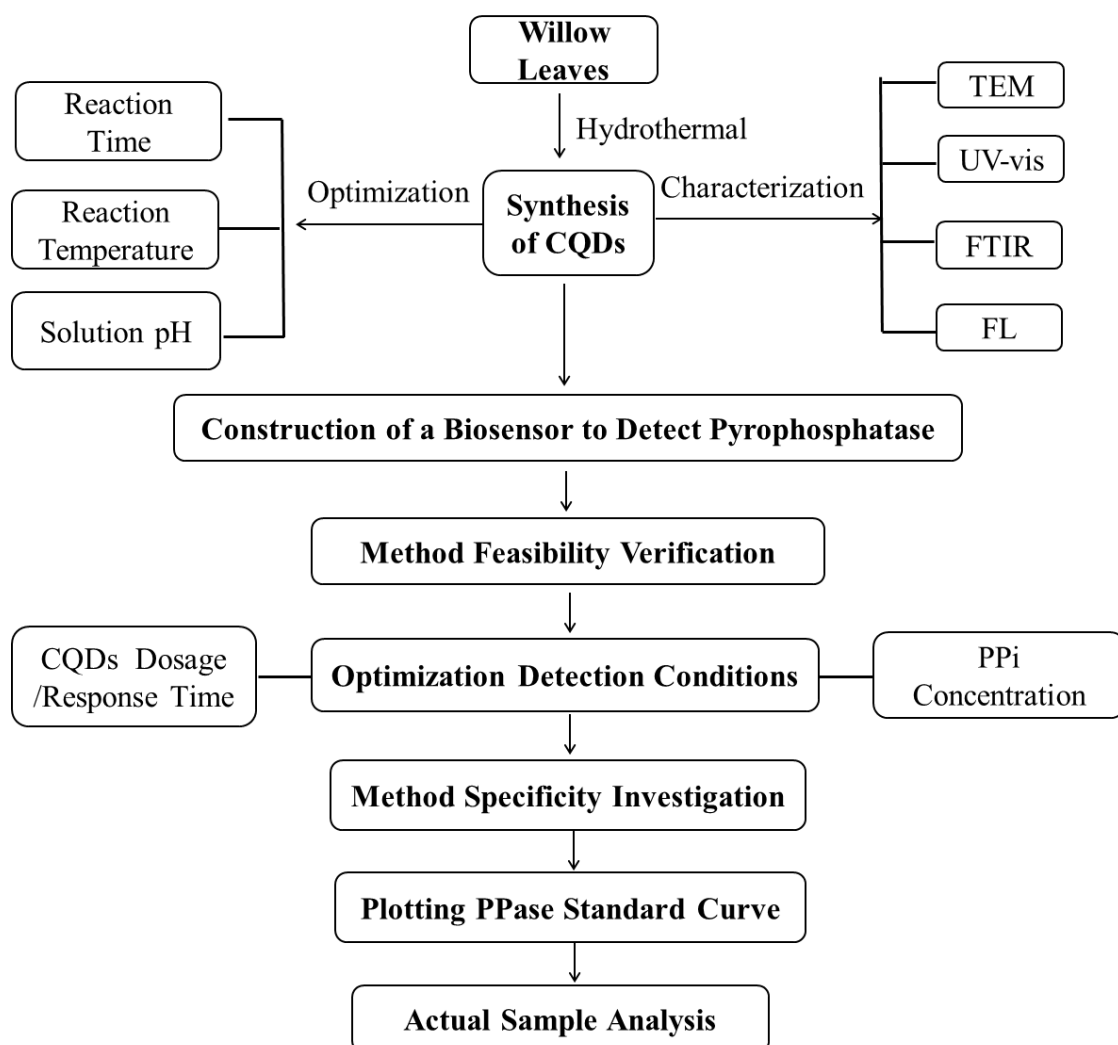


Figure 3.2: Methodology Route for Objective I

i. Synthesis of Willow Leaves-based Biomass CQDs

Willow leaves-based biomass CQDs were produced through a single-step hydrothermal synthesis using dehydrated willow leaves, and the purified product was obtained after centrifugation and dialysis (Figure 3.11).

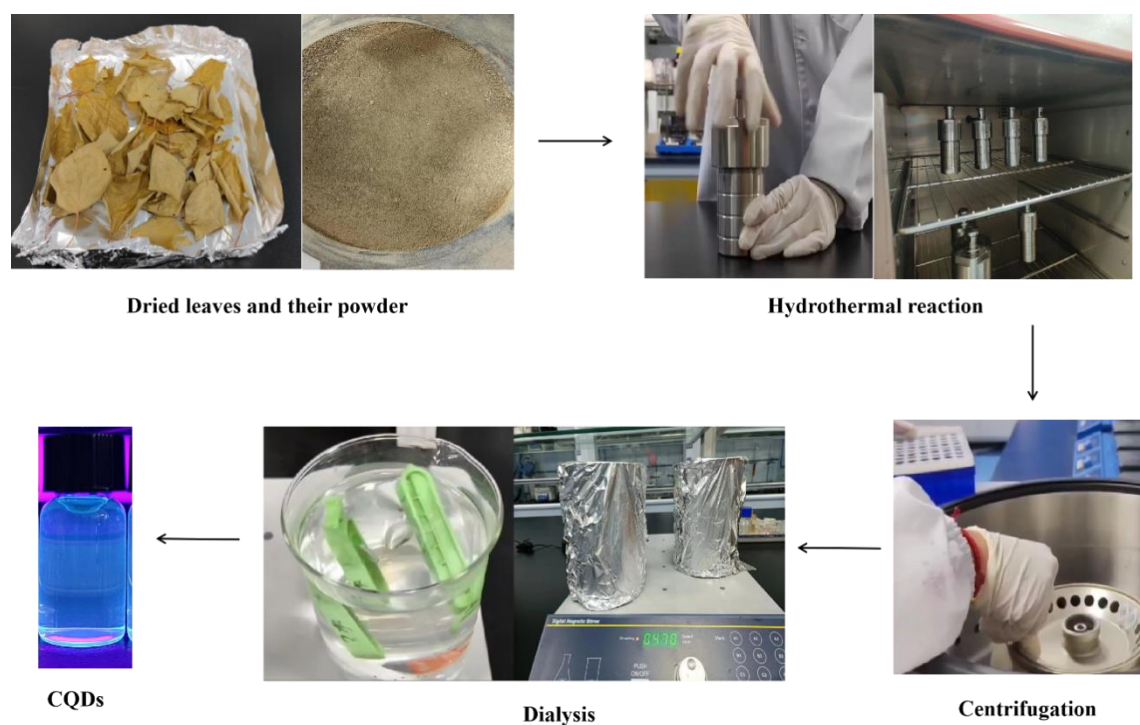


Figure 3.3: Hydrothermal Preparation of Willow Leaves-based CQDs

ii. Construction of the Sensory Method to Detect PPase

Willow leaves-based biomass CQDs were used to construct fluorescence sensors to detect PPase. As shown in Figure 3.4, step 1: when Cu^{2+} was added to the CQDs solution, the fluorescence of the CQDs was quenched, and the fluorescence signal of the system was in the "off" state. Step 2: In the presence of PPI, due to the stronger binding force between PPI and Cu^{2+} , a more stable complex was formed than the CQDs- Cu^{2+} complex. Cu^{2+} was removed from the surface of the CQDs so that the fluorescence of the CQDs could be restored, and the fluorescence signal of the system was "on". Step 3: PPase catalyzed the hydrolysis of PPI to Pi and released Cu^{2+} . The fluorescence of the CQDs was quenched again, and the fluorescence signal of the system was in an "off" state. On this basis, the detection of PPase was achieved with selectivity and high sensitivity.

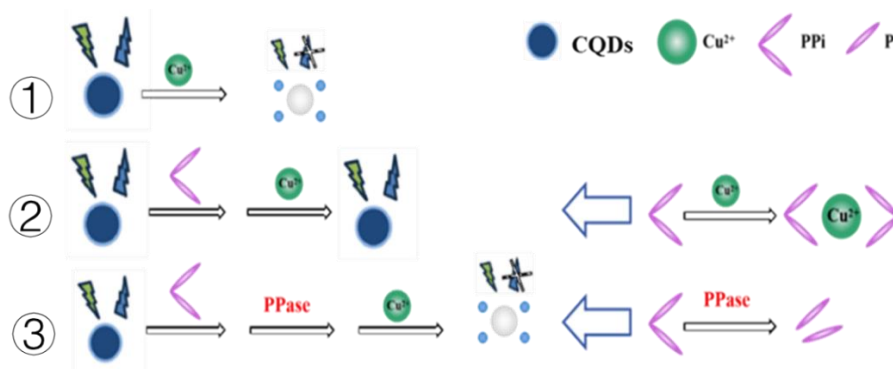


Figure 3.4: Approach for Developing a Fluorescence Detector to Identify PPase

iii. Feasibility verification

The feasibility of this design principle was analysed. First, the fluorescence intensity of the CQDs solution was measured. If the fluorescence intensity of the CQDs solution decreased with the addition of Cu^{2+} , the fluorescence intensity of the CQDs solution tended to increase in the presence of PPI and Cu^{2+} . When PPase was added to catalyze the hydrolysis of PPI, the fluorescence intensity of the system decreased again. PPase activity could be determined by the change in fluorescence intensity.

iv. Effect factors in the synthesis of Willow Leaves-based CQDs

As shown in Table 3.3, the range of the selected experimental conditions for objective 1 was based on previous studies (Thangaraj et al., 2021). It was found that being in these conditions range could induce the reaction more effectively and successfully synthesize CQDs with good fluorescence properties (Palacio-Vergara et al., 2023). After trying different temperatures, different times, different pH, and so on in the synthesis of willow leaves-based CQDs. It was found that this range of conditions could achieve a better balance in terms of reaction rate, product purity, and fluorescence intensity.

(1) Temperature: Six parts of 0.5000 g willow leaves powder were taken and placed in a 50 mL closed Teflon reactor. Subsequently, 30 mL $\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ (pH=7.4) was added, and then the samples were placed in an air-drying oven at 180°C, 190°C, 200°C, 210°C or 220°C for 12 h. Once the reaction finished, the samples were cooled down to room temperature (25°C). The synthesized CQDs solution was labelled successively and poured into a quartz cuvette to measure the fluorescence intensity of the CQDs.

(2) Time: Five parts of 0.5000 g willow leaves powder were taken and placed in a 50 mL closed Teflon reactor. Subsequently, 30 mL $\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ (pH=7.4) was added, and then the samples were placed in an air-drying oven at 200°C for 10 h, 11 h, 12 h, 13 h, and 14 h. Once the reaction finished, the samples were cooled down to 25°C. The synthesized CQDs solution was labelled successively and poured into a quartz cuvette to measure the fluorescence intensity of the CQDs.

(3) pH: Under the conditions in step (1), The temperature for the reaction was kept at 200°C, and the reaction lasted for 12 h. CQDs were synthesized by adding buffer solutions with pH values of 6.2, 6.6, 7.0, 7.4, 7.8, 8.2, and 8.6. Other operations were the same as in step (1). The synthesized CQDs solution was labelled successively and poured into a quartz cuvette to measure the fluorescence intensity of the CQDs.

v. Effect factors in the detection of PPase

As shown in Table 3.3, the range of the selected experimental conditions for detecting PPase was based on previous studies (Zhang et al., 2023). The first is to consider what factors affect PPase hydrolysis of PPi (Lomashvili et al., 2008). Secondly, in the previous experiments on the detection of PPase, it was found that CQDs dosage, response time, and PPi concentration ranges can effectively detect the target with good accuracy and

reliability(Niu et al., 2018). Finally, it was found after preliminary exploratory experiments that within these ranges the assay was able to achieve a better balance of sensitivity, selectivity and stability.

(1) CQDs dosage: 30 μL , 40 μL , 50 μL , 60 μL and 70 μL of CQDs solution were pipetted into a centrifuge tube, 500 μL of 0.2 mol/L buffer solution was added, and the solution was labelled. Then, 50 μL of 5.0×10^{-4} mol/L PPi solution and 50 μL of 5.0×10^{-5} mol/L Cu^{2+} mixture were added to each centrifuge tube, mixed well, and allowed to stand for 15 min. At last, 50 μL of PPase solution was added, and the fluorescence intensity was measured after the reaction at 25°C for 40 min.

(2) Response time: 50 μL CQDs solution was pipetted into a centrifuge tube, 500 μL 0.2 mol/L buffer solution was added, and the solution was labelled. 50 μL 5.0×10^{-4} mol/L PPi solution and 50 μL 5.0×10^{-5} mol/L Cu^{2+} mixture were added to each centrifuge tube, mixed well, and allowed to stand for 15 min. Then, 50 μL of PPase solution was added, and the fluorescence intensity was measured after the reaction at 25°C for 10min, 20min, 30min, 40min, 50min, and 60min.

(3) PPi concentration: 50 μL of PPi solution at concentrations of 1.0×10^{-4} mol/L, 2.0×10^{-4} mol/L, 3×10^{-4} mol/L, 4×10^{-4} mol/L, 5×10^{-4} mol/L, and 5.5×10^{-4} mol/L were removed by a pipette gun, and the obtained solutions were labelled. Then, 50 μL of 5.0×10^{-5} mol/L Cu^{2+} solution was added to each mixture, and the mixture was evenly mixed for 15 min. Six parts of the CQDs solution (50 μL) and buffer solution (500 μL) were removed with a pipette gun, mixed with different concentrations of PPi and Cu^{2+} and incubated for 15 min. Then, 50 μL of the PPase solution was added, and the reaction was carried out at 25°C for

40 min. The fluorescence intensity of the corresponding solution was measured by a fluorescence spectrophotometer.

vi. Quantum yield determination

The quantum yield (QY) was calculated using quinine sulfate as a reference. First, a certain concentration of quinine sulfate solution was prepared with 0.1 mol/L dilute sulfuric acid, which was continuously diluted and adjusted, the absorbance was controlled to be less than 0.05, and the absorbance value (A) of quinine sulfate was determined. The fluorescence emission profiles of the two solutions were scanned with $\lambda_{em}=330-600$ nm. and corresponding area parameters (F) were obtained by integrating them. The obtained data were substituted into equation 3.1 (Egorova et al., 2020; Gao et al., 2023a) to calculate the quantum yield.

$$QY_u = QY_s \times \frac{F_u}{F_s} \times \frac{A_s}{A_u} \quad \text{Equation 3.1}$$

(The symbols u and s represent the material being analyzed and the standard reference, respectively. QY stands for the fluorescence efficiency in %. F represents the peak area of fluorescence, unitless. A indicates the absorbance, no unit).

vii. PPase Standard Curves

The change in fluorescence intensity corresponding to a series of different concentrations of PPase was explored, and a standard curve of PPase was drawn. Three parallel assays were performed at each concentration.

Fifty microliters of CQDs solution and 500 μ L of 0.2 mol/L $\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ (pH=7.4) buffer solution were removed separately with a pipette gun and mixed well. A combination

of 50 μL of a 5.0×10^{-5} mol/L CuCl_2 solution and 50 μL of a 5.0×10^{-4} mol/L pyrophosphate solution was mixed and allowed to incubate for 15 minutes. Subsequently, 50 μL of PPase solution at varying concentrations (0.00, 0.05, 0.1, 0.25, 0.5, and 0.8 U/mL) was sequentially added. The mixture was then labelled and incubated at 25°C for 40 minutes. The fluorescence intensity of the entire system was then assessed using a fluorescence spectrophotometer, and a standard curve was generated based on the fluorescence intensity changes.

viii. Selective investigation and Application of Actual Samples

To assess the accuracy and specificity of the PPase assay, a combination of 50 μL of CQDs, 50 μL of Cu^{2+} solution (5.0×10^{-5} mol/L), 50 μL of PPI solution (5.0×10^{-4} mol/L), and 50 μL of buffer solution (0.7 U/mL) containing trypsin (Try), bovine serum protein (BSA), serum immunoglobulin (IgG), and streptavidin (SA) was prepared at pH 7.4. Following a 40-minute incubation period, the fluorescence intensity of the mixture was measured using a fluorescence spectrophotometer. Compared with the CQDs solution with PPase, the fluorescence intensity of the system was observed.

3.4.2 Detection of Hg^{2+} and GSH

The methodology flow chart for objective II is shown in Figure 3.5.

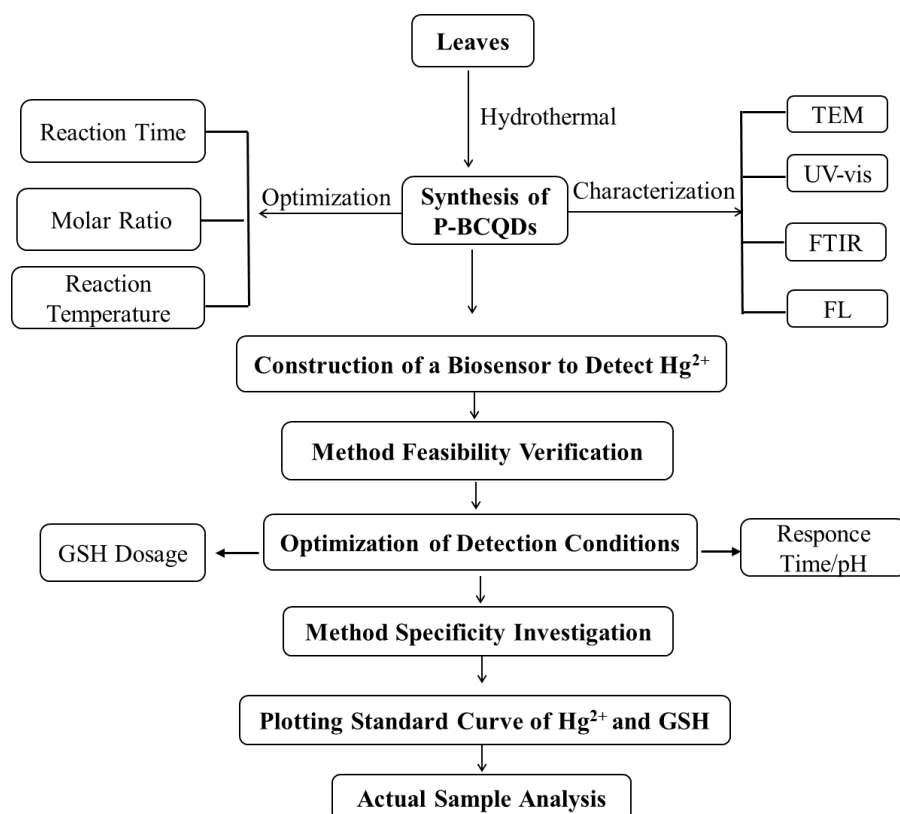


Figure 3.5: Methodology Route for Objective II

i. Synthesis of P-BCQDs

As shown in Table 3.3, the range of experimental conditions chosen for Objective 2 was based on previous studies of elemental doping of modified CQDs (Raju et al., 2020). It was found that CQDs with good fluorescence properties could be synthesized within these ranges of conditions (Kalaiyarasan et al., 2020). After trying different conditions such as different material ratios, different temperatures, and different times. It was found that these condition ranges could achieve a good balance in terms of reaction rate, quantum yield and fluorescence intensity.

P-BCQDs were synthesized using a single-step hydrothermal technique in a 30 mL polytetrafluoroethylene reactor at different dosing ratios (willow leaves powder to sodium pyrophosphate molar ratios of 3:1, 2.5:1, 2:1, 1.5:1, 1:1, 1:1.5, 1:2, 1:2.5, and 1:3), and different reaction times (5-12 h at 1 h intervals) and different temperatures (170-240°C at 10°C intervals) were used to synthesize P-BCQDs. After the reaction, the products were centrifuged at 5000 r/min and 12000 r/min. The centrifuged supernatant was filtered and dialyzed (with a molecular retention of 1000 Da) to obtain an aqueous solution of P-BCQDs.

ii. Construction of the Sensory Method to Detect Hg^{2+}

P-BCQDs with cyan fluorescence were synthesized from willow leaves and sodium pyrophosphate, and the detection principle is shown in Figure 3.6. When Hg^{2+} was added to the P-BCQDs system, a fluorescence burst occurred. When GSH continues to be added, it chelates with Hg^{2+} to form a complex, and the fluorescence of the P-BCQDs system was recovered, which enables Hg^{2+} sensing.

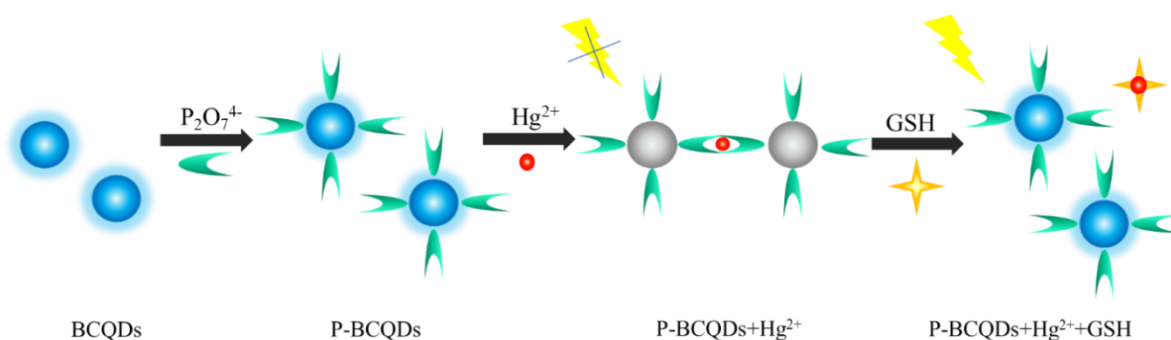


Figure 3.6: Schematic Diagram of the Hg^{2+} Fluorescent Sensor

ii. Effect factors in the detection of Hg^{2+} for P-BCQDs

The range of variables is shown in Table 3.3. Firstly, it is considered that the chemical force between the surface functional groups of CQDs and Hg^{2+} will be affected by system pH and response time (Kaur et al., 2022). Second, it was found in previous Hg^{2+} detection experiments that these response times and pH ranges can effectively detect the target with good accuracy and reliability (Özbek et al., 2023). Finally, preliminary exploratory experiments have found that in these ranges, the detection method is able to achieve a good balance between sensitivity, selectivity and stability.

500 μL P-BCQDs and 2000 μL PBS buffer (pH=7) were added to the centrifuge tube in a total volume of 2.5 mL, and the fluorescence intensity was measured after mixing well with shaking. 500 μL P-BCQDs and 1500 μL PBS buffer (pH=7) were added to the centrifuge tube and mixed homogeneously, and then 500 μL of different concentrations of Hg^{2+} solution were added respectively, and the fluorescence intensity was measured after incubation for 2 min at room temperature, and the value of the change of the fluorescence intensity was calculated (F_0/F), with F_0 denoting the initial fluorescence intensity of the P-BCQDs, and F denoting the fluorescence intensity after the addition of Hg^{2+} . F_0 represents the initial fluorescence intensity of P-BCQDs, and F represents the fluorescence intensity after the addition of Hg^{2+} .

iii. Quantum yield determination

The quantum yield (QY) was calculated using quinine sulfate as a reference, as in Objective I.

iv. Fluorescence stability experiments of P-BCQDs

Stability experiments of the P-BCQDs were carried out by preparing 0.1-1 mol/L NaCl solution and PBS buffer in the range of pH 3-11, and the fluorescence intensity was detected every 30 min by irradiating the P-BCQDs with a UV lamp. The formulated P-BCQDs were stored at 4°C and protected from light to detect the fluorescence intensity every 1 d. The data were recorded and plotted as dot plots.

v. Feasibility experiments of P-BCQDs

Three volumes of the tested solution (1000 μL of PBS buffer and 500 μL of P-BCQDs) were prepared separately. The first portion contained 1000 μL of PBS. The second portion included 500 μL of PBS and Hg^{2+} solution, and the third consisted of 500 μL of Hg^{2+} and GSH.

vi. Selectivity analysis of P-BCQDs

A variety of common ions, including K^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} , Fe^{3+} , Br^- , F^- , Cl^- , CO_3^{2-} , and SO_4^{2-} (10 mmol/L), and a Hg^{2+} concentration of 5 $\mu\text{mol/L}$, were selected for the selectivity experiments. The selectivity of P-BCQDs was analysed in the following way. Five hundred microliters of P-BCQDs and 1500 μL of PBS buffer were added to the centrifuge tube in a total volume of 2 mL, and then 500 μL of Hg^{2+} solution and other interfering substances solution were added separately to detect the fluorescence intensity of different ion solutions at excitation/emission wavelengths of 420/470 nm, respectively. Ion-selectivity experiments were also performed on unmodified CQDs to compare the specificity of P-BCQDs for Hg^{2+} detection.

vii. Hg^{2+} Standard Curves

F_0 was measured by mixing 500 μL of P-BCQDs with 2000 μL of PBS (pH=7). Then, another 500 μL of P-BCQDs and 1500 μL of PBS buffer were added to 500 μL of different concentrations of Hg^{2+} solution, and F was measured. Finally, the changes in fluorescence intensity (F_0/F) were calculated, with F_0 denoting the initial fluorescence intensity of the P-BCQDs and F denoting the fluorescence intensity after the addition of Hg^{2+} .

viii. Fluorescence recovery

The binding of CQDs to metal ions can cause a fluorescence burst, and amino acids can react with metal ions to generate complexes to restore fluorescence. Based on this theory, a variety of amino acids and peptides (Cys, Asp, His, Gln, Lys, Ala, Thr, Leu, GSH, and Arg) were selected for reaction with the P-BCQDs- Hg^{2+} system. The concentrations of the amino acids were all 10 mmol/L. Then, 1000 μL of PBS buffer, 500 μL of P-BCQDs, and 500 μL of Hg^{2+} solution (10 $\mu\text{mol/L}$) were added to the centrifuge tubes in a total volume of 2 mL, 500 μL of the amino acid solution was added to measure the fluorescence intensities and the amino acid with the suitable recovery of fluorescence was selected for the subsequent experiments.

ix. Determination of actual samples

Different concentrations of Hg^{2+} solutions (2, 6, and 10 $\mu\text{mol/L}$) were added to the actual water samples to examine the recoveries of the spiked samples and the accuracy of the method. Then, 1000 μL of PBS, 500 μL of P-BCQDs, and 500 μL of Hg^{2+} solution were mixed with 500 μL of tap water and lake water, and the fluorescence intensity after 2 min was determined.

3.4.3 Detection of Cu(II)

The methodology flow chart for objective III is shown in Figure 3.7.

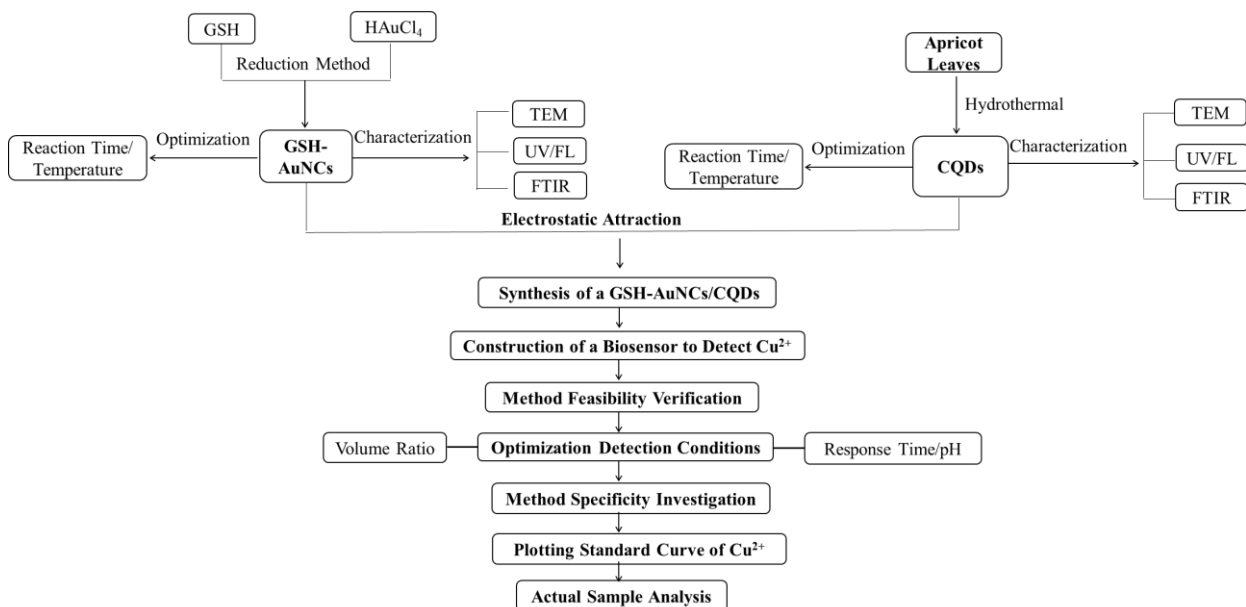


Figure 3.7: Methodology Route for Objective III

i. Synthesis of Apricot Leaves-based CQDs

Apricot leaves-based CQDs were prepared through a single-step hydrothermal synthesis technique. The details are as follows: apricot leaves were rinsed with both tap water and distilled water and dried at 110°C in an electrothermal constant-temperature blast drying oven (DHG-9146A, JING HONG, China). The apricot leaves were subsequently ground into a powder by using a mortar. A total of 0.5000 g of the apricot leaf powder was put into a 50 mL hydrothermal autoclave reactor (KHP50, LI CHEN, China) along with 30 mL of ultrapure water. The reactor was subsequently heated in an oven for 12 h at 200°C. The resulting CQDs solution was subsequently transferred to a high-speed

centrifuge (ROTINA 420, Hettich, Germany) and centrifuged at 12000 rpm/min for 20 min. To further purify the CQDs, the supernatant was filtered through 0.22 μm membranes to eliminate the filter residue, after which the clarified solution was obtained. The mixture was subsequently subjected to dialysis (MW=1000 Da) for 48 h. The purified CQDs solution was subsequently put into a sample bottle and diluted 3 times with phosphate-buffered saline (PBS) (10 mmol/L, pH=7.4) for further experimentation. Subsequently, the synthesized CQDs were stored in a refrigerator at 4°C, after which the material was ready for use.

ii. Effect factors in the synthesis of Apricot leaves-based CQDs

As shown in Table 3.3, the range of selected experimental conditions for Objective 3 was referenced to the willow-based CQDs. After trying different temperatures and different times, it was found that the series of conditions were better balanced in terms of reaction rate and fluorescence intensity.

(1) CQDs reaction temperature: 0.5000 g of apricot leaves powder and 30 mL of ultrapure water were placed inside a hydrothermal autoclave reactor with a capacity of 50 mL and heated in an oven. This procedure was repeated to prepare six identical solutions. The reactions were conducted for 12 h at temperature intervals of 10°C, ranging from 180°C to 230°C. After centrifugation, filtration, and dialysis, the obtained samples were tested for fluorescence intensity using a fluorescence spectrophotometer.

(2) CQDs reaction time: 0.5000 g of apricot leaves powder and 30 mL of ultrapure water were added to the reactor and allowed to react in an oven for eight different durations ranging from 8 h to 10 h at intervals of 1 h. After the reaction, centrifugation, filtration, and dialysis were performed using a dialysis bag to obtain the CQDs. Then, the fluorescence intensity of all the samples was detected by a fluorescence spectrophotometer.

iii. Synthesis of AuNCs

After reacting for 2 min, 10 mL of a 6.0 mmol/L GSH solution and 10 mL of a 4.0 mmol/L chloroauric acid solution were mixed with vigorous stirring at 25°C. The solution changed colour, transitioning from a cloudy, milky white to a transparent, colourless state. Subsequently, the solution was shielded from light using aluminium foil before being stirred in an oil bath at 70°C for 24 h. Then, the obtained liquid was dialyzed (MW=500-1000 Da) for 14 h to obtain the AuNCs solution. Finally, the AuNCs were placed in a 4°C refrigerator for storage, after which the material was ready for further use.

iv. Effect factors in the synthesis of AuNCs

It is known from the literature that reaction time and reaction temperature are crucial in the synthesis of AuNCs (Goswami et al., 2016). Too short reaction time will result in incomplete growth of AuNCs, low yield and small size, and structural instability, while the appropriate reaction time can ensure the full growth of AuNCs and improve the product homogeneity (Nasaruddin et al., 2018). Increasing the reaction temperature can accelerate the reaction rate, but too fast will lead to uncontrolled cluster growth. Different temperatures will also affect the stability and structure of the clusters, as lower temperatures are favourable for the formation of stable structures but slow reactions and higher temperatures may cause structural changes or even decomposition (Sokołowska et al., 2019). In conclusion, the reaction time and temperature need to be carefully considered to obtain gold nanoclusters with uniform size, stable structure and excellent fluorescence properties. In addition, I conducted preliminary exploratory experiments. After trying different temperatures and different time conditions, it was found that this series of conditions could achieve a better balance in terms of reaction rate, product size and fluorescence intensity.

(1) Reaction temperature of the AuNCs: Six round-bottomed flasks containing the same content of GSH and chloroauric acid solution were put into oil baths at different temperatures, such as 60°C, 65°C, 70°C, 75°C, 80°C and 85°C, for the reaction. After dialysis, the fluorescence intensity of the obtained samples was measured.

(2) Reaction time of the AuNCs: A round-bottomed flask containing 30 mL of GSH and chloroauric acid solution was placed in an oil bath at the suitable reaction temperature for eight different times ranging from 20 h to 27 h at intervals of 1 h. After dialysis, the fluorescence intensity of the obtained samples was measured.

v. Construction and effect factors in the detection of the AuNCs/CQDs

The AuNCs and CQDs were mixed at volume ratios of 1:1, 1:1.5, 1:2, and 1:3. The ratios that caused significant changes in the colour gradient upon reaction with Cu(II) were selected. In addition, pH is an important parameter during an experiment, directly impacting the precision and reliability of the experimental findings. The acidity of the probes was adjusted with phosphate buffer solutions at pH 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8 and 8.0. The mixtures were gently shaken to achieve good mixing. A change in the level of fluorescence intensity was observed and subsequently compared. The pH at which the change was most pronounced was selected. To determine the suitable reaction time of the probe, 60 $\mu\text{mol/L}$ Cu(II) was added at the best probe ratio. Subsequently, the change in the fluorescence intensity ratio was promptly measured and recorded over 30 min.

The AuNCs and CQDs synthesized under suitable conditions were mixed at 25°C at a volume ratio of 1:2 for 4 min at pH=7.4. The obtained AuNCs/CQDs solution was stored at 4°C for further experiments.

The electrostatic attraction between gold nanoclusters and CQDs is used to construct a ratiometric fluorescent sensor to detect the content of Cu(II). CQDs were used as reference probes, and GSH-AuNCs were used as response probes. After the addition of Cu(II), the GSH on the surface of the AuNCs and Cu(II) coordinated to cause fluorescence quenching of the AuNCs and the CQDs could be used as reference probes. The addition of Cu(II) did not affect the fluorescence intensity of the CQDs in the reaction system. Finally, the increase in the fluorescence intensity ratio between the AuNCs and CQDs was used to detect Cu(II) (Figure 3.8).

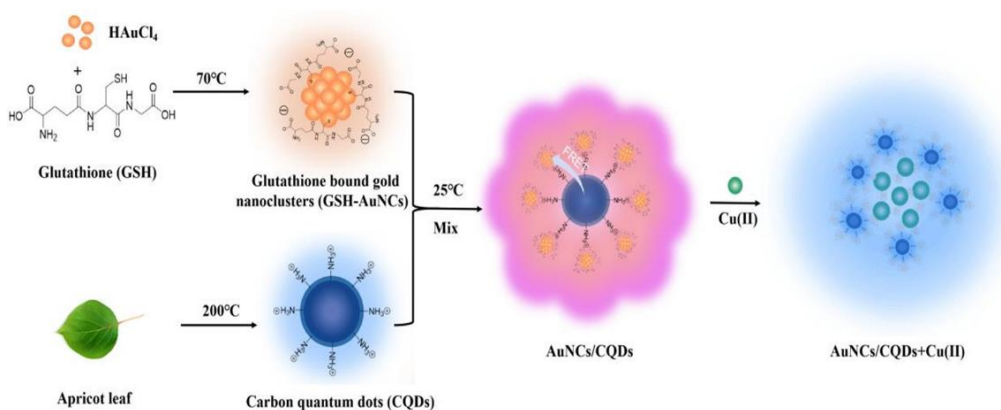


Figure 3.8: Construction of a Ratiometric Fluorescent Sensor to Detect Cu(II)

v. Quantum yield determination

The QY was calculated using quinine sulfate as a reference, as in Objective I.

vi. Feasibility experiment of AuNCs/CQDs

First, two 900 μ L aliquots of the AuNCs/CQDs ratiometric fluorescent probes were placed in centrifuge tubes. Second, 300 μ L of 60 mol/L Cu(II) was added to one of the tubes, and 800 μ L of PBS was added to bring the total volume to 2 mL. Only PBS was added to

the other tube to maintain a consistent volume. Subsequently, fluorescence intensity-wavelength curves were plotted to observe whether the fluorescence intensity ratios changed.

vii. Stability experiment of AuNCs/CQDs

Ten 900 μL aliquots of AuNCs/CQDs were injected into centrifuge tubes and subjected to daylight irradiation for 0-60 h. Afterwards, the fluorescence intensity ratio (I_{450}/I_{568}) was determined.

viii. Selectivity test of AuNCs/CQDs

Briefly, 800 μL of PBS along with 300 μL of 120 $\mu\text{mol/L}$ transition and heavy metal ions (Co^{2+} , Fe^{3+} , Ni^{2+} , Ca^{2+} , Mg^{2+} , Cd^{2+} , Hg^{2+} , Pd^{2+} , Ag^{+} , and K^{+}), as well as some anions (F^{-} , Cl^{-} , Br^{-} , I^{-} , S^{2-} , NO_2^{-} , NO_3^{-} , PO_4^{3-} , SO_3^{2-} , and SO_4^{2-}), were added to 900 μL of the AuNCs/CQDs probes. The fluorescence intensity ratio (I_{450}/I_{568}) was subsequently compared with that of the blank probes.

ix. Plotting of the Cu(II) standard curve

Copper chloride (0.0172 g) was dissolved in a beaker and diluted to 250 mL to obtain a 400 $\mu\text{mol/L}$ Cu(II) solution. The above solution was diluted to obtain different concentrations of the Cu(II) standard solution.

A total of 800 μL of PBS together with 300 μL of a Cu(II) standard solution at different concentrations (0-120 $\mu\text{mol/L}$) was added to 900 μL of the AuNCs/CQDs probe. After 4 min, the change in fluorescence colour upon exposure to UV light was detected. Then, the change in I_{450}/I_{568} was observed by a fluorescence spectrophotometer.

x. Determination of actual samples and spiking recovery experiments

800 μL of PBS and 300 μL of actual water samples were added to 900 μL of AuNCs/CQDs probes. Then, the mixture was mixed and reacted for 4 min. To confirm the method's accuracy and precision, recovery tests were conducted by supplementing an additional 300 μL of Cu(II) standard solution at concentrations of 10, 50, and 90 $\mu\text{mol/L}$ to the previously mentioned solutions. Finally, the recoveries were determined by measuring the I_{450}/I_{568} ratios of the mixed solutions after 4 min.

CHAPTER 4

RESULTS, ANALYSIS AND DISCUSSION

4.1 Willow Leaves-based CQDs as Fluorescent Probes for Highly Sensitive Detection of PPase

In this research, fluorescent CQDs were created via a single hydrothermal method using willow leaves as the carbon source. By utilizing the ability of Cu^{2+} to form complexes with both CQDs and PPI, a fluorescent probe that can switch on and off was effectively developed. This probe demonstrated high selectivity in detecting the presence of PPase.

4.1.1 Effect of Synthesis Conditions on Willow Leaves-based CQDs

The temperature of the reaction is an important factor that affects the production of CQDs (Yao et al., 2022), and the fluorescence performance of CQDs was measured by comparing the fluorescence intensity at different reaction temperatures while other experimental conditions were fixed. As shown in Figure 4.3 (a), the fluorescence intensity first increased and then decreased with increasing temperature. The highest fluorescence intensity was observed at 200°C. The pyrolysis and carbonization process of the carbon source may reach a more desirable state at a temperature of 200°C. At this time, the organic molecules in the willow leaves can be fully decomposed and reorganized to form CQDs with smaller and more uniform sizes, thus enhancing the fluorescence intensity (Katakami et al., 2023). When the reaction temperature was low, the hydrothermal reaction was incomplete, and fewer CQDs were extracted from the willow leaves. With increasing temperature, the hydrothermal reaction was complete, and the number of CQDs produced increased. However, the spacing between the CQDs decreased to a certain degree, increasing the possibility of CQD agglomeration, and decreasing the fluorescence performance of the material (Gao et

al., 2023a; Zhang et al., 2019). Consequently, 200°C was determined to be the most favourable temperature for synthesizing CQDs. The width of the fluorescence curve is closely related to the fluorescence properties of CQDs (Wang et al., 2020). If the fluorescence curve is narrow, it indicates that the CQDs are more concentrated in a specific wavelength range, and may have a higher fluorescence quantum yield and better luminescence stability (Zhao et al., 2022). On the contrary, if the fluorescence curve is wide, it means that the fluorescence emission of CQDs covers a wide range of wavelengths and its monochromaticity is relatively poor. This may lead to a lower fluorescence quantum yield and the luminescence stability may also be affected to some extent (Xian et al., 2022). As shown in Figure 4.3(a), the width of the fluorescence curve was narrowest at 200°C. Consequently, 200°C was determined to be the most favourable temperature for synthesizing CQDs.

The performance of fluorescent probes is significantly affected by the pH. To investigate this effect, the FL intensity was measured before and after the introduction of PPase across a pH range of 6.2 to 8.6. Figure 4.3 (b) shows the variation in the FL intensity of the probe at different pH values, showing an increasing and then decreasing trend. This is because, at lower pH, the probe undergoes protonation, which decreases with increasing pH (Dong et al., 2019) and consequently increases the fluorescence intensity. However, as the pH continues to increase, acidic groups such as carboxyl groups on the surface of CQDs may dissociate, leading to a decrease in fluorescence intensity. It was concluded that the CQDs probe was highly variable and stable at pH 7.4. Therefore, pH 7.4 was evaluated as the suitable pH.

The reaction time is another key factor influencing the generation of CQDs, and the fluorescence performance of the CQDs was measured by comparing it under various reaction times while the other experimental conditions were fixed. Figure 4.3 (c) shows that the fluorescence intensity of the CQDs increased and then decreased over five different periods. The fluorescence colour of the CQDs was the brightest at 12 h. This is because when the reaction time was too short, the hydrothermal reaction was incomplete, and fewer CQDs were produced (Wareing et al., 2021), preventing the fluorescence properties of the CQDs from reaching the suitable level. When the hydrothermal treatment time was too long, the particle size of the CQDs gradually increased due to the increased possibility of agglomeration between them. In nanomaterials, a reduced specific surface area has been observed due to increased size, resulting in a reduction in surface energy (Gan et al., 2023); thus, the fluorescence performance of the CQDs decreases accordingly. In addition, as shown in Figure 4.3(c), at 12 h, the fluorescence curve was not only the highest fluorescence intensity but also a narrower fluorescence curve. It indicated that the CQDs are more concentrated in a specific wavelength range, and may have a higher fluorescence quantum yield and better luminescence stability (Zhao et al., 2022). Therefore, 12 h was determined to be the suitable treatment duration.

The dosage of CQDs is one of the factors affecting the sensitivity and accuracy of the assay. Figure 4.3 (d) shows that when the amount of CQDs added was 30 μL , the fluorescence intensity of the system was weak, and the fluorescence intensity was gradually enhanced with the increase of the amount of CQDs. However, an excessive amount of CQDs may result in some of the fluorescence not all being quenched by the CQDs- Cu^{2+} complex during detection, and may lead to the existence of a detection blind zone in the constructed fluorescence sensor, which does not respond to the presence of the analyte to be detected,

and the detection sensitivity is low, especially when the concentration of the analyte is low. Therefore, the addition of 50 μL of CQD solution is more appropriate.

It is imperative to note that the rapid sensing procedure heavily relies on the reaction time of the CQDs probes, which affects the accuracy of the probe assay (Yan et al., 2023). Figure 4.3 (e) illustrates that the fluorescence intensity of the CQDs exhibited a pattern of initial decline followed by stabilization after the addition of 50 μL of PPase. In 10-30 min, the FL intensity decreased significantly. At 40 min, FL intensity reached a state of equilibrium. This was because the reaction gradually reached dynamic equilibrium with a gradual increase in time, which led to the stabilization of the fluorescence intensity (Li et al., 2024). The investigation determined that the probe exhibited a suitable response time of 40 min.

The fluorescence intensity of the system gradually decreased with the increase in reaction time, indicating that PPase hydrolysis is a continuous process. The fluorescence intensity of the system tends to stabilize after 40 min, indicating that the hydrolysis of PPase is nearly complete. Therefore, the suitable reaction time is 40 min.

PPi is the crucial substrate in the fluorescence sensor constructed in this study, so the concentration of PPi needs to be optimized for the best performance. As can be seen in Figure 4.3 (f), the fluorescence intensity gradually increased with the increase of PPi concentration, which was attributed to the fact that Cu^{2+} coordinated with PPi and released more CQDs (Gao et al., 2023b). The fluorescence intensity was also stabilized when the PPi concentration was $5.0 \times 10^{-4} \text{ mol/L}$. Thereafter, the fluorescence did not increase to a greater extent when the PPi concentration was increased, indicating that the coordination between

PPi and Cu^{2+} had reached a stable state. Thus, the optimized concentration of PPi is 5.0×10^{-4} mol/L.

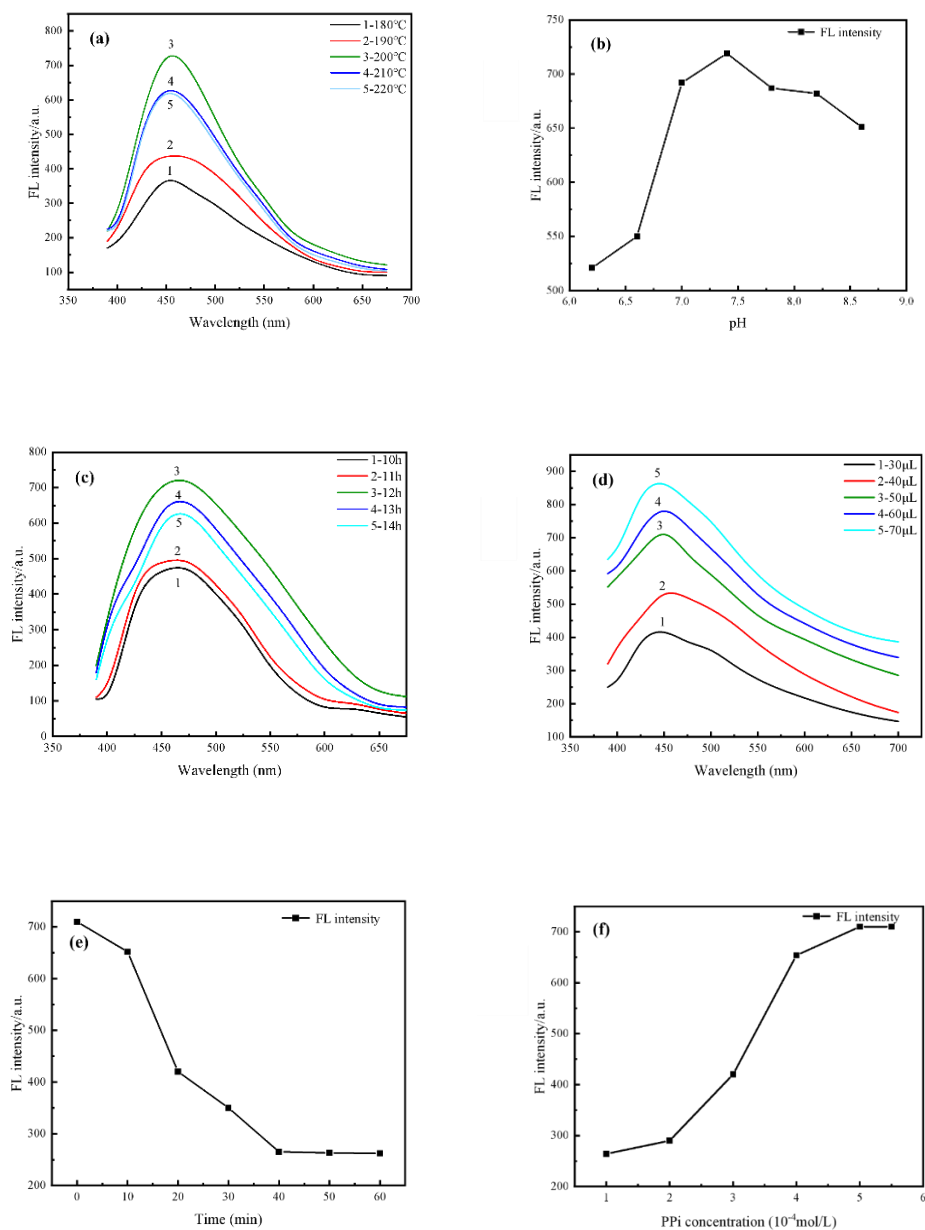


Figure 4.1: Impact of Various Factors on the CQDs, including Reaction Temperatures (a), pH (b), Reaction Times (c), CQDs Dosage (d), Response Times (e), Different Concentrations of PPi on FL Intensity (f)

4.1.2 Characterization of Willow Leaves-based CQDs

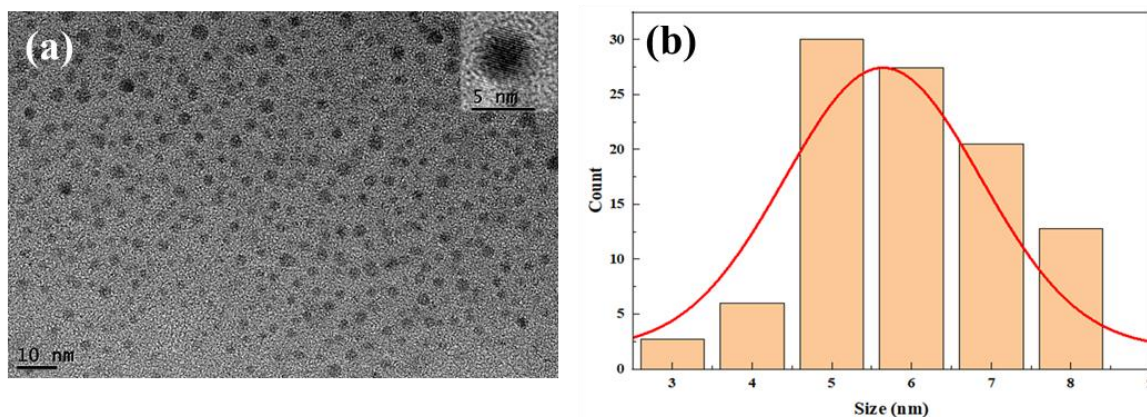


Figure 4.2: Morphological Characterization of Willow Leaves-based CQDs. a: TEM; b: Particle Size Distribution

Figure 4.2a and Figure 4.2b indicate that the synthesised willow leaves-based CQDs possess a spherical morphology and good dispersibility of approximately 5.5 ± 0.5 nm. To understand the elemental composition and distribution ratio of the fluorescent nanomaterials, XPS characterization of the willow leaves-based CQDs was performed via XPS. Figure 4.3a reveals that the main elemental components of the willow leaves-based CQDs are C, N, and O. The characteristic absorption peaks of the functional groups present in the willow leaves-based CQDs were analyzed using FTIR spectra for structural analysis and identification of the samples. Figure 4.3b shows the presence of $\nu_{\text{O-H/N-H}}$, $\nu_{\text{C=O}}$ and $\nu_{\text{C=C}}$ in the FTIR spectra of the willow leaves-based CQDs. The combined results of the XPS and FTIR spectra indicated the successful synthesis of the willow leaves-based CQDs.

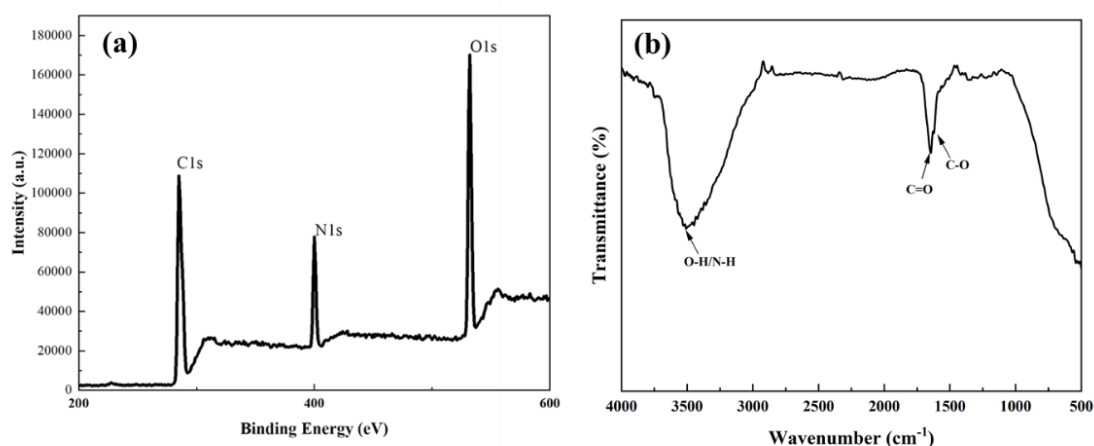


Figure 4.3: XPS Spectra (a) and FTIR Spectrum (b) of the Willow Leaves-based CQDs

4.1.3 Calculation of Fluorescence Quantum Yield

QY, also known as fluorescence efficiency, indicates the ability of a substance to emit fluorescence. According to the above quantum yield equation 3.1, the quantum yield of the reference substance quinine sulfate was 55%, and that of CQDs was 12.1% when $\lambda_{ex}=390$ nm (Table 4.1). This is more than 10% compared to the QY of the biomass CQDs in Table 2.5. It is concluded that biomass CQDs prepared from willow leaves have excellent fluorescence properties.

Table 4.1: QY of Willow Leaves-based CQDs

No.	Name	λ_{ex}/nm	λ_{em}/nm	<i>A</i>	<i>F</i>	QY/%
1.	Quinine sulfate	350	450	0.0307	204541	55
2.	Willow leaves-based CQDs	390	452	0.047	69184	12.1

4.1.4 Feasibility Study

In order to assess the feasibility of this design, an initial evaluation of the fluorescence intensity of the CQDs was conducted. The findings illustrated in Figure 4.4 demonstrate that the fluorescence intensity decreased following the introduction of Cu^{2+} , indicating an effective suppression of CQDs' fluorescence by Cu^{2+} . However, when both PPI and Cu^{2+} were present, the fluorescence intensity of CQDs increased. The subsequent introduction of PPase solution resulted in a reduction in fluorescence intensity. This decline can be attributed to the enzymatic degradation of PPI by PPase, leading to the liberation of Cu^{2+} and the subsequent formation of a complex with CQDs, causing fluorescence quenching. Based on these observations, it can be deduced that this solution exhibits practical potential.

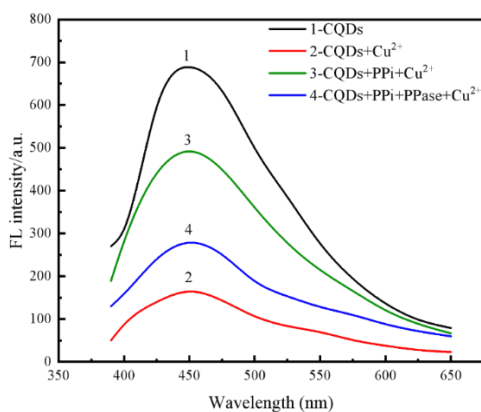


Figure 4.4: Variations in the FL of the CQDs System when Different Components are Added

4.1.5 Plotting the Standard Curve for PPase

The FL intensity of the system decreases as the concentration of PPase increases, as shown in Figure 4.5 (a). This reduction in FL intensity was found to be directly proportional to the PPase concentration ranging from 0 U/mL to 0.8 U/mL. A standard curve for PPase was created using a regression equation of $y = -656.83x + 710.43$ (Figure 4.5 (b)), where x represents the PPase concentration in U/mL and y represents the FL intensity. The linear correlation coefficient for this standard curve was calculated to be $R^2 = 0.9968$, and a limit of detection was determined at 0.1 mU/mL.

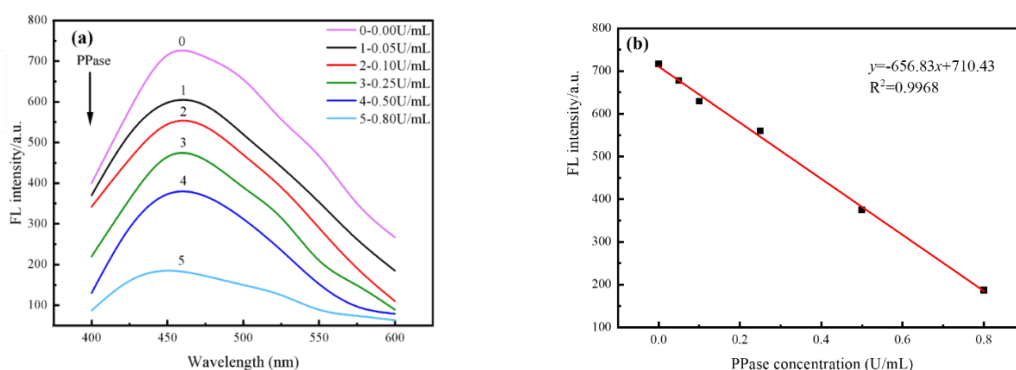


Figure 4.5: FL Spectra (a) and Standard Curve(b) of PPase

4.1.6 Specificity Test

In order to determine the accuracy of the method, different proteins such as Try, BSA, IgG, and SA were selected as control samples for evaluation. The fluorescence intensity of these proteins did not show a notable decrease, except for PPase which did significantly reduce the fluorescence intensity, as shown in Figure 4.6. This is because only PPase can effectively hydrolyze PPI compared to interfering substances, causing the CQDs in the

system to bind to Cu^{2+} and quenching the fluorescence (Zhang et al., 2023). This graph displays the changes in fluorescence intensity after each substance was introduced. The data presented is the average of three measurements. These results suggest that the assay method created in this research demonstrates a strong level of selectivity and specificity towards PPase.

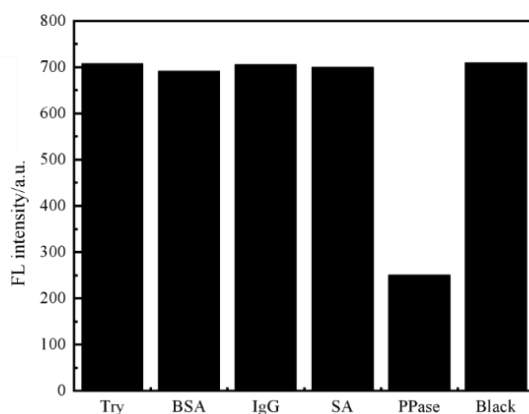


Figure 4.6: Effect of Additional Proteins on the FL Intensity of the CQDs

4.1.7 Recovery Experiment

An experiment was carried out to test a new method for measuring PPase content by recovering a blank CQD solution. The results showed that adding different concentrations of PPase standard solution affected the fluorescence intensity of the system, while the blank solution had no impact on it. The recoveries of the system ranged from 98.7% to 102.3%, as shown in Table 4.2. These findings indicate that the experiment was precise and met the required criteria for testing, making it suitable for detecting human serum samples.

Table 4.2: Recovery Experiment Results of Willow Leaves based-CQDs

No.	Added (U/mL)	Found (U/mL)	Recovery rate (%)	RSD/% (n=3)
1	0	—	—	—
2	0.15	0.148	98.67	2.01
3	0.3	0.308	102.7	1.5
4	0.6	0.595	99.17	2.3
5	0.8	0.818	102.3	3.1

4.2 Determination of Hg^{2+} using a P-doped Willow Leaves-based CQDs Fluorescent Probe

In this study, sodium pyrophosphate-modified CQDs were employed for Hg^{2+} and GSH detection. Based on objective 1, P-BCQDs emitting cyan fluorescence were synthesized by employing willow leaves and sodium pyrophosphate. The method is simple, sensitive, and has good application prospects for detecting Hg^{2+} in water solutions.

4.2.1 Analysis of the Effect of Synthesis Conditions on P-BCQDs

The molar ratio of willow leaf powder to sodium pyrophosphate affects the detection sensitivity and accuracy of ratiometric fluorescent probes. In the same system, the fluorescent probe performance was measured by comparing the fluorescence intensity at different molar ratios. Figure 4.7a shows that when the molar ratio of willow leaves powder to sodium pyrophosphate was 1:3, the colour of the probe changed more obviously under 365 nm UV light. The fluorescent probe changed from blue to cyan and then to blue, and the colour change was obvious and suitable for the detection and analysis of the target. As shown in Figure 4.7b, as the other variables remained unchanged, the fluorescence performance of the synthesized P-BCQDs gradually increased when the molar ratio of willow leaves powder

to sodium pyrophosphate was between 1:1 and 1:3, and the fluorescence performance of the P-BCQDs gradually decreased when the ratio of willow leaves powder to sodium pyrophosphate was between 1.5 and 3:1. From the point of view of reaction kinetics, when the material ratio is 1:3, it can ensure that the carbon source and phosphorus source are fully contacted in the reaction system, which will neither cause insufficient phosphorus doping due to too much carbon source and affect the performance of CQDs (John et al., 2021) nor cause the unnecessary waste or adversely affect the structure of CQDs due to too much phosphorus source (Kalaiyarasan et al., 2020). Therefore, the suitable molar ratio of willow leaf powder to sodium pyrophosphate was determined to be 1:3.

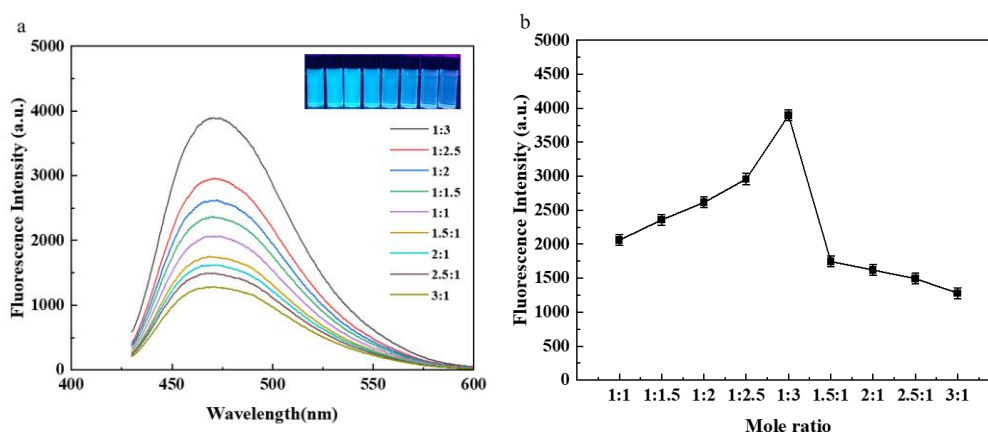


Figure 4.7: Effect of the Raw Material Molar Ratio on the Fluorescence Intensity of P-BCQDs. a: Fluorescence Spectra Plot; b: Linear Relationship Plot

The synthesis of CQDs using willow leaves as a carbon source involves a carbonization process. Since carbonization is a heat-absorbing process, the reaction temperature is a crucial factor that contributes to the fluorescence properties of P-BCQDs (Kang et al., 2020). Figures 4.8a and 4.8b indicate a trend in the fluorescence intensity of P-BCQDs, wherein it initially increases and subsequently decreases as the temperature

increases. The fluorescence intensity of the P-BCQDs synthesized at 210°C was the strongest, and the fluorescence colour in the inset is bright blue. This is because when the temperature was too low, the reaction was incomplete; as the temperature rose, the reaction was gradually completed; if the temperature was too high, the reaction was too violent, and it was difficult to control the reaction process, and may even cause side reactions or decomposition of the products (Yadav et al., 2023). Therefore, 210°C is the ideal reaction temperature.

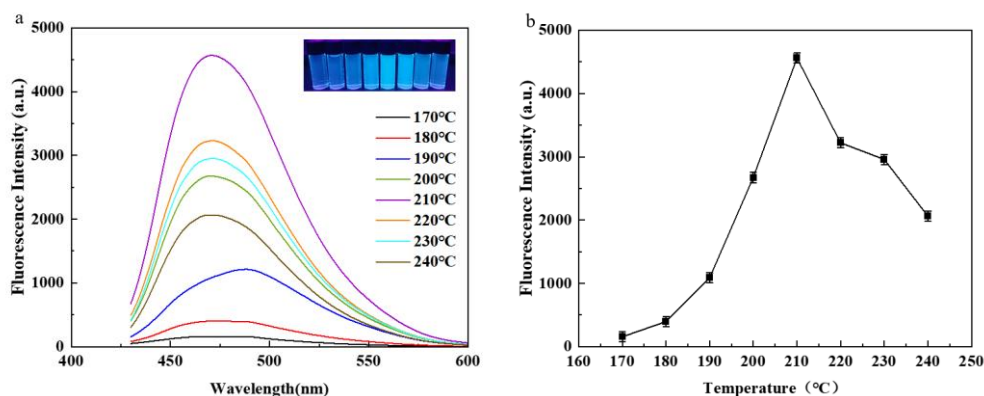


Figure 4.8: Effect of Reaction Temperature on the Fluorescence Intensity of P-BCQDs.
a: Fluorescence Spectra Plot; b: Linear Relationship Plot

The reaction time represents a critical parameter in preparing P-BCQDs, and other experimental conditions were fixed to measure the fluorescence performance of P-BCQDs by comparing the fluorescence intensities at different reaction times. Figure 4.9a shows that the fluorescence of the P-BCQDs was the brightest at 9 h. Figure 4.9b shows that the fluorescence of the P-BCQDs roughly increased and then decreased at eight different periods, and the blue fluorescence intensity was the greatest at 9 h. The fluorescence of the P-BCQDs at 9 h was the greatest. This is because too long a reaction time will lead to excessive

carbonization and destroy the surface structure of CQDs. Too short a reaction time will result in insufficient carbonization of the carbon source, leading to weak fluorescence of the CQDs (Kang et al., 2020). It should be noted that the effect of reaction time on the optical properties of CQDs is temperature-dependent (Singh et al., 2020). Accordingly, a reaction time of 9 h at 210 °C is identified to be the most favourable.

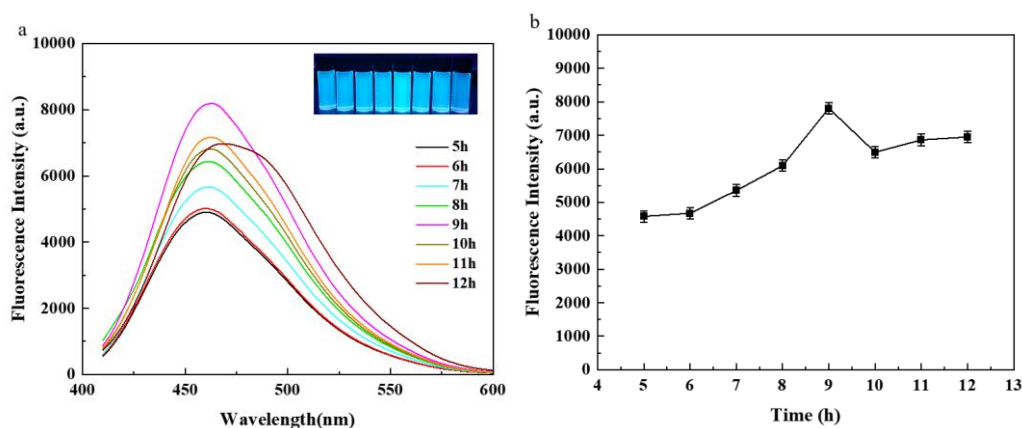


Figure 4.9: Effect of Reaction Time on the Fluorescence Intensity of P-BCQDs. a: Fluorescence Spectra Plot; b: Linear Relationship Plot

4.2.2 Morphological and Structural Analysis of P-BCQDs

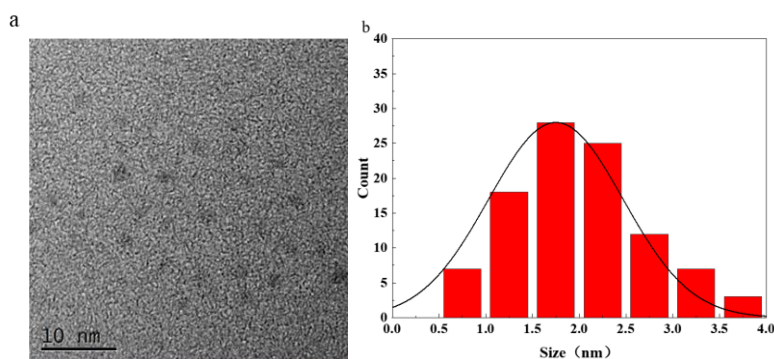


Figure 4.10: Morphological Characterization of P-BCQDs. a: TEM; b: Histogram of the Particle Size Distribution

Figure 4.10a and Figure 4.10b indicate that the fabricated P-BCQDs possess a spherical morphology and good dispersibility of approximately 2.0 ± 0.5 nm.

To understand the elemental composition and distribution ratio of the fluorescent nanomaterials, XPS characterization of the P-BCQDs was performed via XPS. Figure 4.11a illustrates that the main elemental components of the P-BCQDs are C, N, O, and P.

The characteristic absorption peaks of the functional groups present in the P-BCQDs were analysed by FT-IR spectroscopy for structural analysis and identification of the samples. The pressed samples of P-BCQDs and potassium bromide were prepared by the pressed tablet method, and the blank background was deducted from the instrument. Then, the IR spectra of the three samples were collected separately, and the baseline was automatically corrected for spectral retrieval and IR spectrogram analysis. Figure 4.12b revealed that $\nu_{\text{O-H/N-H}}$, $\nu_{\text{C=O}}$, and $\nu_{\text{C=C}}$ appeared in the FTIR spectra of both the BCQDs and P-BCQDs, and $\nu_{\text{P=O}}$ and $\nu_{\text{P-O}}$ appeared only in the spectra of the P-BCQDs. This was consistent with the FTIR of sodium pyrophosphate. As shown in Figure 4.12, an antisymmetric telescopic vibrational absorption peak for P-O bonding ($\nu_{\text{as}}(\text{P-O})$) was observed at 1213 cm^{-1} and a symmetric telescopic vibrational absorption peak for P-O ($\nu_{\text{s}}(\text{P-O})$) was observed at 998 cm^{-1} . The results of the combined XPS and FTIR spectra indicate that phosphorus-doped biomass CQDs were synthesized.

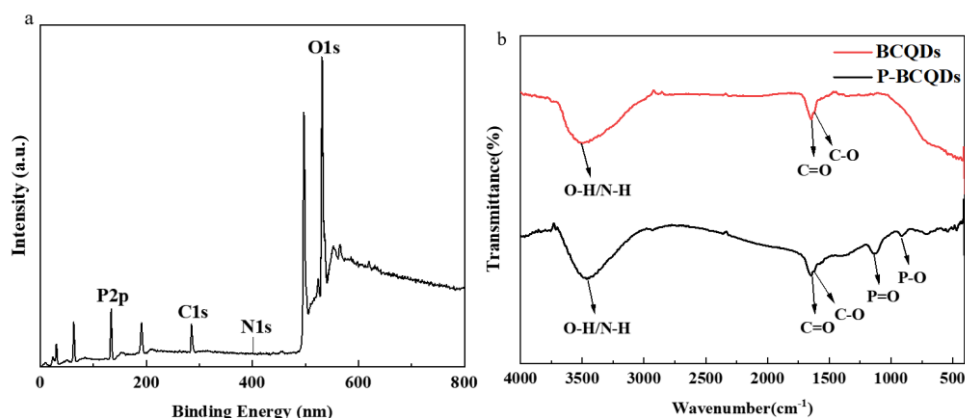


Figure 4.11: XPS Spectra (a) and FTIR Spectrum (b) of P-BCQDs

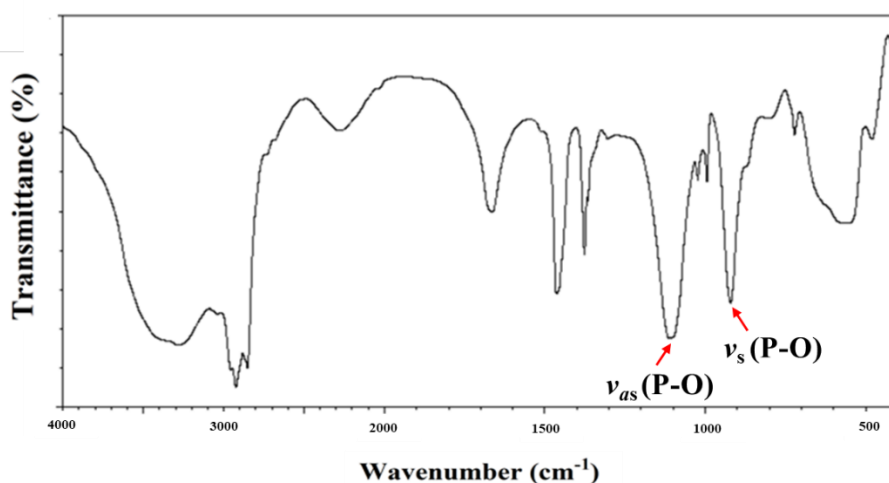


Figure 4.12: FTIR Spectrum of Sodium Pyrophosphate

4.2.3 Optical Characterization of P-BCQDs

As shown in Figure 4.13, the P-BCQDs have UV absorption peaks at 420 nm and 216 nm. The former is the $n-\pi^*$ jump generated by the nitrogen atom. The latter falls into the $\pi-\pi^*$ jump of the aromatic sp^2 structural domain, which may be due to the modifying effect of sodium pyrophosphate.

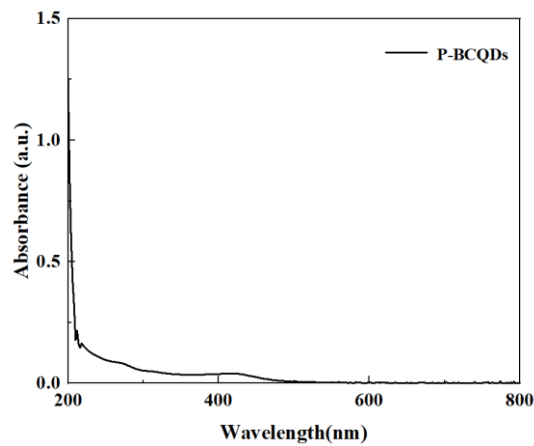


Figure 4.13: UV Absorption Spectra of P-BCQDs

To determine the excitation and emission wavelengths, a fluorescence spectrophotometer was used to characterize the P-BCQDs. The size distribution and energy band structure of CQDs can be evaluated by analysing the broadness and sharp peaks of the emission curves of CQDs at different excitation wavelengths (Takada et al., 1993). When the size of CQDs is more homogeneous, their emission spectra are usually narrower. This is because, in the smaller size range, the quantum limit field effect is more significant, and the energy level distribution of electrons and holes is relatively concentrated, resulting in the emission of photon energy being more single, thus making the emission spectrum narrower (Böddi et al., 1992). On the contrary, if the size distribution of CQDs is broader, CQDs of different sizes have different energy level structures, which will produce emitted photons of different energies at different excitation wavelengths, resulting in a broader emission spectrum. The energy band structure of CQDs determines the position of their emission sharp peak. Different excitation wavelengths can excite the electrons in CQDs to jump from different energy levels to the conduction band and then emit photons with different energies when they return to the valence band (Sun et al., 2000). When the excitation wavelength

changes, if the energy band structure of CQDs is more stable, the position of the emission sharp peak may be relatively fixed; however, if the energy band structure is greatly affected by the excitation wavelength, the position of the emission peak tip may shift. As shown in Figure 4.14a, when λ_{ex} increased from 380 nm to 460 nm, the λ_{em} of the P-BCQDs redshifted from 456 nm to 491 nm. Although the width of the emission curve did not vary much, the sharp peak of the emission was fixed at 470 nm at $\lambda_{ex}=400$ nm, $\lambda_{ex}=410$ nm, and $\lambda_{ex}=420$ nm. The maximum fluorescence intensity is considered at 420.

Hence, the performance was superior at wavelengths of 420/471 nm. Figure 4.14b shows that the synthesized P-BCQDs are transparent to natural light, and cyan fluorescence under UV light.

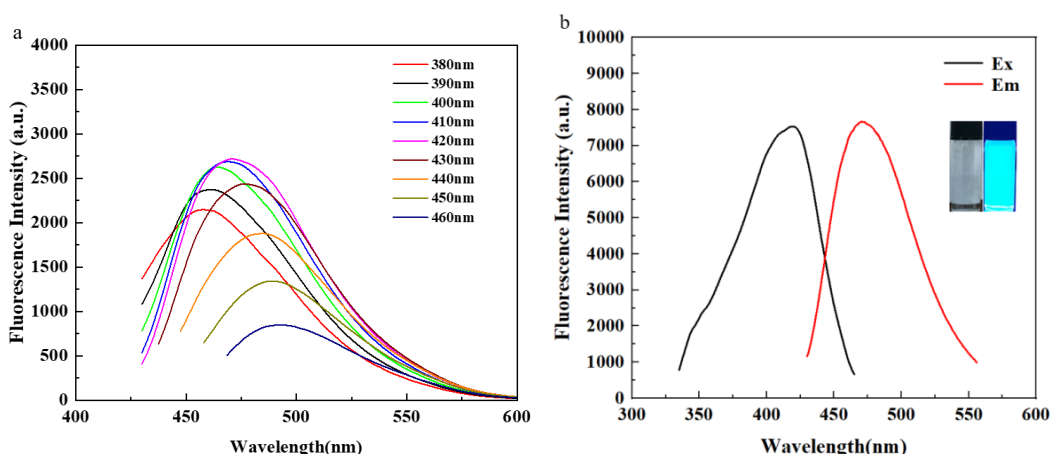


Figure 4.14: Fluorescence Spectra of P-BCQDs. (a) Emission Spectra at Different Excitation Wavelengths; (b) Excitation and Emission Spectra

4.2.4 Calculation of Fluorescence Quantum Yield

QY, also known as fluorescence efficiency, indicates the ability of a substance to emit fluorescence. According to the above quantum yield equation 3.1, the quantum yield of

the reference substance quinine sulfate was 55%, and that of P-BCQDs was 25.4% in Table 4.3. It is more than two times higher than that of BCQDs before modification. It was concluded that P-BCQDs prepared from willow leaves modified with sodium pyrophosphate have better fluorescence properties than BCQDs.

Table 4.3: QY of Willow Leaves-based P-BCQDs

No.	Name	λ_{ex}/nm	λ_{em}/nm	<i>A</i>	<i>F</i>	QY/%
1.	Quinine sulfate	350	450	0.0307	204541	55
2.	Willow leaves-based P-BCQDs	420	470	0.0420	129230	25.4

4.2.5 Stability of Fluorescence Emission from P-BCQDs

Fluorescence stability is a key element in the quantitative analysis of BCQDs. Figure 4.15a shows that the fluorescence intensity of P-BCQDs remains essentially constant as the NaCl concentration increases. This is because the P-BCQDs have a more stable structure, and the functional groups and electronic states on their surfaces are not easily affected by changes in the concentration of external ions (Hebbbar et al., 2023). Therefore, the change of NaCl concentration does not significantly affect the fluorescence of the willow leaves based-CQDs. The fluorescence intensity of the P-BCQDs was unaffected by continuous exposure to 365 nm UV light for 240 min (Figure 4.15b) and storage at 4°C for 8 d (Figure 4.15c). This is because the fluorescence emission of CQDs stems mainly from internal quantum-limited domain effects and surface states, and an increase in irradiation time does not usually have a significant effect on these factors (Ahn et al., 2024). In addition oxygen-containing functional groups on the surface of the P-BCQDs can increase the stability of the CQDs, preventing them from agglomeration or oxidation, and thus keeping their fluorescence

intensity essentially unchanged over the storage time (Arumugam et al., 2018). Figure 4.15d shows that the P-BCQDs were essentially stable under neutral alkaline conditions. This is because P-BCQD particles have numerous hydroxyl groups and carboxyl groups on their surface. Acidic environments may lead to the protonation of these functional groups, altering their interaction with the surrounding medium, which is prone to agglomeration under acidic conditions, causing the fluorescence of P-BCQDs to decrease. The above results suggest that P-BCQDs have good stability, which is favourable for their application in practical production.

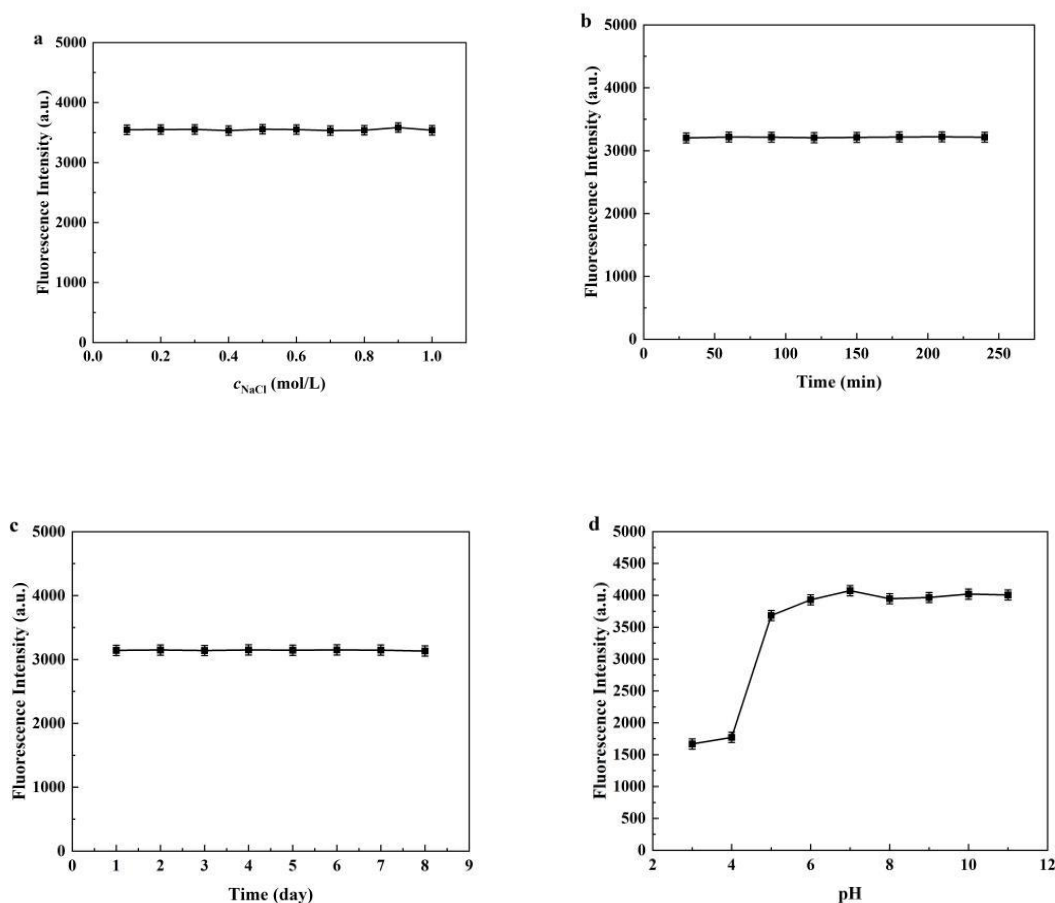


Figure 4.15: Effect of NaCl Concentration (a), UV Irradiation Time (b), Storage Time (c), and pH (d) on the Fluorescence Stability of P-BCQDs

4.2.6 Feasibility Validation

Figure 4.16 shows that the fluorescence intensity decreased after 500 μL of Hg^{2+} was added to 500 μL of P-BCQDs solution, and the fluorescence of the system was restored after the addition of GSH. This is due to the strong reducing property of GSH, and the reaction of GSH with Hg^{2+} -produced complexes, which restored the fluorescence of the P-BCQDs.

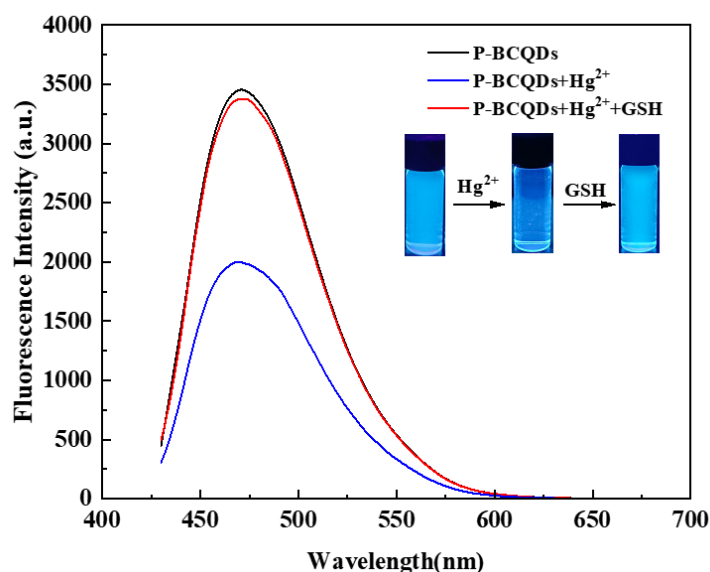


Figure 4.16: Feasibility Validation of P-BCQDs Fluorescent Probes

4.2.7 Selectivity of P-BCQDs for Hg^{2+}

To assay the response of P-BCQDs specifically to Hg^{2+} , the effects of ions such as K^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} , Fe^{3+} , Br^- , F^- , Cl^- , CO_3^{2-} , and SO_4^{2-} on the P-BCQDs were investigated using fluorescence photometry. A comparison of the specificities of the P-BCQDs and BCQDs, as shown in Figure 4.17, revealed that the P-BCQDs probe exhibited strong selectivity for Hg^{2+} . Therefore, P-BCQDs are available for Hg^{2+} detection.

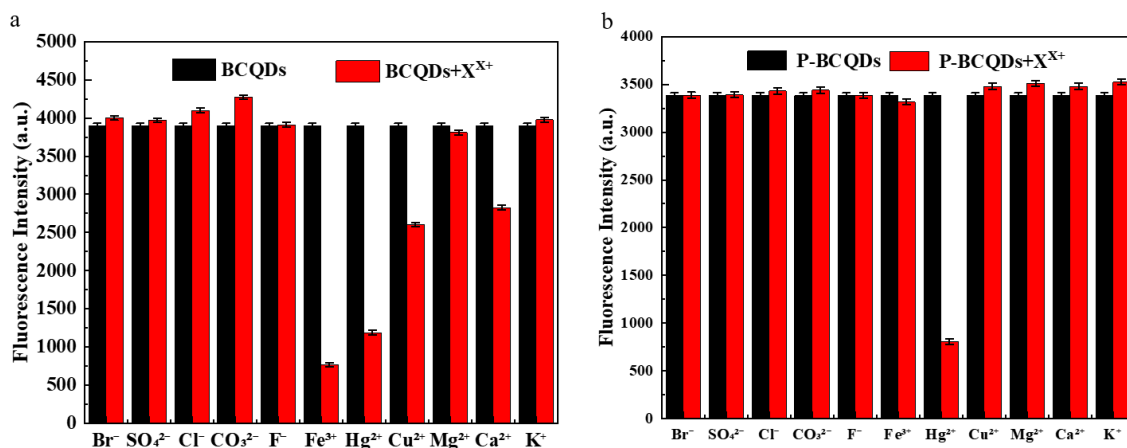


Figure 4.17: The Selectivity of BCQDs (a) and P-BCQDs (b) for Hg²⁺

4.2.8 Response of the P-BCQDs to the Hg²⁺ Concentration

i. Effect of detection conditions

pH and reaction time affect the accuracy and sensitivity of fluorescent probes. A one-way experiment was used to measure the fluorescence performance of the P-BCQDs by comparing the fluorescence intensity under different detection conditions. Figure 18a shows that the bursting efficiency was greatest in pH=7 PBS buffer. This is because changes in pH can change the chemical structure of the fluorescent probe molecule. P-BCQDs have ionizable carboxyl and amino groups on the surface. Under acidic or alkaline conditions, these groups ionized resulting in P-BCQDs not being able to coordinate with Hg²⁺ and the fluorescence of the system not bursting (Kalaiyarasan et al., 2020). In Figure 4.18b, the bursting rate was greatest under the condition of a reaction time of 105 s, which was stabilized at 2 min. This is because the reaction between P-BCQDs and Hg²⁺ usually takes a certain amount of time to reach equilibrium (Karami et al., 2020). If the reaction time is insufficient, P-BCQDs and Hg²⁺ cannot bind sufficiently, resulting in a weak fluorescent

signal and reducing the sensitivity of the fluorescent probe. On the other hand, if the reaction time is too long, side reactions or degradation of the P-BCQDs probe may occur, which may also affect the stability and accuracy of the fluorescent signal. Finally, pH=7 PBS buffer and 2 min proved to be the ideal requirements.

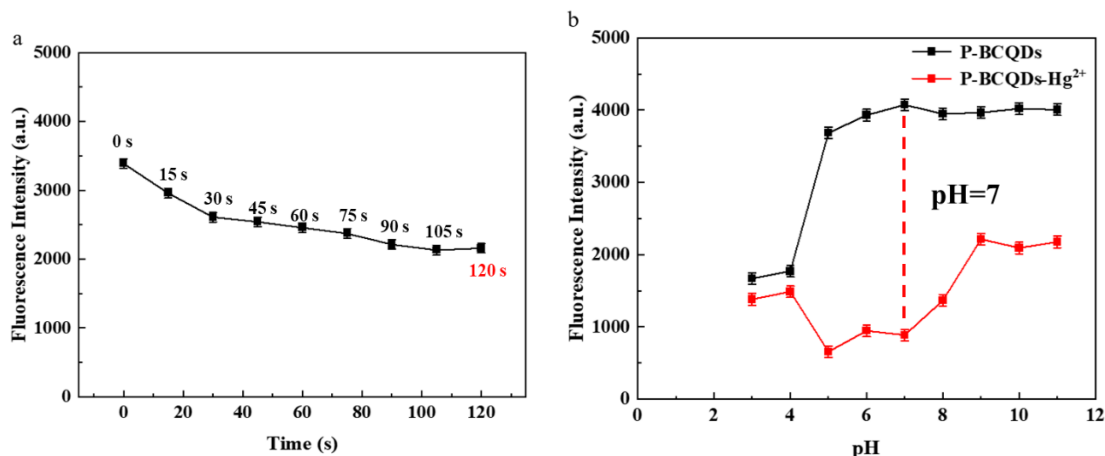


Figure 4.18: Effect of Reaction Time (a) and pH (b) on the Bursting Efficiency of the P-BCQDs-Hg²⁺ System

ii. Plotting the Hg²⁺ standard curve

Based on the principle of fluorescence bursts, to determine the linear range of Hg²⁺, the F_0/F of the P-BCQDs system with different concentrations of Hg²⁺ added was detected by using a fluorescence photometer under suitable experimental conditions. Figure 4.19b shows that the F_0/F of P-BCQDs at 471 nm decreased continuously with increasing Hg²⁺ concentration from 0 $\mu\text{mol/L}$ to 20 $\mu\text{mol/L}$. Figure 4.19a shows that the F_0/F of the P-BCQDs exhibits a strong linear correlation with $c_{\text{Hg}^{2+}}$ (0-20 $\mu\text{mol/L}$). The fitted linear equation was $F_0/F=0.2636x+1.1720$ with an R^2 of 0.9897, and the LOD for Hg²⁺ was 9 nmol/L.

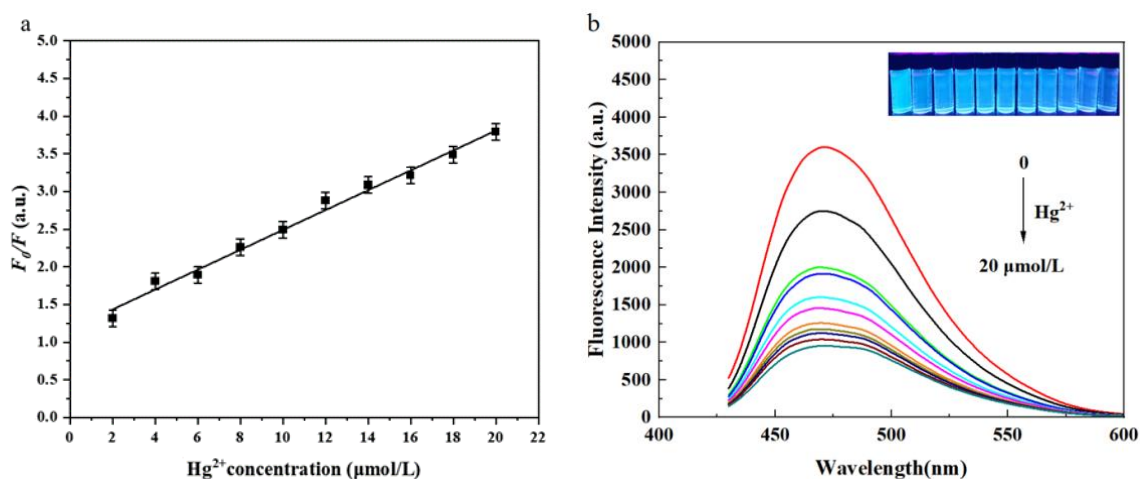


Figure 4.19: Hg^{2+} Standard Curve. a: Variation Curve of F_0/F with Hg^{2+} Concentration. b: Fluorescence Curve

4.2.9 Fluorescence Recovery

A variety of amino acids and peptides, such as Cys, Asp, Gln, Lys, Thr, Ala, Leu, Arg, His, and GSH, were selected and reacted with the P-BCQDs- Hg^{2+} system to investigate whether there was fluorescence recovery in the P-BCQDs system after the metal ions formed complexes with amino acids. Figure 4.20 shows that different amino acids can restore the fluorescence of P-BCQDs- Hg^{2+} . Among them, His and GSH had a similar fluorescence recovery effect. But, in comparison, GSH fluorescence recovery is better. The actual samples I detected were tap water and lake water, which have no amino in them, so GSH was chosen for the fluorescence recovery assay. But in the future, this product is going to be applied to biological samples for detection, I will choose some metal ions to hide His.

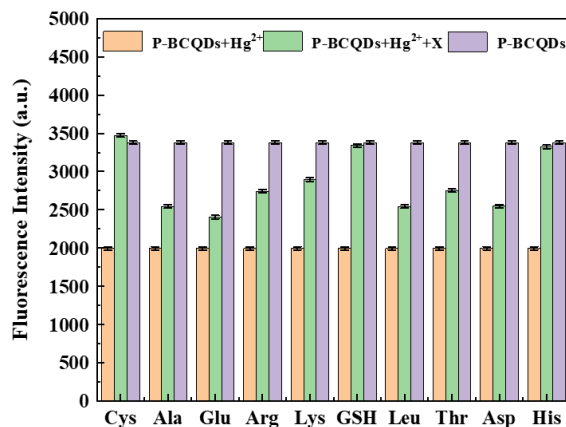


Figure 4.20: Histogram of the Fluorescence Recovery of P-BCQDs

Figure 4.21a shows that the fluorescence of the P-BCQDs-Hg²⁺ system gradually recovered as the concentration of GSH increased from 0 $\mu\text{mol/L}$ to 10 $\mu\text{mol/L}$. The fluorescence recovery of the P-BCQDs-Hg²⁺ burst system showed a good linear relationship with the GSH concentration (0-8 $\mu\text{mol/L}$). The fluorescence recovery regression equation was $F_0/F=0.0526x+1.1936$ with an R^2 of 0.9816 (F_0 indicates GSH, and F indicates no GSH), as shown in Figure 4.21b.

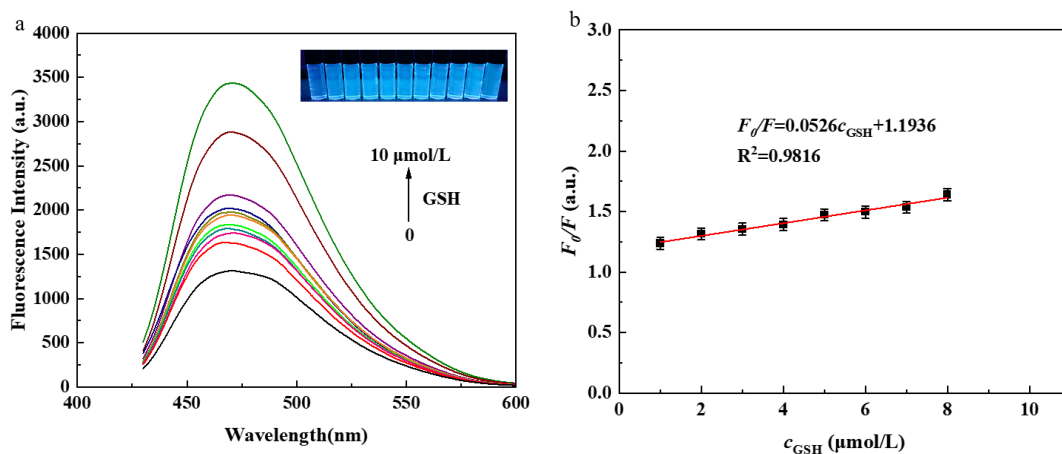


Figure 4.21: GSH Standard Curve. a: Effect of Different Concentrations of GSH on the P-BCQDs-Hg²⁺ system; b: Variation in F_0/F with the GSH Concentration

4.2.10 Detection of Practical Samples

To study the application of P-BCQDs for Hg^{2+} detection in practice, the recoveries were calculated for tap water and lake water after the addition of Hg^{2+} . Table 4.4 shows that the spiked recoveries were 95.0%-105.0%, within the specified range of 90% to 110% (Xu et al., 2012), indicating that the method has good potential for the determination of Hg^{2+} in cells. Spiked recoveries lower than or exceeding 100% may be caused by a variety of reasons, such as insufficient purity of the standard substance, matrix components in the sample affecting the analytical results, operational errors, measurement errors, and so on (Thakur et al., 2022). In the analytical process, it is necessary to strictly control the experimental conditions, standardize the operation procedures, select the appropriate analytical methods and standard substances, and measure the blank samples to ensure the accuracy and reliability of the analytical results (Cordeiro et al., 2018).

Table 4.4: Determination of Hg^{2+} in Actual Samples

Sample	Added ($\mu\text{mol/L}$)	Found ($\mu\text{mol/L}$)	Recovery rate (% , $n=3$)
Tap water	0	0.23	—
	2	2.23	100
	6	6.03	96.7
	10	10.73	105.0
Lake water	0	0.09	—
	2	1.99	95.0
	6	6.09	100

Table 4.4 continued

Sample	Added ($\mu\text{mol/L}$)	Found ($\mu\text{mol/L}$)	Recovery rate ($\%$, $n=3$)
Lake water	10	9.76	96.7

4.3 Visual Detection of Cu(II) Based on an Apricot Leaves-based CQDs Ratiometric Fluorescence Probe

First, positively charged CQDs were synthesized using natural apricot leaves, and then negatively charged AuNCs were prepared by GSH reduction of chloroauric acid (HAuCl_4). Finally, they were mixed at 25°C , and a novel ratiometric type of fluorescent probe of the AuNCs/CQDs was produced by electrostatic gravitational force interactions.

4.3.1 Effect of Synthesis Conditions on Apricot Leaves based-CQDs and AuNCs

i. Reaction temperature for Apricot Leaves based-CQDs

The reaction temperature is one of the key influencing factors for the generation of CQDs (Yao et al., 2022), and other experimental conditions were fixed to measure the fluorescence performance of CQDs by comparing the fluorescence intensity at different reaction temperatures. As reflected in Figure 4.22, the FL intensity first increased and then decreased with increasing temperature. The highest fluorescence intensity was obtained at 200°C , at which point blue fluorescence was emitted. When the reaction temperature was low, the hydrothermal reaction was incomplete, and fewer CQDs were extracted from the apricot leaves. With increasing temperature, the hydrothermal reaction was complete, and the amount of CQDs produced increased. However, the spacing between the CQDs

decreases to a certain degree, the possibility of CQDs agglomeration increases, and the fluorescence performance of the material decreases (Gao et al., 2023a; Zhang et al., 2019). Consequently, 200°C was determined to be the most favourable temperature for synthesizing apricot leaves based-CQDs.

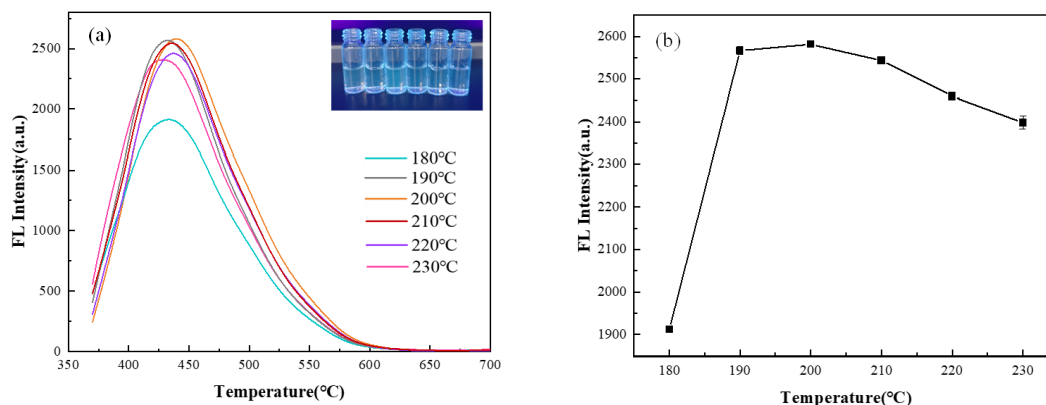


Figure 4.22: Effect of Reaction Temperature During the Preparation of CQDs: (a) Fluorescence Spectra, (b) Dot Line Plots. Reaction Conditions: Apricot Leaf 0.5000 g, 30 mL of Ultrapure Water, Time=12 h, Temperature=180, 190, 200, 210, 220, 230°C

ii. Reaction time of Apricot Leaves based-CQDs

The reaction time is one of the key influencing factors for the generation of CQDs, and other experimental conditions were fixed to measure the fluorescence performance of the CQDs by comparing them under various reaction times. Figure 4.23 shows that the intensity of the CQDs increased and then decreased in eight different periods, with the best blue fluorescence intensity occurring at a reaction time of 12 h. The fluorescence intensity of the CQDs at 12 h in the inset was the brightest, which did not interfere with the colour doping of the apricot leaf itself. This is because when the reaction time is too short, the hydrothermal reaction is incomplete, and fewer CQDs are produced (Wareing et al., 2021),

which prevents the fluorescence properties of the CQDs from reaching the suitable level. When the hydrothermal treatment time is too long, the particle size of the CQDs gradually increases due to the increased possibility of agglomeration between them. In nanomaterials, a reduced specific surface area is observed due to increased size, resulting in a reduction in surface energy (Gan et al., 2023); thus, the fluorescence performance of the CQDs decreases accordingly. Therefore, 12 h was determined to be the suitable treatment time.

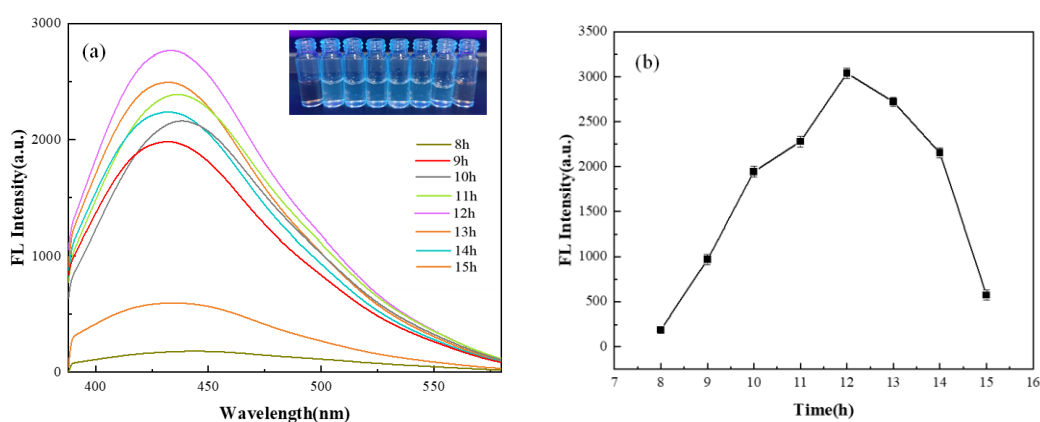


Figure 4.23: Effect of Reaction Time during the Preparation of CQDs: (a) Fluorescence Spectra, (b) Dot Line Plots. Reaction Conditions: Apricot Leaf, 0.5000 g; Ultrapure Water, 30 mL; Time=8, 9, 10, 11, 12, 13, 14, 15 h; Temperature=200°C; λ_{ex} =400 nm

iii. Reaction temperature of AuNCs

The reaction temperature is one of the key influencing factors for the preparation of AuNCs, and other experimental conditions were fixed to measure the fluorescence performance of the AuNCs by comparing the fluorescence intensity at different reaction temperatures. Figure 4.24 shows that the FL intensity of the gold nanoclusters initially increases and subsequently decreases with increasing temperature. The fluorescence intensity of the AuNCs synthesized at 70°C was the strongest, and the fluorescence colour

in the inset is bright orange. Normally, an increase in temperature favours a faster rate of reaction. Since the process of preparing nanogold with chloroauric acid is generally an exothermic reaction, the reaction temperature should not be too high. After preliminary tests, it was found that when the reaction temperature was low, HAuCl_4 was not completely reduced to Au^0 , and the unreduced Au^{3+} was dispersed in the system. With increasing temperature, the reduction reaction is complete, and the amount of produced Au^0 increases, which can form relatively stable aggregates (Guo et al., 2023). However, if the Au^0 concentration increases to a certain extent, the spacing between the Au^0 particles decreases, the possibility of AuNCs agglomeration increases, the number of particles also gradually increases (Chen et al., 2022), and the fluorescence performance of the AuNCs decreases. For that reason, the ideal reaction temperature was chosen to be 70°C .

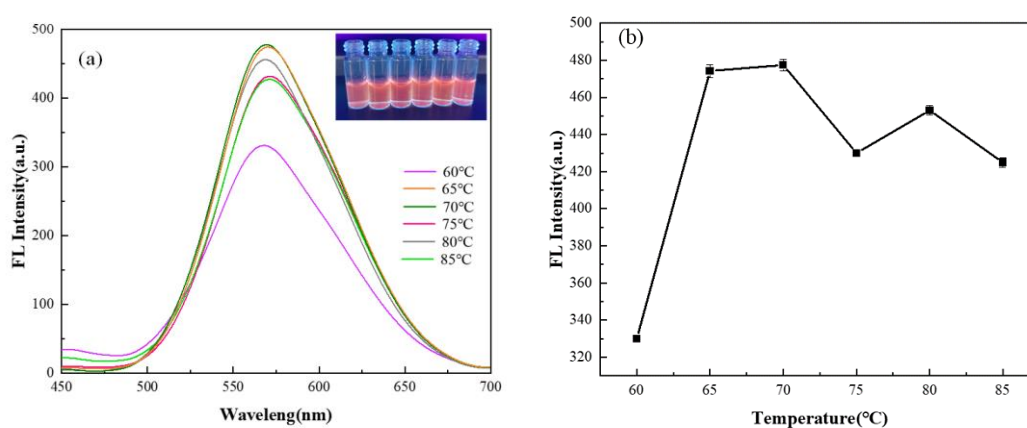


Figure 4.24: Effect of Reaction Temperature during the Preparation of AuNCs: (a) Fluorescence Spectra, (b) Dot Line Plots. Reaction Conditions: GSH, 10 mL; 6.0 mmol/L; HAuCl_4 , 10 mL; 4.0 mmol/L; Time=24 h; pH=7.4; Temperature=60, 65, 70, 75, 80, 85°C

iv. Reaction time of the AuNCs

Reaction time is one of the key influencing factors for the preparation of AuNCs, and other experimental conditions were fixed to measure the fluorescence performance of

AuNCs by comparing the fluorescence intensities at different reaction times. Figure 4.25 shows that the fluorescence intensity initially increased and subsequently decreased as time increased. The synthesized gold nanoclusters have the greatest fluorescence intensity at 24 h because when the reaction time is too short, Au^{3+} is not sufficiently reduced to Au^0 , and the remaining Au^{3+} is evenly distributed throughout the system without undergoing aggregation (Zhou et al., 2012).

Therefore, the fluorescence performance of AuNCs cannot reach a suitable level. The particle size of the AuNCs gradually increased when the hydrothermal time was too long. The larger the size of the AuNCs is, the smaller their specific surface area, and the smaller the surface energy generated (Loynachan et al., 2019); thus, the fluorescence performance of the AuNCs decreases accordingly. As a result, 24 h was identified as the suitable reaction time.

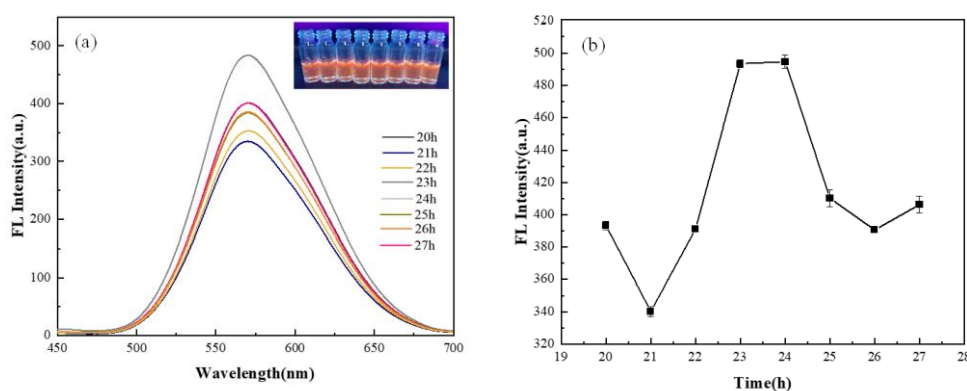


Figure 4.25: Effect of Reaction Time during the Preparation of AuNCs: (a) Fluorescence Spectra, (b) Dot Line Plots. Reaction Conditions: 10 mL of GSH, 6.0 mmol/L of HAuCl_4 , 4.0 mmol/L of HAuCl_4 , Temperature=70°C, pH=7.4, Time=20, 21, 22, 23, 24, 25, 26, 27 h

4.3.2 Construction of the AuNCs/CQDs Ratiometric Fluorescent Probes

It has been reported that the integration of gold nanoparticles with fluorescent materials, including quantum dots, fluorescent molecules, and two-dimensional materials, can induce FRET (Zheng et al., 2021; Yang et al., 2023). As displayed in Figure 4.26, in the AuNCs/CQDs ratiometric fluorescent probe, the CQDs and AuNCs act as energy sources and energy recipients, respectively. The FL intensity of the CQDs was significantly lower than that of the CQDs present alone. Therefore, the energy migrated to the energy acceptor of the AuNCs, which resulted in enhanced fluorescence emitted by the AuNCs to satisfy the experimental conditions for visualization.

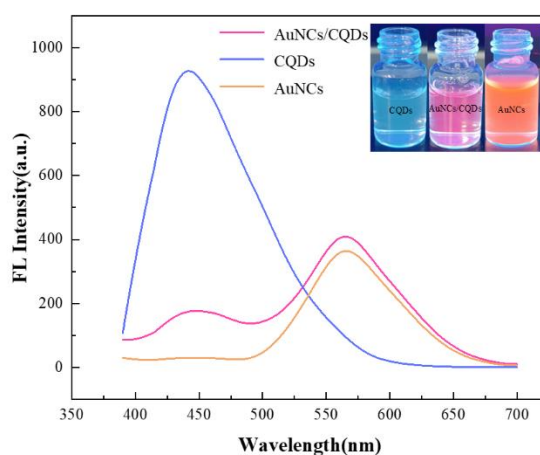


Figure 4.26: Fluorescence Spectra of the AuNCs/CQDs (pink), CQDs (blue), and AuNCs (orange). The Spectral Conditions were as Follows: $\lambda_{\text{ex}}=400$ nm, the Range of λ_{em} was 390-700 nm, the Concentration of the Sample was 0.1 mg/mL, the Width of the Excitation Slit was set at 3 nm, and the Width of the Emission Receiving Slit was 6 nm

Based on the FRET effect, ratiometric fluorescent probes undergo significant fluorescence changes when the analyte is added due to the interaction of the electron-

donating or electron-absorbing group with the analyte (Park et al., 2020). As shown in Figure 4.26, after the introduction of Cu(II) into the AuNCs/CQDs system, the response of the CQDs, as a reference signal, to Cu(II) increased, so the blue fluorescence intensity (at 450 nm) did not change with the addition of Cu(II). When the AuNCs were used as a response signal, the -COOH in the AuNCs combined with Cu^{2+} to form aggregates, and the orange fluorescence (568 nm) of the AuNCs gradually increased. It has been reported that the AuNCs/CQDs ratiometric fluorescent probe is a "Single-Response-Off" sensing probe (Chen et al., 2023). Therefore, in this experiment, Cu(II) was visually quantified through alterations in probe colour and I_{450}/I_{568} . I_{450}/I_{568} is the ratio of the fluorescence intensity emitted by CQDs at 450 nm to that emitted by AuNCs at 568 nm at an excitation wavelength of 400 nm.

v. pH of the AuNCs/CQDs ratiometric fluorescent probes

The performance of fluorescent probes is significantly affected by the pH. To investigate this effect, I_{450}/I_{568} was measured before and after the introduction of Cu(II) across a pH range of 6.6 to 8.0. Figure 4.27 shows the variation in the I_{450}/I_{568} of the probe at different pH values with Cu(II) and showed an increasing and then decreasing trend. This is because, at low pH, the protonation of -COOH on the AuNCs weakens the FRET effect within the probe (Zanetti-Polzi et al., 2022). This result leads to a gradual increase in I_{450}/I_{568} , which occurs with Cu(II) under acidic conditions. In addition, the amino group in CQDs acts as a neutral molecule under alkaline conditions, which similarly reduces the FRET effect (Sapsford et al., 2006), and the addition of Cu(II) also produces comparable results. It was concluded that the AuNCs/CQDs probe was highly variable and stable between pH 7.2 and pH 7.6. Therefore, considering the convenience of actual detection, pH 7.4 was evaluated as the suitable pH.

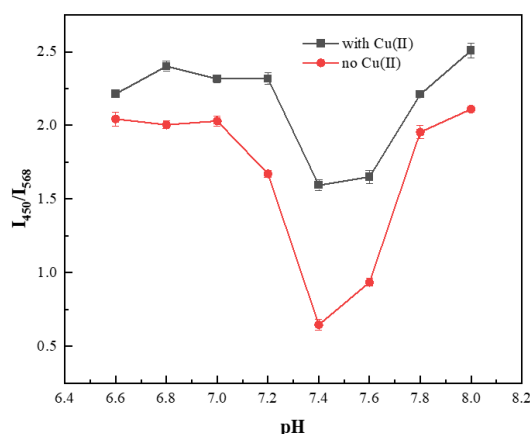


Figure 4.27: Effect of pH on the Preparation of AuNCs/CQDs (the Red Line Represents Cu(II), and the Black Line Represents no Cu(II)). Reaction Conditions: 900 μ L of AuNCs/CQDs ($V_{\text{AuNCs}}:V_{\text{CQDs}}=1:2$), 300 μ L of Cu(II) at 60 mol/L, Temperature=25 $^{\circ}$ C, Time=4 min, pH=6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, and 8.0

vi. The ratio of AuNCs/CQDs ratiometric fluorescent probes

The volume ratio of AuNCs to CQDs affects the detection sensitivity and accuracy of ratiometric fluorescent probes. This is due to the consideration from the perspective of fluorescence performance. After a lot of experimental explorations, it was found that the probes were able to exhibit different energy transfer, fluorescence intensities and colour changes in different volume ratio environments (Park et al., 2020). The probe volume ratio may make the reaction rates of AuNCs and CQDs reach a better balance during the preparation process. Neither too high a proportion of one nanomaterial will lead to too fast a reaction that is difficult to control, nor too low a proportion will lead to too slow a reaction and low yield. This ensures that the preparation process of the probes is efficient and stable. As shown in Figure 4.28(a)-(d), $V_{\text{AuNCs}}:V_{\text{CQDs}}$ ratios of 1:1, 1.5:1, 2:1, and 3:1 were selected for experimental testing. Since the AuNCs/CQDs probe is a “single-response-off” type, the FL intensity of CQDs (on the left) remained constant, but the FL intensity of AuNCs

decreased with the addition of Cu(II). The suitable volume ratio was determined to be 1:2. At this ratio, the colour of the probes showed a more pronounced change under the 365 nm UV lamp. In the inset, the ratio of the fluorescent probe changes from blue to pink, with a larger gradient of colour change, which is more suitable for the detection and analysis of the target. This is because the quantity of AuNCs is a significant contributor to the interaction between the CQDs and AuNCs, which in turn affects the process of fluorescence quenching through FRET (Pyo et al., 2023). When the volume of AuNCs increases or decreases, the excess AuNCs or CQDs will agglomerate and thus fail to disperse homogeneously in the probe system (Lin et al., 2018), and the fluorescence performance of the probes will decrease. When AuNCs and CQDs are combined in a specific ratio, the FRET efficiency is significantly affected. At a volume ratio of 1:2, the distance between AuNCs and CQDs is in a more appropriate range to effectively promote FRET. If the ratio is not appropriate, the distance may be too far or too close. When the distance is too far, the FRET efficiency is low and the effective energy transfer cannot be realized; when the distance is too close, it may cause undesirable phenomena such as fluorescence burst (Dos Santos et al., 2020). With a volume ratio of 1:2, AuNCs act as energy acceptors and CQDs act as energy donors, and the energy transfer between the two can reach a more ideal state so that the probe can fully utilize the FRET effect during the detection process, and improve the sensitivity and accuracy of the detection. Accordingly, a volume ratio of orange-gold nanoclusters to blue-CQDs of 1:2 was finally determined to be the suitable ratio.

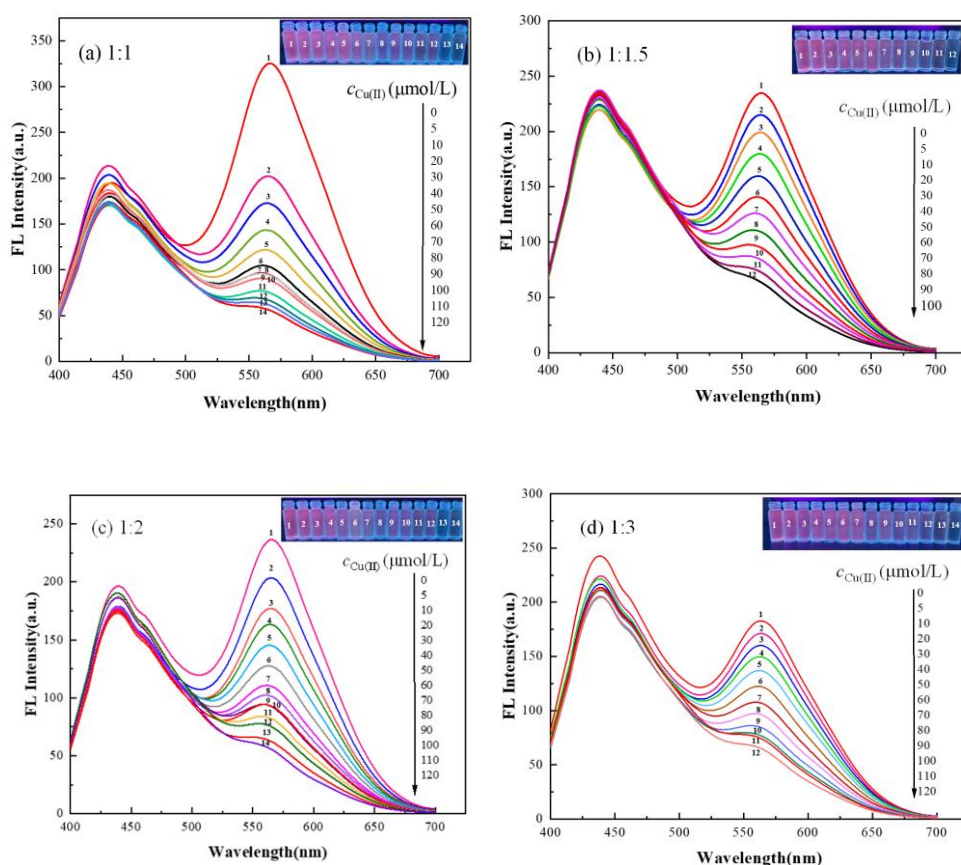


Figure 4.28: Effect of the Volume Ratio of the AuNCs and the CQDs Ratio during the Preparation of the AuNCs/CQDs (a-d). (a) 1:1, (b) 1:1.5, (c) 1:2, (d) 1:3. Reaction Conditions: 900 μL of AuNCs/CQDs, 300 μL of Cu(II) at 0-120 $\mu\text{mol/L}$, 800 μL of PBS at 10 mmol/L, pH=7.4, Temperature=25 $^{\circ}\text{C}$, and Time=4 min, The numbers 1-14 correspond to the concentrations of Cu(II) from 0-120 $\mu\text{mol/L}$.

vii. The reaction time of the AuNCs/CQDs ratiometric fluorescent probe

It is imperative to note that the rapid sensing procedure heavily relies on the reaction time of the AuNCs/CQDs ratiometric fluorescent probes, which affects the accuracy of the probe assay (Li et al., 2023). Figure 4.29 shows that the fluorescence intensity ratio (I_{450}/I_{568}) of the AuNCs/CQDs exhibited a pattern of initial decrease followed by stabilization after the addition of 300 μL of 60 $\mu\text{mol/L}$ Cu(II). At 1-3 min, the I_{450}/I_{568} ratio decreased significantly. After 4 min, I_{450}/I_{568} reached equilibrium, with a value of 1.66. This was because the reaction

gradually reached dynamic equilibrium with a gradual increase in time, which led to the stabilization of the fluorescence intensity ratio (Li et al., 2024). The investigation revealed that the probe exhibited a suitable response time of 4 min.

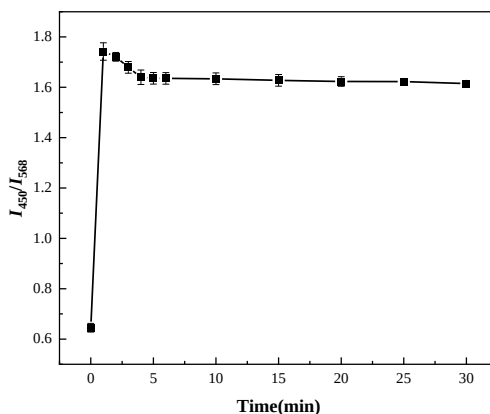


Figure 4.29: Effect of Reaction Time during the Preparation of AuNCs/CQDs. Reaction Conditions: 900 μL of AuNCs/CQDs (1:2), 300 μL of Cu(II), 60 $\mu\text{mol/L}$, 800 μL of PBS, 10 mmol/L, pH=7.4, Temperature=25°C, Time=0, 1, 2, 3, 4, 5, 6, 10, 15, 20, 25, and 30 min

4.3.3 Morphological and Structural Characterization of CQDs

In this study, five characterization methods, including XPS, TEM, XRD, Fourier transform infrared spectroscopy, and UV-visible spectrophotometry, were used to characterize the morphology and structure of the CQDs. Figure 4.30 shows that the peak at 280 nm corresponds to the synthesized CQDs. This phenomenon arises from the valence electron jump of $n-\pi^*$, which occurs in the C=O functional group (Wang et al., 2020) contained in the CQDs. The infrared spectra of the CQDs are illustrated in Figure 4.31, and characteristic peaks appear at 876 cm^{-1} , 1110 cm^{-1} , 1383 cm^{-1} , 1641 cm^{-1} , and 3420 cm^{-1} , which correspond to $\gamma_{\text{N-H}}$, $\nu_{\text{C-O}}(\text{aniline})$, $\nu_{\text{C-O}}^{\text{as}}(\text{carboxyl})$, $\delta_{\text{N-H}} / \nu_{\text{C=O}}$, and $\nu_{\text{C-H/O-H}}$,

respectively (Ronda et al., 2022). Compared with the willow leaves based-CDQs, the peaks at 3400-3500 cm^{-1} become broader and stronger, which may contain intermolecular hydrogen bonds (Liu et al., 2021). Meanwhile, new N-H peaks were added at 1383 cm^{-1} and 876 cm^{-1} (Bouzas-Ramos et al., 2019). Experimental data showed that apricot leaf-based-CQDs are chemically richer, although they also have a similarly large number of surface groups consisting of O, N, and C.

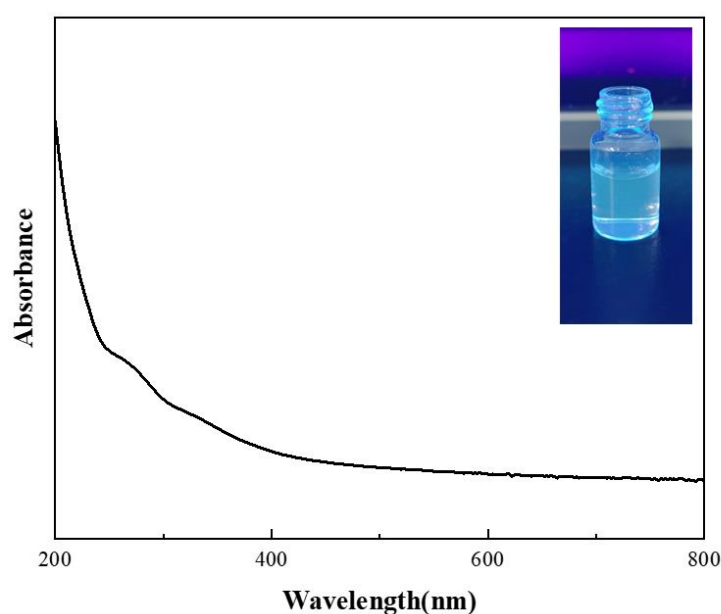


Figure 4.30: UV-visible Absorption Spectra of the CQDs (the inset was taken under a 365 nm UV lamp). The Test Conditions were as Follows: CQDs, 0.1 mg/mL; Temperature, 25 °C; and Scanning Range, 190-800 nm

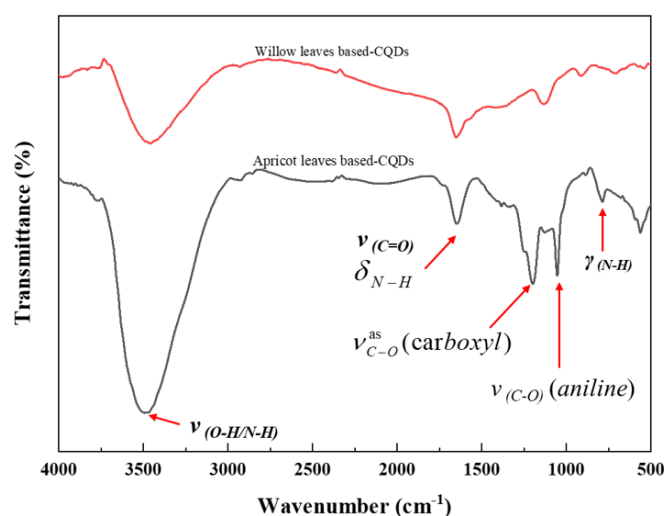


Figure 4.31: Fourier Transform Infrared Spectrum of the CQDs. Test Conditions: 0.1 mg of Freeze-Dried CQDs. The Scanning Range was 500-4000 cm^{-1} by the KBr Pressing Method with a Resolution of 4 cm^{-1}

Figure 4.32(a) shows that the CQDs are relatively uniform in size and well dispersed in water, at approximately 2.39 nm (Figure 4.32(b)). Figure 4.32(c) illustrates a clear broad diffraction peak at 26.6° caused by the (002) crystal plane of graphene (Yang et al., 2024). This suggests that CQDs have a graphene-like layered structure within them, giving CQDs good stability and optical properties. The correlation with the effectiveness of synthesis that the diffraction peak in XRD reflected the effect of heat treatment during the synthesis process, and the suitable reaction temperature and time led to a more pronounced graphene-like structure, resulting in a stronger and narrower diffraction peak (Özbek et al., 2023). In this study, a reaction at 200°C for 12 h led to a stronger and narrower diffraction peak at 26.6° . These findings confirmed that the synthesized substance was CQDs. Figure 4.32(d) shows the full XPS spectrum of the CQDs, which shows that the surface of the CQDs contains C, N, and O. The signature peaks of C, N, and O appeared at 285.08 eV (Ma et al., 2023), 400.08 eV (Xie et al., 2021), and 532.08 eV (Arkin et al., 2023), respectively. These findings

align with the characterization results obtained from the IR spectra. This is because the data from XPS and FTIR are related. XPS can determine the composition and chemical state of surface elements for CQDs. FTIR provides information about the functional groups of the CQDs. Combining the data from XPS and FTIR provides a more comprehensive understanding of the composition, chemical state and chemical structure of CQDs.

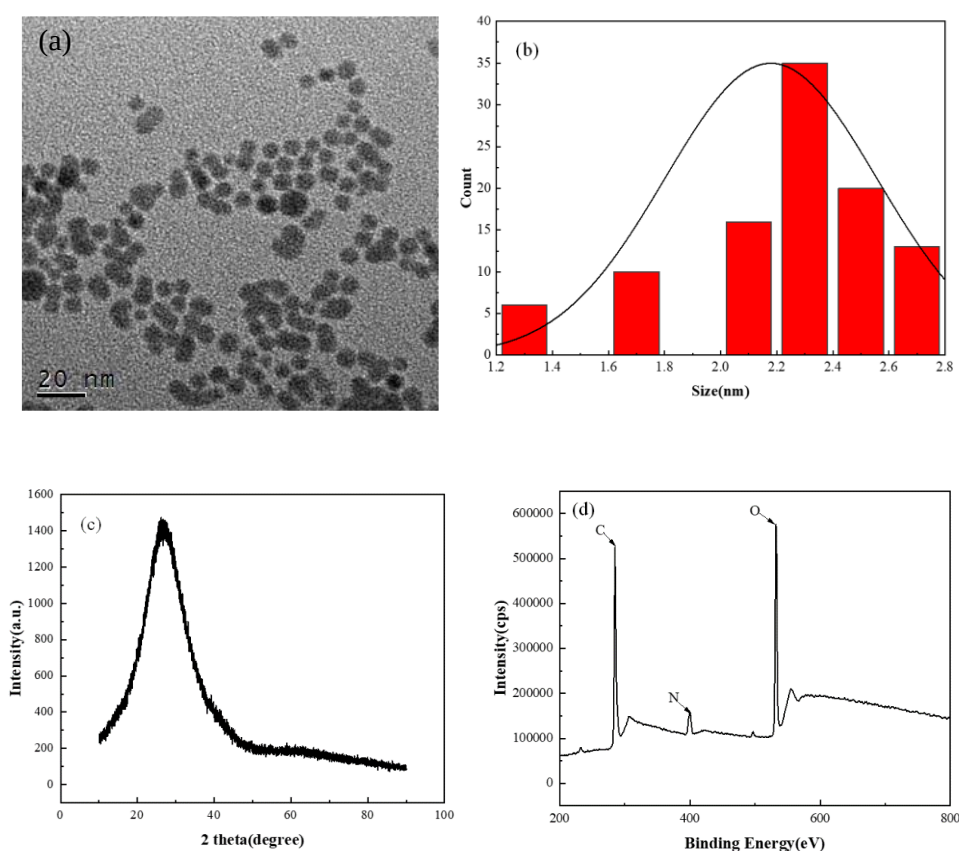


Figure 4.32: TEM (a), Particle Size Distribution (b), XRD(c), and XPS (d) of the CQDs

4.3.4 Morphological and Structural Characterization of the AuNCs

The experiments were carried out to characterize the GSH-AuNCs by five methods: XPS, TEM, XRD, Fourier transform infrared spectroscopy and UV-visible spectrophotometry. As shown in Figure 4.33, the UV-visible spectrum of the AuNCs did not exhibit any visible band, indicating that no sizeable gold nanoparticles were generated in the synthesized AuNCs. Figure 4.34 illustrates the infrared spectrum of the AuNCs, having peaks at 604 cm^{-1} , 1647 cm^{-1} , 2920 cm^{-1} , and 3442 cm^{-1} . These vibrations include Au-N, C=O, C-H, and N-H/O-H bond bending and stretching vibrations (Deng et al., 2015; Lim et al., 2008).

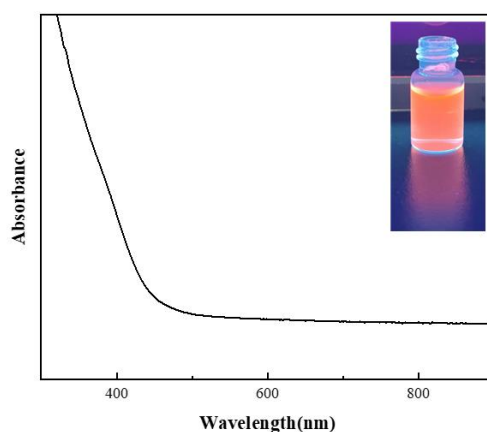


Figure 4.33: UV-visible Absorption Spectra of the AuNCs (the Inset was Taken under a 365 nm UV lamp). The Test Conditions were as Follows: AuNCs, 0.1 mg/mL; Temperature, 25 °C; and Scanning Range, 190-800 nm

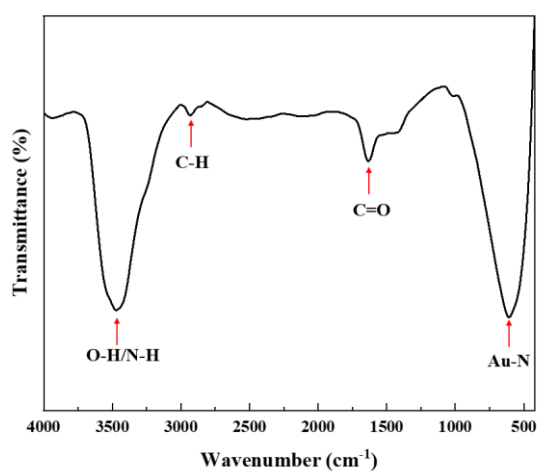


Figure 4.34: Fourier Transform Infrared Spectrum of the AuNCs. Test Conditions: 0.1 mg of Freeze-Dried AuNCs. The Scanning Range was 500-4000 cm⁻¹ by the KBr Pressing Method with a Resolution of 4 cm⁻¹

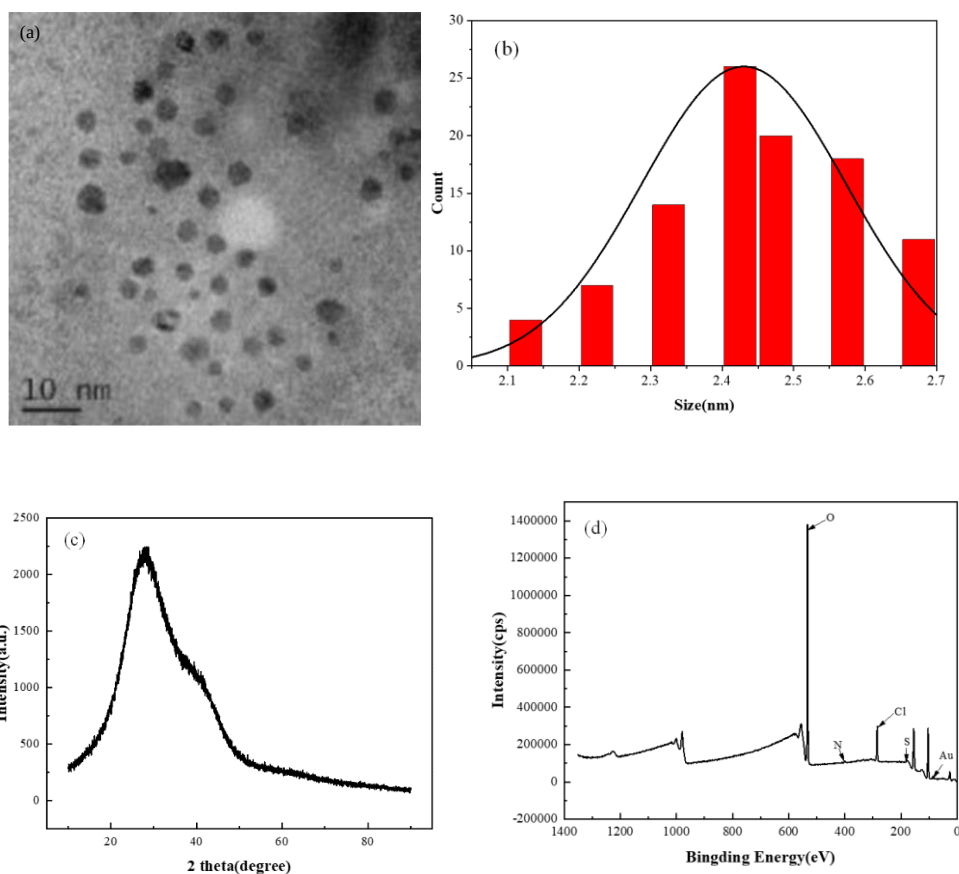


Figure 4.35: TEM (a), Particle Size Distribution (b), XRD (c), and XPS (d) of the AuNCs

Figure 4.35(a) shows that the AuNCs are nearly spherical and are approximately 2.48 nm in size according to the particle size distribution in Figure 4.36(b), which is consistent with the morphology observed via TEM. The 2θ peak at 27.68° in the XRD pattern shown in Figure 4.35(c) indicates that the synthesized AuNCs have a regular crystal morphology. Figure 4.35(d) shows the full-spectrum XPS spectrum of the GSH-AuNCs, which contain several elements, such as C, O, N, Cl, and Au, on their surface. The several corresponding peaks at 1071.09 eV, 284.8 eV, 400.1 eV, 532.88 eV, 198.08 eV, and 84.11 eV are ascribed to the presence of Na, C, N, O, Cl, and Au, respectively (Gao et al., 2022). The above data align with the characterization results obtained from the IR spectra.

4.3.5 Characterization of the Morphology and Structure of the AuNCs/CQDs

Ratiometric Fluorescent Probes

Figure 4.36 shows that the absorption of the AuNCs/CQDs probe in the UV region is attributed to the integration of two different absorption peaks from the AuNCs and CQDs (Peng et al., 2020). In the IR spectra (Figure 4.37), the ratiometric fluorescent probe has peaks at 3460 cm^{-1} , 2880 cm^{-1} , 1650 cm^{-1} , and 547 cm^{-1} . The three-line contrast indicates that the probe contains N-H, O-H, C-H, C=O, and Au-N groups. This suggests that the AuNCs/CQDs probes were successfully synthesized.

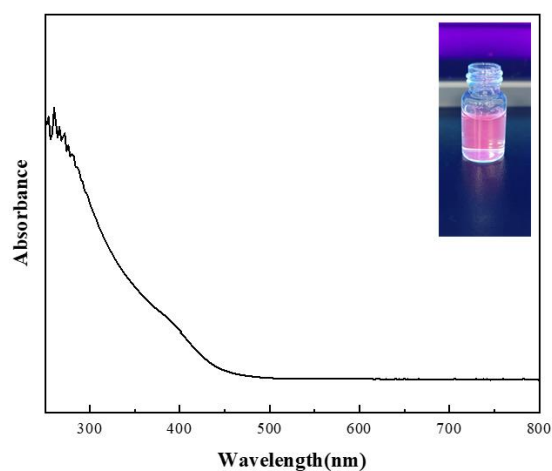


Figure 4.36: UV-visible Absorption Spectra of the AuNCs/CQDs (the Inset was Taken under a 365 nm UV lamp). The Test Conditions were as Follows: AuNCs/CQDs, 0.1 mg/mL; Temperature, 25°C; and Scanning Range, 190-800 nm

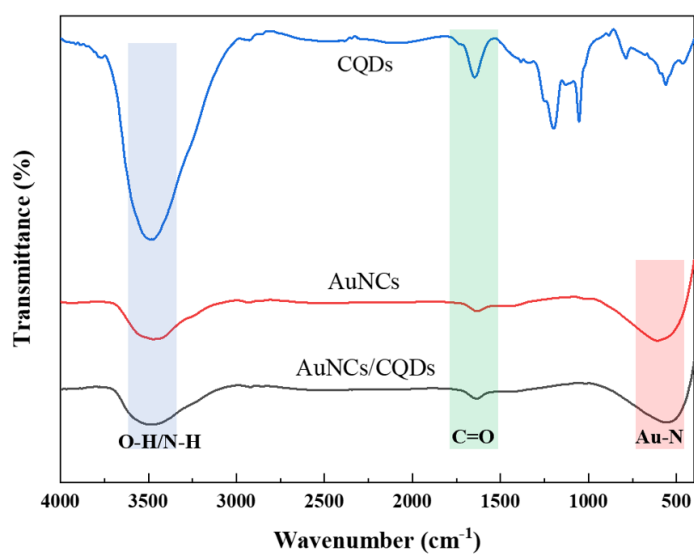


Figure 4.37: Fourier Transform Infrared Spectra of the CQDs, AuNCs, and AuNCs/CQDs. The Test Conditions were as Follows: 0.1 mg each of Freeze-Dried CQDs, AuNCs, and AuNCs/CQDs. The Scanning Range was 500-4000 cm^{-1} according to the KBr Pressing Method with a Resolution of 4 cm^{-1}

Figure 4.38 (a) and (b) demonstrate that the AuNCs/CQDs ratiometric fluorescent probe has a uniform particle size of 8.86 nm due to the binding of AuNCs and CQDs by electrostatic attraction, and the particle size of the AuNCs/CQDs increases. Figure 4.38 (c) shows that the AuNCs/CQDs have a large diffraction peak at $2\theta=27.94^\circ$. The probe has a regular crystal morphology. Figure 4.38 (d) shows the full spectrum of the probe, which contains the major elements possessed by the CQDs and AuNCs, consistent with the functional group elements contained in the infrared spectrum.

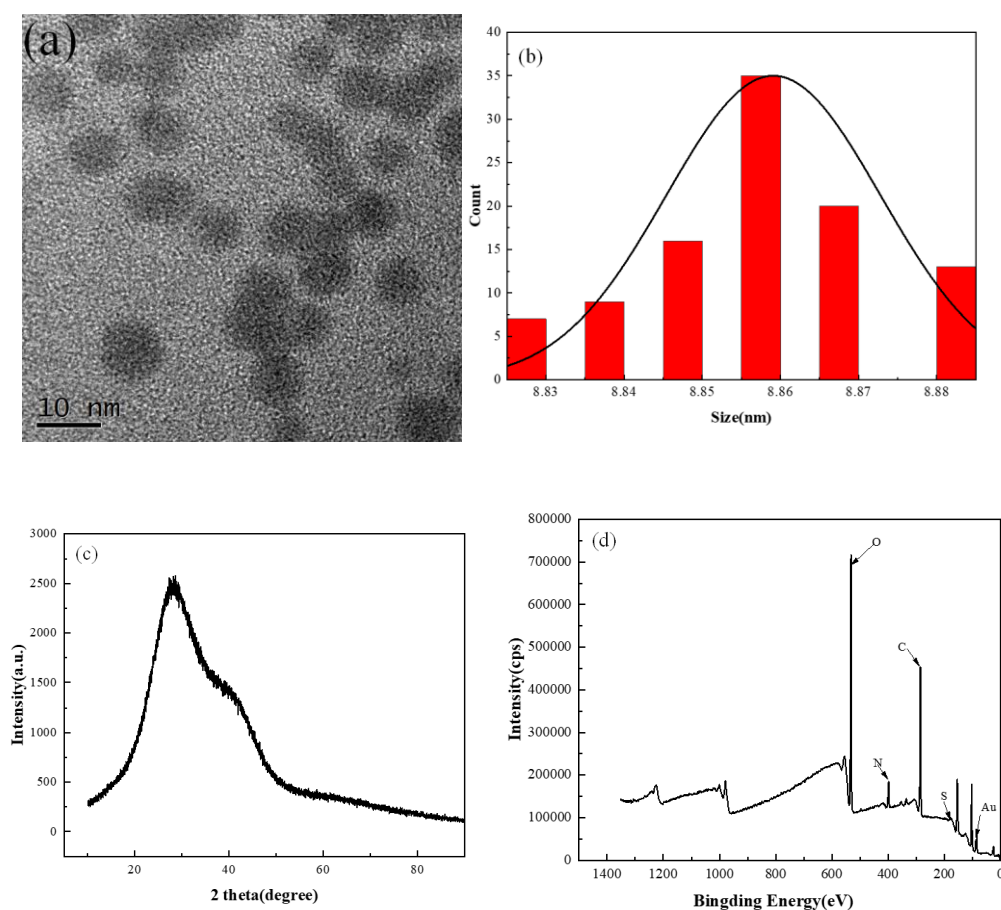


Figure 4. 38: Transmission Electron Microscopy (a), Particle Size Distribution (b), X-ray Diffraction (c), and X-ray Photoelectron Spectroscopy (d) of the AuNCs/CQDs

4.3.6 Optical Properties of the AuNCs/CQDs Ratiometric Fluorescent Probes

As illustrated in Figure 4.39, the full Ex and Em spectra of the AuNCs/CQDs were scanned experimentally with a fluorescence spectrophotometer. These findings reveal that the AuNCs/CQDs probe is a double-emission probe with certain excellent optical properties.

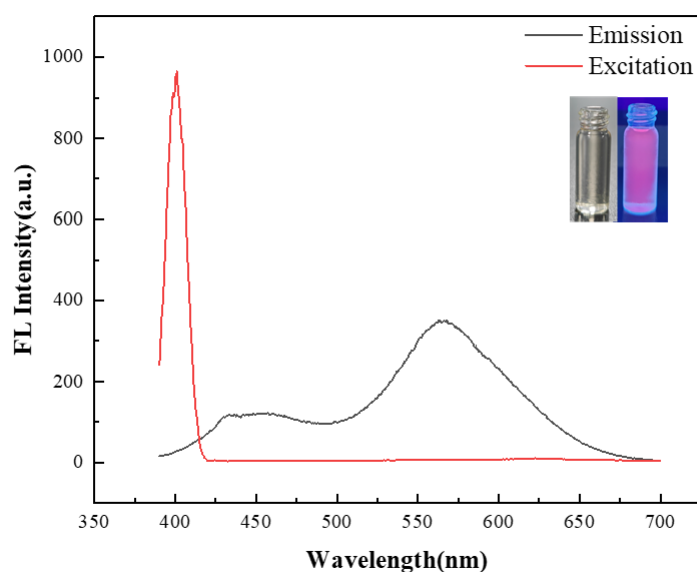


Figure 4.39: Full Excitation and Emission Spectra of the AuNCs/CQDs Probe. Spectral Conditions: the Range for both λ_{ex} and λ_{em} was 390-700 nm, the Concentration of the AuNCs/CQDs Probe was 0.1 mg/mL, the Width of the Excitation Slit was set at 3 nm, and the Width of the Emission Receiving Slit was Set at 6 nm

4.3.7 Quantum Yield

Substituting the collated experimental data into Equation 3.1, the fluorescence quantum yield was 27.6% (Table 4.5) for the biomass CQDs. As shown in Table 4.6, it has the highest quantum yield compared to the previous two biomass CQDs in this study. This is because the carbon source is a crucial factor influencing the quantum yield of biomass CQDs (Han et al., 2024).

Table 4.5: QY of Apricot Leaves-based CQDs

No.	Name	λ_{ex}/nm	λ_{em}/nm	<i>A</i>	<i>F</i>	Y/%
1	Quinine sulfate	350	450	0.0307	204541	55
2	Apricot Leaves-based CQDs	400	450	0.0472	157809	27.6

Table 4.6: Quantum Yields of Three Different Biomass CQDs in this Study

Sample	Quantum yield
BCQDs-WL (BCQDs based on willow leaves)	12.1%
P-BCQDs-WL (P-BCQDs based on willow leaves)	25.4%
BCQDs-AL (BCQDs based on apricot leaves)	27.6%

Figure 4.40 is a characterization of the effect of carbon source type on the degree of carbonation using Raman spectroscopy. In the Raman spectroscopy of biomass CQDs, the peaks at 1356 cm^{-1} and 1592 cm^{-1} correspond to disordered carbon (D peak) and graphitic carbon (G peak) in carbon materials (Kumar et al., 2024). The corresponding I_D/I_G ratios for BCQDs-WL, P-BCQDs-WL, and BCQDs-AL are 1.10, 0.98 and 0.96, respectively. A decrease in the I_D/I_G value implies an increase in the degree of carbonization. The higher the degree of carbonization degree of the biomass CQDs, the higher their quantum yield and the

better the fluorescence performance (Wang et al., 2024). It was concluded that the biomass CQDs prepared from apricot leaves had the most excellent fluorescence properties.

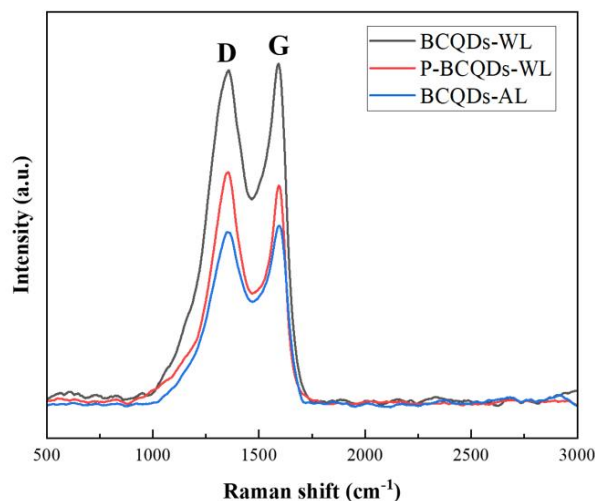


Figure 4.40: Influence of Carbon Source Type on the Degree of Carbonization

4.3.8 Feasibility Testing

The experiments were tested using a gold nanocluster/carbon quantum dot ratiometric fluorescent probe in response to 60 $\mu\text{mol/L}$ Cu(II). Figure 4.41 shows that the emission peak of the CQDs at 450 nm did not change with the addition of 60 $\mu\text{mol/L}$ Cu(II). However, that of AuNCs at 568 nm showed a significant increase with the addition of Cu(II). The phenomenon follows this principle, and the experiment is feasible.

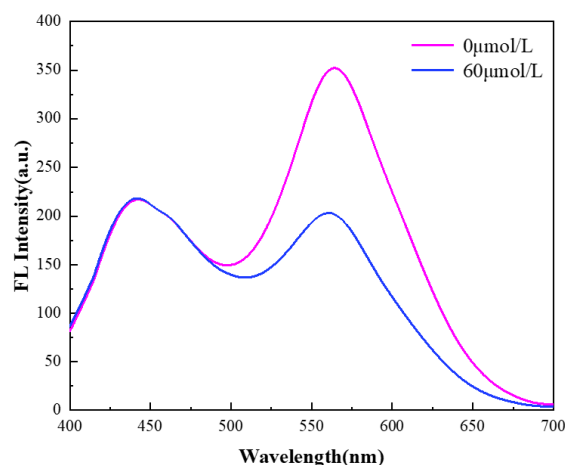


Figure 4.41: Feasibility of the AuNCs/CQDs Probe for Cu(II) Detection (the blue line Represents 60 $\mu\text{mol/L}$ Cu(II), and the Purple Line Represents no Cu(II)). Reaction Conditions: 900 μL of AuNCs/CQDs (1:2), 300 μL of Cu(II), 60 $\mu\text{mol/L}$, 800 μL of PBS, 10 mmol/L, pH=7.4, Temperature=25°C, and Time=4 min. The Range of λ_{em} was 390-700 nm at λ_{ex} =400 nm

4.3.9 Stability Experiments

The stability of ratiometric fluorescent probes affects both the accuracy and sensitivity of a method (Liu et al., 2016). In a common system, the impact of the duration of storage on the stability of fluorescence in AuNCs/CQDs was assessed by determining the I_{450}/I_{568} of AuNCs/CQDs in the range of 0-60 h. Figure 4.42 indicates that the I_{450}/I_{568} of AuNCs/CQDs was stable within 24 h after storage at 4°C and then showed a decreasing trend. This may be due to the agglomeration of AuNCs/CQDs with increasing storage time (Liu et al., 2021). Therefore, the probe is stable for 24 hours, proving its good photostability.

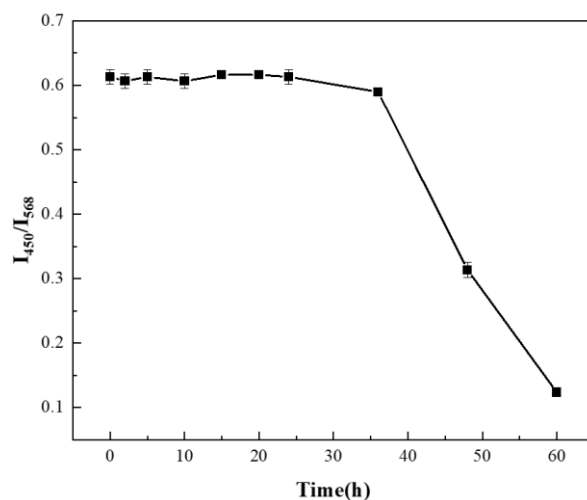


Figure 4.42: Photostability of the AuNCs/CQDs Probe. Reaction Conditions: 900 μL (1:2) of AuNCs/CQDs, 300 μL (60 $\mu\text{mol/L}$) of Cu(II), 800 μL (10 mmol/L) of PBS, pH=7.4, Temperature=25°C, time=0, 2, 5, 10, 15, 20, 24, 36, 48, 60 h

4.3.10 Selectivity of AuNCs/CQDs Probes for Cu(II)

According to the most favourable reaction parameters, Figure 4.43 shows that the I_{450}/I_{568} of the probe was the highest only when 300 μL of 120 $\mu\text{mol/L}$ Cu(II) was added, and the fluorescence intensity ratio changed weakly and was almost unresponsive when 300 μL of 120 $\mu\text{mol/L}$ other ions were added. Therefore, this ratiometric fluorescent probe has proven to have a high degree of selectivity in detecting Cu^{2+} .

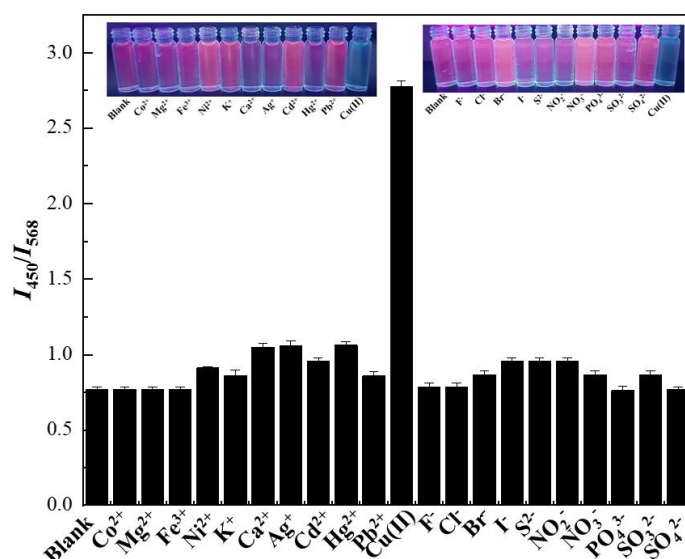


Figure 4.43: Cu(II) Selectivity of the AuNCs/CQDs probe. Inset: Photograph Obtained upon Excitation with a 365 nm UV lamp. Reaction Conditions: 900 μL of AuNCs/CQDs, 300 μL of Cu(II), 120 $\mu\text{mol/L}$, 800 μL of PBS, 10 mmol/L, pH=7.4, Temperature=25°C, and Time=4 min

4.3.11 Plotting of the Cu^{2+} Standard Curve

Figure 4.44 shows that the AuNCs/CQDs ratiometric fluorescent probe was gradually released from the Cu^{2+} solutions at different concentrations. In the inset, the fluorescence emitted by the probe when exposed to UV radiation at a wavelength of 365 nm exhibits a progressive transition in colour, shifting from a pink hue to purple and ultimately to blue. According to the linear relationship curve, the I_{450}/I_{568} of the probe exhibited a strong linear correlation with $c_{\text{Cu}^{2+}}$ (0-120 $\mu\text{mol/L}$). The fitted linear equation was $y=0.0154x+0.6865$ with an R^2 of 0.9982, and the LOD for Cu^{2+} was 0.65 $\mu\text{mol/L}$, which is below the U.S. EPA limit of 20 $\mu\text{mol/L}$.

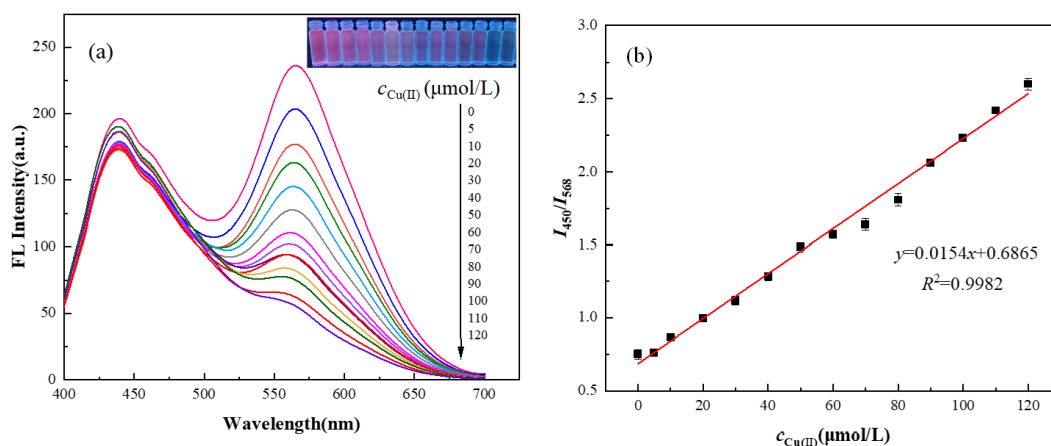


Figure 4.44: (a) Fluorescence Emission Spectra of the CQDs/AuNCs ($\lambda_{ex} = 400$ nm) with the Addition of Cu(II) (0-120 $\mu\text{mol/L}$). Inset: Photograph Obtained upon Excitation with a 365 nm UV lamp. (b) Linear Plotting Relationship between I_{450}/I_{568} and $c_{Cu(II)}$. Reaction Conditions: 900 μL of AuNCs/CQDs, 300 μL of Cu(II) at 0-120 $\mu\text{mol/L}$, 800 μL of PBS at 10 mmol/L, pH=7.4, Temperature=25 $^{\circ}\text{C}$, and Time=4 min

4.3.12 Measurement of Actual Samples and Spiking Recovery Experiments

As shown in Figure 4.45, two copies of the probe added to actual samples can be seen under the UV lamp. The decrease in the intensity of the pink fluorescence was not obvious, which is similar to the colour of the probe. This indicates that the copper ion content was low, and the concentrations were 0.21 $\mu\text{mol/L}$ and 1.97 $\mu\text{mol/L}$. The visualization results further indicate that the AuNCs/CQDs probe possesses high sensitivity for detecting Cu(II) in water. Table 4.7 shows that the spiked recoveries of this method were between 98.3% and 102.3%, with good recovery results. Spiked recoveries lower than or exceeding 100% may be caused by a variety of reasons, such as insufficient purity of the standard substance, matrix components in the sample affecting the analytical results, operational errors, measurement errors, and so on (Thakur et al., 2022). In the analytical process, it is necessary to strictly control the experimental conditions, standardize the operation

procedures, select the appropriate analytical methods and standard substances, and measure the blank samples to ensure the accuracy and reliability of the analytical results (Cordeiro et al., 2018). Table 4.8 summarizes the comparison of the Cu(II) detection data obtained with different ratiometric fluorescent probes. The linear ranges of the probes were wide, and the detection limits met the experimental requirements. Simultaneously, the investigation utilized apricot leaves as a carbon source derived from biomass, thereby demonstrating the benefits of environmental sustainability and the absence of secondary pollutants. Consequently, this method will be promising for the visual detection of intracellular Cu(II).

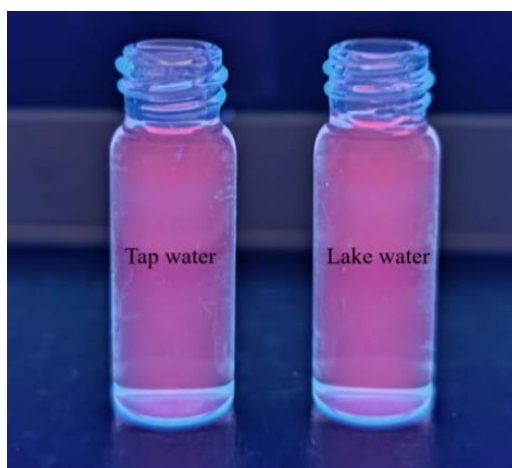


Figure 4. 45: Changes in the Colour of the AuNCs/CQDs Probe after the Addition of Tap Water or Lake Water under a 365 nm UV lamp. Reaction Conditions: 900 μL of AuNCs/CQDs, 300 μL Water Sample, 800 μL of PBS, 10 mmol/L, pH=7.4, Temperature=25°C, and Time=4 min

Table 4.7: Determination of Cu(II) Spiked Recovery in Actual Samples

Sample	Added ($\mu\text{mol/L}$)	Found ($\mu\text{mol/L}$)	Recovery (% , $n=3$)
Tap water	0.00	0.21	-
	10.00	10.20	99.9
	50.00	50.50	100.6
	90.00	88.68	98.3
Lake water	0.00	1.97	-
	10.00	11.81	98.4
	50.00	52.02	100.1
	90.00	94.04	102.3

Table 4.8: Comparison of the AuNCs/CQDs Probe with other Cu(II) Fluorescent Probes

Probe Composition	Linear Range ($\mu\text{mol/L}$)	LOD ($\mu\text{mol/L}$)	Reference
SQDs	20-200	6.78	Huang et al. (2022)
N,S-CQDs	1-100	1	Pizzoferrato et al. (2023)
P-AuNPs	20-60	0.43	Wu et al. (2023)

Table 4.8 continued

Probe Composition	Linear Range ($\mu\text{mol/L}$)	LOD ($\mu\text{mol/L}$)	Reference
CQDs@Cu-IIP	3.9-31 47-157	2.86	Wang et al. (2021)
Au@AgNPs	0.5-100	0.16	Lu et al. (2023)
BSANPs@AgNCs	0-60	1.30	Liu et al. (2022)
kiwi@AuNCs	5-35	1.6	Ma et al. (2023)
N-CDs/AgNCs	0-60	0.13	Yan et al. (2023)
AuNCs/CQDs	0-120	0.65	This work

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Accurate and sensitive detection of metal ions and biomolecules is crucial since they are essential for a wide range of physiological processes. In this study, a hydrothermal method was used to synthesize three types of CQDs fluorescent probes for the detection of PPase, Hg^{2+} , GSH, and Cu(II). A technique for the synthesis of CQDs was devised by using non-toxic and chemically rich willow and apricot leaves as raw materials instead of toxic organics. The quantum yield of CQDs was improved by phosphorus doping and the accuracy of CQD detection was improved by the incorporation of AuNCs.

5.1.1 A technique for the Synthesis of CQDs Fluorescent Probes for PPase Detection

CQDs were produced through a hydrothermal process using willow leaves as raw materials. The synthesis conditions involved a temperature of 200°C, pH of 7.4, and a reaction time of 12 h. Under 365 nm ultraviolet light, the CQDs emitted a light blue fluorescence, with an excitation wavelength of 390 nm and an emission wavelength of 452 nm. The CQDs had a spherical shape, were evenly distributed, and had a quantum yield of 12.1%. A fluorescence sensor was made using these CQDs, showing a good linear range and sensitivity for detecting PPase. The linear equation for detecting PPase within the 0-0.8 U/mL range was $y = -656.83x + 710.43$, with R^2 of 0.9968. PPase recoveries ranged from 98.67% to 102.7%. This method offers high selectivity, ease of use, and cost-effectiveness, making it essential for measuring PPase levels.

5.1.2 Phosphate-doped Biomass CQDs Fluorescent Probes for the Detection of Hg^{2+} and GSH

Phosphorus-doped biomass CQDs were synthesized using a hydrothermal approach, with willow leaves serving as the carbon precursor and sodium pyrophosphate as the modifying agent. P-BCQDs with cyan fluorescence and emission wavelengths of 471 nm were synthesized. The results showed that the fluorescence intensity of the P-BCQDs was highest when the molar ratio was 1:3, the synthesis temperature was 210°C, and the reaction time was 9 h. The obtained P-BCQDs have a spherical structure, uniform dispersion, an average particle size of 1.75 nm, and fluorescence. The quantum yield was 25.4%. The XPS and FTIR results showed that pyrophosphate successfully modified the CQDs. The fluorescence quenching efficiency of the P-BCQDs was the greatest when the pH was 7, and the reaction time was 2 min. Under these conditions, the linear range of Hg^{2+} in the P-BCQDs was 0-20 $\mu\text{mol/L}$, and the detection limit was 9 nmol/L. The recovery rates of Hg^{2+} ranged from 95.0% to 105.0%, with a relative standard deviation was 1.6-2.8% in the actual sample investigation. The fluorescence of the P-BCQDs- Hg^{2+} system gradually recovered as the concentration of GSH increased from 0 to 10 $\mu\text{mol/L}$. The results showed that the established probe for the detection of Hg^{2+} and GSH exhibits good sensitivity in this method.

5.1.3 AuNCs/CQDs Ratio Fluorescence Probes for the Detection of Cu(II)

AuNCs/CQDs ratiometric fluorescent probes were obtained by combining biomass CQDs synthesized at 200°C for 12 h with gold nanoclusters that were synthesized at 70°C for 24 h. The biomass CQDs, with a QY of up to 27.6%, were prepared from apricot leaves for the first time using a hydrothermal method. As a reference, it provides a self-calibrating signal to eliminate background environmental interference. Based on this, the suitable

preparation and detection conditions for ratiometric fluorescent probes were determined. When the pH is 7.4, the volume ratio of CQDs to gold nanoclusters is 2:1, and the reaction time is 4 minutes, the best detection effect is achieved.

The probes displayed distinct emissions at 450 nm and 568 nm. Detection of Cu(II) was quantitatively achieved by visually assessing the colour change and the ratio (I_{450}/I_{568}) between the emissions. GSH-AuNCs with orange fluorescence reacted to Cu(II) with a fluorescence increase, while blue fluorescent CQDs remained unchanged. Throughout the reaction, the probe's colour transitioned from pink to purple to blue in response to different Cu(II) concentrations. The linear range of the AuNCs/CQDs ratiometric fluorescent probe for Cu(II) was 0-120 $\mu\text{mol/L}$, with an LOD of 0.65 $\mu\text{mol/L}$. This research demonstrated the probe's selective ability to detect Cu(II) in water, suggesting practical applications.

5.2 Recommendations

Although substantial progress has been made in the research of CQDs, there are still many problems and challenges.

- i. After the raw materials production, the next step needs to consider whether large-scale production is possible or whether synthesis at 25°C should be considered;
- ii. As the quantum yield increases, so does the detection performance, and it is important to consider whether the current quantum yields are adequate for the assay if it enters a living organism.
- iii. The current detection method is still limited to the solution state, and it is not easy to use, whether it is possible to use a paper-based biosensor, and whether the biosensor can be reused.

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APPENDICES

Appendix A: Journal Publications

1. **W. He**, S. F. Lim, R. Baini, & X. J. Li. (2024). Visual Determination of Copper (II) using a Biomass Sourced Carbon Quantum Dot (CQD) Ratiometric Fluorescent Probe. *Analytical Letters*, 58(2), 228-241.
2. **W. He**, S. F. Lim, Y. Gao, R. Baini, J. Liu, & Y. D. Zheng. (2025). Determination of Folic Acid with a Novel Biomass Sourced Carbon Quantum Dots-Mesoporous Silica Fluorescent Probe. *New Journal of Chemistry*, 49, 1540-1547.
3. **W. He**, S. K. Wu, R. J. Cui, & S. F. Lim. (2024). Preparation of Carbon Quantum Dots Based on Willow Leaves as Raw Materials and Their Use as Fluorescent Probes for High-Sensitivity Detection of Pyrophosphatase. 2023 5th International Conference on Advanced Materials and Ecological Environment, Wuhan, China, 25-27, 2023. *Journal of Physics: Conference Series*, 2679(1): 012009. Institute of Physics Publisher.
4. **W. He**, S. F. Lim, R. Baini, & H. Y. Zhou. (2024). A Fluorescent Sensor Based on P-doped Biomass Carbon Quantum Dots for Selective Detection of Hg^{2+} . *Science of Advanced Materials*, 16(4), 442-449.

Appendix B: Experimental Instruments



Figure 1: Transmission Electron Microscope (JEM-2100, JEOL, Japan)



Figure 2: X-ray Diffractometer Technology (Ultima IV, Rigaku, Japan)



Figure 3: X-ray Photoelectron Spectroscopy (Escalab 250, Thermo Fisher, United States)



Figure 4: FTIR (Nicolet iS5, Thermo Fisher, United States)



Figure 5: Ultraviolet-visible Spectroscopy (GENESYS 50, Thermo Fisher, United States)



Figure 6: Fluorescence Spectrophotometer (F-7000, Hitachi, Japan)



Figure 7: Raman Spectrometer (InVia Reflex, Renishaw, UK)

Appendix C: Samples of Calculations

1. Quantum Yield

Data from the Table 4.1 were brought into Equation 3.1

$$QY = 55\% \times \frac{69184}{204541} \times \frac{0.0307}{0.047} = 12.1\%$$

2. Recovery rate

The recovery rate is the ratio of the found value to the spiked amount when a certain amount of standard material is added to the sample for determination.

$$\text{Recovery rate} = \frac{\text{Found Value}}{\text{Added Value}} \times 100\%$$

Found Value: indicates the concentration detected in the actual sample after the addition of the standard (subtracted a blank value)

Added Value: indicates the concentration of the standard solution of the target

Data from Table 4.4 were brought into the above equation

$$\text{Recovery rate} = \frac{2.23 - 0.23}{2} \times 100\% = 100\%$$

Appendix D: Calibration Data

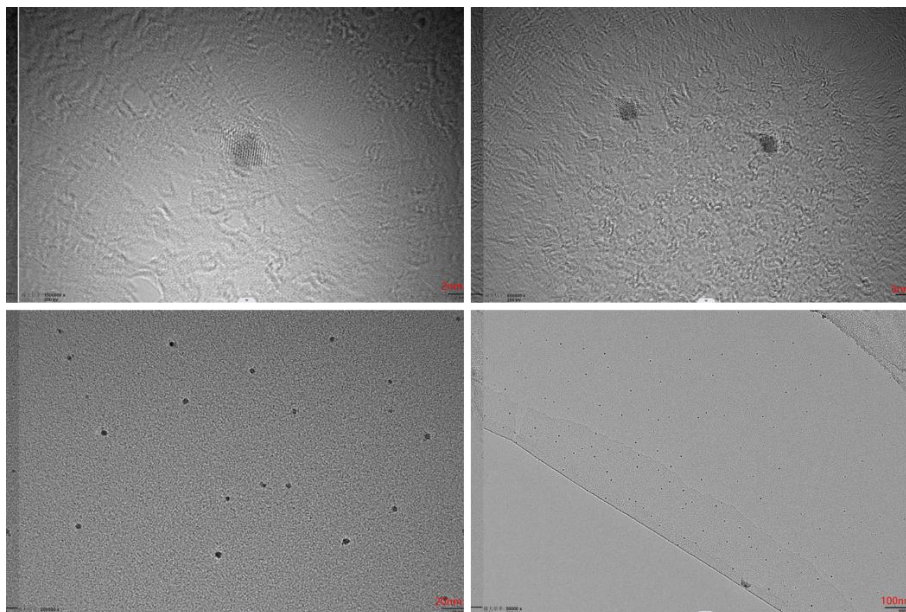


Figure 1: Calibration of TEM using Standard Substance Graphene Quantum Dots

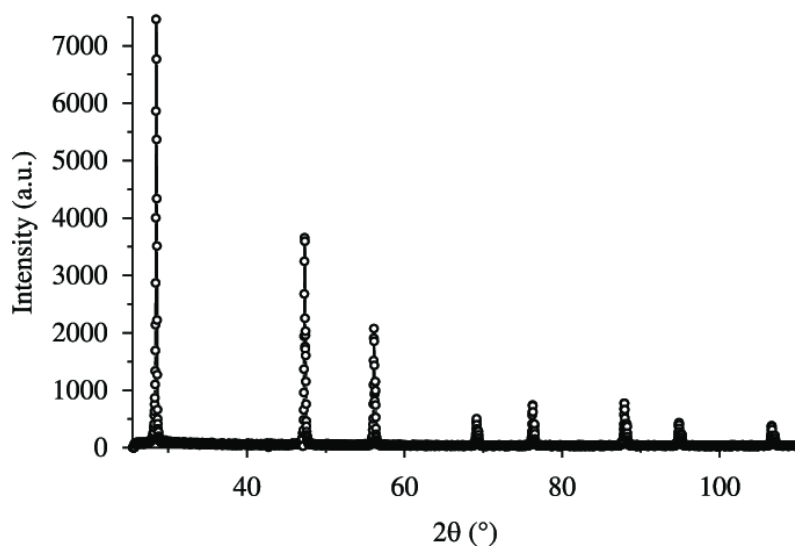


Figure 2: Calibration of XRD using Standard Silicon

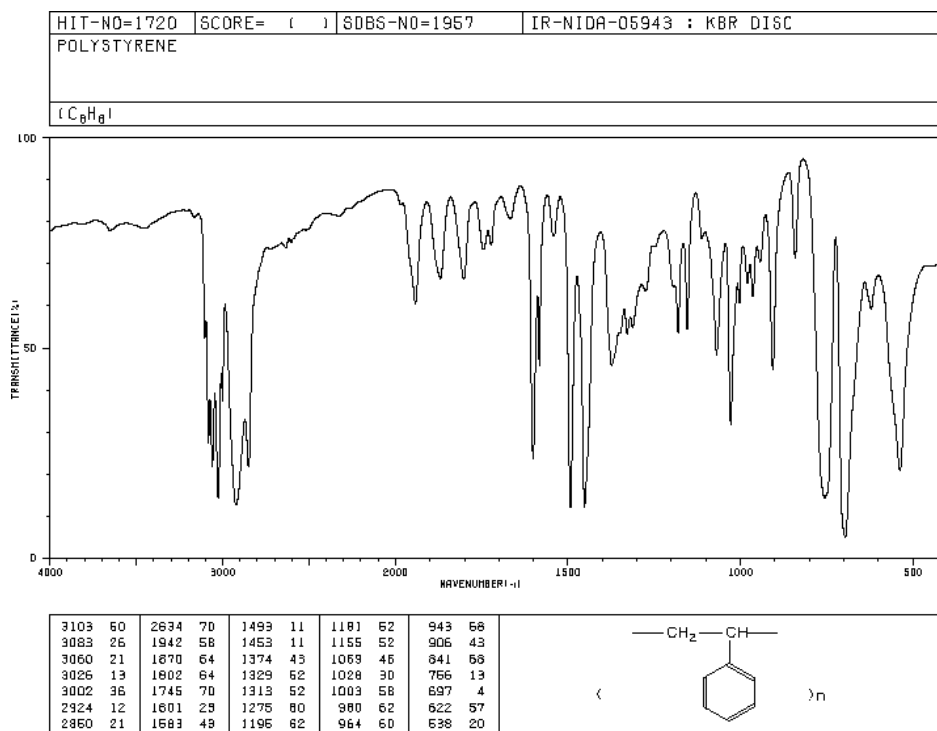


Figure 3: Calibration of FTIR using Standard Polystyrene

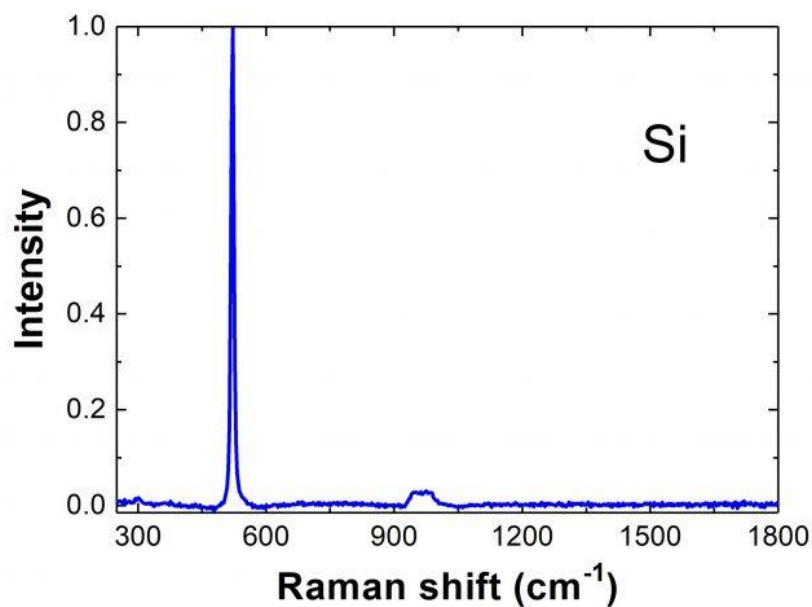


Figure 4: Calibration of Raman using Standard Silicon

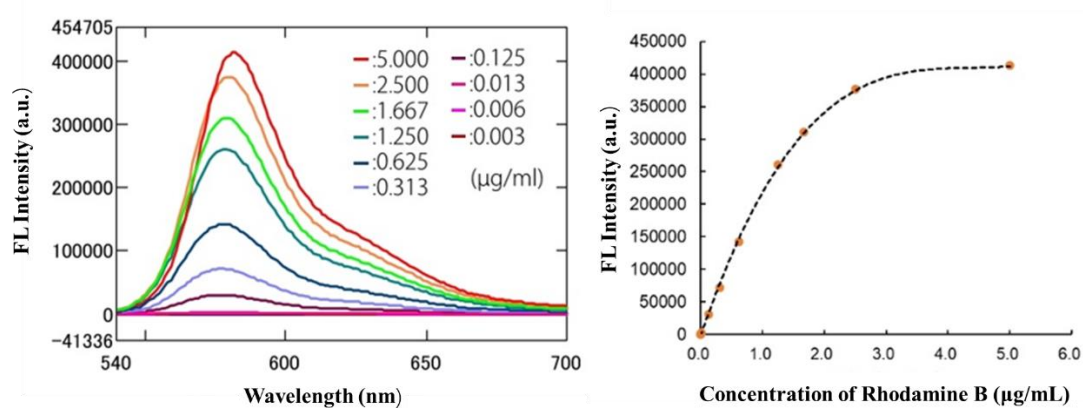


Figure 5: Calibration of Fluorescence Spectrophotometer using Standard Rhodamine B

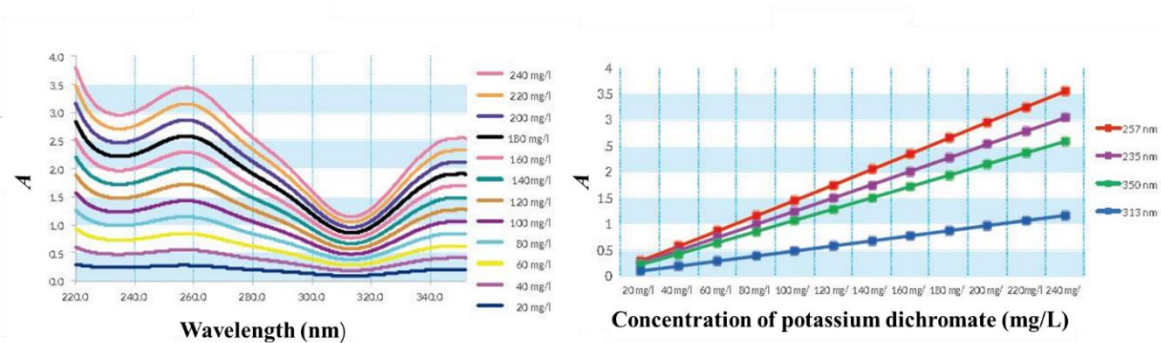


Figure 6: Calibration of UV-vis Spectroscopy using Standard Potassium Dichromate

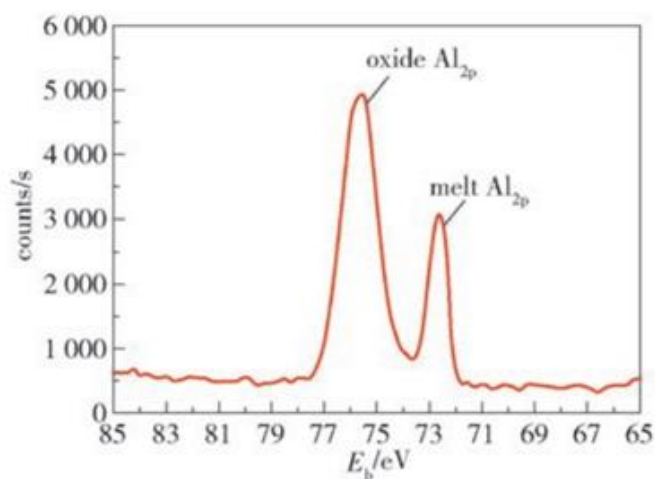


Figure 7: Calibration of XPS using Standard Oxide Al and Melt Al