



Faculty of Engineering

**Development of Graphene Oxide-Based Colorimetric Sensors with
Catalytic Activity for Hg^{2+} and Ag^+ Ions Detection**

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Development of Graphene Oxide-Based Colorimetric Sensors with Catalytic
Activity for Hg^{2+} and Ag^+ Ions Detection

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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

This study aims to construct a novel fast-response and high-sensitivity heavy metal colorimetric sensor based on graphene oxide/nanocomposites with catalytic properties. Eventually, a paper-based colorimetric sensor is developed for rapid on-site detection of heavy metals by using test paper as a solid-phase carrier. By varying the preparation conditions (concentration, temperature, pH and time) for the composites, a simple, unlabelled colorimetric sensing method is proposed to achieve the quantitative detection of Ag^+ . Under optimal conditions, the detection range of Ag^+ by this colorimetric sensing method is 0.5–1000 μM , and the detection limit is 0.5 μM , which is lower than the previously reported detection limit of the unlabelled Ag^+ colorimetric sensing method, indicating increased sensitivity and detection range. Furthermore, incorporating Au into the composite with varying the Au-to-Pt ratio, GO concentration, temperature, time and pH allowed the achievement of a simple and rapid on-site simultaneous detection of Ag^+ and Hg^{2+} . Under the optimal detection conditions, the colorimetric sensor had a detection range of 1–10000 nM for Hg^{2+} , with a detection limit of 1 nM. For Ag^+ , the detection range was 50–100000 nM, with a detection limit of 50 nM. Finally, by depositing a graphene oxide/platinum composite on a paper substrate, we developed a simple, fast, low-cost, naked-eye quantitative, paper-based colorimetric sensor for the simultaneous detection of Hg^{2+} and Ag^+ . The sensor has detection limits of 0.05 and 2.5 μM for Ag^+ and Hg^{2+} , respectively, and detection ranges of 0.05–10 and 2.5–100 μM , respectively. This approach provides not only a basis for on-site screening of heavy metals but also new ideas for the design of portable sensors and cross-applications in other disciplines.

Keywords: Graphene oxide, paper-based, colorimetric sensors, silver ions, mercury ions

Pembangunan alat pengesan kolorimetrik untuk ion Hg^{2+} dan Ag^+ berasaskan grafen oksida yang bersifat pemangkin

ABSTRAK

Kajian ini bertujuan untuk membina satu alat pengesan yang novel dengan tindak balas pantas berasaskan warna dan mempunyai sensitiviti tinggi dalam mengesan logam berat berdasarkan grafen oksida / nanokomposit yang bersifat pemangkin. Hasilnya, satu alat pengesan dalam bentuk kertas dibangunkan untuk digunakan di lokasi bagi pengesanan pantas logam berat dengan menggunakan kertas ujian sebagai pembawa fasa pepejal. Dengan mengubah keadaan penyediaan (kepekatan, suhu, pH dan masa) untuk komposit, kaedah pengesanan yang mudah dicadangkan untuk mencapai pengesanan kuantitatif ion Ag^+ . Di bawah keadaan optimum, julat pengesanan Ag^+ dengan kaedah pengesanan ini ialah 0.5-1000 μM , dan had pengesanan adalah 0.5 μM , yang merupakan had yang lebih rendah daripada had pengesanan yang dilaporkan oleh kajian lain, menunjukkan peningkatan sensitiviti dan julat pengesanan. Tambahan pula, menggabungkan Au ke dalam komposit dengan nisbah Au-ke-Pt yang berbeza-beza, kepekatan GO, suhu, masa dan pH membolehkan pencapaian pengesanan serentak Ag^+ dan Hg^{2+} yang mudah dan cepat di lokasi. Di bawah keadaan pengesanan optimum, alat pengesan ini mempunyai julat pengesanan 1-10000 nM untuk Hg^{2+} , dengan had pengesanan 1 nM. Untuk Ag^+ , julat pengesanan adalah 50-100000 nM, dengan had pengesanan 50 nM. Akhirnya, dengan mendepositkan komposit oksida / platinum grafen pada substrat kertas, satu alat pengesan dalam bentuk kertas telah dibangunkan dengan ciri-ciri mudah diguna, cepat, berkos rendah, mengetahui kuantiti dengan mata kasar, untuk pengesanan serentak Hg^{2+} dan Ag^+ . Alat pengesan ini mempunyai had pengesanan masing-masing 0.05 dan 2.5 μM untuk Ag^+ dan Hg^{2+} , dan julat pengesanan masing-masing 0.05-10 dan 2.5-100 μM . Pendekatan ini

bukan sahaja menyediakan asas untuk pemeriksaan logam berat di tapak tetapi juga idea-idea baharu untuk rekabentuk alat pengesan mudah alih dan aplikasi dalam bidang lain.

Kata kunci: *Grafen oksida, alat pengesan dalam bentuk kertas, alat pengesan berasaskan warna, ion perak, ion merkuri*

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LIST OF ABBREVIATIONS

ICP–MS	Inductively Coupled Plasma–Mass Spectrometry
AAS	Atomic Absorption Spectrometry
XRF	X-Ray Fluorescence spectrometry
NAA	Neutron Activation Analysis
ICP–OES	Inductively Coupled Plasma Emission Spectrometry
AuNPs	Gold Nanoparticles
Fe ₃ O ₄ NPs	Fe ₃ O ₄ Nanoparticles
POD	Peroxidase
GO	Graphene Oxide
TEM	Transmission Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-ray Diffraction
Raman	Raman Spectroscopy
TMB	3,3',5,5'-Tetramethylbenzidine
EPA	the U.S. Environmental Protection Agency
AFS	Atomic Fluorescence Spectrometry
AES	Atomic Emission Spectroscopy,
HPLC	High-performance Liquid Chromatography,
SPR	Surface Plasmon Resonance
LSPR	Local Surface Plasmon Resonance
CYS-GNPs	L-Cysteine-Functionalized Gold Nanoparticles
MOFs	Metal-Organic Frameworks
MB	Methylene Blue

MA-Ag	Carbon Nitride Quantum Dot-doped Mesoporous Melamine Silver
Ag-MnSz/CSPVA	Silver Manganese Disulfide/Chitosan Polyvinyl Alcohol
PDADMAC	Polydalin Dimethyl Ammonium Chloride
PEI	Polyaniline
NOV-LP	Antibody-Modified Graphene/AuNP Hybrids for Colorimetric Detection of Norovirus
HBV	Hepatitis B Virus
CHAs	Graphene/Gold Hybrid Platforms
PDA	Polyacetylene
VOCs	Volatile organic compounds
DA	Dopamine
GSH	Glutathione
CuO: GNS	Cytocompatible CuO: Graphene Nanosphere
Grp-Au NPs	Graphene-gold Nanoparticles
GO-AuNP	the Self-Assembly of Gold Nanorods on Graphene Oxide
ZnFe ₂ O ₄ /rGO	ZnFe ₂ O ₄ -Reduced Graphene Oxide
GOD-GO/MnO ₂	Glucose Oxidase-Conjugated Graphene Oxide/MnO ₂
rGO/PEI/Pd	Reduced Graphene Oxide-PEI-Pd Nanohybrids
Co-His-GQD-G	Cobalt-Graphene Nanohybrid
POCT	Prompt Once Test
PADs	Paper-based Analytical Sensing Devices
LFAs	Lateral Flow Analyses

Dips	Test-Paper Assays
LOD	Limit of Determination
AgNPs	Silver Nanoparticles
μ -PADs	Paper-based Microfluidic Chips
PS	Polystyrene
NCU	Novoulase Unit
ELISA	Enzyme-Linked Immunosorbent Assay
Au-HBNz	Gold nano enzymatic gel
V-Co ₃ O ₄	Vanadium-Containing Porous Cobalt Oxide Nanomaterials
TC	Total Cholesterol
HFABP	Cardiac Fatty Acid Binding Protein
ELISAs	Enzyme-Linked Immunosorbent Assays
RFLFIA	Ratiometric Fluorescent Lateral Flow Chromatography Immunoassay
COVID-19	2019 Coronavirus Disease
SARSCoV-2	Severe Acute Respiratory Syndrome Type 2 Coronavirus
WHO	World Health Organization
AgMBA@Au	Ultrathin Gold-Gold Shells ~ 2 Nm Ag Nanoparticles
MBA	4-Mercaptobenzoic Acid
LFIA	Lateral Flow Immunoassay
SERS	Surface-Enhanced Raman Scattering
ELISA	Enzyme-Linked Immunosorbent Assay
TB	Tuberculosis
MTB	Mycobacterium Tuberculosis
RT-PCR	Reverse Transcription Polymerase Chain Reaction

RPA	Recombinase Polymerase Amplification
HPV-16	Human Papillomavirus Type 16
Fe ₃ C@NCNTs	Nitrogen-Doped Carbon Nanotubes
SAXD	Small-Angle X-Ray Diffraction
WAXD	Wide-Angle X-Ray Diffraction
SAXS	Small-Angle X-Ray Scattering
WAXS	Wide-Angle X-Ray Scattering
EDS	Energy Dispersive X-Ray Spectroscopy
XPS	X-Ray Photoelectron Spectroscopy
USA	the National Institute of Mental Health
GO/PtNPs	Graphene Oxide Nanoplatinum Composites
GO/Au-PtNPs	Graphene Oxide/Au-Pt Nanoparticles
PP	Printing Paper
QFP	Qualitative Filter Paper
QTFP	Quantitative Filter Paper
AF	Acetate Film
GO/Pt@AuNPs	Graphene Oxide/Platinum Composites

CHAPTER 1

INTRODUCTION

1.1 Background of the study

A heavy metal is a kind of metal with a density greater than 5 g cm^{-3} . According to their biological functions, heavy metals are generally divided into essential heavy metals (copper, iron, etc.) and nonessential heavy metals (mercury, lead, etc.). In organisms, even the content of essential heavy metals must be controlled at a very low safe level. With the rapid development of the social economy, heavy metals are increasingly widely used in industrial and agricultural production, and heavy metal pollution in the environment is becoming increasingly serious (Setia et al., 2023). According to China's latest "National Pollution Source Census Bulletin", in recent years, China's average annual emission of heavy metals (lead, mercury, cadmium, chromium, and arsenic-like metals) has reached 182.54 tons. Heavy metal pollution in water amounted to 176.40 tons, accounting for 96.64% of the total emissions of heavy metals (Figure 1.1). Compared with the average annual discharge of heavy metals reported in the "Bulletin of the First National Census of Pollution Sources" in China, the proportion of heavy metals discharged in the aquatic environment has increased by approximately 8.86%, although the total amount of discharge has decreased (Figures 1.1). Ahmed et al. (2020) investigated the heavy metal concentrations in the Langat River by spot sampling (Figure 1.2) and found that the average concentration of lead in the Langat River was $9.99 \pm 1.40 \text{ }\mu\text{g/L}$ and the maximum concentration of lead was $24.93 \text{ }\mu\text{g/L}$, which exceeded the drinking water quality standard of $10 \text{ }\mu\text{g/L}$ set by the Ministry of Health of Malaysia and the World Health Organisation (WHO). g/L limit set by the Ministry of Health of Malaysia and World Health

Organisation. Heavy metals in water can enter organisms through irrigation, ion exchange, or ingestion and are passed through the food chain (Goswami et al., 2023), exposing organisms to different levels of pollution (Zhang et al., 2022a). Heavy metals can be stabilized in aquatic environments and subsequently accumulate in organisms, and heavy metal pollution is characterized by high toxicity, environmental persistence, concealment, and bioaccumulation and is considered to be one of the most harmful pollutants (Jiang et al., 2023). At the top of the food chain, humans are the ultimate organisms involved in heavy metal accumulation (Raheem et al., 2022).

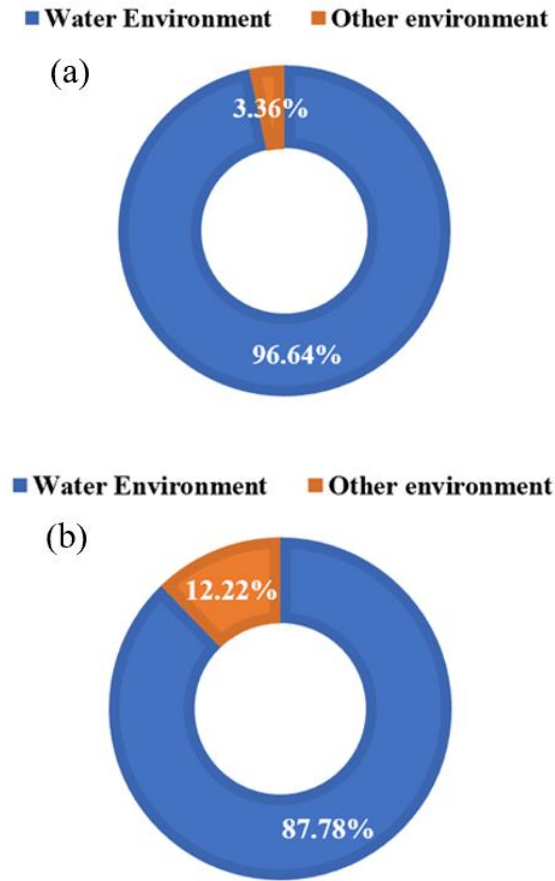


Figure 1.1: Water environmental distribution of heavy metals in China from 2017 to 2020 (a) and 2007 to 2010 (b) (China, 2020)

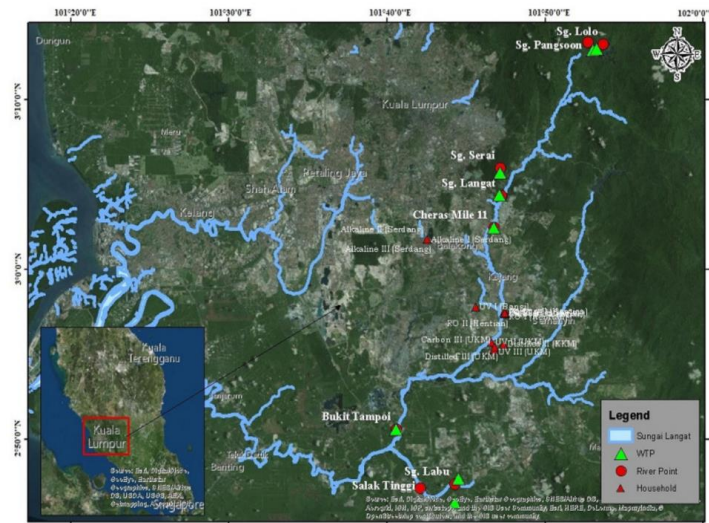


Figure 1.2: Water sampling points at the Langat River, Malaysia (Ahmed et al., 2020)

At present, 50% of the water sources in China are polluted by heavy metal ions. Over 200000 km² of land has been polluted by heavy metals, accounting for more than 1/6 of the total cultivated land area (Zhou et al., 2020). 12 million tons of grain are polluted by heavy metals every year, resulting in economic losses of tens of billion yuan (Yu et al., 2021). In addition, many river basins in countries around the world are facing heavy metal pollution problems, such as the Danube Delta region (Mndrescu et al., 2020), Setiu Wetlands State Park, Malaysia (Talukder et al., 2021), and the Great Lakes Basin of the United States (Li et al., 2022). Therefore, it is urgent to establish a rapid, sensitive, reliable, and low-cost heavy metal ion detection technology that is highly important for food safety, biomedical prevention, and environmental protection.

The most commonly used heavy metal ion detection methods include inductively coupled plasma–mass spectrometry (ICP–MS) (Aa et al., 2020), atomic absorption spectrometry (AAS) (Valasques et al., 2020), X-ray fluorescence spectrometry (XRF) (Zhao et al., 2020), neutron activation analysis (NAA) and inductively coupled plasma emission spectrometry (ICP–OES) (Bozorgzadeh et al., 2020). However, these traditional

detection methods are highly sensitive and specific. However, these methods have several limitations, such as expensive instrumentation, high operating costs, difficulty carrying them, tedious procedures, long processing times, and strict operation requirements for testing personnel. In addition, due to the dependence on detection equipment, traditional detection methods are difficult to use in field detection, and their practicability is poor (Bi et al., 2021; Yılmaz et al., 2021; Ameen et al., 2022). Therefore, the development of rapid (detection time less than 5 minutes), convenient (no need for complex pre-treatment process), low-cost (no need for expensive instrumentation), simple (operation steps are simple), and reliable (detection results are accurate) methods for on-site (outdoor real-time detection) detection is a popular research topic in the field of heavy metal ion detection.

The colorimetric sensing method has the advantages of visualizing detection results, simple operation, and rapid response. It has been widely studied and developed rapidly. Colorimetric sensing uses color changes to visually indicate the presence or concentration of a target, enabling a rapid response (seconds to minutes) for immediate on-site testing, and is particularly suited to application scenarios requiring real-time detection due to its simplicity, low cost, portability and wide applicability. The principle of the colorimetric sensing method is based on the redox reaction between the nano-catalytic material and the tested substance to produce a chemical signal. The color change of the chromogenic substrate is used as an optical signal output to realize the target detection process (Chen et al., 2022a; Xu et al., 2022). Among the numerous nanomaterials, graphene and its derivatives have good catalytic performance and have a unique layered structure, large specific surface area, and active groups on the surface, which provide modification to the active sites for other nanomaterials. It can also be used as a good support material to prepare nanocomposites and has been widely used in the field of colorimetric detection

(Vinotha et al., 2021; Minh et al., 2020; Boruah et al., 2020). Therefore, in this study, graphene oxide was used as a carrier to synthesize stable and efficient catalytic nanomaterials, and a colorimetric detection platform to realize the visualization and rapid field detection of heavy metal ions.

1.2 Problem statement

At present, there are two kinds of colorimetric sensors based on nanomaterials: one is based on the inherent optical properties of nanomaterials and another is based on the catalytic properties of nanomaterials.

Most colorimetric sensors based on the intrinsic optical properties of nanomaterials use gold nanoparticles (AuNPs) to aggregate or disperse in solution, resulting in different colors and detecting the target according to the color change of the solution (Yang, et al., 2020; Lu et al., 2021). However, due to the high surface energy, nanogold easily agglomerates, and the aggregation morphology is difficult to control. Therefore, when detecting heavy metal ions, the anti-interference is weak, which affects the accuracy of the detection of the target heavy metal ions. (Ali et al., 2023; Chadha et al., 2021).

A colorimetric sensor based on the inherent catalytic performance of nanomaterials uses the catalytic activity of nanomaterials to catalyze the color reaction of the substrate to detect the target (Ye et al., 2021). Since the research of Gao (Gao et al., 2007) first demonstrated that magnetic Fe_3O_4 nanoparticles (Fe_3O_4 NPs) have peroxidase (POD) activity, a variety of nanomaterials have been found to have POD activity, which can simulate the catalytic activity of natural enzymes (such as oxidases and peroxidases). For example, precious metal nanomaterials (Li et al., 2021a), carbon nanomaterials (Chen et al., 2022b), and metal oxide nanomaterials (Rashtbari et al., 2023), among others, are utilized.

Nanomaterials with high catalytic performance have an important influence on the sensitivity of contrast color sensing platforms. However, when nanomaterials are used for heavy metal detection, the structural defects resulting from easy stacking and agglomeration limit the further improvement of their catalytic activity and affect the sensitivity of the colorimetric sensing platform (Ye et al., 2021); therefore, it is necessary to modify these nanomaterials. The results showed that the surface charge and other characteristics of the nanomaterials determine their peroxidase catalytic activity (Xia, 2021). Surface modification of nanomaterials can not only effectively reduce the possibility of nanomaterial agglomeration but also provide additional catalytic sites, promote the transfer and transfer of electrons, and improve catalytic efficiency (Khayatian et al., 2020; Minh et al 2020).

In addition, the color changes caused by the above two kinds of heavy metal colorimetric sensing detection methods need to be quantified via spectrophotometry. However, due to the large volume of spectrophotometers, real-time detection of heavy metal ions is difficult to achieve (Ye et al., 2021; Rashtbari et al., 2023). The analytical strip has the advantages of being easy to carry, easy to detect on-site, simple to use, low cost, and amenable to multifunctional analysis (Choudhary et al., 2021; Liu et al., 2021a). Based on the properties of catalases, such as graphene oxide composites, analytical strips of heavy metal ions can be prepared, and the on-site rapid and accurate detection of heavy metal ions can be achieved.

1.3 Hypothesis

i. Graphene oxide (GO) is a derivative of graphene with a layered structure and excellent properties that can be used as a substrate for depositing inorganic nanoparticles.

The nanoparticles can be embedded in layered graphite to prevent graphene aggregation. This is conducive to obtaining composites with stable structure and efficient catalytic performance. PtNPs in GO/PtNPs can react with Ag^+ thereby inhibiting the catalytic performance of GO/PtNPs and enabling specific detection of Ag^+ .

ii. The synergistic effect of various components in the composites can effectively improve the catalytic performance of graphene. The stability and catalytic properties of the composites will affect the stability and sensitivity of the colorimetric sensor. GO/PtAuNPs can react with Ag^+ and Hg^{2+} simultaneously, but the simultaneous detection of both ions can be achieved by the addition of EDTA.

iii. Paper can serve as a carrier for reagents, offering benefits for the advancement of analytical sensing platforms, particularly for rapid testing devices. The design of paper-based sensors will contribute to their application in the rapid on-site detection of heavy metals and quick response following heavy metal contamination accidents.

1.4 Objectives of the research

This study aimed to develop a colorimetric sensor based on graphene oxide composites to achieve rapid and accurate on-site detection of heavy metal ions.

i. To synthesize and characterize the GO/PtNPs for high-efficiency peroxidase-like for achieving optimum detection of silver ions

ii. To synthesize and characterize the GO/PtAuNPs for high-efficiency peroxidase-like for achieving optimum detection of silver and mercury ions

iii. To synthesize and characterize paper based GO/PtAu nanocomposite for high-efficiency peroxidase-like for achieving optimum detection of silver mercury ions

1.5 Scope of the research

The scope of this study mainly focuses on the following elements:

i. Morphological features, surface functional groups, crystal structure, elemental composition of the composites were investigated using transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffractometry (XRD), Raman spectroscopy (Raman), energy scattering spectroscopy (EDS) and ultraviolet-visible (Uv-vis). ii. The GO/PtNPs composites were synthesised using hydrothermal method. The effects of (4 mM), hydrothermal treatment time (6-10 h), and hydrothermal treatment temperature (140-200 °C) on the peroxidase-like catalytic activity of GO/PtNPs composites during preparation. (28 µg/mL), TMB concentration (0.001-1.5 mM), hydrogen peroxide concentration (0-0.04 M), pH (2. 2-8), and reaction time (1-30 min) on the colour change of the system.

ii. The morphological features, surface functional groups and elemental composition of the composites were studied using TEM, FTIR, Raman and EDS. ii. The GO/Au-PtNPs composites were synthesised using a hydrothermal method. iii. 0), GO concentration (0.3-0.7 mM), hydrothermal treatment time (4-12 h) and hydrothermal temperature (160-200 °C) on the peroxidase-like catalytic activity of GO/Au-PtNPs composites during preparation. The effects of composite concentration (1-8 µL), TMB concentration (0.03-4.0 mM), hydrogen peroxide concentration (0.002-0.05 M), pH (3.4-8.0), and reaction time (1-8 min) on the colour change of the system were investigated. The colorimetric sensor was constructed in the solution system to achieve the simultaneous detection of silver and mercury ions bimetallic.

iii. hydrothermal synthesis of GO/Pt@AuNPs composites, QFP, QTFP and AF), composite concentration (8.427-168.531 $\mu\text{g/mL}$), TMB concentration (0.1-10 M), hydrogen peroxide concentration (0.01-10 M), pH (3.5-8), and reaction time (0.5-5 min). The colorimetric sensors were constructed in the solution system for simultaneous detection of silver and mercury ions. Simultaneous detection of silver and mercury ions was achieved.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

This chapter consists of seven main subsections. Among them, Section 2.2 is an Overview of heavy metal pollution in water in which the sources and hazards of heavy metal pollution are described in detail. In Section 2.3, common detection methods and problems of heavy metals are discussed. Among them, the colorimetric detection method is described in detail in Section 2.4. Currently, colorimetric sensing detection can be mainly classified into two categories, which are Colorimetric sensors based on SPR variation and Colorimetric sensors based on nano enzyme catalysis. However, at present, colorimetric sensing detection is limited by the limitation of materials, and the synthetic nanomaterials with poor stability, which affects the reliability and sensitivity of the detection. However, the incorporation of carriers would be a good solution to this problem. Therefore, a good carrier Graphene oxide, and its application in colorimetric detection is introduced in detail in Section 2.5. Section 2.6 introduces paper-based colorimetric sensors, which have a good prospect of application in real-time on-site detection, and details the definition, classification, and application of paper-based colorimetric sensors. Section 2.6 introduces paper-based colorimetric sensors which have promising applications in real-time field detection. The last Section is 2.7, which summarises the Research Gap that exists in this research area nowadays.

2.2 Overview of heavy metal pollution in water

Heavy metals are generally defined as metals with a density greater than 5 g/cm³ or a relative atomic mass greater than 65. These metals typically have high densities, melting points and electrical conductivity. Heavy metals have a wide range of applications in industry, but may also cause environmental pollution problems. Heavy metal pollution refers to environmental pollution caused by heavy metals and their compounds. Heavy metal pollution in water bodies occurs when contaminants containing heavy metals enter a water body, exceeding its self-purification capacity and causing changes in the composition of the water and its properties, thus leading to the deterioration of biological growth conditions in the water environment and causing adverse effects on human life and health. The presence of heavy metals has posed a serious concern for human health over the last few decades due to increasing industrialization and urbanization. Due to the excessive leaching of these nonbiodegradable metals, there has been much discussion about the importance of heavy metals and their detection (Elsagh et al., 2021).

2.2.1 Sources of heavy metal pollution

Heavy metals play a very important role in industrial production. They have been used in many different fields of production and life for thousands of years (Li et al., 2022). For example, as early as 5000 years ago, lead was used in building materials, glass and ceramic pigments, and water pipelines. The ancient Romans used lead acetate to increase the sweetness of wine. Mercury-containing ointment was used to relieve the pain of the children while teething. Cadmium-containing pigments were widely used by Claude Mott in the mid-19th century, and cadmium was widely used in art materials in the early 20th century. Because of the many applications of heavy metals, heavy metals are widely exploited and used in various industrial productions because of social and economic needs.

As a result, an increasing amount of heavy metals are discharged into the environment. Heavy metal resources are the basis and important part of national economic development. On the one hand, they have made great contributions to the rapid development of the social economy. On the other hand, a large number of heavy metals are discharged into the environment, causing severe heavy metal pollution (Wei et al., 2021).

Table 2.1: Main sources of heavy metal pollution (Wu et al., 2022)

Name of heavy metals	Main sources
Ag	Mining, electrical, chemical production, jewelry and metal processing, waste film, etc.
Cu	Mining and metallurgy, electrical, light industry, metal processing, steel production, etc.
Hg	Wastewater from chloralkali, plastics, batteries, electronics, cosmetics, waste medical equipment coal burning, etc.
Zn	Zinc plating industry, automobile manufacturing, machinery industry, electromagnetic industry, etc.
Pb	Lead-containing batteries, gasoline, and pigments, jewelry and glass manufacturing, mining and metallurgy, automobile exhaust emissions, etc.
Cd	Mining and metallurgy, cadmium-containing batteries and plastic waste incineration, phosphate fertilizer manufacturing, and a variety of electronic products production
Cr	Smelting, chromium salt manufacturing, electroplating, metal processing, leather making, paint, pigment, printing and dyeing industry, etc.
Mn	Gravel, mining, welding, dry battery production, dye industry, etc.

Heavy metals enter the environment mainly through human activities. Traditionally, heavy metals can be divided into industrial and agricultural sources. With the acceleration of urbanization, specific urban pollution sources have also emerged (Elsagh et al., 2021). The industrial sources are mainly coal and petroleum. They are the main sources of mercury, lead, cadmium, arsenic, chromium, and other heavy metal pollutants. Mining, mineral processing, smelting, forging, electroplating, processing, coal, and transportation can result in the pollution of a large number of heavy metals. In agricultural production, the uncontrolled use of polluted irrigation, pesticides, and inferior chemical fertilizers can also cause heavy metal pollution, especially from pesticides and chemical fertilizers. Generally, there are different kinds of heavy metals. If heavy metals are not used properly, a large number of heavy metals will enter the environment and cause heavy metal pollution. The development of cities has made it one of the sources of heavy metal pollution. The main sources of heavy metal pollution are listed in Table 2.1 (Jeong, et al., 2021; Yu, et al., 2021; Lna, et al., 2021).

2.2.2 Heavy metal pollution hazards

In recent years, with the intensification of heavy metal pollution in water bodies, heavy metal pollution incidents have occurred frequently. The famous diseases are "Minamata disease" in Japan caused by mercury pollution in 1950 and "painful disease" in Japan caused by cadmium pollution in 1955. In addition, in 2005, the incident of cadmium pollution in the Beijiang River of Guangdong Province in China led to a serious threat to drinking water in three cities downstream of the Beijiang River, namely, Shaoguan, Qingyuan, and Yingde. Furthermore, in 2005, cadmium contamination in the Beijiang River in Guangdong Province led to a serious threat to the drinking water of Shaoguan, Qingyuan, and Yingde cities downstream of the Beijiang River. In 2006, arsenic

contamination in Yueyang County in Hunan Province led to a serious impact on the drinking water safety of 80,000 residents. In 2009, the blood lead concentration exceeded the standard in Wugang City, Hunan Province, resulting in nearly 100 people suffering from lead poisoning. In 2011, the copper and chlorine in drinking water exceeded the standard in Ruichang, Jiangxi Province, resulting in a serious impact on the drinking water safety of construction workers and residents around the site. In 2011, excessive copper and chlorine were incidented in drinking water in Ruichang, Jiangxi Province, resulting in approximately 50 people being hospitalized at the construction site and surrounding residents. The frequent occurrence of heavy metal pollution incidents has caused serious physical and psychological harm to people and is a constant reminder of the seriousness of heavy metal pollution (Wu et al., 2022). Some of Malaysia's rivers and water sources have been affected by heavy metal contamination, including Arsenic, Cadmium, Copper, Lead and Zinc. In April 2019, the Leh River in Upper Perak was exposed to arsenic contamination. In response, the Department of Geology and Minerals Malaysia (DGM) set up a task force to conduct comprehensive water quality tests to determine the exact cause and extent of the contamination. In October 2020, contamination of raw water in the Selangor River led to more than 1,000 districts facing water cuts, affecting millions of customers. Although the type of contaminant was not specified, the incident demonstrated the severity of the water quality problem. In March 2019, the Jinjin River near the industrial city of Pasir Gudang in southern Johor was contaminated with toxic chemical wastes, resulting in the poisoning of more than 2,000 people, including several children. Although heavy metals were not explicitly mentioned, the incident highlights the potential risk to water safety from industrial waste discharges (Ahmed et al., 2020). Excessive heavy metals in water cause immediate damage to aquatic creatures and plants. Heavy metals are

abundant in living organisms. Double metals collect and spread through the food chain, eventually entering the human body and endangering human health indirectly (Proshad et al., 2021). The effects of eight common heavy metals, including Ag, Cu, Hg and Zn, on human health are listed in Table 2.2. The main hazards of heavy metals include neurotoxicity, kidney damage, reproductive system abnormalities, immune system suppression and carcinogenic effects, and long-term exposure may also lead to developmental disorders and chronic diseases. To reduce possible health hazards, the U.S. Environmental Protection Agency (EPA) limits heavy metals in drinking water to 2 to 15 gL⁻¹ (Maleki et al., 2021). Therefore, the detection of trace heavy metals is highly important for environmental monitoring, food safety, and medical and clinical testing. For better monitoring, suitable techniques, such as rapid and sensitive assays, must be developed to prevent heavy metal contamination or to protect the environment and human health.

Table 2.2: Symptoms of excessive heavy metals in the body (Fu et al., 2020; Mitra et al., 2022; Witkowska et al., 2021)

Name of heavy metals	Hazards for Human
Ag	The accumulation of silver in the body may cause argentosis and cause skin discoloration. After accumulating in the human body, it can cause serious damage to cell membranes through extracellular mechanisms, causing skin irritation, stomachache, organ edema, and even death. (Nain et al., 2020)
Cu	Copper deficiency and abnormal copper metabolism can cause many diseases such as Wilson's disease and copper deficiency. (Fagnano et al., 2020)

Table 2.3: continued

Hg	Mercury primarily damages the central nervous system and the brain, leading to symptoms such as limb numbness, dyskinesia, hearing loss, and other effects associated with mercury poisoning encephalopathy. (Santos et al., 2021)
Zn	Zinc abnormality in the human body may be related to various pathological processes, such as Alzheimer's disease, epilepsy, ischemic stroke, etc. (Liang et al., 2021)
Pb	It mainly harms the nervous system, hematopoietic system, and kidneys. It damages the bone marrow, leading to anemia, cerebral hypoxia, brain edema, and motor and sensory abnormalities. (Nain et al., 2020)
Cd	Cadmium in the human body can participate in the formation of cadmium sulfur metalloproteinases, mainly accumulated in the kidney and liver, and damage other organs. (Nain et al., 2020)
Cr	It causes skin congestion, erosion, ulcers, nasal perforations, including skin cancer because it is acidic and inflammatory to the layer of skin, nasal passageways, and digestive tract. It can build up in the kidneys, liver, and lungs.
Mn	It can affect the conduction capacity of synapses, and hyperthyroidism will be caused when excessive. (Miah et al., 2020)

2.3 Conventional methods for heavy metal detection

The pollution of the ecological environment by heavy metals is becoming increasingly serious, posing a serious threat to human health, and the rapid and accurate detection of heavy metals is urgently needed. At present, the analysis methods for heavy metals, such as spectroscopy, mass spectrometry, chromatography, and electrochemistry, are becoming more mature and diversified. As can be seen from Table 2.3, the following analytical and detection methods are currently in common use: Spectroscopic methods,

Mass spectrometry, Chromatography, Electrochemistry. However, these methods are time-consuming and require a high level of sample pretreatment. In recent years, with the continuous innovation and development of science and technology, methods for the analysis and detection of heavy metal ions have been enriched, and simpler, more rapid, and more efficient methods are expected.

2.4 Colorimetric detection methods for heavy metals

Colorimetric sensors are a class of devices that capture the interaction between a recognition element and a target analyte to produce a detection signal and convert it to an optical signal via a conversion element. They have attracted a great deal of interest from researchers due to their advantages of visualizing detection results, simple material preparation, rapid detection, and no need for complex instrumentation. Currently, the main types of heavy metal colorimetric sensors constructed from nanomaterials are those based on surface plasmon resonance (SPR) changes and those based on nano enzyme catalysis (Ye et al., 2021; Takemura et al., 2021; Kongpreecha et al., 2022; Wang et al., 2020c; Yang et al., 2020a; Yang et al., 2021).

2.4.1 Colorimetric sensors based on SPR variation

SPR, or surface plasmon resonance, is a special property of metal nanostructures. The aggregation and breakdown of metal nanostructures can alter SPR, which can be used to create colorimetric sensors. Due mostly to local surface plasmon resonance, certain kinds of metal nanoparticles, such as AuNPs and AgNPs, can display extremely bright colors in the visible spectrum (LSPR) (Ye et al., 2021).

Table 2.4: Advantages and disadvantages of conventional detection methods

Testing methods		Principle	Advantages	Disadvantages	Reference
Spectroscopic methods	Atomic absorption spectroscopy, AAS	Quantification based on the intensity of absorption of the light source by the atoms of the metal element to be measured as they leap from the ground state to the excited state under radiation	Low detection limit, high sensitivity, high precision, good selectivity, fast detection speed, low interference, simultaneous determination of multiple elements	Problems with a selection of multielement light source lamps, complex and demanding for sample pretreatment	(Aquisman et al., 2020)
	Atomic fluorescence spectrometry, AFS	Based on the absorption of a specific frequency of radiation by the base state atomic vapor of a metal element, fluorescence in the form of optical radiation with characteristic spectral wavelengths and intensities is emitted under a certain excitation, and quantitative detection of the content of the metal element is achieved based on the fluorescence intensity.	High sensitivity, wide detection range, good selectivity, small sample size, and simultaneous determination of multiple elements	The sample to be tested is limited by its ability to emit fluorescence and requires a pretreatment to achieve fluorescence analysis.	(Liu et al., 2021b)

Table 2.3: continued

Spectroscopic methods	Atomic emission spectroscopy, AES	The spectrum emitted by the atoms or ions in the sample when they return to their ground state under a certain excitation is used to characterize the sample to be measured, and the content of the metal element to be measured is quantified according to the intensity of its characteristic radiation	Good selectivity, high precision, rapid testing process, wide range of applications	It is not possible to measure multiple elements at the same time, the linear range is not wide enough in practical applications and the sensitivity is not high.	(Vasil et al., 2022)
	UV–Vis spectrophotometry	The colored product is produced by complexation with a chromogenic agent and the characteristic absorption peak corresponding to the absorption intensity is obtained from its absorption spectrum. Quantification of the concentration of the substance to be measured according to the Lamber-Beer law	Simple instrumentation and operation, high accuracy, fast detection, and good repeatability	Low selectivity due to possible overlap of spectral lines, causing interference and introducing operational errors during the conversion of colored substances	(Kulkarni et al., 2015)

Table 2.3: continued

Mass spectrometry	Ion-coupled plasma-mass spectrometry, ICP-MS	The ionization of the sample components into ions with different charge-to-mass ratios, which further enter the mass analyzer and are separated by mass under forces such as electric or magnetic fields to obtain a mass spectrum to quantify the content of the substance to be measured.	Small sample size, wide detection range, low detection limits, high accuracy fast detection, low interference, isotope tracing, and simultaneous detection of multiple elements	Complex instrument structure, time-consuming operation, cumbersome sample handling, and high price	(Xiong et al., 2020)
Chromatography	High-performance liquid chromatography, HPLC	The colored organic reagents will be chelated with the heavy metal elements and further separated and quantified by the column	High efficiency with low sample usage and simultaneous detection of multiple metal elements	The test takes a long time and the colored reagents are usually toxic and can easily cause harm to humans and are not suitable for on-site, rapid testing.	(Chen et al., 2021a)

Table 2.3: continued

Electrochemistry	Amperometric, polarographic, anodic voltammetry, etc.	This analytical method is based on the basic principles and experimental techniques of electrochemistry and the determination of the composition and content of heavy metal ions involved in chemical reactions by measuring physical quantities such as current, conductance, potential, and power in solution	Simple instrumentation, high sensitivity, wide detection range, low detection limits, low cost, and easy to automate	Cumbersome electrode modification, high interference, poor reproducibility, poor stability, easily limited in practical sample detection	(Dai, 2020)
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According to Takemura et al. (2021) LSPR is the outcome of relationships with electromagnetic fields that cause conduction band electrons to collectively oscillate. These nanoparticles are appropriate as probes for colorimetric measurement due to their significant molar extinction coefficients in the visible region (Kongpreecha et al., 2022). The use of SPR nanomaterials for colorimetric sensing generally depends on changes in different optical characteristics brought about by the agglomeration and dispersion of metal nanoparticles.

Monodisperse and aggregated nanoparticles show distinct hues as a result of SPR's properties. It is convenient for measurements to be made with the naked eye since the solution color of the nanoparticles changes when they agglomerate. The characteristics of the particle surface or the solution's composition are what lead to this aggregation process (Zhu et al., 2021). Figure 2.1 shows a graphic that explains the fundamental process of hydrogen bonding or electrostatic interactions between nanoparticles and their environment.

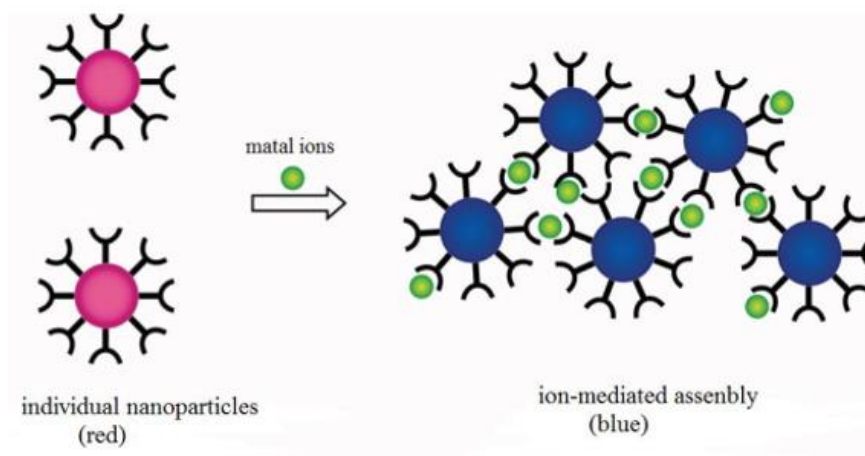


Figure 2.1: Schematic diagram of the functional AuNP colorimetric method for the detection of heavy metals (Saha et al., 2011)

Most of the sensors of this type use AuNPs in solution in an aggregated or dispersed state to show different colors corresponding to the target and detect the target according to the color change of the solution.

For example, Li and Liu reported a method for the determination of Hg^{2+} by unmodified gold nanoparticles and the T- Hg^{2+} -T complex; T-T mismatches can effectively coordinate with Hg^{2+} , resulting in a noncomplementary mismatch of the DNA strand that can also be fully hybridized. In both groups, T-rich well-organized oligonucleotides were mixed with unmodified gold nanoparticles. If Hg^{2+} is present in the sample solution for analysis, the oligonucleotide sequence will form a folded structure and peel off from the surface of the gold nanoparticles. The colloidal stability of the gold nanoparticles is greatly reduced, and the rapid aggregation of particles leads to a color change in the solution, which indicates the presence of Hg^{2+} (Li, et al., 2021; Pei, et al., 2023). Peng et al. (2017) developed an automatic colorimetric sensor based on AgNPs. It is used to detect trace Cu^{2+} in water samples. Compared with that of the colorimetric detection method based on AgNP aggregation, the detection speed of this method is faster, but the detection limit is greater than 0.24 $\mu\text{g/L}$. To improve the detection limit, Memon et al. (2020) used cobalt (II) ions as an ExoIII cofactor and Hg^{2+} -triggered ExoIII auxiliary signal amplification and unmodified AuNPs as indicators. A hairpin loop DNA probe with a thymine-rich recognition terminal was rationally designed, and Hg^{2+} was specifically recognized by the stable T- Hg^{2+} -T structure, which reduced the detection limit of mercury ions and realized the quantitative detection of trace Hg^{2+} with a detection limit of 0.2 nm. The detection mechanism is shown in Figure 2.2.

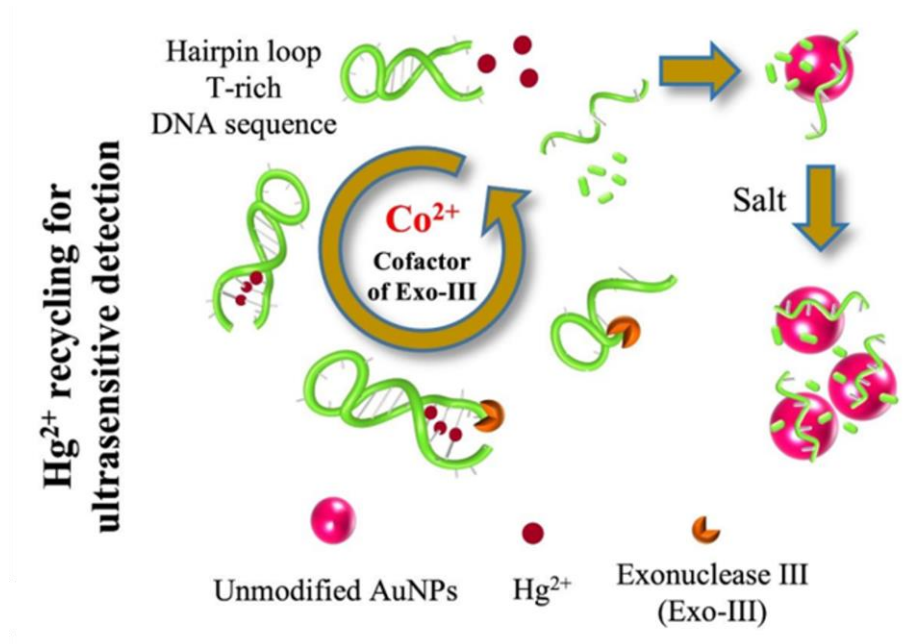


Figure 2.2: Mechanism of Hg^{2+} detection using exo III auxiliary target cycle amplification and unmodified AuNPs as indicators (Memon et al., 2020)

To improve the anti-interference performance of gold nanoparticles, Ling et al. (2020) added Hg^{2+} to coat the growth solution and deposited it on the high-energy surface of the material, which affected the deposition mode of subsequent Au_0 and Au^0 and ultimately led to the formation of defective AuTNPs. The change in morphology is reflected by the wavelength shift of the LSPR, which is proportional to the concentration of Hg^{2+} . This method can further improve the stability of gold nanoparticles and eliminate the interference of Ag^+ . The detection mechanism is shown in Figure 2.3.

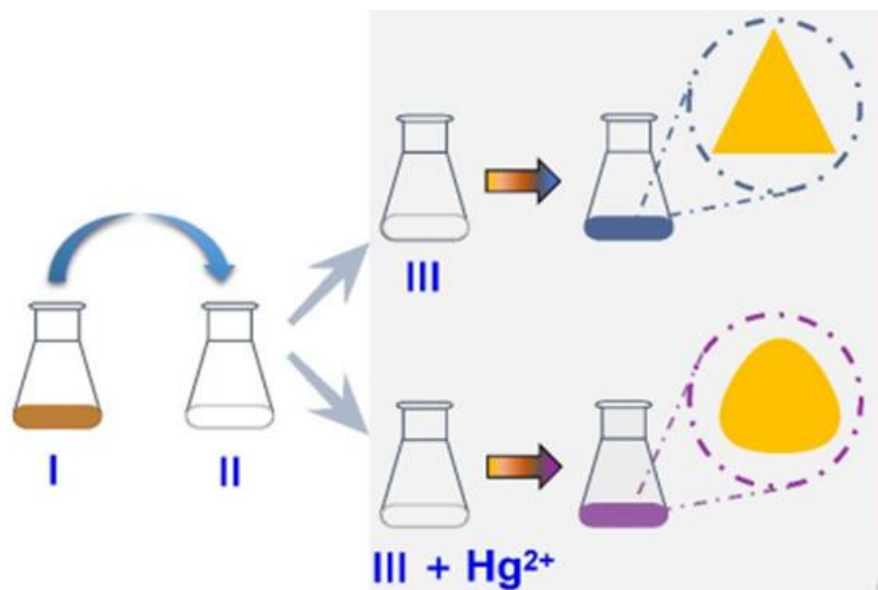


Figure 2.3: Detection mechanism of mercury(II) by target interference during in situ seeding growth of gold triangle nano prisms (Ling et al., 2020)

The stability of nanoparticles has a great influence on the accuracy and resistance to interference during heavy metal detection. When temperature and other interfering ions are present, the detection results are not accurate enough. In addition to the abovementioned sensing strategies, researchers often use DNA, peptides, and amino acids to improve the stability of nanoparticles and improve the accuracy of heavy metal detection (Xue et al., 2008, Chai et al., 2010, Lee et al., 2020, Li et al., 2021).

Lee's team purposefully designed a T-T mismatched DNA strand. The coordination structure of mismatched T and Hg^{2+} was stable, which led to an increase in the temperature of the double-stranded DNA; the detection of Hg^{2+} in an aqueous solution was carried out by changing the color of the gold colloid. The sensitivity of this method can reach the ppb level, while the sensitivity of the conventional color detection method can reach only the micromole level due to the low extinction coefficient (Lee et al., 2020). The Xue team designed and increased the number of mismatched thymines in the functional DNA double

strand to reach 8 pairs. An increase in the number of mismatched bases in the DNA chain will reduce the temperature of unwinding. Detection can be carried out at room temperature. Due to the color change of gold nano sol, the visible detection limit can reach $3\mu\text{mol}\cdot\text{L}^{-1}$ (Xue et al., 2008). Although DNA-modified nanogold colorimetric detection can achieve good sensitivity and selectivity, the detection conditions, such as the reaction temperature, should be controlled accurately. In addition, the design and synthesis of specific thiol-containing DNA sequences that can bind to gold particles are expensive and complex, which makes wide use difficult.

The Chai team detected Hg^{2+} in an aqueous solution using L-cysteine-functionalized gold nanoparticles (CYS-GNPs). A colorimetric response of the CYS-GNPs to Hg^{2+} in aqueous solution was observed. This method has good selectivity for other divalent metal ions for Hg^{2+} detection, with a minimum detection limit of $100\text{ nmol}\cdot\text{L}^{-1}$, but requires the assistance of an ultraviolet light source (Chai et al., 2010). Li et al. (2021) constructed a rapid and accurate mercury ion (Hg^{2+}) biosensor by modifying the surface of gold nanoparticles with polypeptides. The peptide DGPFHR can capture Hg^{2+} with conformational folding. Peptide folding reduces electrostatic repulsion and steric hindrance, thus accelerating the aggregation of AuNPs. In this case, D/C-AuNPs were used as a detection probe for the quantitative analysis of Hg^{2+} , and the linear range was 100-800 nm. As shown in Figure 2.4, compared with that of DNA, the poor stability of amino acids or peptides is an important factor restricting the development of such gold nanoparticle detection methods.

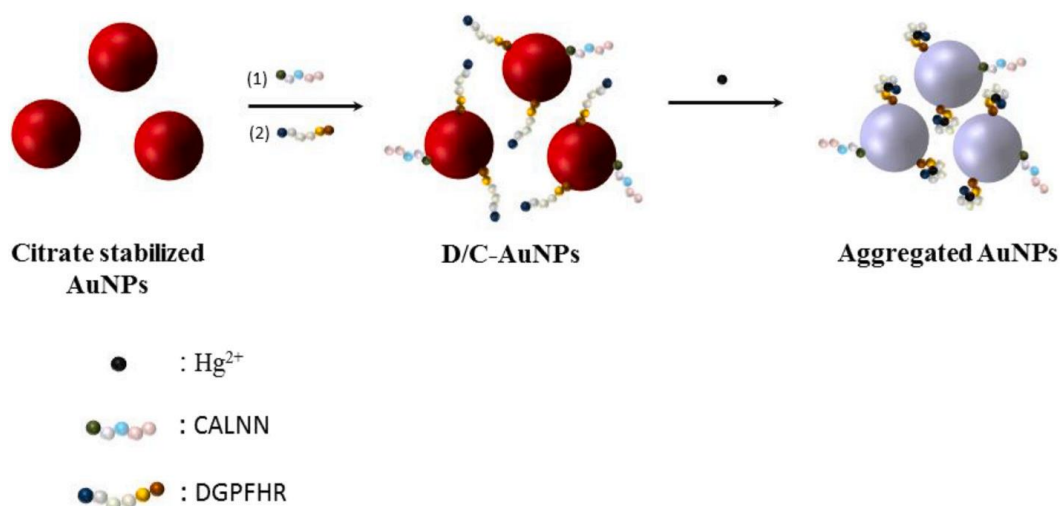


Figure 2.4: Schematic illustrations of Hg^{2+} -induced aggregation in the D/C-AuNPs system (Li et al., 2021a)

2.4.2 Colorimetric sensors based on nano enzyme catalysis

The common enzymatic reactions can be divided into natural enzyme reactions and mimic enzyme reactions. Moreover, there was no significant difference in the catalytic mechanism between the two enzymes. It is difficult to prepare natural enzymes. However, the stability and catalytic activity of the mimic enzyme are excellent. Researchers have found that graphene oxide, precious metals, metal oxides, and other nanomaterials have intrinsic enzyme-like activity and can simulate the catalytic activity of natural enzymes (such as oxidases and peroxidases). Nano-enzymatic catalysis based colorimetric sensors use nanomaterials to react with the target heavy metal ions, which will lead to inhibition or enhancement of the catalytic properties of the nanomaterials, which in turn will cause a change in the colour of the substrate to ultimately achieve the detection of the target heavy metal ions. (Wang et al., 2020d; Yang et al., 2020a; Yang et al., 2021).

Cu(I) 1.28 Cu(II) 0.36Se nanoparticles were created by Yang et al. (2021). These nanoparticles have peroxidase-like activity and are inhibited by Hg(II) but not by Ag(I) .

This special characteristic, in conjunction with the solubility product principle, has made it possible to identify and detect Hg(II). Similar to this, Yang et al. (2020) created a unique colorimetric sensing method for mercury speciation based on gold nanoparticles' simulated peroxidase activity with the help of several reducing agents. Only inorganic mercury can be reduced to Hg^0 in the presence of a weak reducing agent; both inorganic and organic mercury can be reduced to Hg^0 in the presence of a strong reducing agent. The simulated peroxidase activity of gold nanoparticles is significantly increased when mercury is placed on the surface of AuNPs in the form of gold amalgam due to the high affinity between mercury and gold. A variety of mercury forms were identified utilizing 3,3',5,5'-tetramethylbenzidine (TMB) as a substrate based on this effect.

In addition, the specificity of the colorimetric sensor can be improved by the specific recognition of nucleotides and heavy metals. Wang et al. (2020a) reported that Mn_3O_4 nanoparticles (NPS) could catalyze the oxidation of tetramethylbenzidine (TMB). A colorimetric method for the determination of heavy metal ions based on the simulated oxidation activity of Mn_3O_4 nanoparticles (NPS) regulated by oligonucleotides was proposed. Tetramethylbenzidine (TMB) was oxidized in the presence of Mn_3O_4 NPs, and the product was yellow in an acidic solution. It was discovered that Mn_3O_4 nanoparticles adsorbed random oligonucleotides on the regular surface, thereby temporarily impeding TMB oxidation. As a result, the absorbance decreases, and the solution changes to a light green color. The binding of heavy metal ions to thymine is crucial when heavy metal ions, such as Hg(II) or Cd(II), are present. The solution's color changed from light green to yellow as the inhibition disappeared. The presence of heavy metal ions is associated with an increase in absorbance at 450 nm. Figure 2.5 illustrates the mechanism for the detection limits of Hg(II) and Cd(II), which were 3.8 and 2.4, respectively.

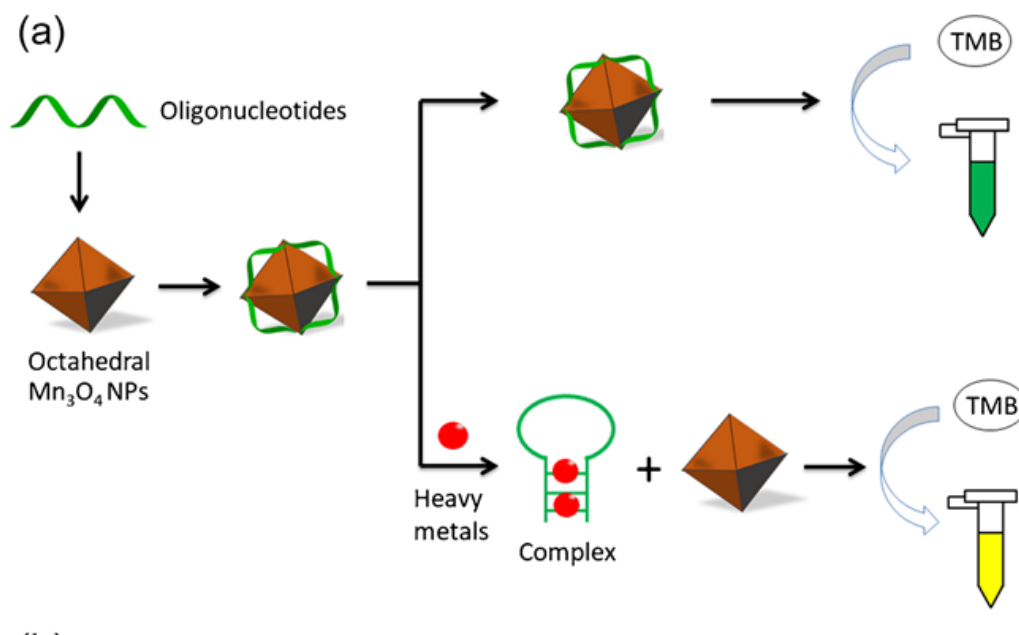


Figure 2.5: Schematic illustration of the method used for heavy metal detection based on the oxidase-mimicking activity of octahedral Mn_3O_4 NPs (Wang et al., 2020a).

However, the small size and large specific surface area of nanomaterials tend to agglomerate, which affects their catalytic performance. The catalytic performance will affect the sensitivity, selectivity, detection limit, linear range, and other detection methods of the color sensor. Therefore, the selection of appropriate carriers to improve the dispersion and catalytic performance of nanomaterials has become a research hotspot. At present, the reported carriers include MOFs, carbon quantum dots, and chitosan (Wang et al., 2020a; Yang et al., 2020b; Li et al., 2021b; Eskandari et al., 2020).

Gold amalgam has demonstrated potential as a nanoenzyme, although there are still few applications for it in real life. The aggregation of gold nanoparticles leads to a decrease in catalytic activity. Therefore, further design of Au NPs is needed to prevent aggregation and increase enzyme activity for the detection of heavy metal ions. Wang (2020b) synthesized hybrid materials of gold nanoparticles and MOFs by using metal-organic frameworks

(MOFs) as carriers (AuNP@MOF). In this method, Au NPs were uniformly immobilized on the surface of Fe-5,10,15,20-tetra (4-carboxyl), and 21 h and 23 h porphyrin MOFs (Fe-TCPPh-MOFs) were successfully synthesized. Based on the Hg^{2+} ions, the Au-catalyzed reduction of methylene blue (MB) was triggered to realize stable detection of Hg^{2+} ions, as shown in Figure 2.6. Furthermore, by modifying the pH, Yang et al. (2020) created and suggested a unique hollow iron oxide that is formed from the metal-organic framework (MOF) @MOF and may mimic the intrinsic activities of oxidase and peroxidase. A new colorimetric transport platform for Cys and Hg^{2+} was created based on the fact that L-cysteine (Cys) inhibits oxidase-like activity and that Cys can regain oxidase-like activity through a unique complexation with mercury (II) ions (Hg^{2+}). Cys concentrations have a linear range of 1 to 25 μM . Hg^{2+} has a linear range of 0.1 to 15 μM . according to Figure 2.7. Li et al. (2021) used a controlled supramolecular self-assembly technique to create carbon nitride quantum dot-doped mesoporous melamine silver (MA-Ag) nanocomposites. Ag@Hg can produce Hg^{2+} , and the nanoflowers made from CNQDs@MA-Ag nanocomposites were shown to have a catalytic action similar to peroxidase. The catalytic effect was specifically enhanced by the alloy. Dual catalytic colorimetry was developed as a quick and efficient method to measure the levels of mercury (Hg^{2+} and Hg^0) in wastewater samples. The Hg^+ and Hg^0 contents in the wastewater samples could be reduced to 0.050 nM and 18.3 nM, respectively. Eskandari et al. (2020) prepared silver manganese disulfide/chitosan polyvinyl alcohol (Ag-MnS₂/CSPVA) nanocomposites for the detection of toxic heavy metals using chitosan as the carrier. The presence of different metal ions does not affect the detection of Hg^{2+} . The results showed that the mercury concentration exhibited a good linear change under the detection limit of 9.0 nM (nanomole level). The literature related to the detection of heavy metal ions by colorimetric

sensing in recent years is listed in Table 2.4. As shown in Table 2.4, based on the detection mechanism, the colorimetric detection of heavy metals can be divided into two categories. One is the detection of targets by SPR changes relying on precious metal nanoparticles. The other is to rely on the enhancement or inhibition of the catalytic properties of the nanomaterials by examples of the target heavy metals.

However, due to the limitations of carriers, the catalytic activity of these sensors is still not high enough, which leads to problems such as long response times, low sensitivities, and narrow detection ranges (Eskandari et al., 2020). And most colorimetric sensors can only detect a single metal, it is difficult to detect two different metals at the same time. Therefore, the development of new nanocarriers is of great significance for the accurate, sensitive and stable simultaneous detection of different heavy metal ions.

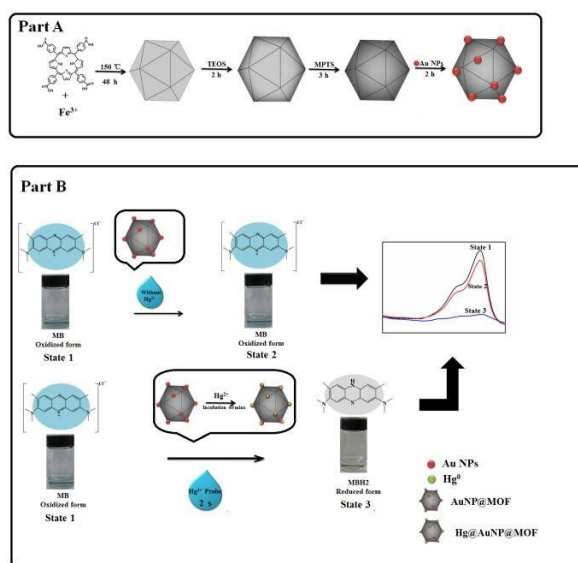


Figure 2.6: (a) Synthesis of AuNP@MOF; (b) schematic illustration of the AuNP@MOF probe for the detection of Hg^{2+} ions (Wang et al., 2020b)

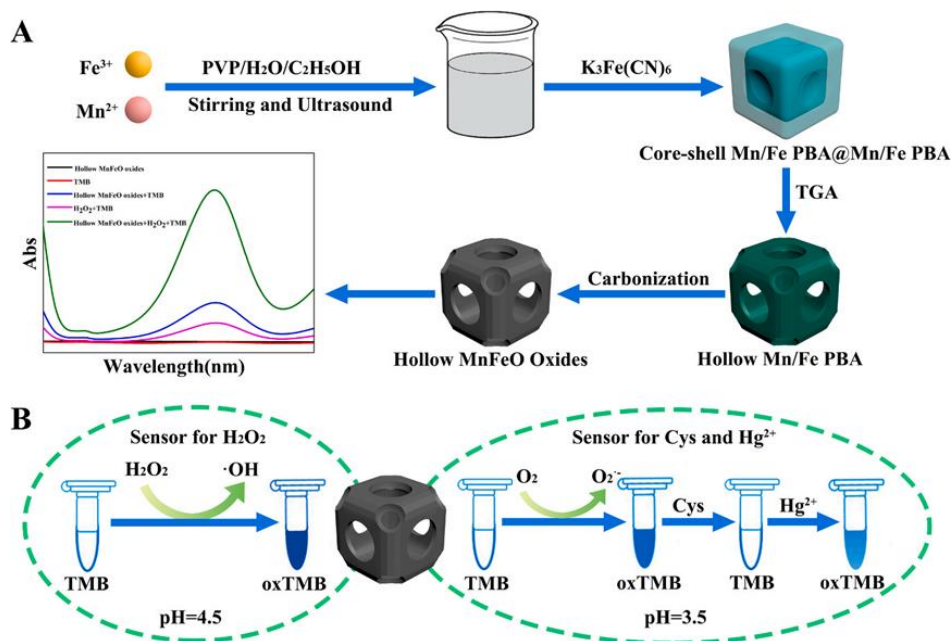


Figure 2.7: (a) Synthesis of hollow MnFeO oxide and (b) a scheme for chromatic detection of biomolecules and Hg^{2+} based on its simulated envelope activities (Yang et al., 2020a)

2.5 Graphene oxide and its application in colorimetric detection

Graphene is the latest member of the family of carbon nanomaterials. Its single-atom two-dimensional materials have a honeycomb structure due to the sp^2 hybrid bonding between its carbon atoms (Wu et al., 2023). Its thickness is only 0.35 nm, and the length of the carbon-carbon bond is approximately 0.142 nm. The cellular lattice of graphene is a lattice of carbon atoms held together by two equivalent σ bonds. Moreover, the outermost layer of each carbon atom has a π orbital perpendicular to the graphene plane, forming a delocalized electronic network, and electrons can move freely in graphene (Carr et al., 2023). Graphene, the fundamental building block of graphite materials in all other dimensions, is a type of carbon atom firmly packed into a two-dimensional honeycomb lattice in a planar monolayer, as seen in Figures 2.8 and 2.9. It can be rolled into one-dimensional nanotubes, layered into three-dimensional graphite, or packaged as zero-dimensional fullerenes. As a result, graphene serves as the building block for all other

Table 2.5: Review of colorimetric sensing of heavy metals in recent years

S.No	Material	Sensor configuration	Metals detected	Range of detection	LOD	Detection time	References
1	AgNPs	SPR changes	Hg(II)	20-100 nM	17 nM	Over 10 min	(Janani et al., 2020)
2	Double peptide modification of AuNPs	SPR changes	Hg(II)	100-800 nM	28 nM	/	(Li et al., 2021a)
3	Tris-base (TB) modified Ag NCs	SPR changes	Hg(II)	5×10^{-15} M- 1×10^{-10} M	0.35 fM	/	(Li et al., 2020)
4	Exonuclease III (EXO-III) assists AgNPs	SPR changes	Hg(II)	0.5-5.0 nM	1 nM	40 min	(Memon et al., 2020)
5	Triangular nano prism AuTNPs	SPR changes	Hg(II)	0.10-40 nM	0.12 nM	20 min	(Ling et al., 2020)
6	AgNps	SPR changes	Fe(II) Fe(III)	5-100 μ M	Fe(II) 1 μ M Fe(III) 5 μ M	1 min	(Dayanidhi et al., 2021)
7	3-(tri methoxy silicon) propyl methacrylate (TMPM)	SPR changes	Hg(II)	20-100 nM	1 nM	/	(Balasurya et al., 2020)

Table 2.4: continued

8	Mn ₃ O ₄ NPs	Nanase catalysis	Hg(II) Cd(II)	Hg(II) 10-200 µg/L Cd(II) 10-100 µg/L	Hg(II) 3.8 µg/L Cd(II) 2.4 µg/L	/	(Wang et al., 2020a).
9	AuNPs	Nanase catalysis	Hg(II) The total mercury	5-100 nM	Machine mercury 1.9 nM The total mercury 0.9 nM	Over 35 min	(Yang et al., 2020a)
10	Cu(I) _{1.28} Cu(II) _{0.36} Se NPs	Nanase catalysis	Hg(II)	10-400 ppb	0.01 ppb	Over 35 min	(Yang et al, 2020b)
11	CNQDs@MA-Ag	Nanase catalysis	Hg(II) Hg ⁰	Hg(II) 0.010-25 µM Hg ⁰ 0.040-140 µM,	Hg ²⁺ 0.050 nM. Hg ⁰ 18.3 nM.	30 min	(Yin et al., 2021)
12	AuNP@MOF	Nanase catalysis	Hg(II)	200-400 pM	103 pM	2 s	(Wang et al., 2020b)
13	Novel paper-based analysis device for ion Selective Polarizing Devices (ISOs)	Chromogenic agent	Ag(I) Hg(II)	Ag(I) 1.92×10 ⁻⁶ - 5.00×10 ⁻³ M Hg(II) 5.74×10 ⁻⁷ - 5.00×10 ⁻⁵ M	Ag(I)1.92×10 ⁻⁶ M Hg(II) 5.74×10 ⁻⁷ M	20 min	(Phichi et al., 2020)
14	Gold-silver nanoparticles	SPR changes	Cd(II)	0.4 ~ 38.6 pM	44 nm	10 min	(Du et al., 2018)

graphite compounds. The most stable six-membered benzene ring found in organic materials, graphene's fundamental structural unit is the perfect two-dimensional nanomaterial.

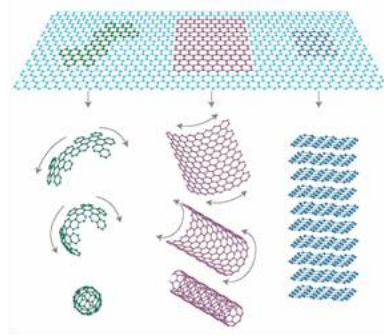


Figure 2.8: Schematic diagram of the morphology and structure of graphene (Geim, 2004)

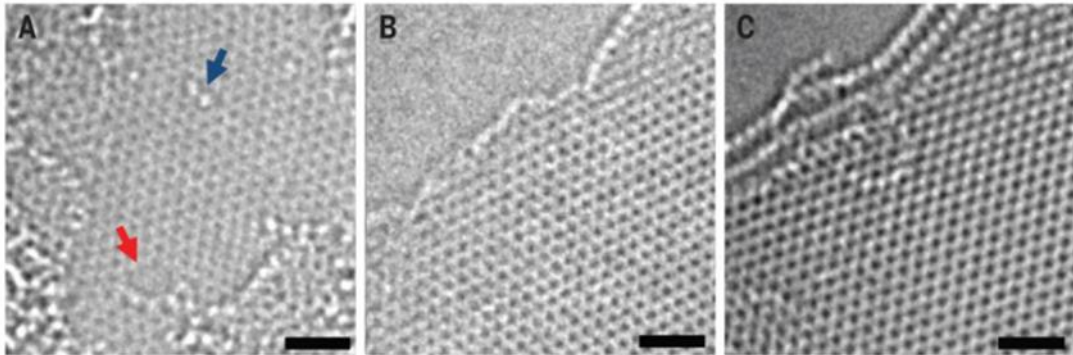


Figure 2.9: High-resolution transmission electron microscopy image of graphene (Voiry et al., 2016)

Based on their unique two-dimensional structure, graphene and its derivatives (such as graphene oxide and nitrogen-doped graphene) have excellent electrical properties and electron transport capacity (Thejas et al., 2022), high flexibility and impermeability (Liu, 2021a), strong mechanical strength (Zhu et al., 2022) and excellent thermoelectric conductivity (Chen et al., 2023). These properties enable graphene and its derivatives to

provide new methods for many fields and even help them make key progress. Therefore, although it has only a short history of more than ten years, graphene has set off an unprecedented research upsurge in many fields. Graphene oxide is a unique branch of graphene.

2.5.1 Structure of graphene oxide

To effectively regulate the basic properties of graphene oxide and explore its potential application value, it is very important to grasp and study its structure. Research on the structure of graphene oxide has been conducted via structural analysis. Although graphite oxide was prepared in the middle of the 19th century, due to its nonstoichiometric composition and strong water absorption properties, its composition and structure have not been determined. In some studies, graphene oxide is considered to be obtained by modifying oxygen-containing functional groups on the sheets and edges of graphene monolayers (Mkhoyan et al., 2010). Most of the precise atomic and electronic structures of graphene oxide are unknown. Moreover, the structures of graphene oxide prepared by different preparation methods are also very different. However, additional details about the structure of graphene oxide, such as the most likely major structural pattern, are still under investigation.

For many years, a large number of theoretical and experimental studies on the structure of graphene oxide have been proposed. As shown in Figure 2.10, starting from the earliest and simplest Hofmann model (epoxy groups appear repeatedly on graphene sheets, and there are no defects on the sheets), pure theoretical models, such as the Ruess model with hydroxyl groups and ether-like groups on folded carbon structures, the Scholz Boehm model with carbonyl groups and hydroxyl groups on folded carbon structures, and

the Nakajima Matsuo model with two graphene sheets connected by vertical sp^3 hybrid C-C bonds and with carbonyl and hydroxyl groups on the sheet, have emerged (Dreyer et al., 2009). Since then, as microscopic imaging techniques have improved, researchers have combined various analytical or imaging techniques to modify and refine these models; for example, Solid-state nuclear magnetic resonance spectroscopy (NMR) has been used. The Lerf-Klinowski model (a non-oxidized benzene ring plane structure combined with a folded six-carbon lipid ring structure containing hydroxyl and ether-like groups) and Dekany model (a cyclohexyl structure containing tertiary alcohol and 1,3-ether combined with a fold containing ketone and quinone structures) was proposed based on FTIR and diffuse Fourier transform infrared spectroscopy network structures), and the Diev-Tour model was proposed based on TEM detection results combined with previous theoretical models (defects in slices, including epoxy groups, hydroxyl groups, carboxyl groups and some sulfur-containing groups left by chemical preparation) (Dimiev et al., 2016; Dreyer et al., 2015). As imaging and compositional analysis techniques continue to evolve, more precise structures will eventually be determined.

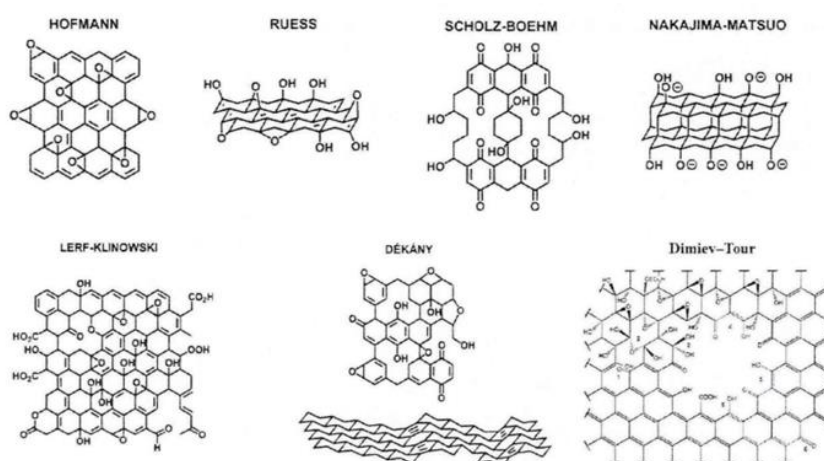


Figure 2.10: Several typical structural models of GO (Dimiev, 2016; Dreyer, 2015)

The cyclohexyl structure of 3-ether combines a fold network structure containing ketone and quinone moieties and the dimmer tour model based on TEM detection results and previous theoretical models (there are defects in the tablet, epoxy, hydroxyl, carboxyl, and some sulfur-containing groups left by chemical preparation, etc.) (Dimiev et al., 2016; Dreyer et al., 2015). With the continuous development of imaging and component analysis technology, a more precise structure will be determined through the continuous efforts of researchers.

However, no matter how the structural details are distributed, the structural diagram of graphene oxide can be constructed by combining theoretical and experimental results, which can be used to analyze the source of its properties and determine its application potential. The widely accepted graphene oxide structure is shown in Figure 2.11. Many sp^2 hybridized carbon atoms and a small number of sp^3 hybridized carbon atoms, coupled to functional groups containing oxygen, form the network structure of hexagonal carbon rings (Sontakke et al., 2021). Among them, oxidation areas can be defined as the sp hybridized carbon atoms that are attached to functional groups containing oxygen, such as carboxyl, epoxy, and hydroxyl groups. The presence of an oxidation region interrupts the ductility of the sp^2 -conjugated network on the graphene chip with the original honeycomb structure, which can be regarded as a nonoxidized region (Petukhov et al., 2021; Rasheed et al., 2021). These sp^3 hybridize sp^2 hybridize structures (Liu et al., 2022a).

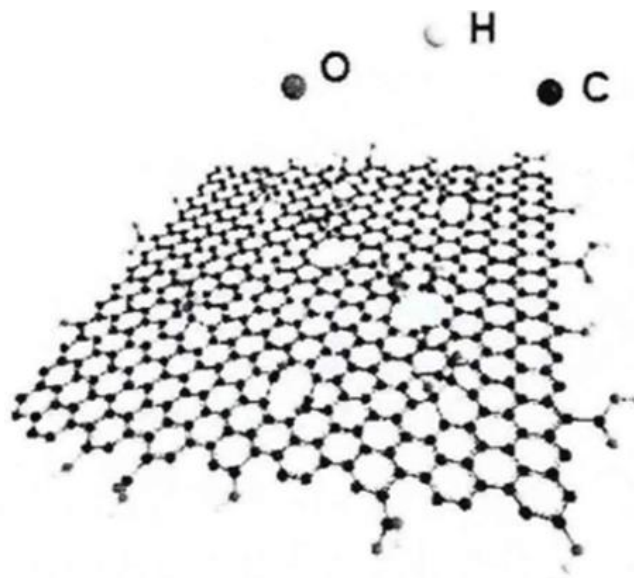


Figure 2.11: Structure of graphene oxide (Wanget al., 2012)

The oxygen-containing functional groups on graphene oxide are primarily attached to graphene-like nanosheets in the form of epoxy and hydroxyl groups, as indicated by the model, analysis, and imaging results above. A small number of oxygen-containing groups also appear at the edge of graphene oxide sheets in the form of carboxyl, carbonyl, phenol, lactone, and quinone groups (Panda et al., 2022). The structure of graphene oxide was determined, which is highly beneficial for experimental design and analysis when graphene oxide is used in different applications.

Since graphene oxide has a large specific surface area, this provides more active sites for adsorption of target analytes. Meanwhile, due to the hydrophilic functional groups contained on the surface, GO has good dispersion in aqueous solution, which helps to improve the detection sensitivity and response speed. In addition, graphene oxide has tunable optical properties, and its absorbance and color change can be regulated by changing the oxidation degree of graphene oxide or introducing different functionalized

modifications. This controllable optical property is particularly important for designing vision sensors based on color change. In addition, the multiple functional groups on the surface of graphene oxide make it easy to combine with other organic small molecules, polymers, metal nanoparticles, and other materials to form composites. These composites tend to exhibit superior sensing properties, such as higher selectivity and faster response time.

In summary, graphene oxide plays a key role in the design and preparation of colorimetric sensors due to its unique structural features, which provides new ideas for the development of new sensing technologies with high performance and low cost.

2.5.2 Properties of graphene oxide

Graphene oxide has many excellent properties in Mechanical, Electrical, Optical, Thermal and Chemical.

2.5.2.1 Mechanical properties

Among graphene materials, graphene has the best mechanical properties (Zhu et al., 2022). Graphene has a strong fracture resistance, which is due to the strong interaction between carbon atoms in graphene sheets through σ bonds (Nariman et al., 2019). According to Young's modulus and fracture strength test, graphene has the highest mechanical strength. Young's modulus is a physical quantity that describes the ability of a solid material to resist deformation. Young's modulus, also known as tensile modulus, is the most common type of elastic modulus or modulus of elasticity. In addition, graphene is characterized by monolayer bonding and excellent flexibility, which provides a mechanical guarantee for practical application.

Compared with those of graphene, during the oxidation process, the oxygen atoms enter the graphene carbon skeleton structure, which changes the connection mode of some carbon atoms, destroys the stability of the carbon skeleton, and affects its mechanical properties. For reduced graphene oxide, although the oxygen atoms were separated from the carbon skeleton and some of the carbon atoms recovered to the sp^2 hybrid structure during the reduction process, there were some defects in the carbon skeleton structure due to the oxidation and reduction reactions during the preparation process. Moreover, some oxygen atoms remain in the reduced graphene oxide, which affects the mechanical properties of the skeleton structure (Jiříčková et al., 2022)

2.5.2.2 Electrical properties

Graphene has the best electrical properties. In the large π -bond structure of graphene, each carbon atom contains unbound π electrons. These nonlocal electrons can move freely on the graphene sheet, which endows graphene with excellent electrical properties. In addition, the unique carrier properties and semi-integer quantum Hall effect of graphene have attracted extensive attention and research (Ahmed et al., 2022).

Compared with those in graphene, the interventions of oxygen atoms in graphene oxide will capture π electrons and restrict the free movement of π electrons, resulting in the fracture of large π bonds in the carbon skeleton, thus affecting its conductivity (Valentini et al., 2023). It has been shown that a graphene oxide layer can be used as a monolithic insulating material (Xue et al., 2021).

2.5.2.3 Optical properties

Among graphene materials, graphene has the best optical properties. Graphene with a single layer has high transmittance, which provides a potential substrate material for the manufacture of practical devices. In addition, graphene also exhibits excellent spectral absorption and nonlinear optical properties, has attracted extensive research, and has good application prospects (Kumar et al., 2021).

GO has different optical properties than Graphene. The reason is that the connections between carbon atoms and carbon atoms and between carbon atoms and oxygen atoms in the graphene oxide structure are different, and the absorption characteristics of light are different. Especially after the intervention of oxygen atoms, the spectroscopic results of graphene oxide also show unique properties. For reduced graphene oxide, the lamellar structure contains not only the characteristic region of graphene oxide with residual oxygen atoms but also the graphene region without oxidation or reduction recovery, and the optical properties are between those of the other two materials (Kvitha et al., 2013; Rasheed et al., 2021).

2.5.2.4 Thermal properties

Among graphene materials, graphene has the best thermal properties. As a stable material, graphene has better thermal conductivity than carbon nanotubes, diamond, and copper. Especially for defect-free graphene sheets, phonon conduction (lattice vibration heat conduction) has excellent diffusion and ballistic conduction properties (Chen et al., 2023).

For defective graphene sheets, the existence of defects will affect the heat transfer between carbon atoms, thus affecting the thermal properties of the materials. Compared with those of graphene oxide, researchers have found that with increasing reduction degree, the in-plane thermal diffusion and thermal conductivity gradually increase. However, the excessive reduction will cause angular deflection and wrinkles in the lamellae, which will affect the thermal conductivity (Thejas et al., 2022).

2.5.2.5 Chemical properties

Graphene is chemically inert due to its structural characteristics. Graphene itself has a large theoretical specific surface area and hydrophobic lipophilic surface, so it can adsorb different types of molecules through π -bond interactions and hydrophobic interactions (Guo et al., 2022).

The introduction of oxygen-containing functional groups not only enhances the hydrophilicity of graphene oxide but also endows graphene oxide with rich chemical properties, which provides a variety of ways to develop graphene oxide-based materials (Georgakilas et al., 2016; Mohan et al., 2018). In addition, graphene-like compounds are prepared by chemical reduction. Graphene oxide is usually prepared first, chemically modified, and subsequently reduced to obtain the target material (Reddy et al., 2022; Gupt et al., 2021).

2.5.3 Synthesis methods for GO

Graphene Oxide (GO) is a two-dimensional material obtained by chemical oxidation of graphite, having a single to several layers of carbon atoms in thickness and containing a large number of oxygen-containing functional groups such as hydroxyl, epoxy

and carboxyl groups. The following are several commonly used synthesis methods for the graphite oxide method (Bamrungsap et al., 2019).

2.5.3.1 Hummers method and modified Hummers method

The Hummers method is one of the most familiar techniques for the preparation of graphene oxide, which involves mixing graphite powder with sulphuric acid and potassium permanganate and carrying out a chemical oxidation reaction at a specific temperature, resulting in a well-dispersed graphene oxide solution. This method is low cost and reproducible and is suitable for mass production. Compared with the original Hummers method, the modified Hummers method improved the selection and dosage of oxidant, and optimised the reaction conditions, such as temperature and time, as well as added surfactants to improve the dispersion of graphite oxides, which improved the quality and yield of graphene oxide (Carr et al., 2023).

2.5.3.2 Brodie method

The Brodie method is an older route to the synthesis of graphene oxide, which is formed by mixing graphite with concentrated nitric acid and then adding a small amount of an oxidising agent, such as hydrogen peroxide, and reacting under mild conditions (Chen et al., 2023).

2.5.3.3 Staudenmaier method

The Staudenmaier method uses concentrated nitric acid and calcium chloride as the reaction medium to oxidise graphite at higher temperatures. This method is capable of generating larger sized graphene oxide flakes, but may result in more defects in the product due to the more vigorous reaction conditions (Guo et al., 2022).

2.5.3.4 Hydrothermal method

Hydrothermal synthesis is an environmentally friendly method, in which the oxidation process can be effectively controlled by reacting graphite and oxidant in a high-pressure, high-temperature hydrothermal reactor, thus obtaining graphene oxide of high purity and high crystallinity (Gupta et al., 2021).

2.5.3.5 Electrochemical oxidation method

Electrochemical oxidation method uses the principle of electrolysis to strip graphene oxide from graphite by oxidation reaction on the anode under the action of electric field. The advantage of this method is that the current density can be directly regulated to adjust the degree of oxidation, enabling precise control of the product properties (Jin et al., 2019).

2.5.4 Methods of Synthesis of GO Composites

There are various methods for the preparation of nanomaterials, which can be divided into vapor-phase methods, solid-phase methods, and liquid-phase methods. Among them, the gas-phase method is mainly suitable for making thin-film nanomaterials, such as NiCoCrAl thin films (Shi et al., 2019) and $\text{Cu}_2\text{ZnSnS}_4$ thin films (Shaikh et al., 2020). The solid-phase synthesis process is dominated by physical reactions, which generally require a more demanding reaction environment, such as high temperature, high pressure, or high mechanical energy. This method has many disadvantages, such as high energy consumption, low efficiency, and easy mixing of impurities. However, the graphene oxide nanocomposites required for this study have high purity requirements. The catalytic properties of the composites will be affected if there are interfering substances in the

system. Therefore, neither gas-phase nor solid-phase methods could be applied to this area of study.

In addition, the liquid-phase method is a typical "bottom-up" method of material preparation. The process starts from a homogeneous solution and controls the chemical reaction process by adjusting the solution type, solution concentration, reaction temperature, pressure, and pH to produce the desired solute, which is then separated from the solvent and used as a precursor to obtaining the desired nanomaterial after drying and heat treatment (Lellala et al., 2021). The main methods are precipitation, hydrothermal, and sol-gel methods.

Among them, the precipitation method dissolves a soluble salt containing one or more target ions into a suitable solvent to form a salt solution of the target ions. When a suitable precipitant is added, hydrolysis or direct precipitation occurs at a certain temperature to form insoluble hydroxides, oxides, or inorganic salts, which are precipitated from the solution. The precipitates are washed, separated, dried, or further sintered to obtain the desired nanomaterials (Tang et al., 2006).

The sol-gel method uses compounds with high chemical word activity (inorganic or metal alcohol salts) as precursors that are uniformly dissolved in a suitable solvent to form a solution of metal compounds. Hydrolysis and condensation reactions occur under the action of catalysts and additives to form stable, transparent sols. The sols are aged and condensed to form gels, which are subsequently dried and sintered to prepare the target product by drying and sintering (Bharate et al., 2021).

The hydrothermal method is a chemical reaction in which water is used as the reaction medium in a high-temperature, high-pressure reaction environment in a reactor.

By adjusting the solution type, solution concentration, reaction temperature, reaction time, pressure, and mineralizing agent, hydrothermal reactions can be used to produce nanomaterials with high purity, good dispersion, and controlled morphology (Zhou et al., 2021). Unlike the above two methods, the preparation using this method in a sealed reactor effectively prevents the occurrence of contamination and facilitates the preparation of high-purity materials.

2.5.5 Characterization methods for GO composites

Material characterization refers to the description or justification derived from the analysis of material structure and property data. Characterization is a form of subjective abstract thinking based on material analysis. It involves explaining and illustrating the intrinsic structure and properties of materials using models, diagrams, or words.

The qualities of materials, which are closely linked to their structure and chemical composition, often determine their applications. The conditions and techniques used during preparation have a significant impact on the chemical composition and structure of the materials. It is important to characterize the basic information about the material and combine it with preparation methods and processes to modulate the properties of the material for application purposes (Subrati et al., 2021).

2.5.5.1 Surface morphology

The surface structure of graphene oxide composites can be visualized by characterizing the surface morphology of the material and obtaining microscopic information about the size, distribution, shape, lattice, and structure of the nanoparticles. The main methods for characterizing the surface morphology of nanomaterials are transmission electron microscopy (TEM) and scanning electron microscopy (SEM). In

particular, electron microscopy (TEM) can be used to directly observe the microstructure and obtain high-resolution atomic images of materials, and a whole set of material structure analyses similar to those of X-ray diffraction can be performed via electron diffraction. Compared to TEM, SEM is mainly used for direct observation of the microscopic surface morphology of materials and is the most difficult method for obtaining images; moreover, this method requires finding the optimal conditions and does not detect small nanoparticles (Sugarthi et al., 2021). Therefore, TEM was chosen to characterize the surface morphology of the graphene oxide composites in this study. However, TEM requires a very thin sample thickness, so it is not suitable for analyzing the surface structure of the paper base. In this case, SEM is preferred. In general, TEM is the best choice if information from the internal structure of the sample is required, while SEM is the first choice when information about the surface of the sample is desired.

According to previous studies (Spurgeon et al., 2021), TEM is used for the following four main purposes: morphological observation, including conventional low-power TEM 2D imaging and 3D reconstruction imaging; crystal orientation identification and crystallinity analysis; atomic structure observation; and chemical composition analysis. SEM is mainly used to observe and analyze the composition, morphology, and structure of the sample surface. Figure 2.12 shows the SEM and TEM characterization image based on cobalt-graphene nanocomposite. The composite has a well-defined three-dimensional structure. The Co nanoparticles are evenly distributed across the graphene sheet, and the average particle size of the nanoparticles is about 35 nm, as demonstrated by the SEM and TEM characterization images. According to Nana et al. (2021), the lattice spacing in the HRTEM image is 0.201 nm, corresponding to the (111) crystal plane of Co nanocrystals.

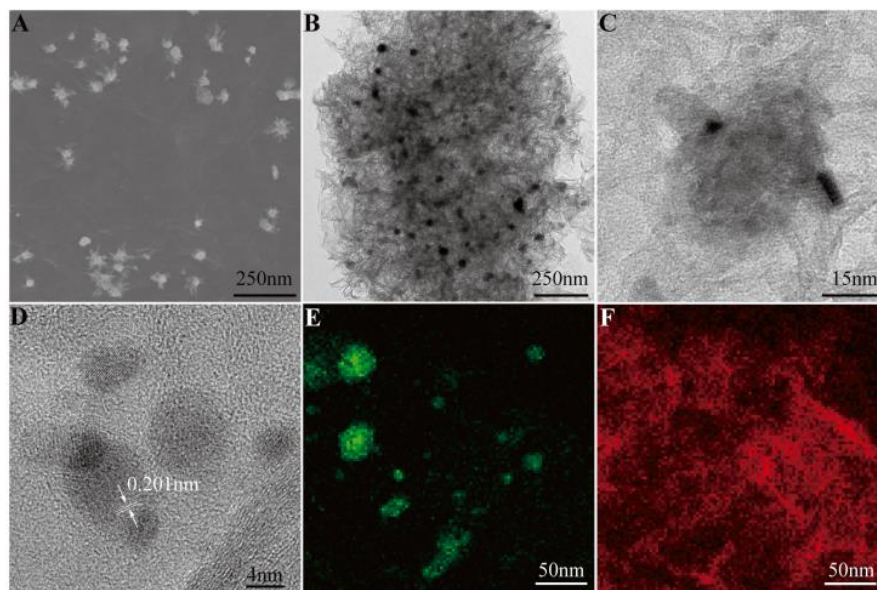


Figure 2.12: SEM (A), TEM (B and C), HRTEM (D), and elemental mapping images of Co (E) and N (F) in Co-His-GQD-G (Nana, et al., 2021)

i. TEM working principle

In transmission electron microscopy (TEM) research, a beam of accelerated, aggregated electrons is projected onto a very thin sample. The electrons collide with the atoms of the sample, causing a change in direction and resulting in stereo angular scattering. When enlarged and focused, bright and dark pictures can be displayed on imaging devices such as fluorescent screens, thin films, and photocoupled assemblies because the magnitude of the scattering angle is correlated with the density and thickness of the sample (Williams et al., 2009). A schematic of the TEM imaging procedure is shown in Figure 2.13.

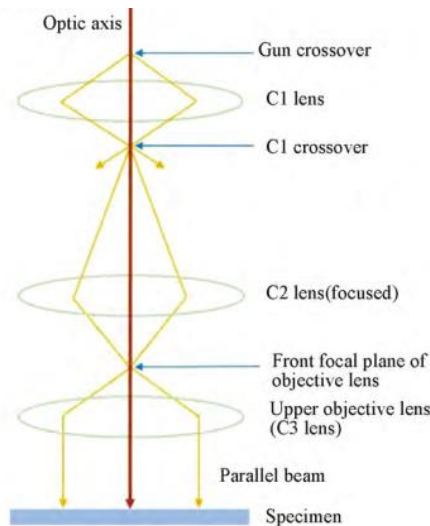


Figure 2. 13: Schematic diagram of TEM imaging (Wllimns, et al., 2009)

ii. TEM instrumentation

The main structure of the TEM instrument includes the following parts: an electron gun, a spotting scope system, a sample stage, an imaging system, and an observation and recording system.

iii. SEM Working Principle

Scanning electron microscopy (SEM) is a technique that involves focusing an electron beam from an electron gun to create a detailed image of the surface of a sample. This allows for scanning the surface of the sample, and by detecting the signals produced by the electrons' actions, one can examine and analyze the structure, morphology, and composition of the surface. Using secondary electrons, backscattered electrons, and distinctive X-ray signals, SEM primarily examines the surface characteristics of the material.

iv. SEM Instrumentation

The scanning electron microscope (SEM) comprises the electronic optical system, vacuum system, computer control system, signal gathering and processing system, signal display and recording system, and other components.

2.5.5.2 Material structure

Obtaining structural information is important for correctly analyzing the properties of materials, determining the mechanism of material synthesis, and optimizing material properties.

Infrared spectrometry and Raman spectroscopy are powerful tools for studying molecular structure and chemical composition and are widely used in materials, chemical, and environmental protection fields due to their rapidity, high sensitivity, and low detection dosage. From the perspective of analytical testing, the use of the two together often provides better information on the molecular structure.

Nitrogen-doped carbon nanotubes ($\text{Fe}_3\text{C}@\text{NCNTs}$) packed with Fe_3C nanoparticles were manufactured by Shang et al. (2019) using a one-step pyrolysis technique. The downstream structures were analyzed using a combination of infrared and Raman spectroscopy. Using Raman spectroscopy, the authors described the degree of graphitization and the structural characteristics of the produced materials (Figure 2.14(a)). The $\text{E}_{2\text{g}}$ vibrations of sp^2 -hybridized graphitized carbon atoms are reflected in the G-band, while the D-band represents the sp^3 defects in carbon, such as amorphous carbon layers and edges, in graphene. As a result, the degree of faults and the degree of graphitization decrease with an increasing $I_{\text{D}}/I_{\text{G}}$ value, which represents the intensity ratio between the D-

band and G-band. The FTIR infrared spectrum also shows oxygen-containing functional groups (Figure 2.14(b)). All four peaks, except for the C=C peak, weakened as the calcination temperature increased from 600°C to 1000°C, suggesting that the growth of the graphene structure was associated with the decomposition of unstable O and N species.

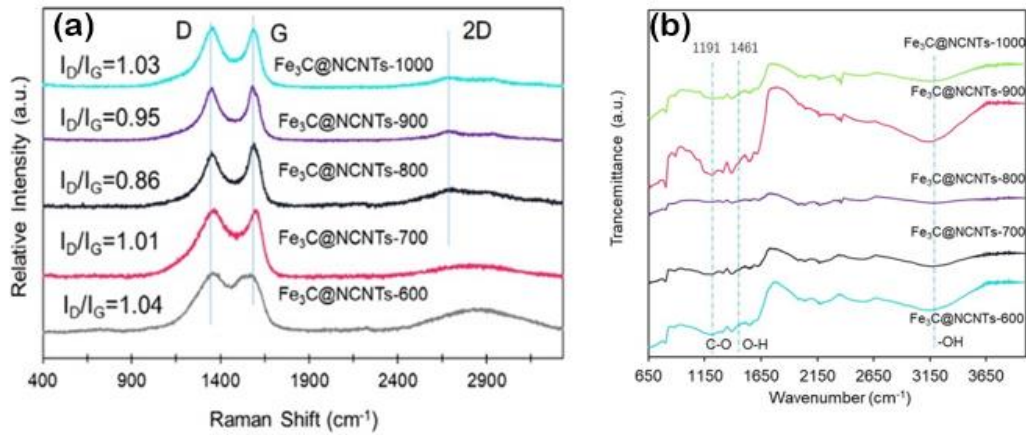


Figure 2.14: Raman spectra (a) and FTIR infrared spectra (b) of Fe₃C@NCNTs synthesized at 600-1000°C (Shang, et al., 2019)

i. Detection principle

Infrared spectroscopy: Electromagnetic radiation's energy is similar to the difference in vibrational or rotational energy between molecules when it interacts with material molecules, causing the molecules to move from low to high energy levels. Because of this, material molecules absorb certain wavelengths of electromagnetic radiation. By determining the intensity of radiation at various wavelengths, one can obtain infrared absorption spectra. In addition, molecules that absorb infrared radiation undergo a transition in vibrational and rotational energy levels. Molecular vibrational rotation spectroscopy is another name for infrared spectroscopy (Ozaki et al., 2021).

When light strikes a material, photons in the Raman spectrum collide with electrons within the molecule. If this collision is inelastic, some of the photon energy is transferred

to the electrons, causing the frequency of the scattered light to differ from the frequency of the incident light. The resulting spectrum is known as the Raman spectrum, and this scattering phenomenon is referred to as Raman scattering (Wang et al., 2020d).

ii. Testing instruments

Fourier transform infrared spectrometers mainly consist of a light source, Michelson interferometer, detector, computer, etc. Compared with dispersive infrared spectrometers, Fourier transforms infrared spectrometers have the advantages of high resolution, high wavenumber accuracy, fast scanning rate, wide spectral range, and high sensitivity (Ozaki et al., 2021).

A dispersive Raman spectrometer is composed of an excitation light source, an external optical path system, a multigrating monochromator, a detector, and other systems. The laser light emitted from the light source is incident on the sample after monochromatic treatment; the scattered light is collected by the concave mirror and irradiated onto the grating through the slit. The scattered light is collected by a concave mirror and irradiated to the grating through the slit. The grating is rotated continuously so that scattered light of different wavelengths can enter the detector through the exit slit in turn, and the Raman spectrum is subsequently obtained by amplification and display (Wang et al., 2020e).

2.5.5.3 Crystallinity

It is important to characterize the crystal structure of the material to obtain information such as grain size, grain orientation, symmetry, and atomic properties of the material, and to quickly realize not only the identification of the physical phase but also the analysis of the microstructure of the material through database comparison.

Currently, the crystal structure of materials is mainly determined via X-ray diffraction (XRD) characterization, which yields a wealth of crystalline and amorphous structural information. The X-ray diffraction data will not only provide crystal structure information, including cell size, symmetry (point group, space group), and atomic properties (atomic species, atomic coordinates, occupancy, atomic displacement factor, etc.) but also grain size, grain orientation, and microstructure information, including layer dislocation, twinning, domain structure, etc. X-ray diffraction will also obtain the atomic radial distribution function or atomic pair distribution function of amorphous materials, which will be used to analyze the material. X-ray diffraction also provides the atomic radial distribution function or atomic pair distribution function of amorphous materials, which can be used to analyze the atomic local coordination characteristics of materials. Owing to the easy-to-understand principle of X-ray diffraction and relatively simple instrument structure, X-ray diffraction is widely used in materials, physics, chemistry, medicine, metallurgy, mining, and other fields.

A diffraction angle within 5° is considered small-angle X-ray diffraction (SAXD). A diffraction angle ranging from 5° - 90° is called wide-angle X-ray diffraction (WAXD). These are collectively referred to as XRD (Zhang et al., 2006).

If the irradiated specimen has a nonperiodic structure with different electron densities, the secondary X-rays will not interfere, and this phenomenon is called diffuse X-ray diffraction (scattering for short). In this case, when the diffraction angle is within 5° , the process is called small-angle X-ray scattering (SAXS). A diffraction angle ranging from 5° to 90° is called wide-angle X-ray scattering (WAXS) (Zhang et al., 2006).

Thus, diffraction corresponds to the phenomenon of coherent diffraction caused by the interaction of periodic structures such as lattices with X-rays, while scattering corresponds to the change in electron density of X-rays before and after transmission through the sample.

In this study, the graphene oxide material has a periodic structure; therefore, we used XRD for characterization. The C(002) peak in graphite oxide has a diffraction angle of approximately 15.4° (Nana et al., 2021); therefore, the diffraction angle chosen for characterization via XRD was greater than 5° , and WAXD was used for characterization.

i. Principle of X-ray diffraction

X-rays are electromagnetic waves with extremely short wavelengths that are alternating oscillating electromagnetic fields. When X-rays irradiate a material, the electrons outside the nucleus of the atoms that make up the material oscillate under the effect of the alternating electric field, creating a new source of oscillation (scattering source). The new scattering source scatters the incident electromagnetic waves in all directions. Since the scattered wave has the same vibrational frequency as the incident wave, the two waves are coherent. If the material to be measured is a crystal, the newly formed scattering source is also periodic due to the periodic structure of the crystal. The phase difference between the scattered waves of the periodic scattering source is the same; thus, a strong interference phenomenon occurs between them, i.e., Bragg diffraction (Shi et al., 2014).

ii. Instrumentation

There are various forms of X-ray diffractometers with different applications, but their basic components are very similar. Four major sections make up the system: a ray detector, a sample with a sample position adjustment mechanism system, a high-stability X-ray source, and a diffraction map processing and analysis system. The physical diagram of the XRD results is displayed in Figure 2.15.



Figure 2.15: XRD physical diagram

The most important use of XRD in the study of material synthesis is for phase identification, which supports the successful synthesis and fundamental properties of materials. Phase identification is used to solve the "what" problem of the material to be measured and is the most common method for structural analysis of materials with known structures. Any phase will be described by a unique set of crystal structure data (including parameters such as lattice constants, space groups, and atomic properties). Since the lattice

constant determines the peak position of the diffraction peak and the atomic properties determine the peak intensity, both will uniquely determine the X-ray diffraction pattern. Therefore, each phase has only one unique set of X-ray diffraction patterns, which are characterized by a "fingerprint". The standard X-ray diffraction patterns of all known structures are grouped to form a powder diffraction database, referred to as a PDF (power diffraction file) card. Identifying and matching the diffraction patterns of test results and PDF cards (search-match) has become the most common method of physical identification. Figure 2.16 shows the Mn_3O_4 NPS. XRD spectra.

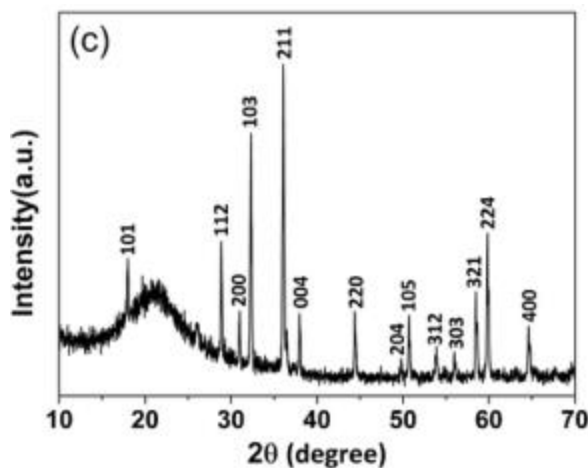


Figure 2.16: XRD result of octahedral Mn_3O_4 NPS. (Wang et al., 2020c)

2.5.5.4 Chemical state analysis

The chemical state includes information such as the bonding state of the atoms, the electronic distribution state, and the band gap width. By analyzing the chemical state of a material, it is possible to determine the elemental composition, valence state, and ratio of different valence states. Currently, chemical characterization methods include energy-dispersive X-ray spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS). XPS

can obtain additional information about the chemical state of a material and can be used to analyze the elemental composition, quantitative analysis of elements, structural analysis, etc. However, XPS requires a large amount of sample and is unable to measure liquid samples at low concentrations (Nefedov et al., 2023). Compared with XPS, EDS can analyze the composition of experimental samples and requires a small amount of samples for determination. In addition, EDS can be used in conjunction with TEM (e.g., Figure 3.9).

In addition, EDS has the following advantages: high sensitivity, a wide range of applications, and simultaneous determination of multiple elements. Because the material synthesized in this study is synthesized in the form of a low-concentration liquid and because compositional analysis of the material is needed, EDS was chosen to characterize the chemical state of the synthesized GO composite in this study.

i. Working principle of EDS

The sample is bombarded with a fast-moving electron beam. The electrons collide with the elemental nuclei and outer electrons in one or more elastic and inelastic collisions. Furthermore, some electrons bounce off the sample's surface, while the remaining electrons enter it. They lose kinetic energy over time before being stopped and absorbed by the material. Only about 1% of the incident electron energy is used to excite the sample and generate various signals during this process, with over 99% of the incident electron energy being converted into thermal energy. Among them, the characteristic X-rays are high-energy electron excitations of the inner layer of the atom's electrons, so that the atom is in an unstable state and the outer layer of electrons fills the inner layer of the space so that the atom tends to reach a stable state. In the process of the leap, the characteristic energy and wavelength of electromagnetic radiation are directly released, i.e., the

characteristic X-rays. The characteristic X-ray energies emitted by different elements are not the same, the use of characteristic X-ray energies is different, and elemental analysis is called the energy dispersion method. The spectrometer used is called an EDS (Figure 2.17).

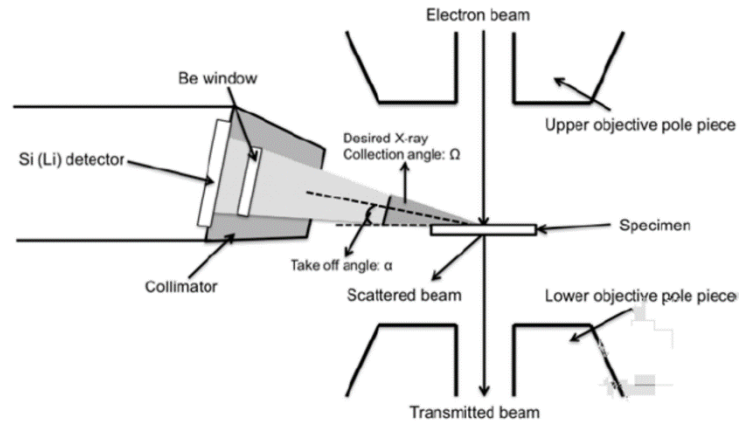


Figure 2.17: A schematic drawing of a cross-section of the EDS detector and objective lens in the TEM stage (Kempa et al., 2011)

ii. Instrumentation

The multichannel pulse analyzer and the Si(Li) semiconductor detector, also known as the lithium-drift silicon semiconductor detector, are the two primary components of the X-ray energy spectrometer. Thousands of electron volts of incident electron beam energy are used to irradiate the sample and excite characteristic X-rays. These X-rays are directed onto the Si(Li) semiconductor detector through a beryllium window. This process ionizes the silicon atoms and generates a significant number of electron-hole pairs, which is directly proportional to the energy of the X-ray. This is because an X-ray photon can create an electron-hole pair with a minimum average energy of ϵ ; therefore, $N = \Delta E / \epsilon$ represents the number of electron-hole pairs it can generate. N will rise in proportion to the incident X-ray photon's energy. Various elements emit X-rays with different energy levels, and these varying energies of X-rays lead to different amounts of electron-hole pairs being generated (Guo, 2021). Figure 2.18 displays an EDS physical diagram.



Figure 2.18: EDS physical diagram

iii. EDS spectral curve

Commonly utilized X-rays have an energy range of 0.2–20.48 keV. A range of 20 eV corresponds to each channel site if there are a total of 1024 channel sites. Low-energy X-rays are associated with few channel sites, whereas high-energy X-rays are associated with many channel sites. It is possible to determine the X-ray intensity of each element by counting the number of X-rays captured at different locations. Next, the energy spectrum curve of X-rays, displayed on the X-Y recorder or cathode ray tube, shows the number of pulses and the pulse height curve. If used in conjunction with TEM, qualitative analysis of the X-ray energy spectrum consists mainly of element identification and analysis of the element distribution. X-ray spectra are collected from a microregion, and the peak position of each spectral line is determined. The characteristic X-rays of each experimental spectral line can be identified by consulting the energy table of standard characteristic X-rays, and the elements contained in the microregion can be obtained (Guo et al., 2021).

2.5.6 Application of graphene oxide in colorimetric detection

Graphene and graphene oxide (GO) have the unique ability to adsorb nucleic acids, proteins and peptides. They can also recognise single-stranded DNA (ssDNA) from double-stranded DNA (dsDNA) and have strong fluorescence quenching ability. And it is possible to functionalize graphene oxide surfaces by grafting amine and sulfhydryl groups, $\pi - \pi$ stacking, electrostatic interactions, and surface plasmon resonance (SPR). For the development and production of biosensors and colorimetric sensors, graphene is a suitable substrate material. Combining graphene's exceptional properties with a simple, rapid, and efficient colorimetric detection technique can lead to the creation of a more sensitive and rapid colorimetric sensing platform for the detection of target substance.

2.5.6.1 Detected proteins

Graphene can be functionalized to enhance the selectivity of graphene-based materials by facilitating the adsorption of proteins and nucleic acids. Because the aggregation of AuNPs on a graphene oxide surface can lead to a color change in the solution, Bamrungsap proposed a colorimetric strategy for heparin detection. The probe utilized in this study was polydiallyl dimethyl ammonium chloride, or PDADMAC (Figure 2.19). The resulting solution was red because the combination of PDADMAC and graphene prevented AuNPs from aggregating on the surface of the graphene. In the presence of heparin, heparin and PDADMAC can combine to form a stable polymer. Heparin colorimetric detection is achieved by gold nanoparticles self-assembling on the surface of graphene, which turns the solution purple (Bamrungsap et al., 2019).

Circular aptamers have been shown by Li et al. (2019) to bind to graphene through $\pi - \pi$ interactions. Isothermal amplification occurs when the circular aptamer binds to

graphene and changes configuration upon encountering the target protein. The rolling loop amplification reaction that the cyclic aptamers coupled to graphene produce on the paper surface is what causes the peroxidase activity to be amplified, as illustrated in Figure 2.20. In order to identify proteins, the reaction can also catalyze the oxidation of TMB, resulting in a visible color change in the solution.

Wiriyachaporn et al. developed a GO/AuNP hybrid by combining graphene with metal ions. Through the self-assembly of AuNPs on the surface of graphene oxide, protamine was identified using colorimetry. As illustrated in Figure 2.21, the aggregation of AuNPs causes the solution's color to change from red to dark purple when they come into contact with graphene oxide nanosheets through electrostatic interactions. Protamine can be added to the solution to prevent AuNP aggregation, maintain AuNP dispersion, and cause the solution to turn red again to facilitate detection (Wiriyachaiporn et al., 2019).

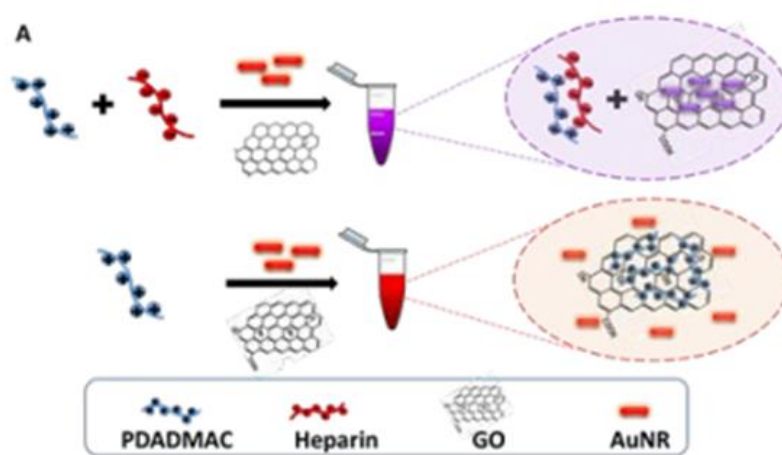


Figure 2.19: PDADMAC was detected by heparin probe colorimetry (Bamrungsap et al., 2019)

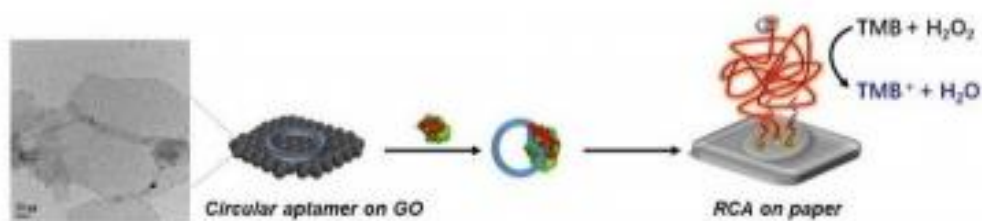


Figure 2.20: Schematic illustration of the graphene oxide circular aptamer-based assay on bioactive paper (Li et al., 2019)

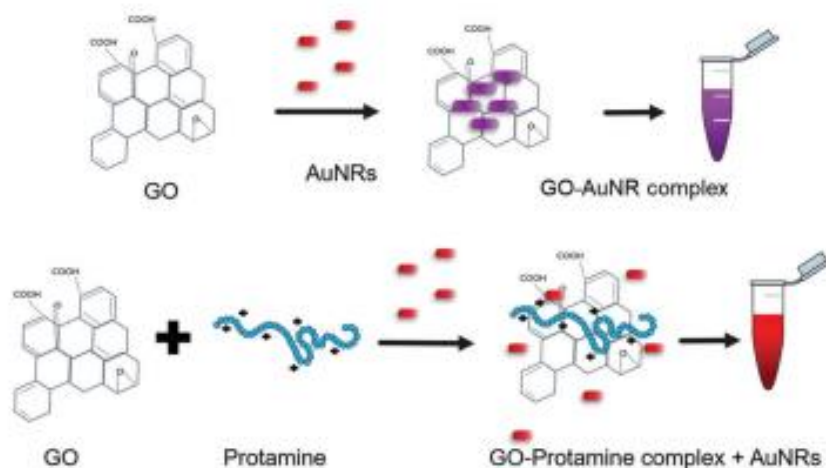


Figure 2.21: Schematic illustration of the protamine detection principle for a colorimetric sensor based on the self-assembly of AuNRs on graphene oxide (Wiriyaichaiyorn et al., 2019)

2.5.6.2 Detection of metal and nonmetal ions

Colorimetry can be utilized on a sensor platform that is based on two-dimensional materials to detect metal ions. Zhang et al. (2017) dispersed Pd NPs on the surface of graphene by using polyaniline (PEI) as a bridge between metal ions and graphene. The nanohybrid's peroxidase-mimicking activity was significantly increased in the presence of Hg²⁺, which promoted the oxidation of TMB and resulted in a dark blue color change. Nanohybrids have lower detection limits and increased activity for Hg²⁺ detection compared to standard Pd NPs. Using a solvothermal technique, Borthakur and colleagues

developed a graphene oxide nanocomposite modified with cobalt sulfide (CoS) to detect Hg^{2+} in water (Borthaku et al., 2017).

According to reports, hemin-modified graphene oxide can be used in conjunction with DNA probes to detect a variety of analytes, such as metal ions, DNA, and other small molecules (Tao et al., 2013). Figure 2.22a illustrates how heme and graphene combine via DNA-mediated synthesis to produce distinct optical signals based on the target substance's concentration, enabling quick and easy colorimetric detection of a range of analytes.

Functionalized graphene can be used for colorimetric detection of non-metallic ions as well as metal ions. Li et al. (2013) developed a rapid and sensitive sensor for detecting fluoride in water. They achieved this by utilizing silicon-based Spiro pyran dyes that have a specific affinity for fluorine ions, allowing them to self-assemble on graphene oxide (GO). The Si-O bond breaks due to a nucleophilic substitution process between dye molecules and fluorine ions, as illustrated in Figure 2.22b. The outcome was the opening of the closed spirogyra structure, which caused the solution's color to change from colorless to orange-yellow. The sensitivity increases, the range of change shortens, and the detection limit of fluorine ions decreases with the addition of graphene oxide.

Liu et al. (2023) showed that the peroxidase-like activity of bare $\text{Ti}_3\text{C}_2\text{T}_x$ MNR@Au was weak, but was significantly enhanced in the presence of Hg^{2+} , effectively catalysing the oxidation of colourless substances (e.g. o-phenylenediamine) to coloured products. The oxidation product of o-phenylenediamine has a significant reduction current and is sensitive to the concentration of Hg^{2+} . Based on this, a new method for the detection of Hg^{2+} by highly sensitive homogeneous voltammetry is proposed, which is fast, sensitive and quantifiable.

Lv et al. (2023) showed that Poly-C-containing DNA strongly adsorbs on the GO surface and enhances the physical adsorption between the two. Hg^{2+} specifically binds to single-stranded DNA containing phosphorothioate cleavage sites and fluoresces according to the FRET principle. The limit of detection of the Poly-C PS 1 site sensor is up to 1.11 nM. The Poly-C PS 1 site sensor has a detection limit of 1.11 nM and the detection limit is up to 2.5 nM.

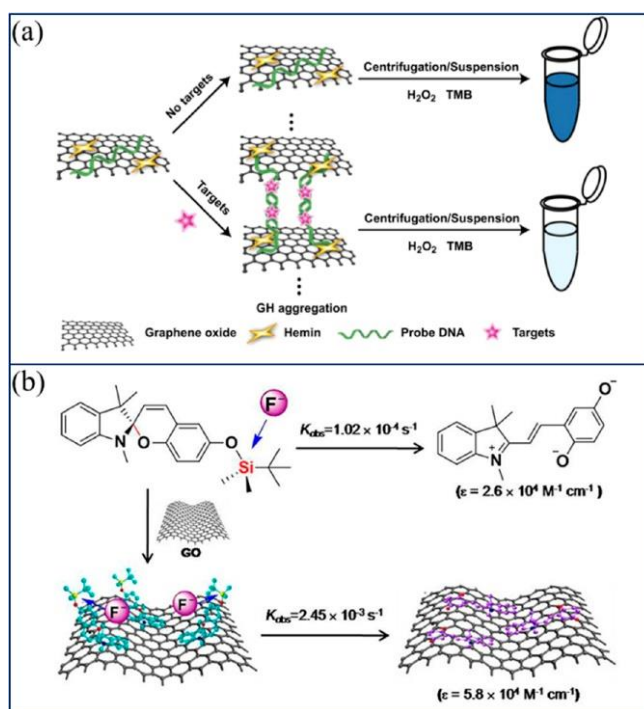


Figure 2.22: (a) Schematic diagram of the Hg^{2+} detection procedure (Tao et al., 2013); (b) Determination of fluoride content by functional graphene (Li et al., 2013)

2.5.6.3 Detection of viruses and bacteria

In the past, colorimetric pathogen and viral detection has extensively relied on sensing systems constructed with functional nanomaterials. Graphene and its hybrid materials have been extensively employed in the field of virus detection.

The production of graphene/AuNP hybrids modified with antibodies for the colorimetric detection of norovirus (NOV-LP) was demonstrated (Ahmed et al., 2017). As shown in Figure 2.23, AuNPs are attached to the surface of graphene, which is conducive to the combination of antibodies with graphene/AuNP hybrid materials and the formation of nanoprobe after binding with virus antibodies. The TMB/H₂O₂ system detects viruses more quickly and sensitively when NOV-LP is added because it causes the TMB solution to turn blue. In a different instance, Guo et al. (2011) created heme-modified graphene nanosheets using π - π interactions and a wet chemical approach. Hepatitis B virus (HBV) can be detected using simple, practical, and environmentally friendly colorimetric platforms made of graphene-based hybrid materials.



Figure 2.23: Antibody-modified graphene/AuNP hybrid for the detection of norovirus (nov-lp) (Ahmed et al., 2017)

On a sensing platform utilizing 2D materials, colorimetric detection of bacteria can also be achieved through the specific binding of bacteria to antibodies or ssDNA aptamers. Kaushal et al. (2020) for instance, described the fabrication of a colorimetric sensor based on GO and gold nanoparticles (AuNPs) that, when combined with antibodies, can quickly detect.

Wu et al. (2017) conducted a study in which they synthesized ZnFe₂O₄-RGO hybrid nanostructures. These nanostructures have the potential to be used as powerful

peroxidase analogs for colorimetric detection of salmonella typhimurium. As a result, one biotin aptamer was utilized as a capture probe, and the aptamer was coupled to the ZnFe₂O₄-RGO hybrid as a single probe. A sandwich structure was created after incorporating the bacterial target. The ZnFe₂O₄-RGO enzyme exhibited significant peroxidase-like activity, leading to a clear color change of the TMB/H₂O₂ system to dark blue. In a buffer solution, salmonella typhimurium had a detection limit of 11 CFU/mL and a detection range of 11-1.10× 10⁵ CFU/mL.

It is crucial for the colorimetric detection of the management of specific diseases that Kaushal and his associates have developed a recent, versatile, and efficient colorimetric technique that uses ssDNA aptamers to modify graphene oxide encapsulated in AuNPs (Gupta et al., 2021). To enhance the binding of aptamers to graphene, graphene oxide was deposited on the surface of AuNPs, as illustrated in Figure 2.24. Furthermore, high-affinity E8 aptamers were selected, and AuNPs coupling was employed to attach them to the graphene surface. When bacteria are present, the aptamers attach to the target bacteria's cell surface and uptake AuNPs, leading to the aggregation of AuNPs and a color change. The unaided eye can clearly see that the solution has changed from red to blue. The solution remained crimson, and the AuNPs were still distributed in the absence of the target bacterium.

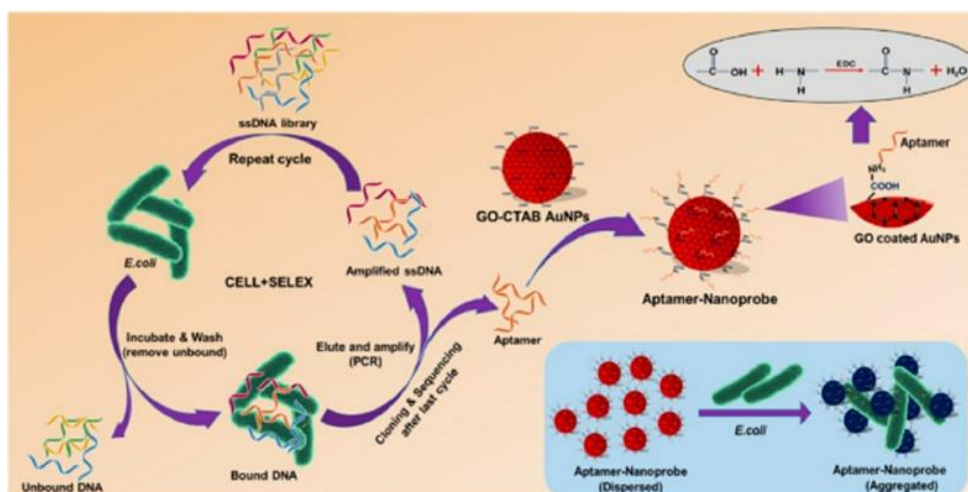


Figure 2.24 Colorimetric detection of aptamer binding with graphene oxide-coated AuNPs. (Kaushal et al., 2020)

2.5.6.4 Detection of DNA/RNA

MiRNAs are genetic indicators of numerous illnesses. MiRNAs can be quickly and effectively identified, which is highly advantageous for the detection and management of various disorders. Conventional miRNA detection techniques are expensive and complex. Based on graphene, Lee (2017) developed a highly sensitive network technique for colorimetry-based miRNA detection. MiRNAs are captured through networks, and a miRNA/graphene composite network was constructed to improve the colorimetric properties of the resulting material. The use of bimolecular information markers and miRNAs for strand exchange improved the change in color during colorimetric detection, as shown in Figure. 2.25a.

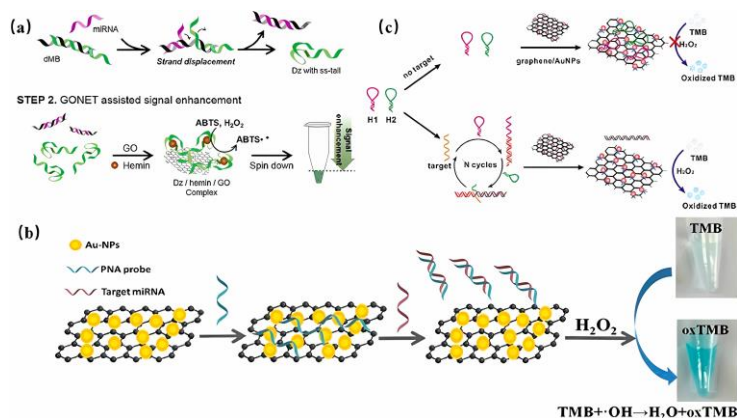


Figure 2.25: 2D material colorimetric sensing of DNA and RNA: (a) Colorimetric detection of graphene by a miRNA network. (Lee et al., 2017) (b) Detection of graphene/AuNP hybrid materials by miRNA (Zhao et al., 2017). (c) DNA detection of graphene/gold hybrid materials (Chen et al., 2017)

Using a graphene/AuNP hybrid surface and the spontaneous adsorption of PNA-21, Zhao et al. (2017) developed a label-free colorimetric sensor for miRNA detection. Similar to graphene/AuNPs catalysis, the contact between the PNA probe and graphene completely deactivates peroxidase, as demonstrated in Figure 2.25b, which significantly reduces the catalytic oxidation of TMB by H₂O₂. As a result, the introduction of miRNA initiated the hybridization reaction between ssPNA-21 and miRNA, leading to the detachment of PNA from graphene/AuNP and causing it to change from colorless to blue. This restored the catalytic activity of graphene/AuNP.

In order to achieve label-free DNA detection, Chen (2017) suggested an assembly method for graphene/gold hybrid platforms (CHAs) based on targeted catalytic hairpin assembly. Without a target, H1 and H2 cannot be constructed, as illustrated in Figure 2.25c. The catalytic activity of H1 and H2 was reduced due to their adsorption on graphene/gold hybrid materials. As a result, TMB oxidation was prevented. Targeted DNA is added,

causing hairpin construction, H1-H2 assembly, and a decrease in the material's binding affinity for graphene/gold hybrids, resulting in a color change from colorless to blue.

2.5.6.5 Small-molecule detection

Effective detection of small molecules can also be achieved with colorimetric biosensors based on two-dimensional materials, such as biological small molecules, organic compounds, and small-molecule pharmaceuticals. For instance, using graphene/gold hybrid materials and the adjustable peroxidase activity of oxytetracycline aptamers, Yuan et al. (2017) devised a colorimetric approach for the detection of oxytetracycline. Combining these two elements can prevent graphene/gold hybrid materials from exhibiting peroxidase-like activity, as they have the ability to adsorb single-stranded DNA through π - π interactions. Following the addition of oxytetracycline, the complex's activity was automatically restored for detection.

Wang et al. (2013) utilized graphene as a transparent and efficient carrier, and a polyacetylene (PDA)-based colorimetric sensor was employed as a high-performance detector for volatile organic compounds (VOCs) by observing the color change of the solution from blue to red. The technique offers a strong colorimetric signal, quick response time, and high sensitivity. Low levels of VOCs can be detected using it.

CuO and graphene were hybridized by Sharma et al. (2017) to create CuO-GO nanospheres for colorimetric H_2O_2 detection. H_2O_2 can also be used to detect cholesterol because it is produced during the oxidative breakdown of cholesterol. By embedding MnO_2 nanoparticles in bovine serum albumin (BSA)-coated graphene oxide, Lee et al. (2019a) reported the synthesis of graphene oxide/ MnO_2 hybrid materials, which can be employed as a novel enzyme with oxidase activity. A dual sensor platform based on

plasma separation and GOD-GO/MnO₂ combination was constructed, as depicted in Figure 2.26. Colorimetry was utilized to determine the glucose concentration without requiring pretreatment of blood samples. Bromograpene was created by Singh and his colleagues for colorimetric measurement of glucose and H₂O₂. The outcomes demonstrated that the breakdown of H₂O₂ and the oxidation of TMB, which turns the solution blue, might be catalyzed by peroxidase-like activity.

Through plasma-mediated hybridization, Rostami and colleagues combined graphene nanosheets with AgNPs to create a colorimetric sensor. In human serum samples, the sensor performed well in detecting glutathione (GSH) and dopamine (DA) (Sr et al., 2020). DA was identified because the shape of the graphene/AgNPs hybrid changed during the etching process, and the color of the solution shifted from green to red. AgNPs then underwent glutathione-induced aggregation, which resulted in a decrease in the absorption intensity of the AgNPs' SPR band. The solution changes color from red to gray, making GSH visible to the naked eye.

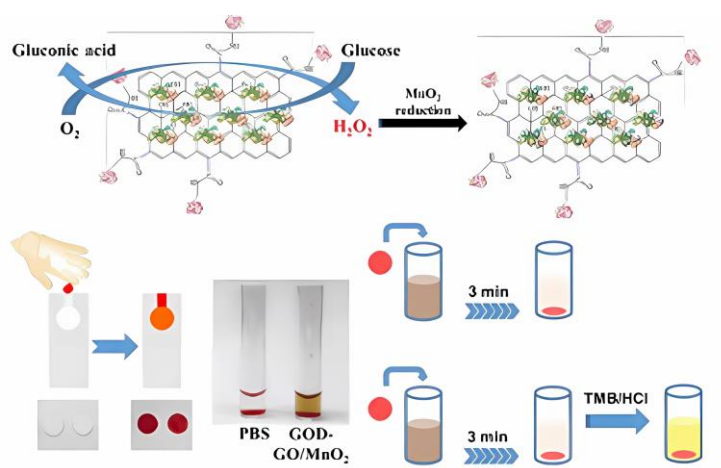


Figure 2.26: Determination of glucose concentration by oxidase graphene oxide/manganese dioxide 2 direct colorimetry (Lee et al., 2019b)

Table 2.6: Review of colorimetric sensor detection methods constructed based on graphene in recent years

S.No	Material	Sensor configuration	Targets detected	Range of detection	LOD	Detection time	References
1	Brominated graphene (GBR)	Nanase catalysis	H ₂ O ₂ Glucose	H ₂ O ₂ 0.50-5.00 mM Glucose 40-100 mM	H ₂ O ₂ 0.417 mM Glucose 28.41 mM	30 min	(Singh et al., 2017)
2	Cytocompatible CuO: Graphene nanosphere (CuO: GNS) composite	Nanase catalysis	Cholesterol H ₂ O ₂	Cholesterol 0.1 mM - 0.8 mM H ₂ O ₂ 0.01-0.1 mM	Cholesterol 78 μ M H ₂ O ₂ 6.88 μ M	40 min	(Sharma et al., 2017)
3	graphene-gold nanoparticles (Grp-Au NPs)	Nanase catalysis	Norovirus-like particles (Novo-LPS)	100 pg/mL-10 μ g/mL	92.7 pg/mL	180 min	(Ahmed et al., 2017)
4	the self-assembly of gold nanorods on graphene oxide (GO-AuNP)	Nanase catalysis	Protamine	100-700 ng/mL	6.3 ng/mL	30 min	(Wiriyachai porn et al., 2019)

Table 2.6: continued

5	the coating of graphene oxide (GO) layer on AuNPs.	SPR changes	<i>E. coli</i> Salmonella typhimurium	<i>E. coli</i> 10 ³ -10 ⁹ CFU Salmonella typhimurium 10 ² -10 ⁹ CFU	<i>E. coli</i> 10 ³ CFU Salmonella typhimurium 10 ² CFU	5 min	(Kaushal et al., 2021)
6	ZnFe ₂ O ₄ -reduced graphene oxide (ZnFe ₂ O ₄ /rGO)	Nanase catalysis	Serum Typhimurium	11~1.10×10 ⁵ CFU/mL	11 CFU/mL	180 min	(Wu et al., 2017)
7	glucose oxidase-conjugated graphene oxide/MnO ₂ (GOD-GO/MnO ₂)	Nanase catalysis	Glucose	25-300 mg/dL	3.1 mg/dL	11 min	(Lee et al., 2019a)
8	GO	Nanase catalysis	MiRNA	10 ⁻⁹ -10 ⁻⁷ M	10 ⁻⁹ M	115 min	(Lee et al., 2019b)
9	GO	Enzymatic catalysis	Platelet-derived growth factor (PDGF)	0.001 nM-10 nM)	100 pM	145 min	(Li et al., 2019)
10	CoS/reduced porous graphene oxide nanocomposite	Nanase catalysis	Hg(II)	2-100 nM	14.23 nM	30 min	(Borthakur et al., 2017)

Table 2.6: continued

11	Reduced Graphene Oxide-PEI-Pd Nanohybrids (rGO/PEI/Pd)	Nanase catalysis	Hg(II)	0.1-25 nM	0.39 nM	8 min	(Zhang et al., 2017)
13	self-assembly of gold nanorods and graphene oxides	SPR changes	Heparin	20 ~ 140 ng/mL	104 ng/mL	45 min	(Bamrungs ap et al., 2019)
14	cobalt-graphene nanohybrid (Co-His- GQD-G)	Nanase catalysis	Chlorpyrifos	2-20 ng/mL	2 ng/mL	70 min	(Nana et al., 2021)

As shown in table 2.5, the above introduction and discussion reveal that graphene oxide, which contains other biosensor probes (such as metal nanoparticles, enzymes, and aptamers), is more suitable for use as a colorimetric biosensor platform. For the preparation of colorimetric biosensors based on TMB/H₂O₂ or SPR-based biosensors, the catalytic activity of graphene oxide nanoparticles is very limited. To overcome this problem, hybrid graphene oxide composites can be synthesized by coupling with other nanoscale biosensor probes, such as metal nanoparticles, nanorods, and metal oxide nanoparticles. The sensitivity and selectivity of the biosensor are improved. However, the colorimetric sensors constructed above still use larger instruments, such as spectrophotometers, in their use, making it difficult to achieve instant detection. Immediate detection is the analysis of a sample for a desired test outside the laboratory, close to or in the place where the test is needed. This approach provides rapid test results close to the sample and allows for immediate action. The key driver is that decision-making can be delayed when the sample is sent to the laboratory. This results in increased equipment purchase and maintenance costs, staff training, connectivity to laboratory information systems, quality control, and other tasks. Therefore, accurate, rapid, and timely on-site assays are important for clinical diagnostics, environmental monitoring, and food safety management.

2.6 Paper-based colorimetric sensors and their application in colorimetric sensing detection

Paper is an inexpensive material made of cellulose or nitrocellulose that serves as an immobilized carrier for reagents and offers advantages for the development of analytical sensing platforms, especially for prompt once test (POCT) devices (Lewiński et al., 2021; Noviana et al., 2021). For example, paper-based analytical sensing devices (PADs) enable

in situ analysis of a wide range of targets because many recognition probes, such as ligands, antibodies, and aptamers, can be easily immobilized in cellulose matrices (Guan et al., 2021; Yun et al., 2023). Lateral flow analyses (C), test-paper assays (Dips), and microfluidic PADs (μ -) are usually used for PADs. (Xiong et al., 2023), which have had great impacts on clinical diagnostics, environmental monitoring, and food safety management (Shalaby et al., 2023; Ranveer et al., 2023).

2.6.1 Classification of paper-based colorimetric sensors

Paper-based colorimetric sensors can generally be divided into three categories, namely Paper method, Microfluidic paper substrates, Lateral Flow Chromatography Strips (LFAS).

2.6.1.1 Paper method

The test paper method usually involves fixing the color developer that reacts with the target on filter paper of a specific shape by soaking and drying, and only drops of the sample to be tested containing the target are added to the test paper. The reaction is compared with the standard color rendering card after a certain period, thus achieving quantitative detection of the target. For example, Shrivastava et al. (2020) reported a smartphone paper-based sensor impregnated with cetyltrimethylammonium bromide-modified silver nanoparticles (AgNPs/CTAB) (e.g., Figure 2.27) for the determination of Fe^{3+} in water and plasma samples. Fe^{3+} was determined by utilizing a smartphone and processing the change in signal intensity after the AgNPs/CTAB were deposited on a paper substrate through ImageJ software. The linear range of this method was 50-900 $\mu\text{g/L}$ with a limit of determination (LOD) of 20 $\mu\text{g/L}$. Shrivastava et al. (2021) reported inkjet-printed paper-based silver nanoparticles (AgNPs) colorimetric sensor for the on-site determination

of mercury ions in ambient water samples using a smartphone and a color detector app (e.g., Figure 2.28). The paper-based colorimetric assay has a linear range of 40-1200 μgL^{-1} and a detection limit of 10 μgL^{-1} . Paper-based sensors are simpler, faster, more user-friendly, less expensive, and smaller than approaches requiring sophisticated analytical gear, making them ideal for on-site target determination.

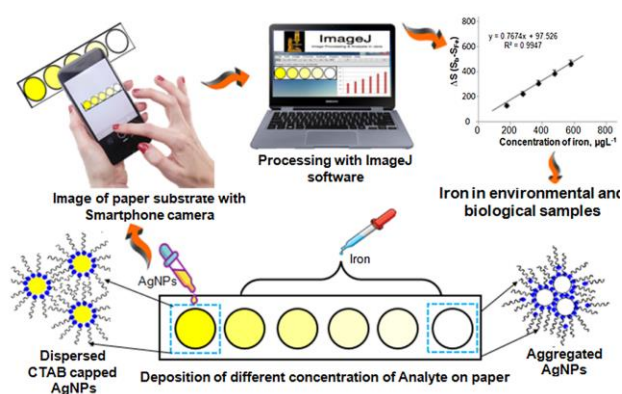


Figure 2.27: Schematic diagram demonstrating the use of a smartphone paper-based sensor followed by processing in ImageJ software to determine the calibration curve and total iron concentration in unknown environmental water and biological samples (Shrivastava et al., 2020)

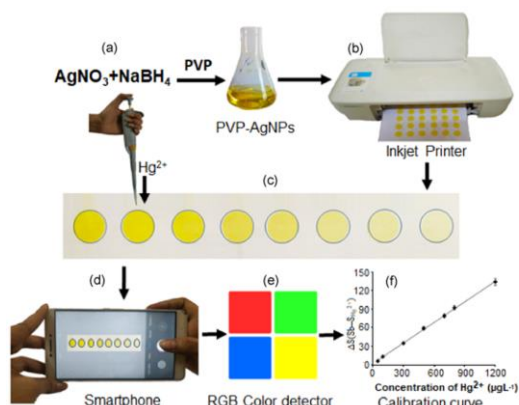


Figure 2.28: Schematic diagram for the synthesis of (a) PVP-AgNPs, (b) inkjet-printed PVP-AgNPs on a paper substrate, (c) deposited Hg^{2+} on printed paper with different concentrations of Hg^{2+} by micropipette, (d) recorded from a smartphone, (e) measured for color intensity via an RGB color detector app and (f) from a standard calibration curve (Shrivastava et al., 2021)

2.6.1.2 Microfluidic paper substrates

Paper-based microfluidic chips (μ -PADs) are a type of microfluidic chip in which paper is used as a substrate material to prepare a miniature all-analysis platform, whereby hydrophobic barriers are formed by combining hydrophobic materials with hydrophilic paper fibers via several different processing techniques, which in turn allows the processing of flow channels that can control the direction of fluid flow (Fakhri et al., 2020). Because paper is porous and hydrophilic, it offers an ideal medium for creating microfluidic channels. Currently, most such devices are fabricated by forming hydrophobic barriers on porous paper sheets using wax or photoresist patterns. On μ -PADs, a wide variety of detectors can be successfully constructed, including those based on optical and spectroscopic systems, and other detectors based on electrical measurements (Figure 2.29). Three key concepts can be used to summarize a detector's working principle: (i) selective element recognition; (ii) conversion of information from the captured target or transducer element into an output signal; and (iii) digital processing of the converted response, which is commonly and universally recognized. For example, Somvanshi et al. (2022) developed a novel paper-based single-input-channel microfluidic device using polystyrene (PS) particle-modified aptamers to modify AuNPs, which allows for the simultaneous detection of a wide range of whole-cell foodborne bacteria and quantitative readout via image analysis. This microfluidic paper-based multiplexed adaptive sensor can simultaneously detect *E. coli*. The use of AuNPs (PSeAu) improved the sensitivity of the analytical signals and produced colorimetric results due to the aggregation of nanoparticles when the analyte was present. The design of the microfluidic paper-based device is shown in Figure 2.30. Akarapipad et al. (2022) reported that μ -PADs, 3D-printed holders, and smartphone cameras were integrated into a portable analytical tool for the detection of severe acute

respiratory syndrome 2 (SARS-CoV-2) in diluted saliva samples (Figure 2.31). The required immunoassays in the sensing zone were based on fluorescent antibody-coupled particles ($\varnothing=0.5 \mu\text{m}$; concentration = $0.02 \mu\text{g} \mu\text{L}^{-1}$). The authors detected SARS-CoV-2 spike proteins well ($1 \text{ fg} \mu\text{L}^{-1}$), with good specificity and a fast analysis time (15 min).

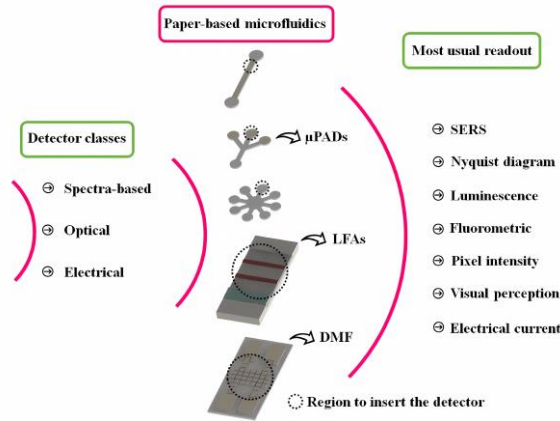


Figure 2.29: Schematic representation of most usual detector classes that can be utilized in paper-based microfluidics (Silva-Neto et al., 2023)

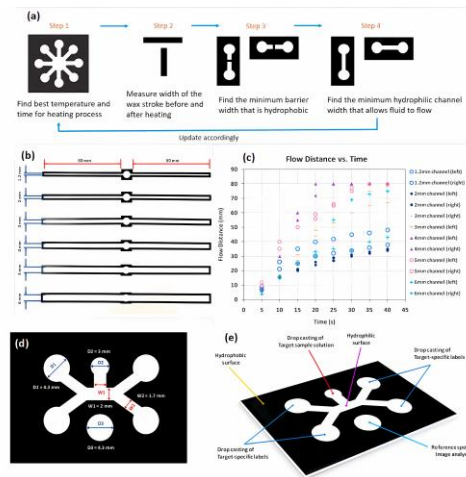


Figure 2.30: Experimental flowchart for optimization of the wax printing process, (b) in-wax channels of different widths for optimization of the flow rate, (c) flow rate versus time plot for increasing channel width, and (d-e) optimized design for the paper device (Somvanshi et al., 2022).

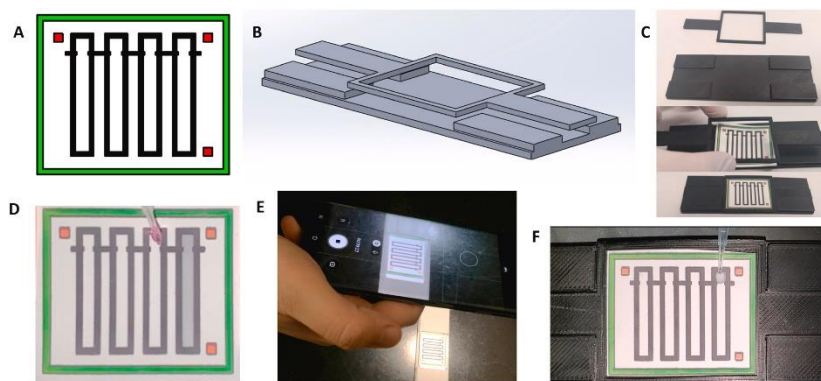


Figure 2.31: Flow profile assay of SARS-CoV-2 on a paper-based microfluidic chip. (A) Paper-based microfluidic chip design containing a green edge and three red squares for recognizing the chip area in automated flow distance measurement. (B) The chip holder and the chip lock. (C) A paper-based microfluidic chip was placed into a chip holder to flatten the chip. (D) 4 μ L of the sample was loaded directly onto the square inlet (top) of each channel and dried for 10 min. (E) A smartphone camera was held just above the chip to view the chip area and start recording the video. (F) 4 μ L of Ab-particles were loaded, and the liquid flow on the paper microfluidic chip was recorded with the smartphone camera (Akarapipad et al., 2022)

2.6.1.3 Lateral Flow Chromatography Strips (LFAS)

A nitrocellulose membrane (NC membrane), a sample pad, a binding pad, an absorbent pad, and a PVC backing are the standard components of lateral flow chromatography test strips (Parolo, 2020). Capture molecules, such as antibodies, can be deposited on nitrocellulose membranes to create test and control lines through hydrogen bonding or electrostatic interactions, as illustrated in Figure 2.32. There are two main types of mechanisms by which LFAs achieve target detection: sandwich-type and competition (or inhibition) types. In a sandwich-type LFA, a beacon molecule reacts with a target analyte to form a beacon-analyte complex, which then continues to migrate along the fluid flow. Through the interaction between the analyte and the corresponding capture molecule, a detection line is formed for determining the presence or absence of the target analyte. It

is possible for labeled molecules devoid of the analyte to cross the test line and be subsequently captured by a different type of capture molecule, thus creating a control line. In a competitive LFA produces conjugated particles are produced, making it difficult to retain them on the detection line. This is because the analyte competes with the captured molecules for binding sites on the detection line, and the beacon molecules may react with the captured molecules deposited on the test and control lines. Conjugated particles can be captured on the test and control lines in the absence of analytes. The binding modes are different, resulting in two opposed color trends. As the number of targets increases, the T-line coloration deepens in the sandwich type and gradually becomes lighter in the competition type (Zhihui et al., 2023).

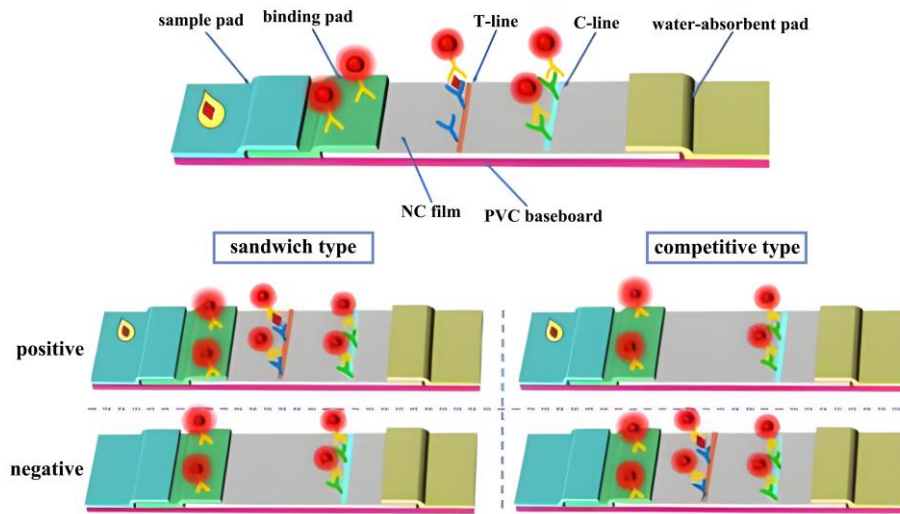


Figure 2.32: Schematic illustration of the main components and the two combination methods of LFAs (Zhihui et al., 2023)

For example, He et al. (2021) developed a lateral flow chromatography platform using nanoparticles as luminescent probes for the simultaneous detection of three periodontitis biomarkers, namely, matrix metalloproteinase-8, interleukin-1 β , and tumor

necrosis factor- α , in saliva, with limits of detection of 5.455, 0.054, and 4,439 ng/mL, respectively. Fluorescence lateral chromatography assays, test kits, and signal detecting equipment (e.g., Figure 2.33) were used by Rong et al. (2021) to streamline the process of various bedside diagnostics and enable on-site high-throughput screening for infectious diseases in settings with limited resources. Serum samples from patients with and without infections showed signs of infectious illnesses (Fig. A). HIV antibodies, hepatitis B, and hepatitis C were among the infectious diseases that were reported. Like the enzyme-linked immunosorbent assay (ELISA), the limits of detection (LODs) ranged from 0.1 to 0.22 Novoulase Units (NCU) mL⁻¹, and the detection time was rapid (20 min). Reproducibility is the main area that may be improved (Relative Standard Deviation ranged from 7.2% to 14.1%).

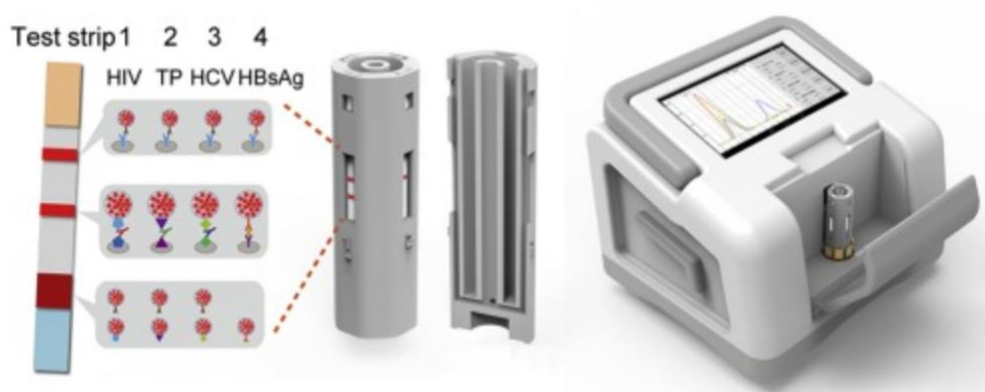


Figure 2.33: Schematic illustration of LFAs and portable equipment for detecting infectious diseases via POCT (Rong et al., 2021)

2.6.2 Applications of paper-based colorimetric sensors

Currently, paper-based colorimetric sensors can be applied to four main areas: ion detection, molecular detection, nucleic acid analysis, and protein detection.

2.6.2.1 Ion detection

Metal ions, especially heavy metal ions, can be enriched in the human body through various pathways, such as the diet, leading to neurological disorders and many other diseases. Therefore, accurate determination of heavy metal ions in daily drinking water and food is important for protecting the environment and human health. A sensitive in-situ mercury sensing platform was developed by Ko et al. (2021) to facilitate easy and efficient monitoring of mercury levels in lake water samples. Encapsulating Au nanoparticles in a PEG hydrogel to form a gold nano enzymatic gel (Au-HBNz) and integrating it into a polymer membrane-based colorimetric device resulted in the construction of a straightforward and useful mercury detection system, as illustrated in Figure 2.34. The blue product oxTMB was produced by catalytically oxidizing TMB through the modulation of Au-HBNz to demonstrate strong peroxidase-like activity upon the addition of Hg^{2+} ions. Based on this mechanism, a liquid-phase colorimetric sensing system was constructed. The system was applied to a paper-based sensing device in which a water-absorbent pad provided the driving force for the reaction, allowing the target to reach the Au-HBNz region and triggering the reaction. With a detection limit of $1.10 \text{ ng}\cdot\text{mL}^{-1}$, strong linearity ($R^2 = 0.96$), and a wide detection range of $0.008 \sim 20 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$, a smartphone was utilized for the quick and easy detection of Hg^{2+} . As a result, the developed sensor can monitor Hg^{2+} in real-time with great selectivity and efficiency, and a smartphone may be used to quantitatively detect the target. However, the low selectivity and the addition of extra corrosive H_2O_2 limit its further application on the market. In addition, Liu et al. (2017) prepared dual-color carbon dot ratio fluorescent test paper,

which produces fluorescent color changes depending on the dose of the target, to achieve semiquantitative detection of copper ions (Cu^{2+}). As shown in Figure 2.35, in this study, radiometric fluorescent test paper was prepared by using a mixture of r-CDs and b-CDs (1:7) as ink and spraying it on paper. The paper evolved from blue to orange-red with increasing Cu^{2+} concentration, and the Cu^{2+} dose discrimination was as low as 25 nM. The results reported in this study imply the advantages of the unique optical properties of nanomaterials and the use of a ratio signal readout, which increases the reliability of the results and provides a new methodology for the development of visually detectable test strips for environmental and medical testing, as well as for promising application.

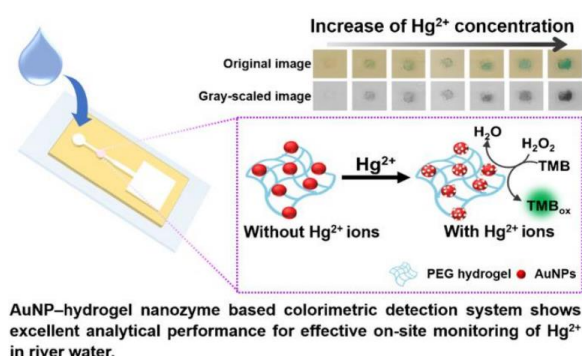


Figure 2.34: Schematic diagram of the Au NP-PEG hydrogel (Au-HBNZ) enzyme paper-based colorimetric detection method for the Hg^{2+} system (Ko et al., 2021)

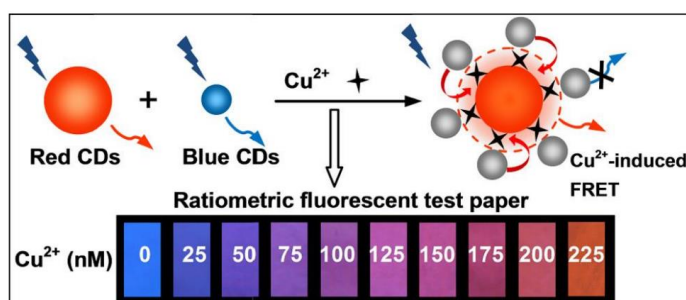


Figure 2.35: Schematic illustration of dual-colored carbon dot ratiometric fluorescent test paper for the semiquantitative assay of copper ions (Liu et al., 2017)

2.6.2.2 Molecular detection

Utilizing a simple soft template technique, Wong et al. (2022) developed vanadium-containing porous cobalt oxide nanomaterials ($\text{V-Co}_3\text{O}_4$) that served as peroxidase mimics for the concurrent colorimetric evaluation of total cholesterol (TC) and glucose in whole-blood samples on 2D μ -PADs. As shown in Figure 2.30, $\text{V-Co}_3\text{O}_4$ has a large surface area and bimetallic catalytic activity, indicating that it has good catalytic activity for ABTS and TMB. A 2D μ -PAD was prepared using a simple wax stamping and cutting technique for simultaneous colorimetric reactions of glucose and TC. The method involves the addition of a drop of whole-blood sample to the sample zone constituted by a blood separation membrane, and the H_2O_2 generated by the enzymatic reaction is further oxidized by $\text{V-Co}_3\text{O}_4$ to generate $^{\bullet}\text{OH}$, which induces the generation of colored products from ABTS and TMB. The amount of green and blue products in the glucose and TC systems, respectively, was proportionate to the amount of H_2O_2 that was produced. The new 2D μ -PAD does not require extensive sample pretreatment steps because it has a small sample volume (15 μL of whole blood) and a fast analysis period (~ 5 min). The new $\text{V-Co}_3\text{O}_4$ integrated with the 2D μ -PAD proved to be a groundbreaking portable device with practical applications for preclinical testing and point-of-care diagnostics for unskilled end-users, not only for the determination of glucose and TC but also for other significant biomarker assays (Figure 2.36). Its advantages include simplicity, low cost, long-term stability, and simultaneous detection.

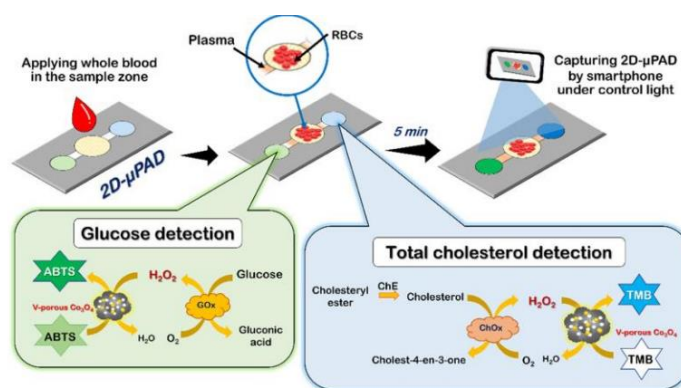


Figure 2.36: Schematic illustration of vanadium-doped porous cobalt oxide for simultaneous total cholesterol and glucose determination in whole blood based on a simple two-dimensional paper-based analytical device (Wong et al., 2022)

2.6.2.3 Protein detection

The most common cause of unexpected cardiac mortality is acute myocardial infarction. For this reason, prompt and early warning systems are critical to the diagnosis and management of CVD; however, clinical tests for signs and symptoms are inadequate given their low specificity. Because of this, identifying cardiac biomarkers is a more reliable method to diagnose an acute myocardial infarction (Tilea et al., 2021). As early as 1.5 h following the onset of symptoms, heart fatty acid binding protein (HFABP), a biomarker of cardiac damage, can be detected at a threshold of 6.2 ng/mL. Currently, the most commonly used methods for the clinical detection of HFABP are enzyme-linked immunosorbent assays (ELISAs) or latex assays (Zheng et al., 2023). These techniques yield accurate results but require specialized equipment, trained testers, and short turnaround times. Additionally, they are not suitable for use in emergency situations or when untrained patients self-test at home. For the sensitive and quick quantitative detection of HFABP, Wang et al. (2021) designed a radiometric fluorescent lateral flow

chromatography immunoassay (RFLFIA) strip. As shown in Figure 2.37, RFLFIA strips work on the principle of radiometric changes in fluorescence signals and the internal filtration effect of gold nanoparticles, which combine to capture and detect antibodies by mixing the fluorescence emitted by two composite nanoprobe. Ratio analysis using a customized smartphone-based portable analysis unit enabled quantitative detection of HFABP with a lower limit of detection of $0.21 \text{ ng}\cdot\text{mL}^{-1}$. Furthermore, the RFLFIA strips produced a visually discernible color change from green to red around the threshold concentration of HFABP (6.2 ng/mL), enabling highly accurate and reproducible semi-quantitative diagnosis by the naked eye. The analytical performance of RFLFIA fluorescent strips is superior to that of ordinary fluorescent strips due to the unique advantages of the radiometric fluorescence method. Moreover, RFLFIA fluorescent strips are comparable to clinical latex agglutination tests and have great potential for immediate detection and home self-testing for diagnosis, which provides feasible technological support for the quantitative detection of other disease markers.

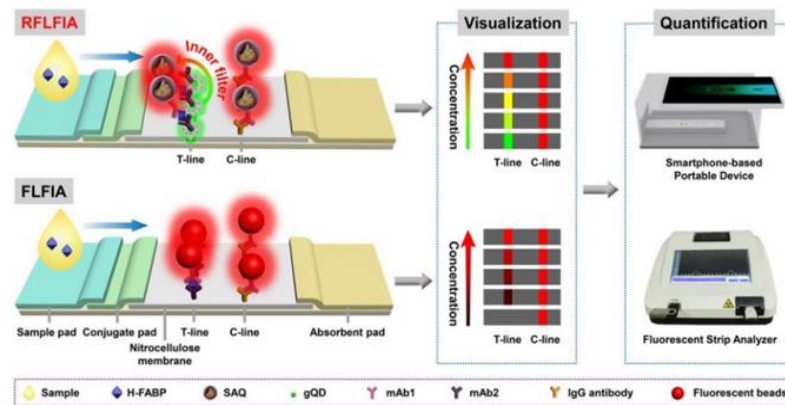


Figure 2. 37: Schematic illustration of the RFLFIA and traditional FLFIA for visual and quantitative detection of H-FABP (Wang et al., 2021)

The severe acute respiratory syndrome type 2 coronavirus (SARS-CoV-2), which causes the 2019 coronavirus disease (COVID-19), was declared a global pandemic by the World Health Organization (WHO) in March 2020. It has had a significant impact on people's lives, health, society, and economy. To reduce the rate of cross-infection, patients must be appropriately placed under quarantine control or seek medical attention in the early stages of positivity. Immunoglobulin assays are crucial for diagnosing the progression of SARS-CoV-2 infection, and the level of SARS-CoV-2 IgG is one of the most important indicators. By using a ligand-assisted epitaxial growth method, Liang et al. (2022) created ultrathin gold-silver shells Ag (AgMBA@Au) with a size of 2 nm embedded with 4-mercaptobenzoic acid (MBA). These components were subsequently incorporated into a lateral flow immunoassay (LFIA) (Figure 2.38) for the dual-mode detection of SARS-CoV-2 IgG using colorimetric and surface-enhanced Raman scattering (SERS). AgMBA@Au combines the exceptional optical properties of silver with the surface chemical benefits of gold. Furthermore, the nanogap between the gold shell and the silver core significantly enhances the Raman signal. AgMBA@Au identifies and binds to SARS-CoV-2 IgG after being modified with a human anti-mouse antibody. SARS-CoV-2 spike protein on the T-cell then binds to the IgG antibodies. Qualitative analysis was performed by visual inspection of the T-line color, and quantitative analysis was performed using the SERS signal, which increased the sensitivity of the measurement by 4 orders of magnitude (limit of detection 0.22 pg-mL^{-1}). When the concentration of other proteins was 10-20 times greater than that of the neocoronavirus IgG, it was almost impossible to produce a distinguishable signal. In the end, this approach was used to test 107 clinical serum samples. The outcomes demonstrated significant improvement compared to colloidal gold test paper and showed good agreement with enzyme-linked immunosorbent assay (ELISA)

kits. As a result, there is significant therapeutic potential for this dual-mode LFIA in identifying and monitoring early immune responses to SARS-CoV-2.

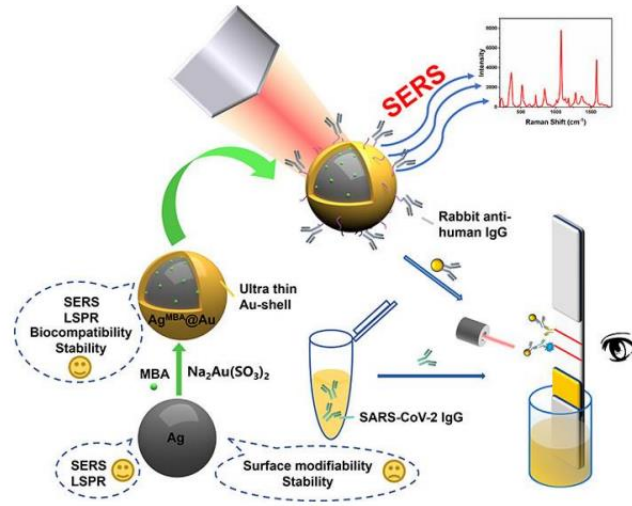


Figure 2.38: Schematic diagram of the synthesis of AgMBA@Au NPs and the test strips for SERS detection of SARS-CoV-2 IgG (Liang et al., 2022)

2.6.2.4 Nucleic acid analysis

A major threat to people's health, tuberculosis (TB) is a chronic infectious disease caused by mycobacteria, specifically *Mycobacterium tuberculosis* (MTB), sometimes referred to as "tubercle bacilli." The issue still pertains to public health. The extensive use of conventional *Mycobacterium tuberculosis* DNA detection techniques is limited in low-resource environments due to the requirement for sophisticated infrastructure and skilled workers, which escalates diagnostic complexity and costs. The development of a portable analytical sensor is urgently needed to address these problems. Zhou et al. (2020a) utilized a thermometer in a paper-based device to develop a cost-effective photothermal biosensing method for the quantitative detection of genetic infections, such as MTB DNA. The DNA capture probe was easily immobilized with a single surface modification step, as illustrated

in Fig. 2.39. By utilizing DNA sandwich hybridization to attach oligonucleotide-functionalized Au NPs onto the test paper, the chromogenic substrate TMB was oxidized, resulting in oxTMB with a high photothermal conversion efficiency. This oxTMB was then used for photothermal biosensing of MTB DNA under 808 nm laser irradiation. Target DNA can be quantitatively detected on paper under ideal circumstances by using an inexpensive thermometer as a signal recorder. This approach delivers higher sensitivity without the issue of color interference compared to traditional colorimetric methods, all without the need for expensive analytical equipment. The highly specific detection of genomic DNA provided further confirmation of the method's validity. This is the first photothermal biosensing method for quantitative nucleic acid analysis using microfluidics and a thermometer. It will serve as a new source of inspiration for the development of affordable, simple, miniature photothermal diagnostic platforms that can be utilized for the quantitative point-of-care detection of various diseases.

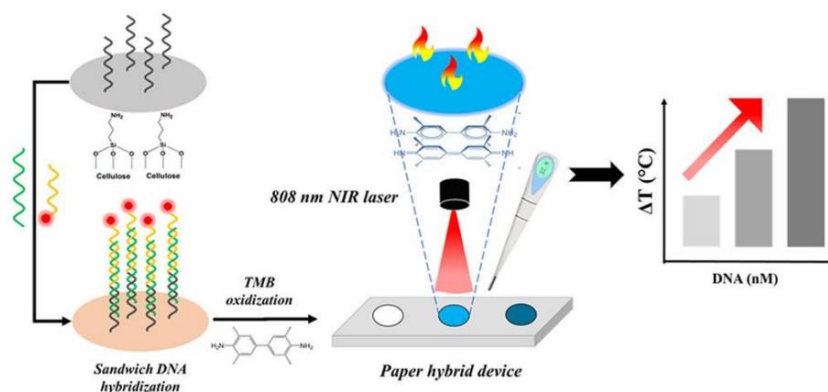


Figure 2.39: Schematic illustration of the working principle of AuNP-mediated photothermal biosensing of MTB target DNA on a paper-based device (Zhou et al., 2020b)

Reverse transcription polymerase chain reaction (RT-PCR) is the gold standard nucleic acid amplification technique for identifying this novel coronavirus. However,

POCT does not allow for RT-PCR, which requires a significant amount of time, large instruments, and specialized knowledge. Meng et al. (2022) successfully integrated FeS₂ nano enzymatic strips with recombinase polymerase amplification (RPA) for simple nucleic acid amplification and subsequent colorimetric signal enhancement. This was based on the high peroxidase-like activity of the FeS₂ enzyme to develop POCTs for SARS-CoV-2 (e.g., Figure 2.31). Significantly, the results for clinical samples of human papillomavirus type 16 (HPV-16) agreed 100% with earlier RT-PCR results, demonstrating the method's adaptability and dependability. The findings imply that non-enzymatic-based nucleic acid detection holds significant promise for application in COVID-19 and other virus diagnoses. Notably, the RPA plays a major role in the specificity of the system. As a result, the primers used for RPA must be carefully designed to prevent primer-dimer formation, which could lead to false-positive results. Furthermore, for practical, extremely sensitive, and specific POCTs, an integrated device that combines sample processing, RPA, and nanoenzymatic detection needs to be taken into consideration.

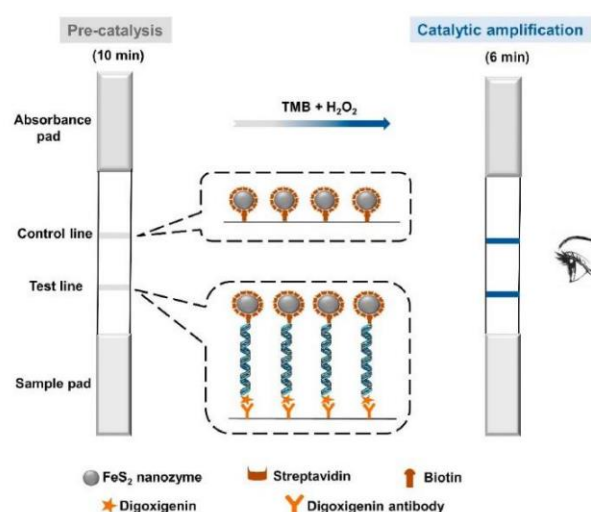


Figure 2.40: Schematic illustration of enzyme strips for nucleic acid detection (Meng et al., 2022)

Colorimetric sensing based on paper is widely utilized in the investigation of various substances, including biomolecules and heavy metals. The primary working principle of this technology is the color shift of the nanoparticles that are included in the paper matrix as active components. Precious metal nanoparticles (Ag, Au, and Cu) are commonly utilized in paper-based devices. When the target analyte interacts with the paper-based device, the nanoparticles change color. The color shift that occurs on the paper sensor is then converted into a digital image using additional software or a digital camera device, such as a portable desktop scanner. The color observed on the paper strip indicates the concentration of the target analyte, which can be determined using a color detection program or the ImageJ software (<https://imagej.nih.gov>). Compared to organic reagents, utilizing nanoscale materials as sensing elements has the advantages of increased stability, stronger extinction coefficients, and higher detection sensitivity for the target analyte (Shrivastava et al., 2021; Shrivastava et al., 2020). Therefore, paper-based colorimetric sensors are simple, efficient, and inexpensive. There is no need for large equipment or instruments or specialized personnel to operate the process. The analysis system is easy to miniaturize and portable, no external drive pump is needed, and the paper is foldable and easy to store and transport. Moreover, the paper base is biocompatible and can immobilize biomolecules such as enzymes, proteins, and DNA, enabling rapid on-site detection of targets.

2.7 Summary

With the development of the social economy, a large amount of wastewater containing heavy metal pollution is discharged into the environment, which poses an increasingly serious threat to human health and the ecological environment. Therefore, it is of environmental importance to establish a rapid, sensitive, reliable, and low-cost heavy metal ion detection technology. Conventional analytical methods have difficulty meeting

current detection needs due to defects such as expensive instruments, high operating costs, difficulty carrying analytes, cumbersome and time-consuming procedures, and strict operational requirements for detectors. As an emerging detection technology, nanomaterial-based colorimetric sensors with rapid response times, low sensitivities, and simple operation procedures for homogeneous systems provide new ideas for the detection of heavy metals. However, this type of sensor is limited by the carrier, and the structural defects of easy stacking and agglomeration when applied to heavy metal detection limit the further improvement of its catalytic activity, resulting in a long response time, insufficient sensitivity, and narrow detection range. It is also hard to realize the simultaneous detection of two different heavy metal ions. In contrast, graphene and its derivatives have a unique layered structure, large specific surface area, and active groups on the surface to provide modified active sites for loading other nanomaterials, making them good support materials for the preparation of nanocomposites; additionally, these materials have been widely used in the field of colorimetric sensing and detection. (Vinotha et al., 2021; Minh et al., 2020; Boruah et al., 2020). In addition, most of the color changes in the currently reported colorimetric sensing assays for heavy metals need to be quantified by spectrophotometers; however, the large size of spectrophotometers makes it difficult to meet the demand for instantaneous detection of heavy metal ions (Li et al., 2016; Feng et al., 2017). While analytical test strips have the advantages of easy portability, easy field detection, simple operation, low cost, and multifunctional analysis (Ye et al., 2021), little research has been reported on colorimetric analytical test strips that can be used for the field detection of heavy metals. Therefore, in this study, graphene oxide was used as a carrier to synthesize structurally stable and catalytically efficient noncatalytic materials, construct a colorimetric sensing detection platform, and prepare test strips for the analysis

of heavy metal ions based on the performance of graphene oxide composites such as hydrogen peroxidase to achieve rapid and accurate detection of heavy metal ions in the field.

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter, the experimental framework, material synthesis, material characterization, UV–Vis method for heavy metal detection, imaging and analysis, Michaelis-Menten equation, and experimental design are presented. Details about the discussion are provided in Sections 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, and 3.8. Finally, a summary of this chapter is presented in Section 3.9.

3.2 Experimental framework

This section describes the experimental framework of this study to clarify the correspondence between the research content and the research objectives, as shown in Figure 3.1. This study involves three main steps: the synthesis of graphene oxide/nonplatinum composites for detection of Ag; the improvement of the sensitivity for the simultaneous detection of two different heavy metals; and design and application of heavy metal colorimetric sensors using test strips for heavy metal analysis.

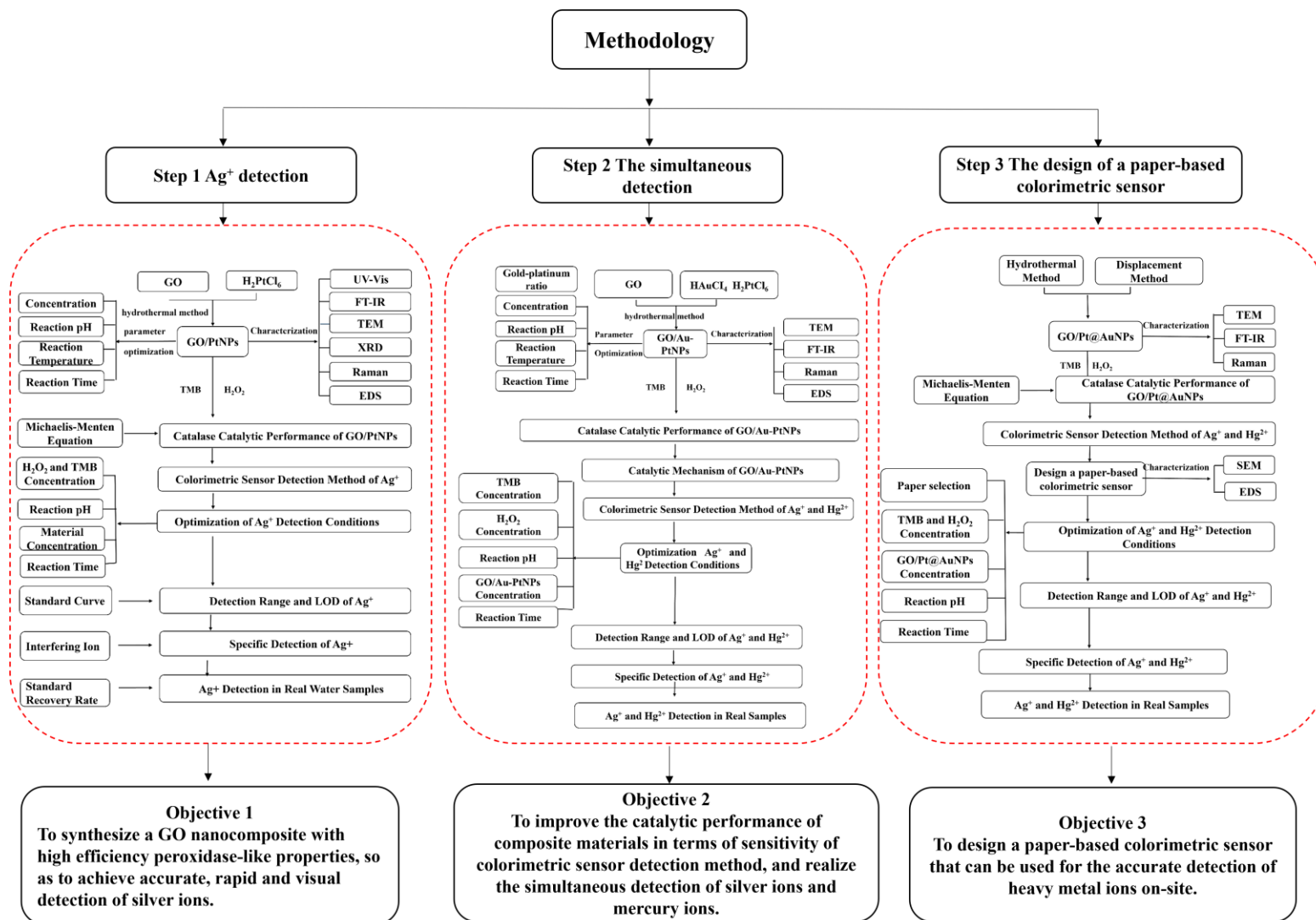


Figure 3.1: Research methodology flowchart

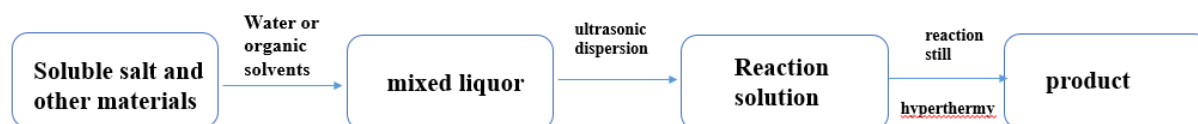


Figure 3.2: Main steps in the synthesis of graphene oxide composites by hydrothermal methods

3.3 Material characterization

3.3.1 Surface morphology

The composites produced in this study were based on graphene oxide. Due to the short electron wavelength, transmission electron microscopy achieved significantly higher resolution compared to optical microscopy, with a magnification of 0.1–0.2 nm. Therefore, the fine structure of the synthesized graphene oxide composites, including the size, distribution, and microstructure, was observed via transmission electron microscopy; thus, transmission electron microscopy was chosen to characterize the surface morphology of the synthesized GO composites. However, the characterization of the paper base is too thick, and TEM requires the sample to be very thin, so SEM is preferred. The steps are as follows.

To observe graphene oxide nanocomposites using TEM, the samples need to be dispersed in pure water and homogenised by sonication. A suitable amount of the dispersion was taken and dropped on an ultrathin support film (copper mesh with 300 mesh aperture) and allowed to dry at room temperature. Observation was performed using a transmission electron microscope with an accelerating voltage of 200 kV. Appropriate magnification (100,000~1,000,000 times) was selected and the image was imaged in low-dose mode to minimise damage to the sample by the electron beam so as to obtain a clear image of the microstructure.

To observe the nanoparticles on the surface of the filter paper using scanning electron microscopy (SEM), the sample first needs to be fixed on the sample stage and dried at the freeze-fracture or critical point. Set the working voltage of the SEM to 5-15 kV, adjust the working distance and vacuum according to the sample characteristics, choose the appropriate magnification (5,000~50,000 times), and carefully observe and record the morphological features of the nanoparticles.

3.3.2 Material structure

In this study, the Raman spectra of graphene oxide, nanoparticles (e.g., gold nanoparticles and platinum nanoparticles), and graphene oxide composites were analyzed to characterize the vibrational and rotational energy levels before and after the synthesis of graphene oxide composites and to analyze the successful synthesis of graphene oxide composites by using the "fingerprint" characteristics of Raman shifts. The infrared spectra of graphene oxide and graphene oxide composites were compared and analyzed to determine the synthesis mechanism and properties of the composites. The steps for Raman and IR measurements were shown below:

When using Raman spectroscopy to characterise liquid graphene oxide nanocomposites, a laser with a wavelength of 785 nm should be selected as the excitation source with a power of about 5-10 mW to avoid thermal effects on the sample. A long working distance objective lens (50X) is used to focus the incident light into the liquid sample in a non-contact manner. The exposure time is typically set to 10-30 seconds and the number of accumulations can be adjusted according to the signal-to-noise ratio. The collected spectra should be in the range of 800-2200 cm^{-1} , focusing on the D-band ($\sim 1350 \text{ cm}^{-1}$) and G-band ($\sim 1600 \text{ cm}^{-1}$) to analyse the material properties.

To characterise graphene oxide nanocomposites using Fourier transform infrared spectroscopy (FTIR), the samples need to be dried and pressed or coated on KBr sheets. The spectrometer was set to have a resolution of not less than 4 cm⁻¹ and a scanning range of 400-4000 cm⁻¹. 32 scans were accumulated for each sample to improve the signal-to-noise ratio and to analyse the changes in functional groups of the materials and their composites.

3.3.3 Crystallinity

In this study, the successful synthesis of the composites was supported by measuring the peak position, peak width, and peak intensity of the diffraction peaks of graphene oxide, platinum nanoparticles, gold nanoparticles, nickel nanoparticles, and their composites. In addition, the changes in the XRD diffraction peaks of the graphene oxide/composite before and after the assay were further analyzed to determine the mechanism involved.

Characterisation of graphene oxide nanocomposites using X-ray diffraction (XRD) required uniform spreading of the samples on a sample holder. A Cu-K α radiation source was used with a wavelength of 0.15406 nm, a scanning range of 5° to 80°, a step size of 0.02° and a scanning rate of 2° per minute. This enabled the detection of the crystal structure and phase composition of the material, in particular to observe the typical intercalation peaks of graphene oxide and the formation of new phases in the composite.

3.3.4. Chemical state analysis

In this study, we synthesized graphene oxide/composite materials, and the synthesized materials were all liquid at low concentrations; therefore, we chose EDS to analyze the elemental species and relative contents on the surface of the synthesized

composite samples to further support the successful synthesis of the composite materials and subsequent analysis of the catalytic mechanism.

Characterisation of graphene oxide nanocomposites using energy dispersive X-ray spectroscopy (EDS) requires that the samples are first properly cemented or coated on a conductive substrate. Under scanning electron microscopy (SEM), a suitable accelerating voltage (e.g., 15 kV) is selected and the sample is scanned at a fixed point or area. The acquisition time is set to 30-60 seconds to obtain qualitative and quantitative analysis data of carbon, oxygen and other elements to assess the chemical composition of the material and its distribution.

3.4 Michaelis-Menten equation

An enzyme reaction's rate of initiation with respect to substrate concentration is expressed by the Michaelis-Menten equation, which is a rate equation. When the substrate concentration in an enzymatic reaction is in the intermediate range, the reaction is a mixed-order reaction with respect to the substrate. At low substrate concentrations, the reaction is a first-order reaction. The reaction transitions from a first-order reaction to a zero-order reaction as the substrate concentration increases.

$$\frac{1}{V} = \frac{K_m}{V_{\max}[S]} + \frac{1}{V_{\max}} \quad \text{Equation 3.3}$$

The Michaelis-Menten equation, or K_m value, represents the reaction rate when the enzyme is saturated with the substrate. $[S]$ stands for the substrate concentration. This equation, also known as the Michaelis-Menten equation, is derived based on the assumption that a steady-state reaction condition exists. The substrate concentration at which the reaction velocity V reaches $1/2V_{\max}$ (i.e., $K_m = [S]$) is the physical significance of

the K_m value. The unit is typically mol/L, which is solely defined by the nature of the enzyme and is unrelated to the enzyme concentration. Different enzymes can be identified by their K_m values (Wang et al., 2022; Chen et al., 2021b).

3.4.1 Significance of the Michaelis-Menten equation

The substrate concentration limits the enzyme's activity when it is significantly lower than K_m , at which point the reaction rate is proportional to the substrate concentration. The reaction rate approaches V_{max} when the substrate concentration is significantly higher than K_m . At this point, the enzyme activity is saturated, and the reaction rate is not significantly affected by further increases in the substrate concentration. The K_m value indicates how well an enzyme is suited to its substrate. According to Wang et al. (2022) and Chen et al. (2021), an enzyme with a small K_m value has a strong affinity for the substrate, enabling it to achieve a higher reaction rate even at lower substrate concentrations. Conversely, an enzyme with a large K_m value has a weak affinity for the substrate and requires a high substrate concentration to achieve a high reaction rate.

3.4.2 Methods for the determination of K_m and V_{max} values

The K_m and V_{max} values can be obtained by fixing the enzyme concentration in the reaction and then analyzing the starting velocities at several different substrate concentrations. However, it is difficult to determine the K_m or V_{max} values directly from a plot of starting velocity against substrate concentration because the curve is asymptotic as it approaches V_{max} . Therefore, it is common to use the transformed form of the Michaelis-Menten equation to find the K_m and V_{max} values. A commonly used transformed form of the Michaelis-Menten equation is the Lineweaver-Burk equation, also known as the double inverse equation. A straight line can be obtained by making a $1/V$ graph against $1/[S]$. The

absolute value of $1/K_m$ can be obtained from the intercept of the line with the x-axis; $1/V_{max}$ is the intercept of the line with the y-axis. Double inverse graphing is intuitive and easy to understand and provides an easily recognizable graph for enzyme inhibition studies (as shown in Figure 3.3).

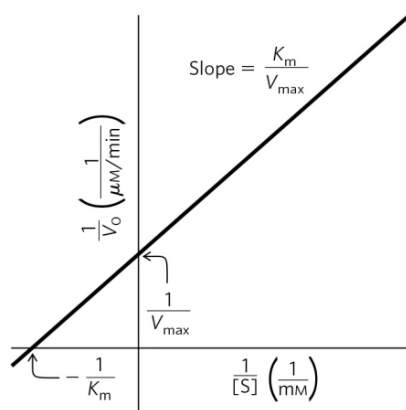


Figure 3.3: Image of the Lineweaver-Burk equation (Nelson et al., 2008)

The discipline of analyzing the rate of enzymatic processes and the various factors influencing the rate is known as enzymatic kinetics. In kinetics, the Michaelis-Menten equation is crucial. The development of the Michaelis-Menten equation, which shifted enzymology research from a qualitative description stage to a quantitative study stage, can be considered a pivotal moment in the field. Therefore, the catalytic kinetics of biological and bio-like enzymes are frequently described by the Michaelis-Menten equation. In this study, we synthesized graphene oxide nanocomposites with peroxidase-like properties. We used the Michaelis-Menten equation to describe the catalytic kinetic process of the synthesized graphene oxide nanocomposites (peroxidase-like). The affinity between our synthesized graphene oxide nanocomposites and the substrate was obtained by measuring the obtained Michaelis-Menten constant K_m and comparing it with the Michaelis-Menten constants reported in other studies.

3.5 Imaging and analysis

3.5.1 The method of collect the paper image

In order to reduce the error, the paper base was placed in the same position inside the identical dark box for each photo. The HUAWEI Mate 20 X mobile phone was secured in a fixed position at the top of the dark box to capture photos in a stationary mode, and the phone's flash was utilized to provide light for each shot.

3.5.2 Image analysis

In this study, to quantify the ability of the designed analytical test strips to be detected, a digital camera was used to take pictures of the color development of the test strips, and the images were subsequently digitized by ImageJ software to split the original images into R (red), G (green) and B (blue) image channels to construct quantitative curves of color greyscale values and target concentrations for quantitative analysis of the targets (Shrivastava et al., 2020).

The steps for using ImageJ software were as follows:

- i. The target image to be analyzed was opened with ImageJ.
- ii. "Image" and "Type" were clicked to convert the image to 8-bit grayscale (0-255).
- iii. "Edit" and "Invert" were clicked to invert the gray values.
- iv. The "ROI Manager", "Analyse", "Tools", and "ROI Manager" were clicked. Tools", "ROI Manager".

v. The measurement parameters were set. Click "Analysis" and "Settings", and then check "Average gray value" and "Threshold limit".

vi. The area was selected with the MagicWand tool. The "Magic Wand Tool" button is double-clicked in the toolbar to adjust the mode and tolerance; the smaller the tolerance value is, the closer the selected color is.

vii. The "ROI Manager" clicks the "Measure" button to obtain the average gray value.

3.6 Experimental design

In this study, relying on the H_2O_2 -TMB chromogenic system, a total of three colorimetric sensors were constructed: an Ag^+ colorimetric sensor based on a graphene oxide/nano platinum composite, simultaneous Hg^{2+} and Ag^+ colorimetric sensors based on graphene oxide/Au-Pt nanoparticles and a paper-based colorimetric sensor based on a graphene oxide/platinum composite for simultaneous detection of Hg^{2+} and Ag^+ . This study aimed to construct a colorimetric sensing method for the on-site, rapid, and accurate detection of heavy metal ions.

3.6.1 Ag^+ colorimetric sensor based on a graphene oxide/nanoplatinum composite

3.6.1.1 Chemicals

Silver nitrate (AgNO_3), citric acid ($\text{C}_6\text{H}_8\text{O}_7$), disodium hydrogen phosphate (Na_2HPO_4), hydrogen peroxide (H_2O_2), chloroplatinic acid (H_2PtCl_6) and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Tianjin Daimao Chemical Reagent Co. Ltd. (China), and graphene oxide was purchased from Shenzhen Qihang Technology Co. Ltd. (China). All the metal salt solutions (0.1 M) were prepared from the corresponding

metal salts in deionized water. All glassware was cleaned by rinsing several times with tap water and then rinsing with high-purity water. High-purity water obtained using a water purifier (WP-UPT-20, China) was used for reagent preparation. 0.1 N hydrochloric acid and 0.1 N sodium hydroxide, both of analytical grade and obtained from Tianjin Damao Chemical Reagent Co. Ltd. (China), were used to adjust the pH.

3.6.1.2 Synthesis of GO/PtNPs

As mentioned in Section 3.4, hydrothermal method was used to prepare GO/PtNPs. First, 1.25 mL of graphene oxide (0.5 mg/L) and 0.3 mL of H_2PtCl_6 solution (10 mM) were mixed homogeneously with 10 mL of ultrapure water. Then, the pH of the system was adjusted to 9.5 with sodium hydroxide (0.1 mol/L) before adding ultrapure water to 12.5 mL. The mixture was then sonicated for 6 h before being transferred to a hydrothermal reactor (LC-KH-50, LICHEN, China) at 180°C for 8 h. From this process, graphene oxide nanoplatinum composites (GO/PtNPs) were obtained.

The parameters (pH of the mixture, concentration of H_2PtCl_6 solution, synthesis temperature, and synthesis time) affect the size, shape, dispersion, and catalytic ability of GO/PtNPs. Therefore, the parameters were varied to determine the ideal conditions. The effects of the pH of the mixed solution (8, 8.5, 9, 9.5, 10, and 10.5), concentration of H_2PtCl_6 solution (0.08, 0.16, 0.24, 0.32, and 0.4 mM), synthesis temperature (150, 160, 170, 180, 190, and 200°C) and reaction time (6, 7, 8, 9, and 10 h) on GO/PtNP synthesis were investigated.

Five microliters of the synthesized GO/PtNPs, 40 μL of TMB (1 mM), and 4 μL of H_2O_2 (10 M) were mixed and then fixed to 2000 μL with a citrate buffer solution at pH=5.

The absorbance values were measured using a UV-1750 spectrophotometer (Shimadzu, Japan) at 652 nm after 5 min of reaction.

3.6.1.3 Characterization of the synthesized GO/PtNPs

UV–Vis spectra were obtained using a UV-1750 spectrophotometer from Shimadzu Co., Ltd., of Japan. First, dispense GO or GO/PtNPs in water. Set the scanning range to be in the 200-700 nm range according to the experimental requirements (generally GO absorbs in the UV region and PtNPs may have plasma absorption peaks in the visible region). Place the GO or GO/PtNPs solution in a cuvette and place it in the sample chamber of the instrument for scanning. The change in absorbance with wavelength is recorded. Then, observe the characteristic absorption peaks of GO and PtNPs. GO will usually show an absorption peak at about 230 nm due to $\pi \rightarrow \pi^*$ electron leaps, while PtNPs may show a plasma absorption peak in the visible region. Fourier transform infrared (FTIR) spectroscopy was performed with a Nicolet iS5 (USA) in the range of 4000-400 cm^{-1} to observe the changes in the surface groups of GO and GO/PtNPs. A LabRAM HR confocal micro-Raman spectrometer (France) was used to analyze the changes in GO surface defects before and after synthesis. The crystal structures of GO and GO/PtNPs were evaluated using a PANalytical X-ray diffractometer (XRD) (Netherlands). The shape and size of the prepared GO/PtNPs were analyzed using a JEOL-JEM2100F (Japan) transmission electron microscope (TEM) at 200 kV. An SM-7500F (Japan) X-ray energy spectrometer (EDS) was used for the elemental studies.

3.6.1.4 Study of the catalytic kinetic parameters of GO/PtNPs

A fixed amount of GO/PtNPs (0.047 $\mu\text{g/mL}$), H_2O_2 , and TMB was placed in a 5 mL test tube. The concentrations of H_2O_2 and TMB were varied from 0.005 to 0.04 M and

from 0.01 to 1.5 mM, respectively. Then, a citrate buffer solution with a pH varying between 2.2 and 8 was added to 2000 μL . The absorbance values were measured using a UV-1750 spectrophotometer (Shimadzu, Japan) at 652 nm. The measurement time was also varied between 1 and 5 min.

3.6.1.5 Detection ability of the synthesized GO/PtNPs for Ag^+ ions

Specificity and anti-interference analysis. Ag^+ , Zn^{2+} , Cr^{6+} , Cu^{2+} , Ni^{2+} , Al^{3+} , Cd^{2+} , and Ca^{2+} solutions were added to 1000 μL of citric acid buffer solution at pH 5 and 2 μL of GO/PtNP solution. After mixing well for 10 min, 4 μL of H_2O_2 (10 M) and 40 μL of TMB (1 mM) were added to the mixture, which was subsequently fixed to 2000 μL of a citric acid buffer solution at pH 5. The absorbance values were measured using a UV-1750 spectrophotometer (Shimadzu, Japan) at 652 nm after reacting for 5 min.

Sensitivity analysis. GO/PtNPs (2 μL) were mixed with Ag^+ solution (0.5–1000 μM) and citric acid buffer solution (1000 μL , pH=5) until homogeneous for 10 min. Then, 4 μL of H_2O_2 (10 M) and 40 μL of TMB (1 mM) were added to 2000 μL of citric acid buffer solution (pH 5). After reacting for 5 min, the absorbance values were measured at 652 nm, and standard curves were drawn.

Spike-and-recovery experiment. The practicability of the proposed method was studied by adding different concentrations of Ag^+ to various environmental water samples (i.e., tap water, pond water, and river water). Tap water samples were collected from the municipal pipe network in Jinan, Shandong Province, China; pond water was collected from Jinan, Shandong Province, China; and river water samples were collected from the Xiuyuan River in Jinan, Shandong Province, China. The collected water samples were filtered through a filter membrane with a pore size of 0.22 μm , and Ag^+ (1, 10, 100, 500

μM) standard solutions were added to the actual water samples. The proposed colorimetric sensing method based on graphene/nano platinum was used for determination, the experimental results were recorded, and the recovery rate was calculated.

3.6.2 Simultaneous Ag^+ and Hg^{2+} colorimetric sensors based on graphene oxide/Au-Pt nanoparticles

3.6.2.1 Reagents

Mercury nitrate ($\text{Hg}(\text{NO}_3)_2$), silver nitrate (AgNO_3), disodium hydrogen phosphate (Na_2HPO_4), hydrogen peroxide (H_2O_2) and citric acid ($\text{C}_6\text{H}_8\text{O}_7$) were purchased from Tianjin Damao Chemical Reagent Co. Chloroauric acid ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$) and chloroplatinic acid (CPA) were purchased from Tianjin Mysco Chemical Co. Graphene oxide was purchased from Shenzhen Qihang Technology Co. 3,3',5,5'-Tetramethylbenzidine (TMB) was purchased from Shanghai McLean Biochemical Technology Co. All metal salt solutions (0.1 M) were prepared by dissolving the respective metal salts in deionized water. All glassware was thoroughly cleaned through multiple rinses with tap water followed by subsequent rinses with deionized high-purity water. High-purity water, obtained through the use of a water purifier (WP-UPT-20, China), was used for the preparation of reagents. Analytical grade 0.1 mol/L hydrochloric acid and 0.1 mol/L sodium hydroxide were used to manipulate the pH. The chemicals utilized in this study were procured from Tianjin Damao Chemical Reagent Co., Ltd., located in China.

3.6.2.2 Synthesis and optimization of GO/Au-PtNPs composites

A total of 2.5 mL of graphene oxide (GO) aqueous solution at a concentration of 0.5 mg/mL, 0.5 mL of $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ at a concentration of 10 mM, 0.5 mL of $\text{HPtCl}_4 \cdot 6\text{H}_2\text{O}$ solution at a concentration of 10 mM and 0.9 mL of NaOH solution at a

concentration of 0.1 M were mixed uniformly. After the pH of the system was set to 8.5, the mixture was subjected to ultrasonication at 25°C for 2 h and subsequently placed in a hydrothermal reactor to react at 180°C for 9 h. This process produced the GO/Au-PtNP composite.

Various parameters affect the size, structure, dispersion, and catalytic ability of GO/Au-PtNPs. Therefore, to determine the ideal conditions, the pH of the reaction system (8.0, 8.5, 9.0, 9.5, and 10.0), the ratios of the loading metals Au and Pt (1:2, 3:4, 1:1, 4:3, and 1:2), the concentration of graphene oxide (0.3, 0.4, 0.5, 0.6, and 0.7 mg/mL), the hydrothermal reaction time (4, 6, 8, 10, and 12 h), and the hydrothermal reaction temperature (160, 170, 180, 190, and 200°C) were optimized. Only one of the parameters was changed at a time, and the others were kept constant.

3.6.2.3 Characterization of GO and GO/Au-PtNPs

The morphology of the GO/Au-PtNPs was characterized by transmission electron microscopy (TEM) on a JEOL-JEM2100F (Japan) instrument at a voltage of 200 kV. The changes in the surface defects of GO and GO/Au-PtNPs before and after synthesis were analyzed by a confocal micro-Raman spectrometer (LabRAM HR type). Elemental analysis was carried out with an SM-7500F (Japan) X-ray energy spectrometer (EDS). FTIR spectra were obtained in the range of 400–4000 cm⁻¹ by a Nicolet iS5 (USA), and the changes in the surface groups of GO and GO/PtNPs were obtained.

3.6.2.4 Optimization of the detection conditions for the GO/Au-PtNPs colorimetric sensor

A total of 892 μL of citric acid buffer solution at $\text{pH} = 5$, 6 μL of GO/Au-PtNPs, and 100 μL of TMB solution at a concentration of 0.5 mM were evenly mixed. Finally, 2 μL of 30% H_2O_2 solution was added, after which the mixture was shaken evenly and reacted at room temperature for 5 min. The absorbance was measured at a wavelength of 652 nm.

During the reaction, various parameters influence the color change of the system, which is very important for contrast color sensors and affects the detection limit and sensitivity of the sensor. To determine the ideal conditions, the pH (3.4, 4.0, 5.0, 6.0, 6.4, 7.0, 7.4, and 8.0), reaction time (1, 2, 3, 4, 5, 6, and 7 and 8 min), GO/Au-PtNPs (1, 2, 3, 4, 5, 6, 7, and 8 μL), TMB concentration (0.3, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mM), and H_2O_2 concentration (0.2, 0.4, 0.6, 1.0, 2.0, 3.0, 4.0, and 5.0 M) were optimized.

3.6.2.5 Development of a colorimetric sensor and its application in practical samples

Detection method for Hg^{2+} via colorimetric sensing. To initiate the experiment, a mixture of 6 μL of GO/Au-PtNPs and 882 μL of citric acid buffer solution at a pH of 5 was combined with 10 μL of Hg^{2+} solution at various concentrations (0.001, 0.002, 0.01, 0.05, 0.1, 0.5, 1, 8, and 10 μM). The solution was thoroughly mixed and subsequently incubated at a temperature of 20°C for 10 min. Subsequently, 100 μL of TMB solution at a concentration of 0.5 mM and 2 μL of H_2O_2 at a concentration of 30% were added. The total volume was adjusted to 1000 μL using a citric acid buffer solution. The mixture underwent a reaction for 5 min before the absorbance was measured at a wavelength of 652 nm.

Ag⁺ colorimetric sensing detection method. Six microliters of GO/Au-PtNPs and 882 μL of citric acid buffer solution at $\text{pH} = 5$ were added to 10 μL of Ag⁺ solution at different concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 μM). The solution was thoroughly mixed and incubated at 20°C for 10 min. Then, 100 μL of TMB solution at a concentration of 0.5 mM and 2 μL of H₂O₂ at a concentration of 30% were added. The volume was then adjusted to 1000 μL using a citric acid buffer solution. The mixture was allowed to react for 5 min before the absorbance was measured at 652 nm.

Ag⁺ and Hg²⁺ were detected simultaneously in actual samples. Ten microlitres of campus lake water sample, tap water, and serum were added to different concentrations of Ag⁺ (10 nM, 100 nM, 1000 nM) and Hg²⁺ (10 nM, 100 nM, 1000 nM) by the standard addition method. 882 μL of citric acid buffer solution at $\text{pH} = 5$ and 6 μL of GO/Au-PtNPs were added to the actual sample and incubated at 20°C for 10 min after being evenly mixed. Then, 100 μL of TMB solution at a concentration of 0.5 mM and 2 μL of H₂O₂ at a concentration of 30% were added. The volume was fixed to 1000 μL with citric acid buffer solution, and the mixture was reacted for 5 min. The absorbance value under the joint influence of Ag⁺ and Hg²⁺ was determined at 652 nm.

EDTA was added to the actual sample after 10 μL of the standard was added to mask the Hg²⁺ in the sample. 882 μL of citric acid buffer solution at $\text{pH} = 5$ and 6 μL of GO/Au-PtNPs were added to the system for incubation at 20°C for 10 min. Afterward, 100 μL of TMB solution at a concentration of 0.5 mM and 2 μL of H₂O₂ at a concentration of 30% were added, and the volume was fixed to 1000 μL with citric acid buffer solution. The mixture was allowed to react for 5 min. The absorbance value under the sole influence of

Ag⁺ was determined at 652 nm. The recovery rate (Zhang et al., 2020a) was calculated by using the established standard curve equation shown in Equation (3.3).

$$\text{Recovery rate (\%)} = 100 \times (C_{\text{mean detected}} / C_{\text{spiked ion}}) \quad \text{Equation 3.4}$$

Specificity and Anti-interference. 10 μL metal solution at a concentration of 1 μM was added to 6 μL of GO/Au-PtNPs and 882 μL of citrate buffer solution at pH = 5. The solution was mixed homogeneously and then incubated for 10 min at 20°C. After shaking, 100 μL of TMB solution at a concentration of 0.5 mM and 2 μL of 30% H₂O₂ solution were added. The solution was fixed to 1000 μL . After mixing evenly for 5 min, the absorbance was measured at 652 nm.

3.6.3 A paper-based colorimetric sensor based on a graphene oxide/platinum composite for simultaneous detection of Hg²⁺ and Ag⁺

3.6.3.1 Chemicals

Disodium hydrogen phosphate (Na₂HPO₄), hydrogen peroxide (H₂O₂), silver nitrate (AgNO₃), and citric acid (C₆H₈O₇) were obtained from Tianjin Damao Chemical Reagent Co. Chloroauric acid (HAuCl₄·4H₂O) and chloroplatinic acid (CPA) were obtained from Tianjin Mysco Chemical Co. Graphene oxide was purchased from Shenzhen Qihang Technology Co. 3,3',5,5'-tetramethylbenzidine (TMB) was acquired from Shanghai McLean Biochemical Technology Co. All metal salt solutions (0.1 M) were prepared by dissolving the respective metal salts in deionized water. Heavy metal reagents commonly employ metal chlorides, except for AgNO₃. All glassware was thoroughly cleaned through multiple rinses with tap water followed by additional rinses with deionized high-purity water. High-purity water, obtained with a water purifier (WP-UPT-20, manufactured in China), was used for the preparation of reagents.

3.6.3.2 Preparation of graphene oxide/platinum composites

A mixture was prepared by combining 2.5 mL of a graphene oxide solution (GO) at a concentration of 0.5 mg/mL, 0.5 mL of a $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ solution at a concentration of 10 mM, and 0.9 mL of a NaOH solution at a concentration of 0.1 M. The aforementioned system was diluted to a volume of 25 mL using high-purity water. Subsequently, the mixture was subjected to sonication at a temperature of 25°C for 4 h. Afterwards, a hydrothermal reaction was carried out at 180°C for 6 h. The resulting hydrothermal products were subsequently cooled to room temperature, leading to the formation of graphene oxide/platinum nanoparticles (GO/PtNPs).

The composites of GO/PtNPs, comprising 25 mL of solution containing 10 mM $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$, were thoroughly mixed. The mixture was sonicated at a temperature of 25°C for 20 minutes. Subsequently, the mixture was subjected to magnetic stirring at the same temperature for a period of 24 h, resulting in the formation of GO/Pt@AuNPs.

3.6.3.3 Preparation and optimization of paper-based colorimetric sensors based on GO/Pt@AuNPs composites

Four different types of paper, namely, printing paper (PP), qualitative filter paper (QFP), quantitative filter paper (QTFP), and acetate film (AF), were utilized in the experiment. A hole punch with a 10 mm diameter was used to create circular papers with a 10 mm diameter. One microliter of the GO/Pt@AuNPs composite (67.413 $\mu\text{g/mL}$) and 6 μL of TMB solution at a concentration of 6 mmol/L were applied to the surface of the paper. A H_2O_2 solution with a concentration of 1 mol/L at pH 4.0 was gradually added to the surface of the paper mentioned above. Subsequently, color development was observed for 1 min before imaging. The study involved assessing the colour consistency and

measuring the greyscale value of the test paper photographs using ImageJ software. Based on these measurements, a suitable paper base was chosen for the development of the paper-based colorimetric sensor.

In addition to the influence of paper type on the construction of paper-based colorimetric sensors, several parameters also affect the uniformity of the colour, sensitivity, and background value of the sensor. Therefore, to determine the optimal detection conditions, various parameters were investigated for their impact on the performance of the colorimetric sensor. These parameters included the color development time (0.5, 1, 2, 3, 4, and 5 min), the concentration of the GO/Pt@AuNPs composite (8.427, 16.853, 33.706, 67.413, 101.119, 134.825, and 168.531 $\mu\text{g/mL}$), the concentration of H_2O_2 (0.001, 0.01, 0.1, 1, 2, 4, 6, and 10), the pH of the color development solution (3.5, 4, 4.5, 5.5, 6.0, 6.5, 7.5, and 8), and the concentration of the TMB solution (0.1, 0.5, 1, 2, 4, 6, 8, and 10 M). These parameters were investigated to determine their influence on the performance of the colorimetric sensor. The optimal detection conditions for this paper-based sensor were ultimately determined.

3.6.3.4 Characterization of the GO/Pt@AuNPs composites and paper bases

FTIR spectroscopy was conducted using a Nicolet iS5 (USA) in the range of 4000-400 cm^{-1} to analyze the surface groups of GO and GO/Pt@AuNPs. A LabRAM HR confocal micro-Raman spectrometer (France) was used to analyze the changes in the surface defects of GO before and after synthesis. The shape and size of the prepared GO/Pt@AuNPs were analyzed and characterized using a JEOL-JEM2100F transmission electron microscope (TEM) from Japan operating at 200 kV. An SM-7500F scanning electron microscope and X-ray energy spectrometer (EDS) and Scanning Electron

Microscope (SEM) from Japan were utilized to examine the surface structure and elemental composition of the deposited materials on paper before and after the study.

3.6.3.5 GO/Pt@AuNPs composite paper-based colorimetric sensors for Hg^{2+} and Ag^+ detection

Specificity and interference analysis. A 10 mm circular paper base was coated with 1 μL of the GO/Pt@AuNPs composite at a concentration of 67.413 $\mu\text{g/mL}$ and 6 μL of TMB at a concentration of 6 mmol/L. The paper was used to detect Hg^{2+} and Ag^+ ions. Seven microliters of Ni^{2+} , Cr^{6+} , Zn^{2+} , Cu^{2+} , Cd^{2+} , Na^+ , K^+ , or Al^{3+} solution at a concentration of 10^{-5} mol/L was added dropwise to the paper mentioned above, after which the mixture was allowed to react for 5 min. The dried test paper was treated with an 8 mol/L H_2O_2 solution, after which the solution was added dropwise. The paper was then photographed for 1 min, and the gray value was measured using ImageJ software.

Construction of Ag^+ and Hg^{2+} paper-based colorimetric sensors. A 10 mm circular paper base was coated with 1 μL of the GO/Pt@AuNPs composite at a concentration of 67.413 $\mu\text{g/mL}$ and 6 μL of TMB at a concentration of 6 mmol/L. 7 μL of Hg^{2+} at concentrations of 10^{-4} , 10^{-5} , 7.5×10^{-6} , 2.5×10^{-6} , 10^{-6} , 5×10^{-7} , 10^{-7} , 5×10^{-8} , 10^{-8} , or 10^{-9} mol/L was added dropwise to the paper substrate mentioned above. Alternatively, 7 μL of Ag^+ at concentrations of 10^{-4} , 10^{-5} , 7.5×10^{-6} , 2.5×10^{-6} , 10^{-6} , 5×10^{-7} , or 10^{-7} mol/L was added dropwise to the abovementioned paper base and allowed to react for 5 minutes. H_2O_2 solution at a concentration of 8 mol/L was added dropwise onto the dried paper base mentioned above. The color was allowed to develop for 1 minute, after which the sections were photographed. The gray values were processed using ImageJ software to create a standard curve showing the change in gray values in relation to the concentration of Hg^{2+} .

Detection of Ag^+ and Hg^{2+} in real samples. 1 μL of the GO/Pt@AuNPs composite at a concentration of 67.413 $\mu\text{g/mL}$ and 6 μL of TMB at a concentration of 6 mmol/L were deposited on a 10 mm circular paper base. Hg^{2+} at concentrations of 0.1, 1, and 10 $\mu\text{mol/L}$ and Ag^+ at concentrations of 10, 50, and 500 $\mu\text{mol/L}$ were standardized to match the concentrations of the actual samples. 7 μL of the above solutions was added dropwise onto the paper. 7 μL of the above solution was added dropwise onto test paper and allowed to react for 5 minutes. H_2O_2 solution, at a concentration of 8 mol/L, was added dropwise to the previously dried test paper. The color was allowed to develop for 1 min before being photographed.

EDTA was added to the system to chelate Hg^{2+} , and the gray value of the paper base was determined in the presence of Ag^+ alone. EDTA solution was added to the mixture of actual samples containing Ag^+ and Hg^{2+} separately, and the mixture was thoroughly reacted. 7 μL of the reaction solution was added dropwise onto the paper base mentioned above and allowed to react for 5 minutes. H_2O_2 solution at a concentration of 8 mol/L was added dropwise to the previously dried test paper. The color was allowed to develop for 1 min before being photographed. All the paper-based photographs mentioned above were processed for gray values using ImageJ software. The concentrations of Hg^{2+} and Ag^+ were then calculated using the standard curves of Hg^{2+} and Ag^+ to determine the recovery rate. The recovery rate can be derived using the following in Equation 3.3 (Zhang et al., 2020b).

3.7 Summary

In summary, this chapter begins by showing the correspondence between the Experimental Framework and the objectives. The contents related to the material synthesis,

material characterization, UV-vis light for heavy metal detection, Michaelis-Menten equation, imaging, and analysis are subsequently presented. Finally, the experimental design of this study is explained in detail, including three phases corresponding to the three research objectives of this study.

CHAPTER 4

AG⁺ COLORIMETRIC SENSOR BASED ON GRAPHENE OXIDE/NANO-PLATINUM COMPOSITE

4.1 Overview

Chapter 4 describes the Ag⁺ colorimetric sensor based on graphene oxide/nano-platinum composite. The main topics covered were graphene oxide/nano-platinum composite material. The main topics included: synthesis and characterization of graphene oxide/nano-platinum composite, optimization of the synthesis conditions of graphene oxide/nano-platinum composite, optimization of the detection conditions of the colorimetric sensor, catalytic kinetic analysis of graphene oxide/nano-platinum composite, and analysis of the limit of detection (LOD), sensitivity, range, and specificity of the colorimetric sensor for Ag⁺. range and specificity of the colorimetric sensor for Ag⁺. In Chapter 4 a graphene oxide nanocomposite with efficient peroxidase-like properties was synthesized and achieved accurate, rapid, and visual detection of silver ions.

4.2 GO/PtNPs characterization

TEM, Raman, FT-IR, XRD, EDS, and Uv-vis were used to obtain detailed characterisation information of GO/PtNPs.

4.2.1 TEM characterization analysis

Graphene oxide thin/nano platinum composites were synthesized by a one-step hydrothermal reaction in solution. In this process, the chloroplatinic acid precursor is directly reduced to Pt NPs, which are anchored on the graphene surface mainly through defects and oxygen functional groups (Satar et al., 2019; Zhao et al., 2021a). Due to the

residual oxygen-containing groups on the graphene surface, the obtained hybrid can form a well-dispersed aqueous colloid with good stability (Shuang et al., 2017), which is conducive to its application in colorimetric sensing detection. Figure 4.1 (A) and (B) show that platinum clusters were fixed on the surface of the graphene oxide. The Pt NPs are nearly spherical and distributed between graphene oxide sheets, with particle sizes ranging from 2 to 3 nm. Figure 4.1 (C) shows the lattice stripes of Pt NPs, whose average crystal plane spacing is 0.2 nm, that is, 2.0 Å, corresponding to the {200} and {111} sides of the platinum face-centered cubes.

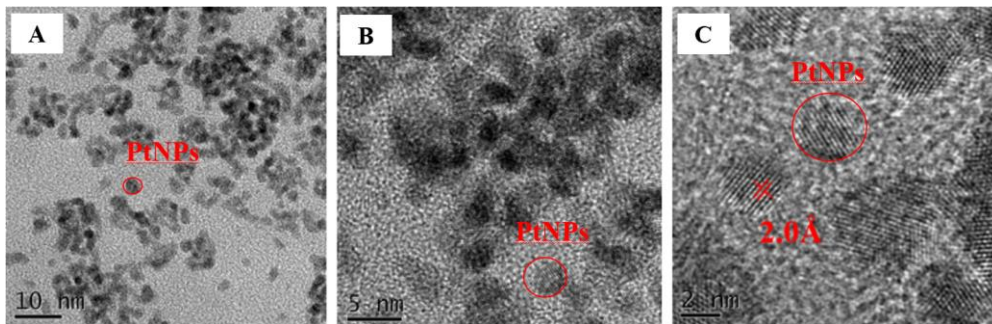


Figure 4.1: GO/PtNPs TEM images; (A) 10nm, (B) 5nm, and (C) 2nm

4.2.2 Raman characterization analysis

Raman spectrum analysis also confirmed the formation of Pt NPs on the graphene sheets (Figure 4.2). Figure 2 shows that graphene oxide has two characteristic peaks in the range of 1200–1800 cm^{-1} , namely, the D peak at 1369 cm^{-1} and the G peak at 1614 cm^{-1} . The D peak represents the disorder-induced feature of carbon materials, as the ordered structure of graphene is destroyed after oxidation (Tamalampud et al., 2019). The G peak corresponds to the E_{2g} phonon vibration in the center of the Brillouin region and is an intrinsic Raman mode of graphene (Artur et al., 2019; Muniyalakshmi et al., 2020). Partial chemical reduction of GO can cause structural changes, as evidenced by the shift of the D

and G bands to lower wavenumbers and the increase in the Raman spectral intensity ratio (I_D/I_G) of the D and G bands (Shu et al., 2019). This difference may be due to the partial reduction of GO, where a large number of sp^3 -hybridized carbon atoms are partially deoxygenated to reform new sp^2 -hybridized regions. The reformed sp^2 regions are smaller than those of GO, so the average size of the sp^2 regions of the reduced graphene decreases with increasing number of sp^2 regions. This is reflected in the Raman spectra as the I_D/I_G . The Raman spectra of PtNPs-GO show that the characteristic peaks of the D and G bands are at 1336 cm^{-1} and 1593 cm^{-1} , respectively, and that the intensity ratio (I_D/I_G) is enhanced (Shu et al., 2019). The peaks can also be seen to be slightly redshifted compared with those of pristine GO, and the I_D/I_G ratio increases from 0.89 to 1.09. The slight increase in the I_D/I_G ratio suggested that the average size of the sp^2 region of GO decreased during the preparation of Pt NPs-GO. Therefore, the shifted band and enhanced I_D/I_G indicate that GO was partially reduced during GO/PtNPs preparation.

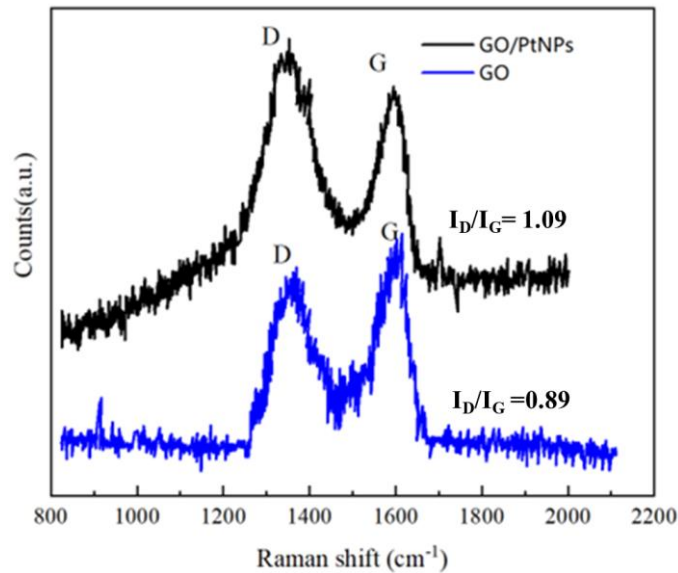


Figure 4.2: Raman spectra of GO and GO/PtNPs

4.2.3 FT-IR characterization analysis

As shown in Figure 4.3, graphene oxide mainly has characteristic peaks at 1053 cm^{-1} , 1227 cm^{-1} , 1616 cm^{-1} , and 1732 cm^{-1} . The C-O-C telescoping vibration peak at 1053 cm^{-1} may exist, the face-bending vibration of CH may exist at 1227 cm^{-1} , and the peaks at 1616 cm^{-1} and 1732 cm^{-1} may be attributed to C=O stretching vibrations. In the spectra of GO/PtNPs, there may be a C-O-C telescopic vibration peak at 1131 cm^{-1} and a C=C telescopic vibration peak at 1583 cm^{-1} . The disappearance of the characteristic C=O telescopic vibration peak at 1732 cm^{-1} may be due to the reduction reaction during the synthesis of the composites. The C=O groups may have been reduced to C-OH or C-H during the hydrothermal process (Ray et al., 2020). The decrease in the characteristic peak of oxygen-containing functional groups was reportedly due to the partial reduction of oxygen during the hydrothermal reaction of graphene oxide (Muniyalakshmi et al., 2020).

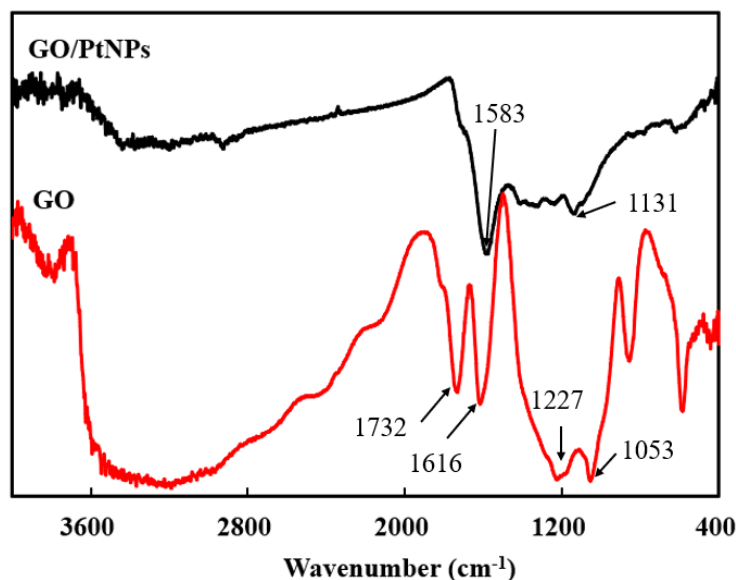


Figure 4.3: FT-IR spectra of GO and the GO/PtNPs

4.2.4 XRD characterization analysis

For precious metal platinum nanoparticles, XRD is a good means to analyze the crystal structure. A comparison of the XRD patterns of pristine graphene oxide and GO/PtNPs is shown in Figure 4.4, which reveals an obvious C (002) peak at approximately 10.1° . The enhanced characteristic peak at 23.1° indicates that GO is reduced to a certain extent. This is mainly due to the fact that the unreduced GO shows a broad peak at about 10° in the XRD pattern, which is due to the large number of oxygen-containing functional groups (e.g., carboxyl, hydroxyl, and epoxide groups) resulting in an increase in the interlayer spacing. This peak reflects the disordered structure of GO. The peak near 23.1° , on the other hand, is a characteristic peak of graphene or partially reduced GO, which corresponds to the (002) crystal plane and reflects the carbon layer spacing information. When GO is reduced, the oxygen-containing functional groups are reduced and the layer spacing is narrowed, approaching the layer spacing of graphene, which makes the peak at 23.1° more obvious or even enhanced. This indicates that GO was partially reduced. Moreover, if GO is completely reduced, there should be a weak and wide peak at 26.6° (Legge et al., 2018; Mitra et al., 2021). However, as shown in Figure 4, there is no peak at 26.6° , which again indicates that GO is only partially reduced rather than completely reduced. The existence of GO is an important reason why the aqueous solution of the composite can maintain good dispersion for a long time. Furthermore, the d-spacing of the three diffraction peaks located at 40.2° , 47.2° and 67.3° corresponding to the Pt nanoparticles were calculated by Bragg's equation to be 0.224 nm, 0.192 nm, 0.139 nm, which corresponds to the (111), (200), and (220) facets of the face-centred cubic lattice of Pt, respectively (Mitra et al., 2020), which proves that the Pt nanoparticles are fully loaded on the graphene oxide surface.

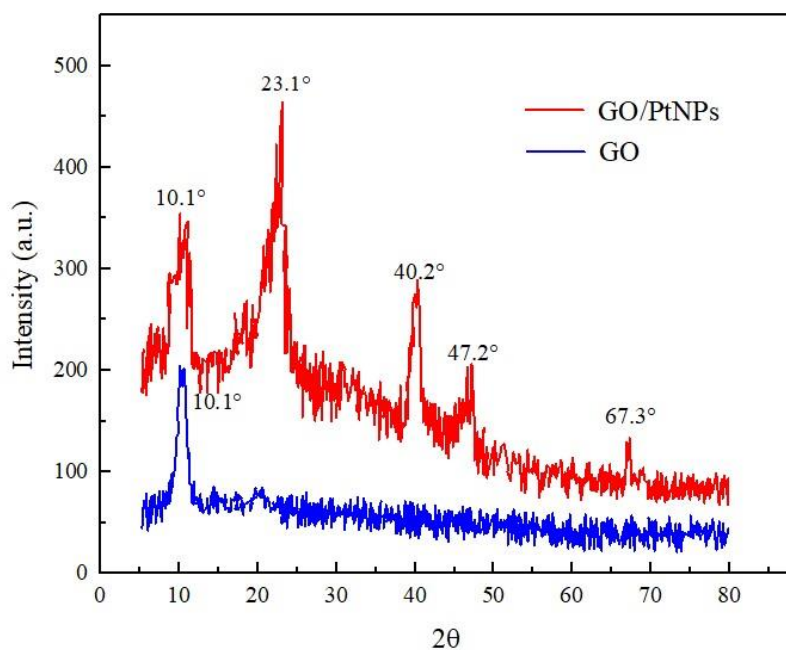


Figure 4.4: XRD chromatograms of GO and the GO/PtNPs

4.2.5 EDS characterization analysis

EDS analysis of GO and GO/PtNPs in Figure 4.5 shows that GO contains only two elements, carbon and oxygen, compared to GO/PtNPs. GO/PtNPs contain platinum in addition to carbon and oxygen elements and the content of the main three elements of this material is presented below: 1.74% carbon, 97.37% oxygen, and 0.89% platinum, which further indicates that the platinum nanoparticles are immobilized on the surface of the GOs and that the composite material of GO/PtNPs is still rich in oxygen-containing functional groups.

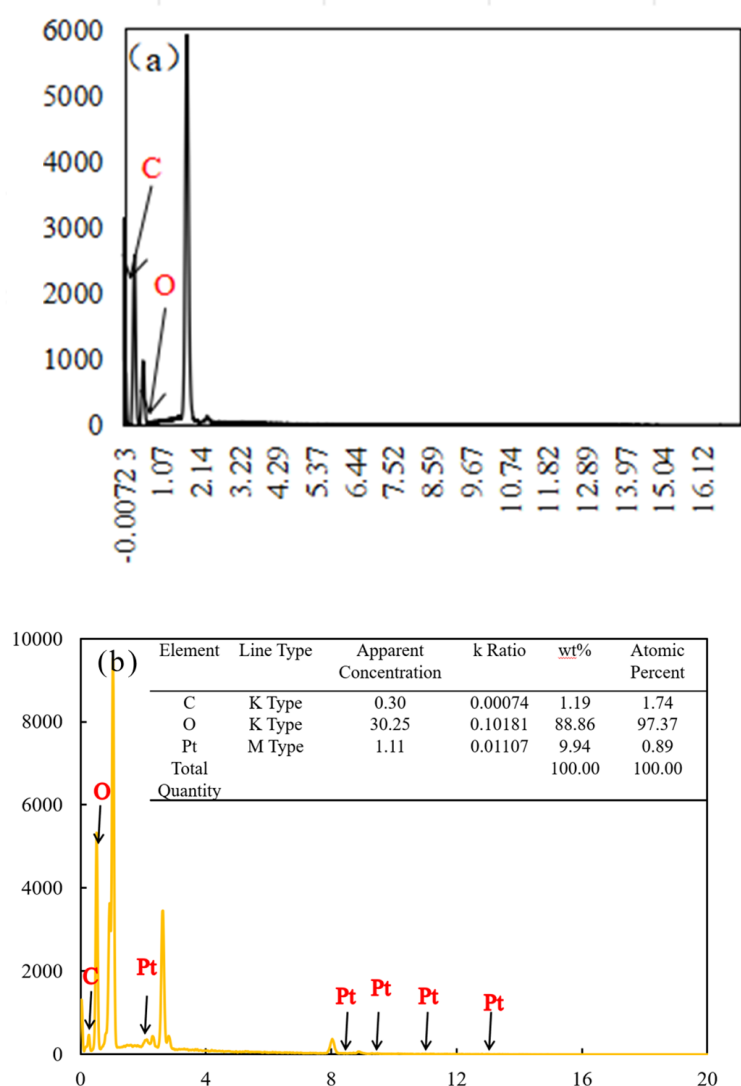


Figure 4.5: EDS chromatogram of (a) GO; (b) GO/PtNPs

4.2.6 Ultraviolet-visible characterization analysis

The UV–Vis absorption spectra of the graphene/platinum nanoparticles and graphene oxide are shown in Figure 4.6. GO had a relatively strong characteristic peak at a wavelength of 231 nm, while GO/platinum NPs had a strong characteristic peak at 267 nm in addition to 231 nm, which was attributed to the reduction of GO. This indicates that the

amount of graphene oxide in the graphene/platinum nanoparticle composites is partially reduced.

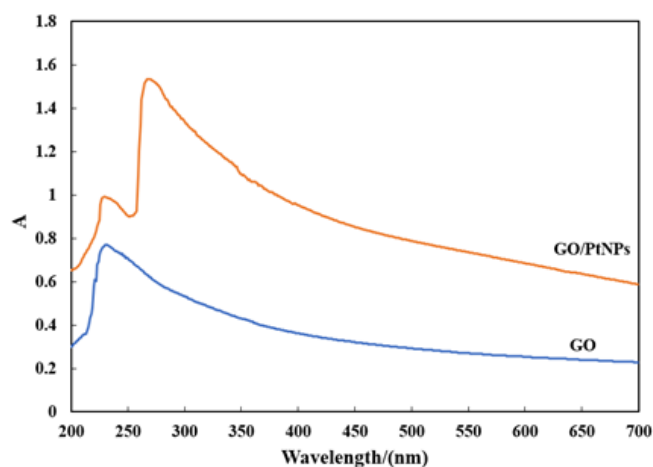


Figure 4.6: Ultraviolet-visible spectral characterization of GO and GO/PtNPs

4.3 Optimization of GO/PtNPs preparation conditions

The catalytic activity of the prepared GO/PtNPs complex is directly related to several influencing factors. The effects of pH, H_2PtCl_6 concentration, hydrothermal treatment time, and hydrothermal temperature on the peroxidase-like catalytic activity of the GO/PtNPs complex during preparation were investigated. The experimental results are shown in Figure 4.7. Figure 4.7A shows the activity test results for catalysts prepared at different pH values. It can be seen that the catalytic activity of GO/PtNPs increases with the increase of pH value, reaching a maximum when the pH value is 9.5, and decreasing with the increase of pH value starting at pH 9.5. This is because the Pt^0 precursor can be directly reduced to Pt nanoparticles and grown on the graphene surface under alkaline conditions due to the presence of defects and oxygen-containing functional groups on the surface of graphene oxide lamellae during hydrothermal reactions (Yoo et al., 2009). Moreover, in a weakly alkaline environment, the concentration of OH^- ions in solution is higher. This can

facilitate the reduction of platinum ions because OH⁻ can act as a ligand to stabilise the intermediate oxidation state of platinum, thus facilitating the transformation of platinum ions to zero-valent platinum (Pt⁰). The reaction equation is shown in Equation 2.1. In addition, under alkaline conditions, platinum ions may form more stable complexes that are more readily reduced to metallic platinum than simple platinum salts. Once platinum ions are reduced to zero-valent platinum atoms, they tend to aggregate to form nanoscale particles.

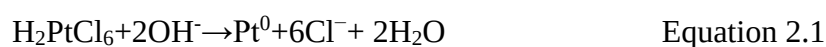


Figure 4.7B shows the activity test results for the catalysts prepared with different concentrations of H₂PtCl₆. The catalytic activity of GO/PtNPs increases with increasing H₂PtCl₆ concentration and reaches a maximum when the concentration of H₂PtCl₆ is 0.24 mM. However, the catalytic activity decreases with increasing H₂PtCl₆ concentration greater than 0.24 mM. This is due to the reduction of Pt⁴⁺ to Pt⁰ during the hydrothermal reduction process and in situ growth into platinum nanoclusters (refer to the TEM images in Figure 4.8). However, the number of platinum ions in solution increases at too high a concentration of H₂PtCl₆, which leads to more platinum atoms rapidly aggregating during the reduction process. The increased collision frequency between platinum ions at high concentrations leads to a decrease in the number of nuclei formed and an increase in the growth rate of each nucleus, which results in an increase in the size of the final platinum nanoparticles formed. In addition, too high a concentration of platinum ions may also lead to fusion between particles, further increasing the size of the particles. Thereby reducing the reactivity of the catalyst (Yuhan et al., 2019).

As shown in Figure 4.7C, the catalytic performance of GO/PtNPs increases from 6 to 9 h and then decreases with increasing reaction time. This shows that the best catalytic

performance of GO/PtNPs was achieved after 9 h of hydrothermal reaction. This is because when the hydrothermal treatment time is too short, Pt^{4+} is not sufficiently reduced to Pt^0 , and the remaining Pt^{4+} is dispersed in the system and not immobilized on the GO surface (Yoo et al., 2009). When the hydrothermal treatment time is too long, the size of the Pt nanoparticles gradually increases. This is due to the fact that the prolonged reaction promotes the reduction of more platinum ions and the continuous growth of already formed nanoparticles. At the same time, the prolonged reaction increases the inter-particle interactions and promotes agglomeration phenomena, which further leads to an increase in particle size. It was reported that a larger nanomaterial reduces the specific surface area and the surface energy generated (Yuhan et al., 2019); thus, the catalytic activity of the composites decreases.

As shown in Figure 4.7D, the catalytic activity of GO/PtNPs increased and then decreased at a turning point of 180 °C. This is therefore the optimum hydrothermal synthesis temperature. When the temperature of the hydrothermal reaction decreases, Pt^{4+} is not reduced to Pt^0 . At higher temperatures, the Pt^0 loaded on the surface of graphene oxide increases continuously. Consequently, the spacing between the graphene oxide lamellae increases, which effectively reduces the possibility of agglomeration. This contributed to the increase in the catalytic activity of the material (Yuhan et al., 2019).

Therefore, through the optimization of the experimental conditions used in the preparation of GO/PtNPs, the ideal pH was determined to be 9.5, the concentration of H_2PtCl_6 was 0.24 mM, the hydrothermal treatment time was 9 h, and the reaction temperature was 180 °C.

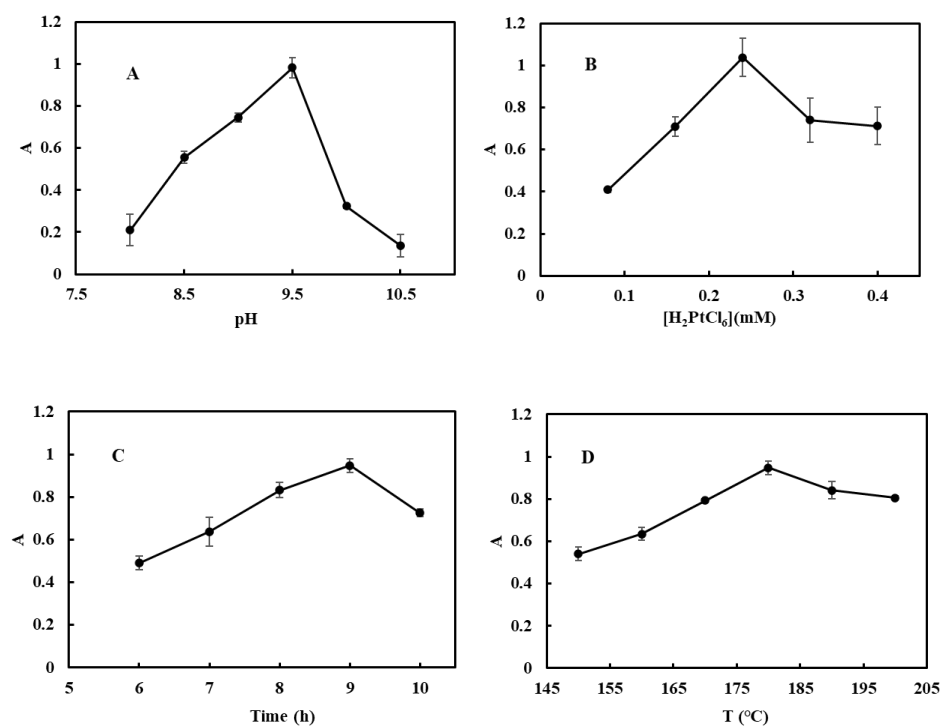


Figure 4.7: The changes of the catalytic activity (absorbance) with respect to variations in (A) pH value during the preparation of GO/PtNPs at 180°C, 0.24 mM H_2PtCl_6 , 8 h; (B) H_2PtCl_6 concentration at 180°C, pH 9.5, 8 h; (C) Hydrothermal reaction time at 180°C, 0.24 mM H_2PtCl_6 , pH 9.5; and (D) Reaction temperature at 0.24 mM H_2PtCl_6 , pH 9.5, 9 h

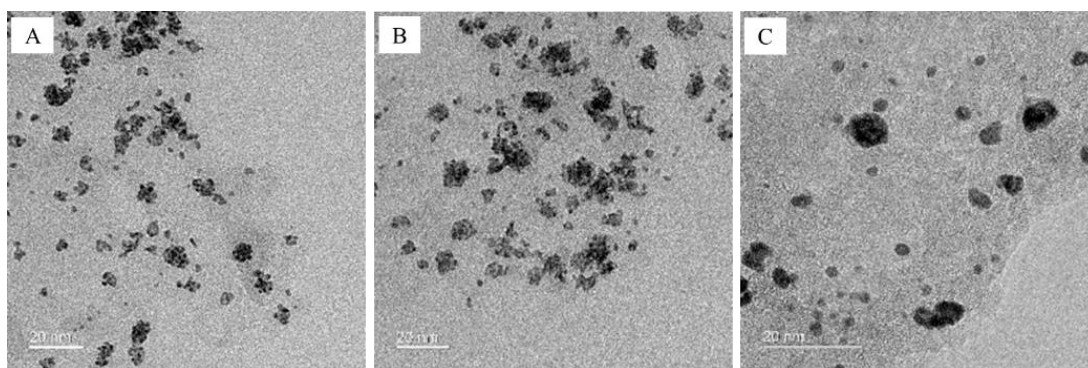


Figure 4.8: Characterization of GO/PtNPs: (A) to (C) Chloroplatinic acid concentrations of 0.16, 0.24, and 0.32 mM, respectively

4.4 Analysis of catalytic kinetics

The synthesized GO/PtNPs have catalase-like properties. With H_2O_2 and TMB as substrates, the catalytic activity of GO/PtNPs was investigated under optimal conditions by using the theory and method of enzyme kinetics. In a certain range of substrate concentrations, the reaction follows typical Michaelis–Menten kinetics (Shi et al., 2011), as shown in Figure 4.9. From the above equation it can be seen that this is a linear equation with respect to $1/v$ (inverse of the initial reaction rate) and $1/[S]$ (inverse of the substrate concentration). Therefore, the change in substrate concentration over 5 min (initial reaction rate V) can be calculated by varying the H_2O_2 and TMB concentrations. and $[S]$ in the equation represents the concentrations of H_2O_2 and TMB. Therefore, a series of values for $1/V$ and $1/[S]$ can be obtained. The K_m and $V_{\max}$ values can be calculated and obtained by linear fitting. The kinetic data were obtained by changing the concentration of one substrate and fixing the concentration of the other, and the results were applied to the double reciprocal of the Michaelis–Menten equation, as shown in equation 3.3

K_m and $V_{\max}$ were obtained from Lineweaver Burk diagrams (Table 1). The K_m value of GO/PtNPs under H_2O_2 conditions is greater than that of enzymes such as GBR (Ray et al., 2017) (Table 4.1), which indicates that the activity of GO/PtNPs under H_2O_2 conditions is greater. In addition, a relatively small K_m value was obtained by changing the concentration of TMB. This indicates that TMB has a stronger affinity for GO/PtNPs than for other materials.

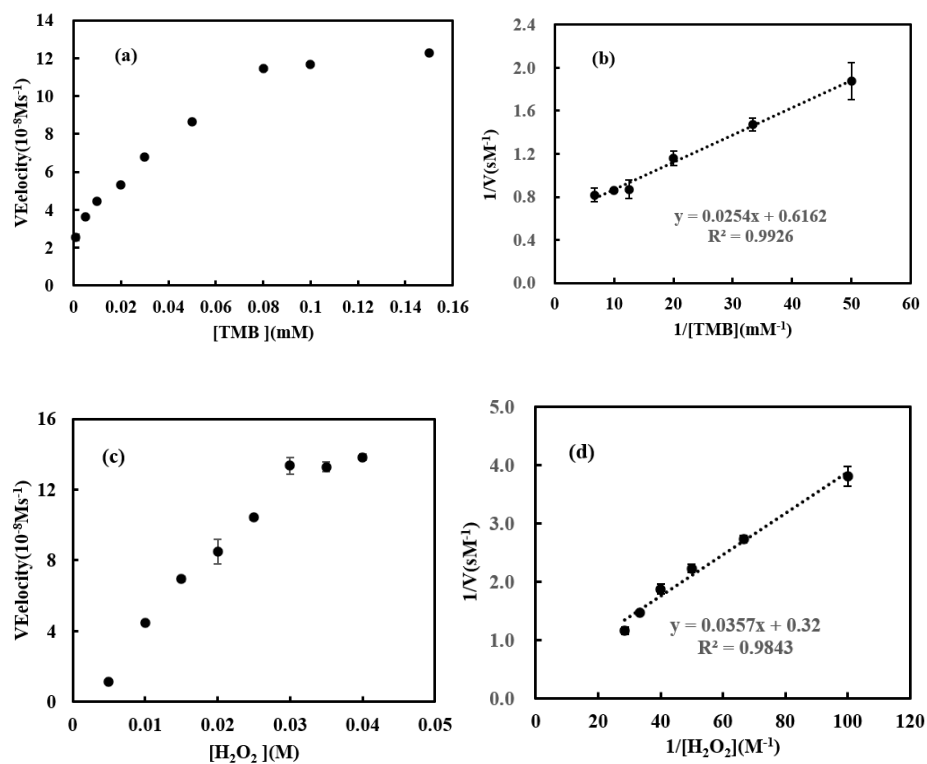


Figure 4.9: Steady-state kinetic measurements and catalytic mechanism of GO/PtNPs (a-d). (a) Relationship diagram between initial velocity and TMB concentration; (c) relationship diagram between initial velocity and H₂O₂ concentration; (b, d) catalytic kinetic fitting diagram of GO/PtNPs activity when one substrate (H₂O₂ or TMB) concentration is fixed and the other substrate concentration changes

Table 4.1: Comparison of apparent Michaelis constants (k_m) and maximum reaction rates (V_{max}) between GO/PtNPs and other catalases

Catalyst	Substance	V_{max} [$10^{-8} \cdot M \cdot s^{-1}$]	k_m [mM]	Reference
GO/PtNPs	TMB	16.23	0.041	This study
	H ₂ O ₂	31.25	111.56	
GBR	TMB	0.68	0.83	(Ray et al., 2017)
	H ₂ O ₂	3.60	10.98	
CoS-p-rGO	TMB	5.0	0.3380	(Borthakur et al., 2017)
	H ₂ O ₂	12.2	0.1114	
Co-ferrocene metal-organic framework	TMB	29.7	27.63	(Fang et al., 2020)
	H ₂ O ₂	\	\	
	H ₂ O ₂	\	\	

Table 4.1: continued

Catalyst	Substance	$V_{\max} [10^{-8} \cdot \text{M} \cdot \text{s}^{-1}]$	$k_m [\text{mM}]$	Reference
	H ₂ O ₂	\	\	
Iridium oxide nanoparticles	TMB	179	19.27	(Zhen et al., 2020)
	H ₂ O ₂	\	\	
Fe-N/C single-atom nanozyme	TMB	59.8	0.97	(Qc et al., 2020)
	H ₂ O ₂	\	\	
Co-His-GQD-G	TMB	38	0.66	(Li et al., 2021b)
	H ₂ O ₂	\	\	

4.5 Optimization of test conditions

The background colour of the colorimetric sensor affects the sensitivity and detection limit of the constructed colorimetric sensor, in order to obtain the appropriate colour, the optimization was carried out from the following aspects: composite material concentration, TMB concentration, Hydrogen peroxide concentration, pH, reaction time.

4.5.1 Composite material concentration

Figure 4.10 and Figure 4.11 show that with increasing concentration of the graphene oxide/nanoplatinum composite, the trend gradually increases at first and then reaches a plateau at an absorbance of 1.5. This is because the higher the concentration of GO/PtNPs is, the more catalase-like catalytic sites there are in the reaction system. Therefore, it can more effectively catalyze the decomposition of hydrogen peroxide to produce hydroxyl radicals to oxidize TMB into oxTMB, and the more obvious the color change is (Liu et al., 2012); that is, the greater the absorbance. However, the human eye can most easily

distinguish the colour change when the absorbance values are between 0.4 and 0.6 (Figure 4.11). It can be observed that the absorbance value is closest to this range when the concentration of GO/PtNPs is $0.05 \mu\text{g/mL}$. In addition, GO/PtNPs contain platinum, a precious metal, and the cost increases as the amount of GO/PtNPs increases. Therefore, a concentration of $0.05 \mu\text{g}\cdot\text{mL}^{-1}$ was selected for the experiment is $0.05 \mu\text{g}\cdot\text{mL}^{-1}$ as the detection condition for Ag^+ .

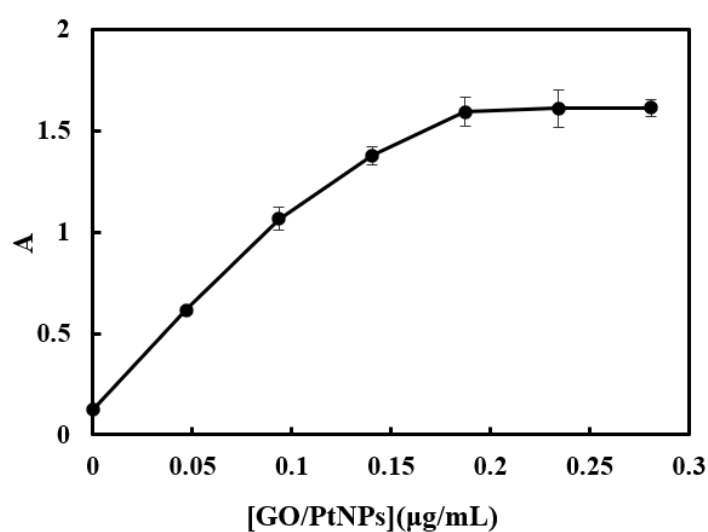


Figure 4.10: The changes of the catalytic activity (absorbance) with respect to composite material concentration during color development

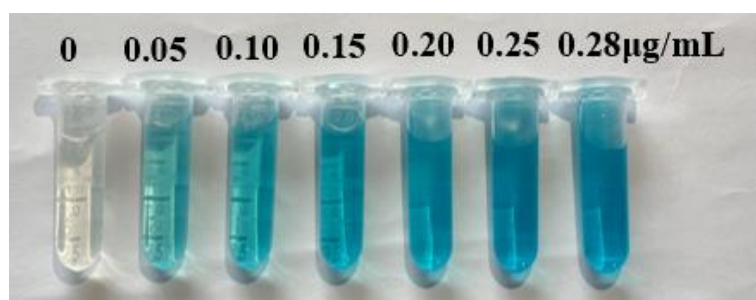


Figure 4.11: Images of color development during the optimization of the composite concentration

4.5.2 Reaction time

Figure 4.12 and Figure 4.13 show that the absorbance is proportional to the reaction time. The absorbance plateaued after 20 min. As the reaction time increased, more GO/PtNPs could catalyze H_2O_2 , and more hydroxyl radicals generated from decomposition would oxidize TMB into oxTMB (Liu et al., 2012); thus, the absorbance also increased. However, the longer the reaction time was the greater the time cost of detecting Ag^+ . If A is too large, the colours will be too dark and the human eye will not be able to distinguish between the colour changes, while if A is too small, the magnitude of the colour changes will be small, making the detection range narrower. Therefore, the acceptable value of A is 0.4-0.6, and therefore, 5 minutes was chosen as the detection time.

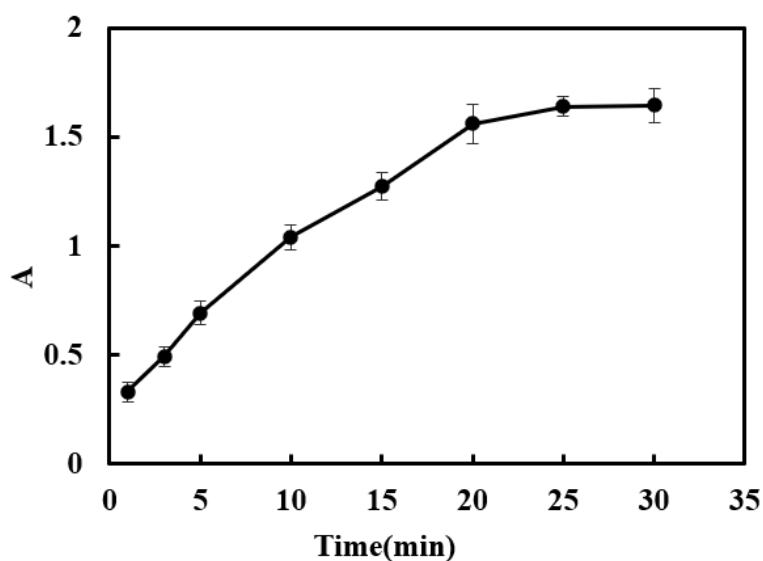


Figure 4.12: The changes of the catalytic activity (absorbance) with respect to reaction time during color development. Reaction conditions: 0.047 $\mu\text{g/mL}$ GO/PtNPs, 0.02 M H_2O_2 , 0.02 Mm TMB, pH=5, t=1, 3, 5, 10, 15, 20, 25, 30 min, $\lambda=652$ nm

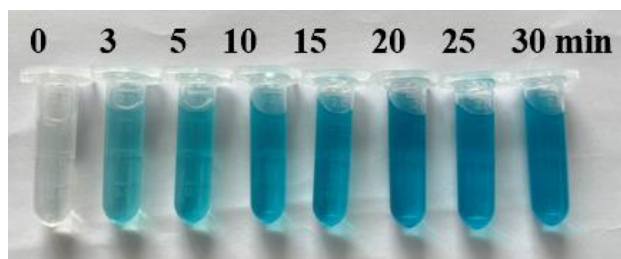


Figure 4.13: Images of color development during the optimization of reaction time

4.5.3 pH

Figure 4.14 and Figure 4.15 show that the absorbance increases from pH 2 to pH 5, where the absorbance reaches a maximum value of 0.676. The reason is that GO/PtNPs, as catalase-like materials, are strongly affected by pH, similar to other enzymes. When the pH is between 4 and 5, this kind of catalase has the highest catalytic activity (Li et al., 2021c; Liu et al., 2012). With this pH, more hydroxyl radicals decompose, increasing the oxidation ability of TMB and thus increasing the absorbance. And the oxidized TMB product is a blue quinonimine intermediate. Under strong acidic conditions, this intermediate protonates and undergoes a structural change to form a more stable yellow compound which is actually 3,3',5,5'-tetramethyl-2-(1H)-benzimidazole. The blue quinonimine form is more stable under weak acidic conditions, making the color developing system blue. This finding is consistent with the results of TMB color conditions reported in the literature (Liu et al., 2012). Therefore, a reaction system with a pH of 5 was selected as the detection condition for Ag^+ .

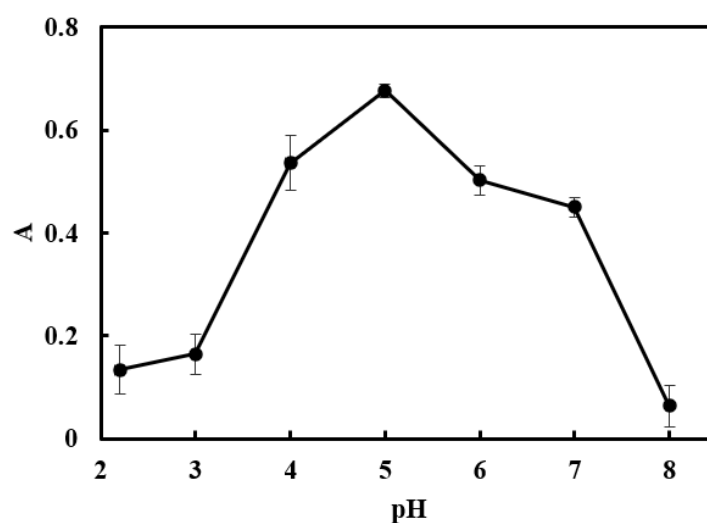


Figure 4.14: The changes of the catalytic activity (absorbance) with respect to pH during color development. Reaction conditions: 0.047 $\mu\text{g/mL}$ GO/PtNPs, 0.02 M H_2O_2 , 0.02 mM TMB, pH=2.2, 3, 4, 5, 6, 7, 8, $t=5$ min, $\lambda=652$ nm



Figure 4.15: Images of color development during the optimization of pH

4.5.4 Hydrogen peroxide concentration

As shown in Figure 4.16 and Figure 4.17, the absorbance values of the system tended to increase with increasing hydrogen peroxide concentration before stabilizing above 0.035 M. This was mainly due to the increase in H_2O_2 around the catalytically active sites of the GO/PtNPs with increasing hydrogen peroxide concentration, which decomposed to produce more $-\text{OH}$ and thus accelerated the color development of TMB (Li et al., 2021d; Liu et al., 2012). However, according to the color development shown in

Figure 4.17, the detection was satisfactory when the H_2O_2 concentration was 0.02 M. Because the absorbance value at this point is closest to 0.4-0.6, the colour is neither too dark nor too light. This is because if the colour is too dark our eyes have no way of distinguishing the colour change and we cannot use our eyes to achieve detection. However, if the colour is too light, the detection limit of the colorimetric sensor will be affected.

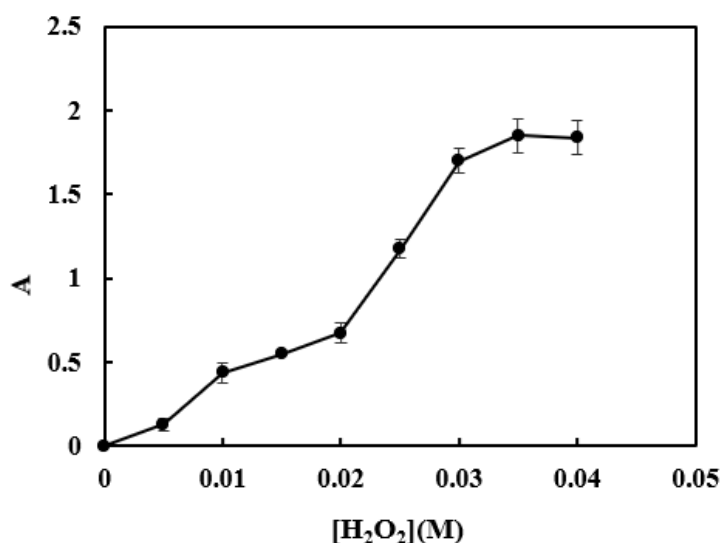


Figure 4.16: The changes of the catalytic activity (absorbance) with respect to H_2O_2 concentration during color development. Reaction conditions: 0.047 $\mu\text{g/mL}$ GO/PtNPs, 0.005 H_2O_2 , 0.01 H_2O_2 , 0.015 H_2O_2 , 0.02 H_2O_2 , 0.025 H_2O_2 , 0.03, 0.035, 0.04 M, 0.02 Mm TMB, pH= 5, t=5 min, $\lambda=652$ nm

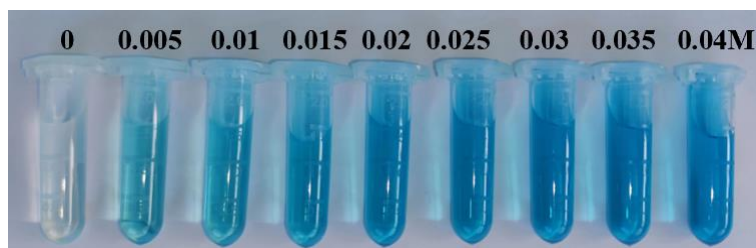


Figure 4.17: Images of color development during optimization of the H_2O_2 concentration

4.5.5 TMB concentration

As shown in Figure 4.18, the absorbance value continued to increase with increasing TMB concentration. However, at a concentration of 0.02 mM, the absorbance was 0.485, which was between 0.4 and 0.6 and met the requirements of the assay (Figure 4.19). Moreover, this concentration avoids the phenomenon of a high background value caused by the system being too dark in color. Therefore, the TMB concentration in the subsequent experiments was chosen to be 0.02 mM.

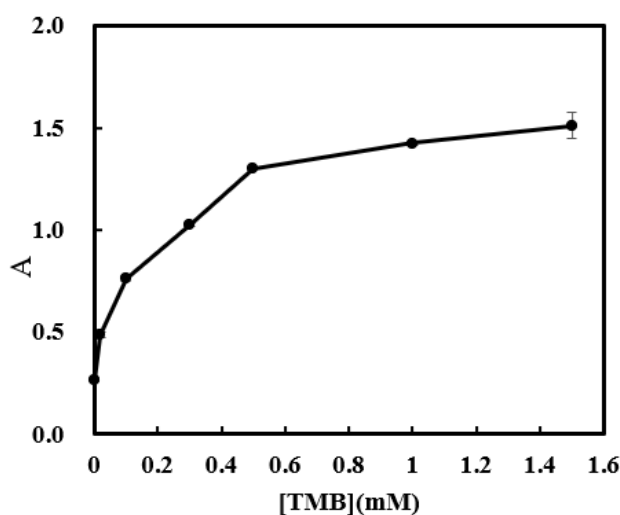


Figure 4.18: The changes of the catalytic activity (absorbance) with respect to TMB concentration during color development. Reaction conditions: 0.047 $\mu\text{g/mL}$ GO/PtNPs, 0.02 M H_2O_2 , 0.001, 0.02, 0.1, 0.3, 0.5, 1.0, 1.5 mM TMB, pH=5, $t=5$ min, $\lambda=652$ nm

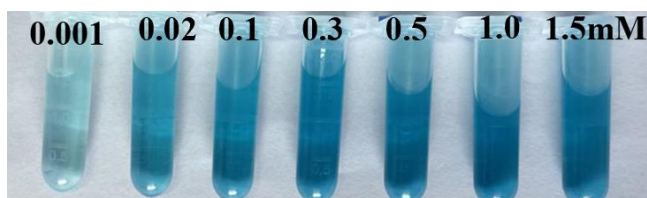


Figure 4.19: Images of color development during the optimization of the TMB concentration

4.6 Specific detection of different metal ions

To evaluate the selectivity of Ag^+ detection based on the GO/PtNP colorimetric sensor, when other conditions were unchanged, Zn^{2+} , Cr^{6+} , Cu^{2+} , Ni^{2+} , Al^{3+} , Cd^{2+} , and Ca^{2+} were selected as interfering ions to investigate the specificity of the colorimetric sensing platform. As shown in Figure 4.20, the absorbance of the reaction system with interfering ions was close to that of the blank group, and there was no obvious inhibition of the properties of the GO/PtNPs catalase. However, the absorbance of the reaction system with Ag^+ decreased significantly, indicating that Ag^+ significantly inhibited the catalytic ability of the GO/PtNPs. This is due to the fact that silver ions are able to form stable complexes on the surface of graphene oxide and be reduced to silver metal due to their strong complexing ability and easy acceptance of electrons, whereas other heavy metal ions do not have the same complexing tendency or stability. In addition, silver ions were able to cover the active sites of platinum nanoparticles more efficiently, preventing TMB and hydrogen peroxide from contacting them, thus significantly altering the catalytic activity. The positive electropositive nature of silver ions and their size make them more readily adsorbed on negatively charged graphene oxide surfaces, in contrast to other metal ions that may not be readily adsorbed due to inappropriate state of charge or size, and do not cover the active sites of platinum in the same manner. These properties allow silver ions to uniquely influence catalytic reactions in a way that other metal ions cannot (Liu et al., 2019; Chang et al., 2016). Therefore, when Ag^+ is present in the reaction system, it will inhibit the catalytic effect of GO/PtNPs on H_2O_2 , resulting in a significant reduction in the absorbance of the reaction system.

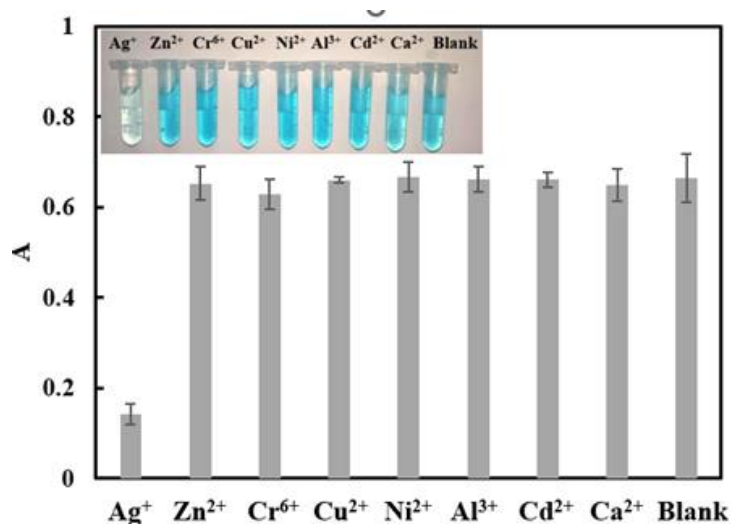


Figure 4.20: The changes of the catalytic activity (absorbance) with respect to different metal ions on graphene oxide/nano platinum peroxidase activity. Reaction conditions: 0.047 $\mu\text{g/mL}$ GO/PtNPs, 0.02 M H_2O_2 , 0.02 Mm TMB, pH=5, t=5 min, $\lambda=652$ nm

4.7 Actual water sample detection

As shown in Figure 4.21A, as the measured concentration of Ag^+ increased, the color change in the reaction solution gradually weakened. Figure 4.21B shows the absorbance change curve of the GO/PtNPs-based colorimetric sensing method under different Ag^+ concentrations at a wavelength of 652 nm (the characteristic absorption wavelength of oxTMB). It can be seen from the figure that the change in solution absorbance weakens with increasing Ag^+ concentration. This is because Ag^+ will absorb nonspecific ions on the surface of the composite material so that the active sites on the composite material cannot be completely exposed, thus inhibiting the peroxidase-like activity of the composite material (Chang et al., 2016). Figure 4.21C shows the concentration of Ag^+ (0.5–1000 μM) and the absorbance of the solution after the system had reacted for 5 min. The concentration of Ag^+ ranged from 0.5–1000 μM , which indicated a good exponential relationship with the absorbance of the system, and the linear fitting equation from Figure 4.21D is shown in Equation 4.1.

$$A = -0.1565 \log[Ag^+] - 0.2922 \quad \text{Equation 4.1}$$

This provides a basis for the quantitative analysis of Ag^+ . The limit of quantification (LOQ) of this sensing method is $0.5 \mu\text{M}$, which is the lowest concentration of the sample that can be detected in the experiment.

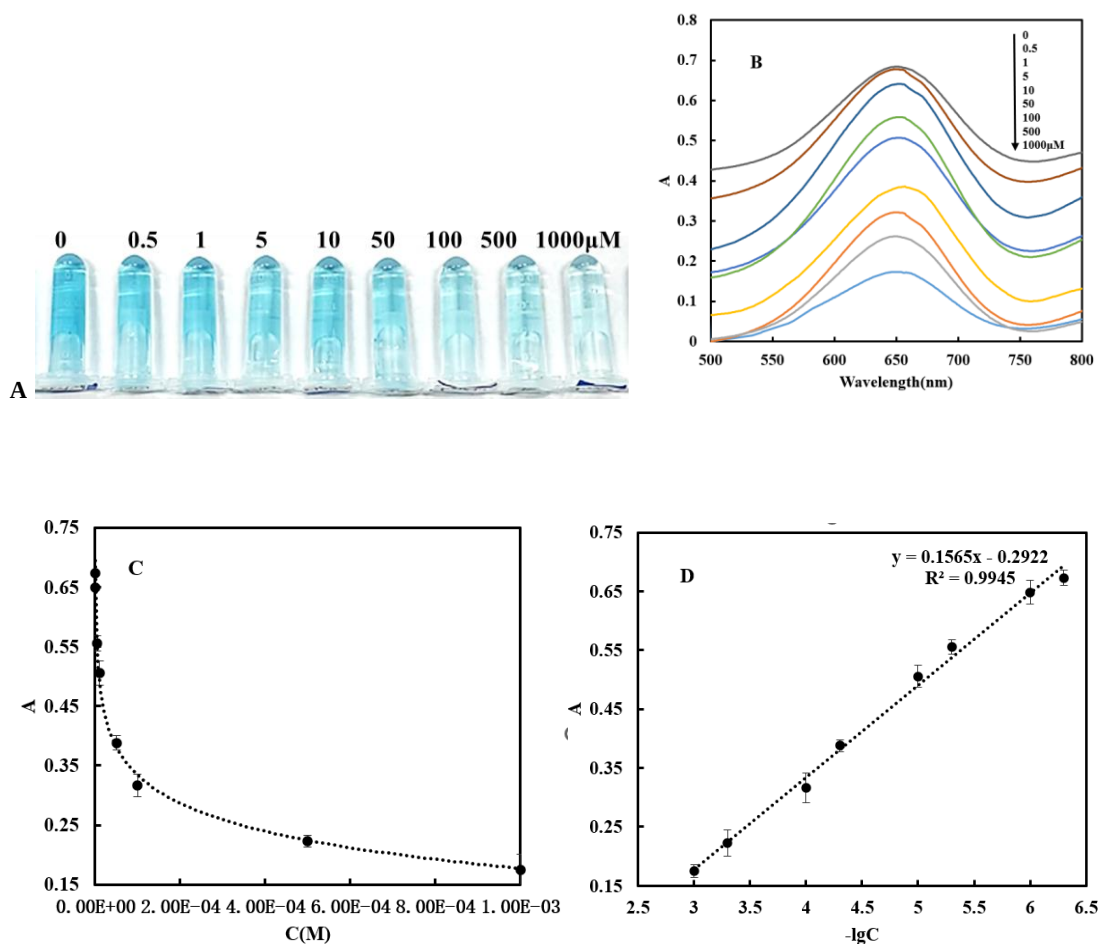


Figure 4.21: (A) Photos of the color change in the solution used in the detection system at different concentrations of Ag^+ . (B) Colorimetric sensing method based on the absorbance of GO/PtNP mixture versus time for different concentrations of Ag^+ . (C) Relationships between the absorbance and concentration of Ag^+ . (D) Linear fitting curve between the absorbance and $\log [Ag^+]$. Reaction conditions: $0.047 \mu\text{g/mL}$ GO/PtNPs, 0.02 M H_2O_2 , 0.02 Mm TMB, $\text{pH}=5$, $t=5 \text{ min}$, $\lambda=652 \text{ nm}$

To explore its reproducibility, the Ag^+ concentration was adjusted to 0.5, 1, or 10 μM . The relative standard deviations (RSDs, $n=3$) of the obtained results are 5.58%, 1.95%, and 6.16%, respectively, which proves that the sensing method has good reproducibility. In addition, Ag^+ samples with different concentrations were prepared, and the accuracy of the sensing method was investigated via a spiking recovery experiment. Based on the obtained linear fitting equation, the sample Ag^+ concentration was calculated from the solution absorbance. The calculation results are shown in Table 4.2. The recoveries of the method were 96.46%~104.57%, within the range of 90%~110% with acceptable errors. These findings indicate that the accuracy and precision of this sensing method are good and that the method can be used for the quantitative detection of Ag^+ in actual water samples.

Table 4.2: Detection of Ag^+ in actual water samples

Type of water sample	Sample No	Dosage(μM)	Measured quantity (μM)	Recovery rate (%)
Tap Water	1	1	1.02±0.02	102.20±1.87
	2	10	10.46±0.48	104.57±4.82
	3	100	101.77±4.31	101.77±4.31
	4	500	493.63±18.82	98.73±3.76
Pond Water	1	1	0.99±0.05	99.32±4.58
	2	10	9.90±0.12	98.98±1.19
	3	100	102.27±4.30	102.27±4.30
	4	500	493.70±20.75	98.74±4.15
River Water	1	1	0.99±0.04	98.81±3.86
	2	10	9.66±0.24	96.61±2.40
	3	100	103.28±4.37	103.28±4.37
	4	500	482.30±31.21	96.46±6.24

As shown in Table 4.3, the detection range of silver ions based on this colorimetric platform was 0.5–1000 μM . Compared with sensors of the same kind, the detection range is wider, and the application conditions are wider. Moreover, the detection limit was 0.5 μM , which is more sensitive than that of the same kind of colorimetric sensor.

Table 4.3: Performance comparison of several silver ion sensors

Core materials	Test method	Detection scope (μM)	Detection limit (μM)	Reference
(E)-1-((pyridine-4-yl)Methylene) Thiosemicarbazide	Colorimetric sensor	2.2	2.2-15	(Sahu et al., 2020)
MNTPZ	Colorimetric fluorescence sensor	1.03	1.03-30	(Dongare et al., 2020)
HPEI-AuNPs	Colorimetric sensor	10.3	10.3-315	(Bian et al., 2020)
Bulk Ion-selective Optodes	Colorimetric sensor	1.92	1.92-500	(Phichi et al., 2020)
Silatrane	Colorimetric sensor	0.141	0.141-1	(Singh et al., 2019)
GO/PtNPs	Colorimetric sensor	0.5	0.5~1000	This study

4.8 Ag^+ ion detection mechanism

The principle of the colorimetric sensor is based on the redox reaction between the nano catalytic material and the substance to be tested to generate a chemical signal, which is outputted as an optical signal through the color change of the color-developing substrate, thus realizing the detection of the target (Chen et al., 2022a; Xu et al., 2022). The

synthesized GO/PtNPs have superior peroxidase-like properties that can catalyze the decomposition of hydrogen peroxide. The hydroxyl radical generated from decomposition can oxidize the hydrogen peroxide substrate 3,3',5,5'-tetramethylbenzidine (TMB), which can change from a colorless substance to a blue product. When the system contains Ag^+ , it mainly reacts with Pt^0 on the surface of graphene oxide in a redox reaction to produce silver-platinum bimetallic nanoparticles (Ag@PtNPs), resulting in poor affinity for the substrate TMB. This inhibits the catalytic activity of the composite material, leading to a lighter color and lower absorbance of the system. The higher the concentration of Ag^+ was, the lighter the color of the system was, and the lower the absorbance was. Qualitative analysis of Ag^+ can be achieved by the naked eye through color changes. In addition, according to Lambert's Beer's law, the quantitative relationship between the absorbance and concentration of Ag^+ can be established, and the quantitative concentration of Ag^+ can be determined. This mechanism of the Ag^+ colorimetric sensor is shown in Figure 4.22.

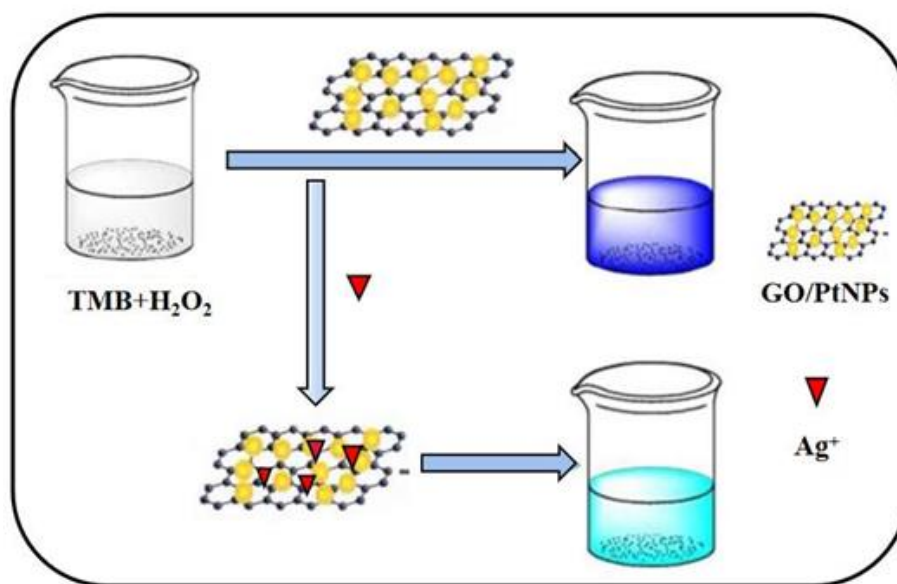


Figure 4.22: Schematic illustrations of the Ag^+ ion colorimetric sensor based on GO/PtNPs

4.9 Chapter Summary

In this paper, GO/PtNPs were synthesized using a one-step hydrothermal method and visualized using the catalase-like nature of the material and the non-specific adsorption of Ag^+ on the composite surface. The GO/PtNPs were evaluated by TEM, Raman, and XRD characterization. The weak redshifts in the D-bands and G-bands under Raman characterization indicate that the graphene oxide is only partially reduced, and the unique face-centered cubic structure characteristic peaks of noble metal Pt appeared in the XRD characterization, suggesting that the Pt nanoparticles are fully loaded on the surface of graphene oxide. The catalytic activity of GO/PtNPs was investigated using enzyme kinetic theory and methodology with H_2O_2 and TMB as substrates.

By optimizing the synthesis conditions of GO/PtNPs, it was found that the composites with good catalytic properties could be obtained under the conditions of pH 9.5, H_2PtCl_6 of 0.25 mM, reaction temperature of 180 °C, and reaction time of 9 h. The composites with good catalytic properties could be obtained by the optimization of the synthesis conditions of GO/PtNPs. The composite concentration, reaction time, and pH value of the system were optimized during the detection process. The concentration of GO/PtNPs was 0.005 $\mu\text{g}\cdot\text{mL}^{-1}$, the reaction time was 5 min, and the pH value of the system was 5 as the detection conditions of Ag^+ .

Finally. Compared with the effect of eight metal ions, the method has good selectivity for Ag^+ . Ag^+ was simultaneously detected at different concentration gradients and in real water samples such as tap water, pond water, and river water. The results showed that the detection range was 0.5-1000 μM with a detection limit of 0.5 μM , and the recoveries ranged from 96.61% to 104.57% with a detection time of 5 min for tap water,

pond water, and river water. Compared with similar sensors, it has a wider detection range, higher sensitivity, and wider application conditions, and has broader application prospects.

CHAPTER 5

SIMULTANEOUS Hg²⁺ AND Ag⁺ COLORIMETRIC SENSORS BASED ON GRAPHENE OXIDE/AU-PT NANOPARTICLES

5.1 Overview

Chapter 5 describes simultaneous Ag⁺ and Hg²⁺ colorimetric sensors based on graphene oxide/Au-Pt nanoparticles. Based on 4.2, graphene oxide/Au-Pt nanoparticles with stronger catalytic properties were synthesized using the synergistic interaction between Au nanoparticles and Pt nanoparticles to improve the sensitivity of Ag⁺ detection. Because, GO/Au-PtNPs have higher catalytic properties compared to GO/PtNPs. Therefore, to achieve the same absorbance value in the detection system, GO/Au-PtNPs are used in less amount, and a higher detection sensitivity for Ag⁺ can be achieved. In addition, the simultaneous detection of Hg²⁺ was achieved at the same time. Therefore the chapter's main contents include the synthesis and characterization of graphene oxide/Au-Pt nanoparticles composites, optimization of the detection conditions of this colorimetric sensor: catalytic kinetic analysis of graphene oxide/Au-Pt nanoparticles, detection limits of this colorimetric sensor for Ag⁺ and Hg²⁺, sensitivity, detection range and specificity analysis.

5.2 Analysis of the results of optimization of the synthesis conditions of GO/Au-PtNPs

In order to obtain GO/Au-PtNPs with high catalytic performance, the synthetic materials were optimised in terms of the following aspects: the system pH, the ratio of

loaded metal gold to platinum, GO concentration, hydrothermal temperature, hydrothermal treatment time.

5.2.1 Analysis of the optimization results for the system pH

As shown in Figure 5.1, when other variables were held constant, the catalytic properties of the synthesized GO/Au-PtNPs gradually increased with increasing catalytic activity and increasing pH during hydrothermal heating at pH = 8.0–8.5, and the synthesized GO/Au-PtNPs exhibited the highest catalytic activity at pH = 8.5. With a further increase in pH, the catalytic activity of the composites gradually decreases. Under the weakly alkaline environment, HAuCl_4 and H_2PtCl_6 are more easily reduced to form Pt nanoparticles, promoting their uniform distribution and formation. The weakly alkaline condition favours the reduction process of platinum ions and gold particles, which enables the platinum and gold nanoparticles to be more uniformly distributed in the material. Meanwhile, graphene oxide has better dispersion under weakly alkaline conditions, avoiding aggregation in acidic or neutral environments and contributing to the uniform deposition of Pt and Au nanoparticles on its surface. In addition, the weakly alkaline condition facilitates the bonding between the Pt and Au nanoparticles and graphene oxide, so that the Pt and Au particles form a stable attachment on the surface of graphene oxide, which improves the effective use of the catalytic sites, and thus enhances the overall catalytic activity of the composites. This environment resulted in a tighter bonding between Pt and graphene oxide, which further enhanced the catalytic performance of the materials (Zhao et al., 2016).

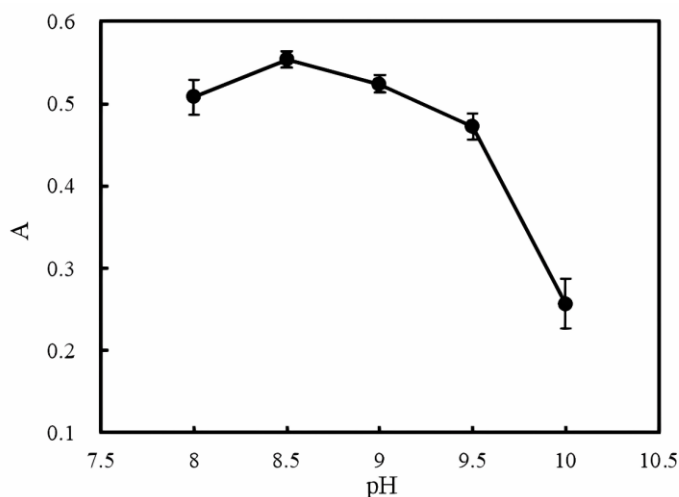


Figure 5.1: The changes of the catalytic activity (absorbance) with respect to system pH for the hydrothermal process of GO/Au-PtNP composites

5.2.2 Analysis of the optimization results of the ratio of loaded metal gold to platinum

As shown in Figure 5.2, the best catalytic activity of the composite material was obtained when the gold: platinum ratio of the loading metal was 1:1 when the gold: platinum ratio was optimized. This is because the gold nanoparticles are well dispersed on the graphene oxide surface when the ratio of gold to platinum in the composite material is 1:1. However, since gold nanoparticles are usually larger than platinum nanoparticles, the inconsistency of the particle sizes intensifies with the increase of the gold content, which increases the interaction force between the particles and tends to trigger agglomeration phenomena (Zhao et al., 2016; Zhang et al., 2020b), and the catalytic activity of the composite material will decrease.

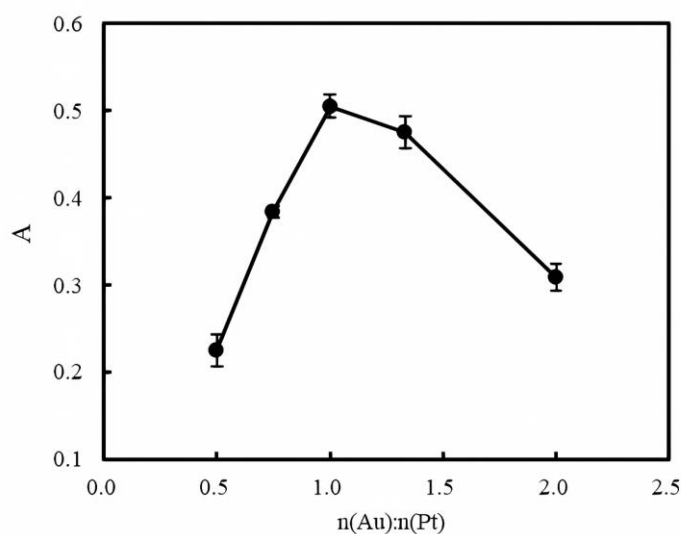


Figure 5.2: The changes of the catalytic activity (absorbance) with respect to the gold-platinum ratio in GO/Au-PtNPs composites during the hydrothermal process

5.2.3 Analysis of the optimization results for the GO concentration

As shown in Figure 5.3, the catalytic performance of the synthesized composites increased and then decreased with increasing GO concentration, and the best catalytic activity was achieved when the GO concentration was 0.5 mg/mL. This is due to the high chemical stability and large specific surface area of GO, which contains defects and oxygen-containing functional groups on the surface and can provide nucleation sites for the growth of metal nanoparticles (Wang et al., 2017); thus, gold and platinum nanoparticles are loaded on the surface of GO. However, aggregation also occurs when the concentration of GO is too high (Zhang et al., 2020b), which reduces the number of active sites on the surface of GO and prevents better loading of gold and platinum nanoparticles, and the catalytic activity of the composites decreases accordingly.

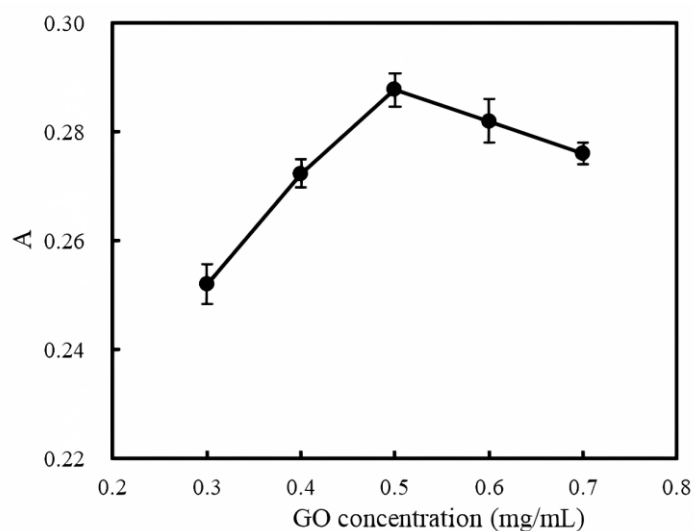


Figure 5.3: The changes of the catalytic activity (absorbance) with respect to the graphene oxide concentration in GO/Au-PtNP composites during the hydrothermal process

5.2.4 Analysis of the hydrothermal treatment time optimization results

As shown in Figure 5.4, the catalytic performance of GO/Au-PtNPs increased with increasing reaction time, and the catalytic activity of the synthesized composites slightly decreased after 10 h of reaction. This is because when the hydrothermal treatment time is too short, Au^{3+} and Pt^{4+} are not sufficiently reduced to Au^0 and Pt^0 , respectively, and the remaining Au^{3+} and Pt^{4+} are dispersed in the system and not immobilized on the surface of GO (Zhao et al., 2016); thus, the catalytic performance of the composites cannot reach the optimum level. When the hydrothermal treatment time is too long, the particle size of the gold and platinum nanoparticles gradually increases. When the hydrothermal treatment time is too long, the particle size of the gold and platinum nanoparticles gradually increases. This is since in hydrothermal reactions, as the reaction time is prolonged, the precursor ions have more opportunities to diffuse and aggregate to form larger particles. At the same time, due to the high surface energy of small nanoparticles, they tend to grow by

aggregation to reduce the surface energy and promote the formation of larger particles. In addition, the nucleus undergoes a growth process during the early stages of formation, and prolonged heating provides sufficient energy for the nucleus to continuously absorb surrounding atoms or molecules, further increasing the particle size. Together, this series of processes results in a larger nanoparticle size with time (Schmidt et al., 2022; Xuan et al., 2022; Liu et al., 2022b; Rudra et al., 2022). Due to the larger size of the nanomaterials, the smaller their specific surface area is, the smaller the surface energy generated (Qu et al., 2023); thus, the catalytic activity of the composites decreases accordingly. Due to the larger size of the nanomaterials, the smaller their specific surface area is, the smaller the surface energy generated (Qu et al., 2023); thus, the catalytic activity of the composites decreases accordingly.

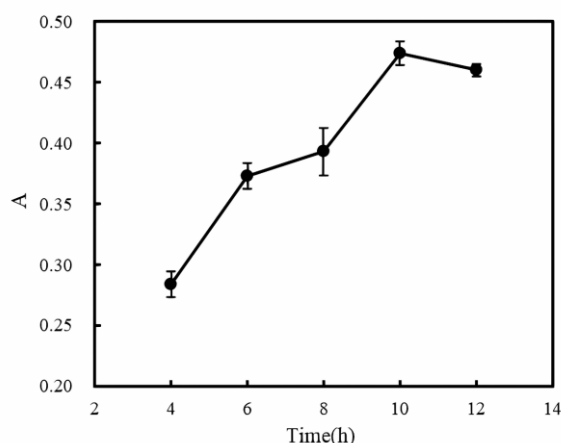


Figure 5.4: The changes of the catalytic activity (absorbance) with respect to hydrothermal treatment time for the hydrothermal process of GO/Au-PtNP composites

5.2.5 Analysis of hydrothermal temperature optimization results

As shown in Figure 5.5, the catalytic activity of GO/Au-PtNPs tended to increase and then decrease with a gradual increase in the one-step hydrothermal reaction temperature. A hydrothermal temperature of 180 °C was the optimum hydrothermal synthesis temperature. When the temperature of the hydrothermal reaction is low, Au^{3+} and

Pt^{4+} are not reduced to Au^0 or Pt^0 , and the unreduced Au^{3+} and Pt^{4+} are dispersed in the system and cannot be fixed on the surface of graphene oxide; The increase of Au^0 and Pt^0 loaded on the graphene oxide surface with the increase of temperature can effectively reduce the possibility of agglomeration of graphene oxide. This is mainly due to the fact that when Au^0 and Pt^0 nanoparticles are loaded onto the graphene oxide (GO) surface, they occupy the positions between the GO lamellae, form a physical barrier, and produce a spatial site-barrier effect, preventing the GO lamellae from re-stacking and thus preventing agglomeration. In addition, these metal nanoparticles change the charge state of the GO surface, enhancing the negative charge or forming a new charge distribution, resulting in electrostatic repulsion between GO layers, further preventing the layers from re-stacking. The metal nanoparticles also act as ‘pillars’ to increase the GO layer spacing and provide mechanical support, which helps to maintain the dispersion of GO layers and reduce the possibility of agglomeration (Zhao et al., 2016; Zhang et al., 2020b). As a result, the catalytic activity of the material was also increased.

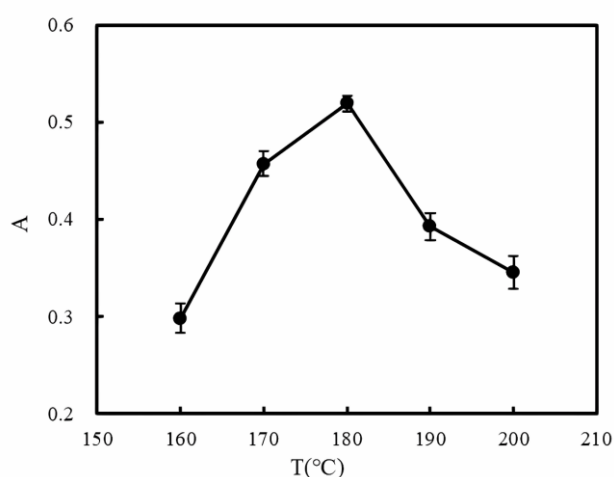


Figure 5.5: The changes of the catalytic activity (absorbance) with respect to hydrothermal temperature for the hydrothermal process of GO/Au-PtNP composites

5.3 Characterization of GO and GO/Au-PtNPs

TEM, Raman, FT-IR, and EDS were used to obtain detailed characterisation information of GO and GO/Au-PtNPs.

5.3.1 Transmission electron microscopy (TEM).

TEM can reveal the size, shape, size, and crystal lattice of materials. GO and GO/Au-PtNPs were characterized by a JEOL-JEM2100F transmission electron microscope, and the results are shown in Figure 5.6. Figure 5.6a shows that GO has wrinkles on its surface and slightly curled edges, indicating that GO can overlap each other in a disorderly manner in solution (Jin et al., 2019; Sun et al., 2018). The formation of a disordered multilayer frame structure provides additional active sites for precious metals. Figures 5.6b and 5.6c show the lattice of nanoparticles loaded on the surface of graphene oxide. From the distribution of the lattice, it can be inferred that the graphene oxide surface may be loaded with two types of nanoparticles, in which the spherical gold nanoparticles are in the middle and the platinum nanoparticles are dispersed outside the gold nanoparticles. The gold nanoparticles had a lattice spacing of approximately 0.226 nm and a particle size of approximately 6 nm. The surrounding light gray particles are platinum nanoparticles with a lattice spacing of 0.218 nm and a particle size of approximately 2–3 nm (Ye, et al. 2022).

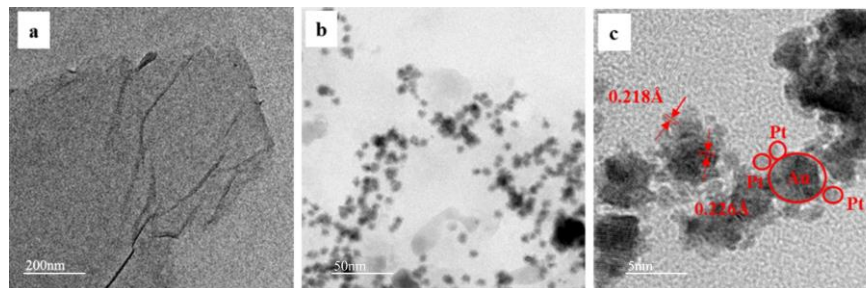


Figure 5.6: (a) 100,000x magnification, (b) 100,000x magnification, and (c) 400,000 HRTEM maps of the GO/Au-PtNP composites

5.3.2 Raman spectroscopy

Raman spectroscopy can provide structural information on graphene materials. As shown in Figure 5.7, both the GO and GO/Au-PtNP composites exhibited two characteristic peaks, the D-band and G-band. The intensity of the D-band peak represents the asymmetry of the sample structure, and the intensity of the G-band peak represents the symmetry of the sample structure (Wang et al., 2020b). The ratio of the Raman spectral intensity (I_D/I_G) of GO is 1.19, and the ratio of the I_D/I_G of the GO/Au- composite is 1.24. This indicates that the I_D/I_G ratio of the composite is greater than that of GO. This phenomenon can be attributed to the partial reduction of graphene oxide, which leads to the partial deoxygenation of a substantial number of sp^3 hybridized carbon atoms. Consequently, new sp^2 hybridized regions are formed. The reformed sp^2 regions exhibit smaller dimensions than the sp^2 regions found in graphene oxide. Therefore, with an increase in the number of sp^2 regions, the average size of the sp^2 regions in the reduced graphene decreases. This phenomenon is observed in the Raman spectra, where there is an increase in the I_D/I_G ratio (Kasztelan et al., 2021).

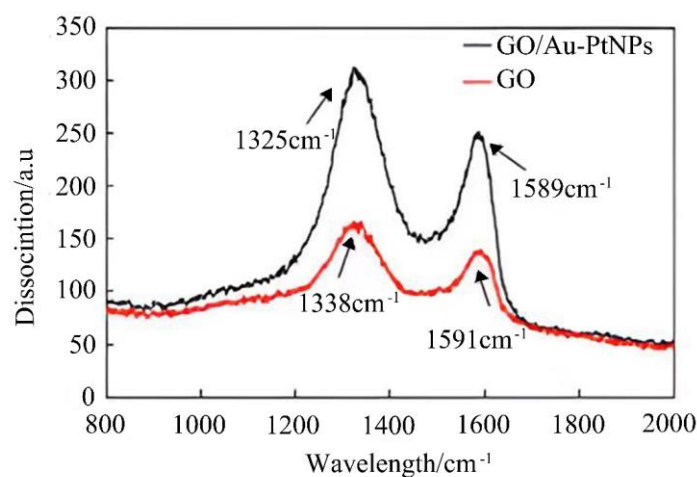


Figure 5.7: Raman characterization of GO and the GO/Au-PtNPs composites

5.3.3 EDS characterization

To understand the composition and distribution ratio of elements in the composite, GO/Au-PtNPs were characterized by using a JSM-7500F cold field emission electron microscope. As shown in Figure 5.8, GO contains only two elements, carbon and oxygen, but the GO/Au-PtNPs composite also contains gold and platinum, with an atomic percentage of 4.35 for platinum and 4.69 for gold. Therefore, the ratio of gold to platinum is 1.08. Thus, according to the semiquantitative analysis, the ratio of gold to platinum was close to 1:1, indicating that the GO/Au-PtNPs composite material was successfully synthesized.

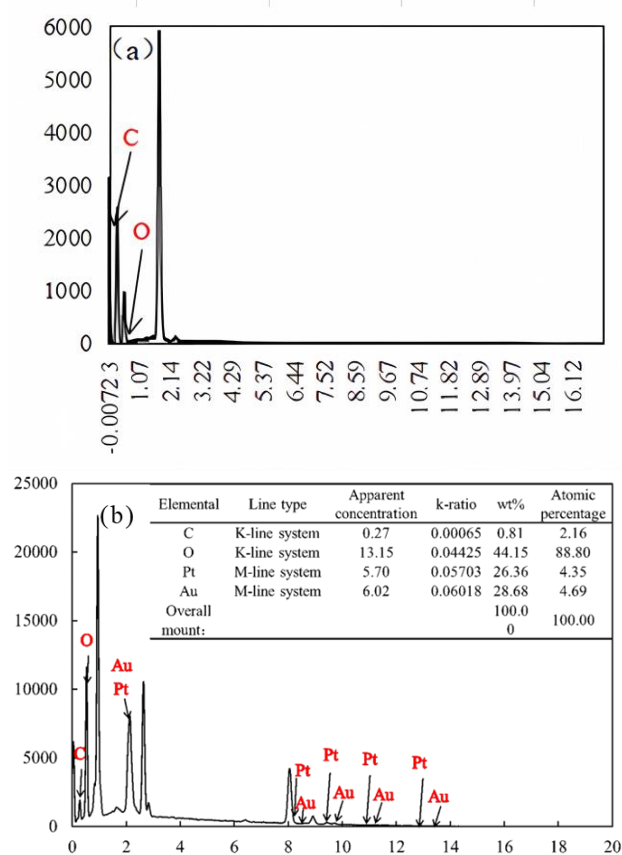


Figure 5.8: EDS characterization map of the (a) GO, (b) GO/Au-PtNPs composites

5.3.4 FT-IR Analysis

To analyze the changes in the surface functional groups of GO before and after material synthesis, the FT-IR spectra of GO and GO/Au-PtNPs were analyzed by a Nicolet iS5 Fourier transform infrared spectrometer. As shown in Figure 5.9, compared with that of GO, the absorption peak of the GO/Au-PtNPs composite at 1583 cm^{-1} is attributed to C=C, and the stretching vibration peak is blueshifted, indicating that the molecular vibration frequency of the synthesized GO is increased. GO has characteristic peaks at 1053 cm^{-1} , 1227 cm^{-1} , 1616 cm^{-1} and 1732 cm^{-1} . There may also be a C-O-C stretching vibration peak at 1053 cm^{-1} , a plane bending vibration peak at 1227 cm^{-1} , and stretching vibrations at 1616 cm^{-1} and 1732 cm^{-1} . The characteristic peak at 1732 cm^{-1} attributed to the C=O stretching vibration of the GO/Au-PtNPs composite disappears, which may be due to the reduction reaction (Muniyalakshmi et al., 2020) during the synthesis of the composite.

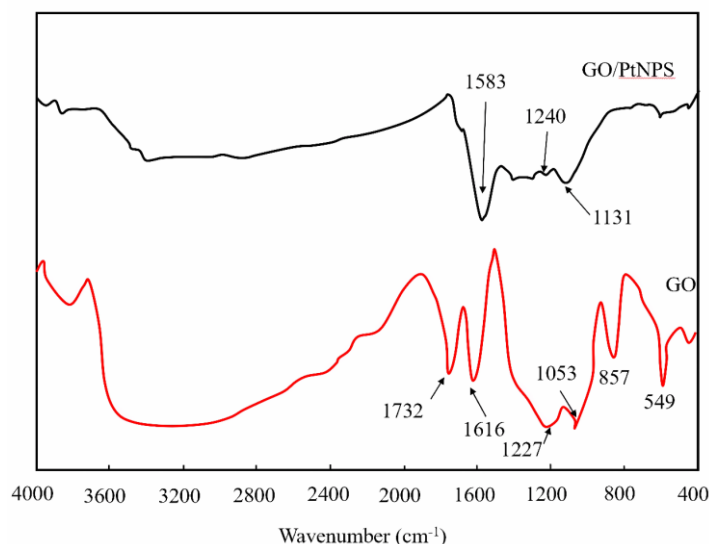


Figure 5.9: FT-IR characterization of GO and GO/Au-PtNPs composites

5.4 Analysis of the Detection Conditions for Ag⁺ and Hg²⁺ Colorimetric Sensors

For a colorimetric sensor, the range of color change and background color directly affects the sensitivity of the sensor, so the possible influencing factors in the color development process, including the pH, reaction time, composite material concentration, H₂O₂ concentration, and TMB concentration, are optimized.

5.4.1 Effect of pH in the system

As shown in Figure 5.10, when other variables are held constant, the catalytic activity of GO/Au-PtNPs first increases and then decreases with a gradual increase in pH. This is because, on the one hand, TMB produces a blue quinoneimine intermediate upon oxidation. Under strong acidic conditions, this intermediate protonates to form a stable yellow compound, 3,3',5,5'-tetramethyl-2-(1H)-benzimidazolone. Whereas, the blue quinone imine is more stable when pH = 5 under weakly acidic conditions, making the system blue in colour. On the other hand, this under weakly acidic conditions, the GO/Au-PtNPs composites catalyse H₂O₂ more efficiently and produce $\cdot$ OH more effectively to oxidise the TMB substrate, which results in a blue colouration of the solution system as evidenced by an increase in absorbance (Zhao et al., 2020). Therefore, a system pH = 5 was selected for the subsequent experiments.

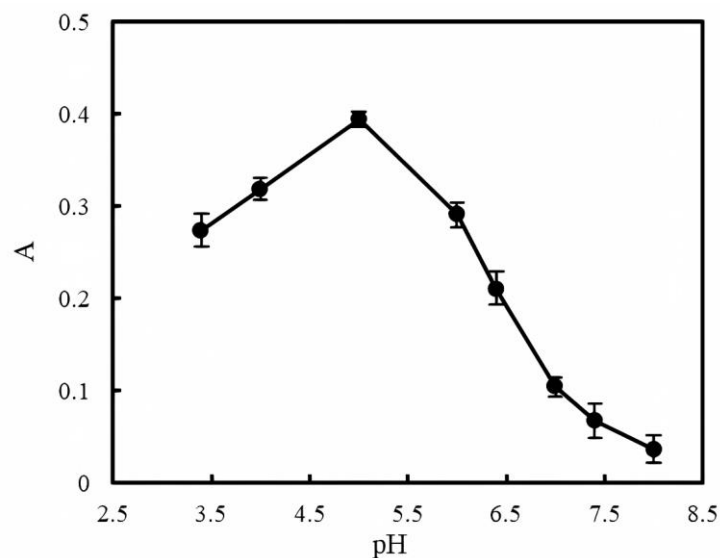


Figure 5.10: The changes of the catalytic activity (absorbance) with respect to the system pH during catalysis. Reaction conditions: pH=3.4, 4.0, 5.0, 6.0, 6.4, 7.0, 7.4, and 8.0; reaction time=5 min; GO/Au-PtNPs=6 μ L; TMB concentration=0.5 mM; H_2O_2 concentration=0.02 M; λ =652 nm

5.4.2 Effect of reaction time

As depicted in Figure 5.11, when the reaction time was 1–5 min, the absorbance of the system increased drastically, and when the reaction time was greater than 5 min, the absorbance values were approximately 0.55. This is because with increasing time, the system gradually reaches catalytic dynamic equilibrium, and the absorbance tends to be constant (Liu et al., 2019; Li et al., 2021). Therefore, a reaction time of 5 min was selected as the optimal reaction time for the system.

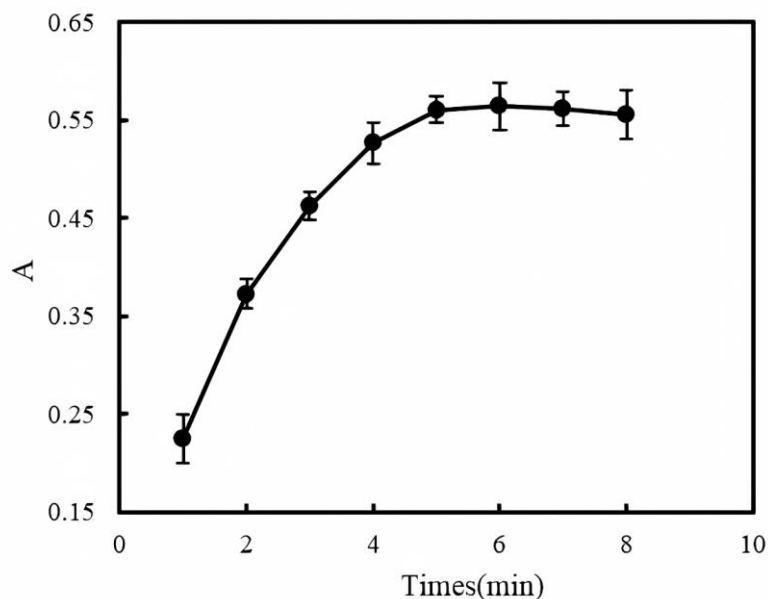


Figure 5.11: The changes of the catalytic activity (absorbance) with respect to reaction times in catalytic processes. Reaction conditions: pH= 5.0; reaction time=1, 2, 3, 4, 5, 6, 7, and 8 min; GO/Au-PtNPs 6 μ L; TMB concentration=0.5 mM; H_2O_2 concentration=0.02 M; λ =652 nm

5.4.3 Analysis of the influence of the amount of GO/Au-PtNPs material added

As shown in Figure 5.12, when the volume of GO/Au-PtNPs is between 1 and 6 μ L, the absorbance increases, revealing that the catalytic activity of the system gradually increases. The system reached catalytic equilibrium at 6 μ L, where the absorbance was 0.557. This is because, with the increasing volume of GO/Au-PtNPs, more active sites can be provided, which accelerates the oxidation rate of H_2O_2 . When the volume of GO/Au-PtNPs reaches a certain value (6 μ L), H_2O_2 in the system is completely decomposed, impeding further reaction in the system that causes no change in the absorbance value. Therefore, a GO/Au-PtNPs of 6 μ L was selected as the optimal volume for the system.

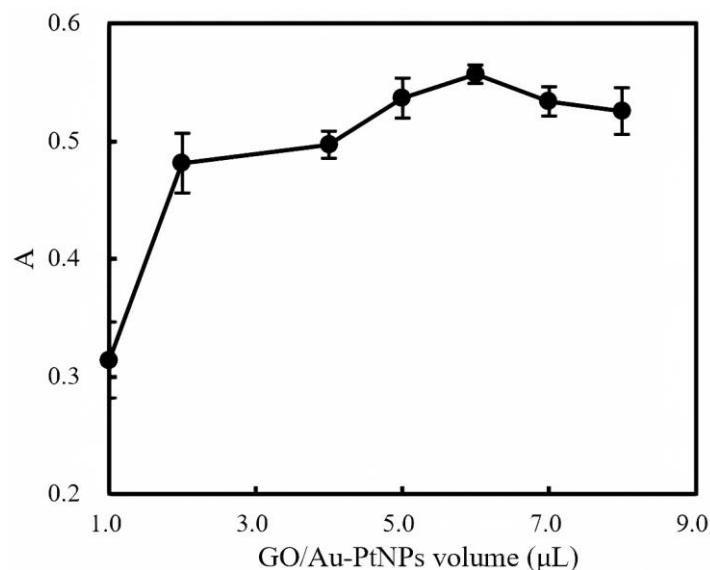


Figure 5.12: The changes of the catalytic activity (absorbance) with respect to the GO/Au-PtNPs concentration in catalytic processes. Reaction conditions: pH= 5.0; reaction time=5 min; volume of GO/Au-PtNPs (1, 2, 4, 5, 6, 7, and 8 μL); concentration=0.5 mM; concentration=0.02 M; and wavelength=652 nm

5.4.4 Effect of H_2O_2 concentration

As shown in Figure 5.13, when the concentration of hydrogen peroxide was 0.002–0.02 M, the absorbance changed perpendicularly. This indicates that with increasing hydrogen peroxide concentration, the H_2O_2 concentration around the catalytic active site of GO/Au-PtNPs increases, and the decomposition rate of H_2O_2 accelerates, resulting in more $\cdot\text{OH}$, which in turn accelerates the color development of TMB. When the concentration of H_2O_2 reaches 0.02 M, the absorbance is 0.558, and the catalytic activity of the system is the highest. When the concentration of H_2O_2 in the system is greater than 0.02 M, the absorbance tends to plateau. Therefore, a H_2O_2 concentration of 0.02 M was selected as the optimal condition for the system.

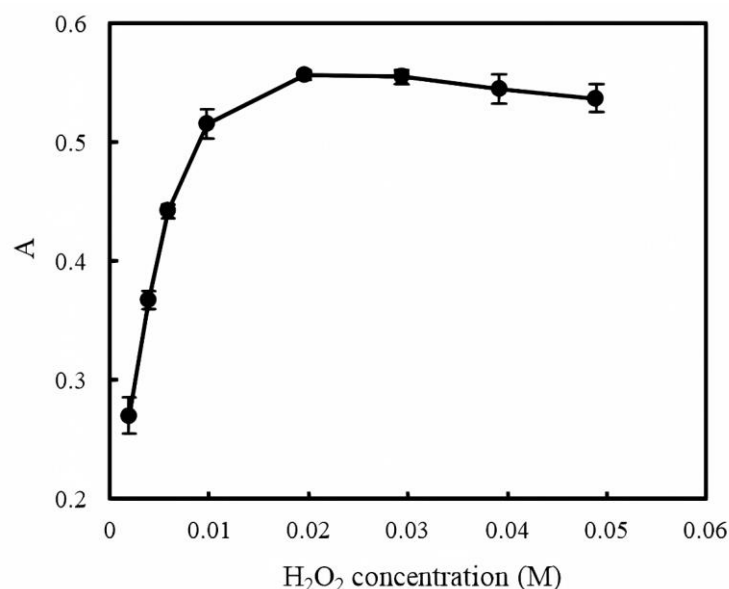


Figure 5.13: The changes of the catalytic activity (absorbance) with respect to the H₂O₂ concentration during catalysis. Reaction conditions: pH= 5.0; reaction time=5 min; GO/Au-PtNPs volume=6 μ L; TMB concentration=0.5 mM; H₂O₂ concentration=0.002, 0.004, 0.006, 0.01, 0.02, 0.03, 0.04, 0.05 M; λ =652 nm

5.4.5 Effect of the TMB substrate concentration

As shown in Figure 5.14, the catalytic activity of GO/Au-PtNPs in the system increased with increasing TMB substrate concentration. Even at the lowest TMB concentration of 0.5 mM, the absorbance value is 0.536, which meets the detection requirements; Because the absorbance value at this point is 0.4-0.6, the colour is neither too dark nor too light. This is because if the colour is too dark our eyes have no way of distinguishing the colour change and we cannot use our eyes to achieve detection. However, if the colour is too light, the detection limit of the colorimetric sensor will be affected. Moreover, this approach can avoid situations in which the background value is high due to the dark color of the system (Shen et al., 2021). Therefore, a TMB concentration of 0.5 mM was selected as the best condition for the system.

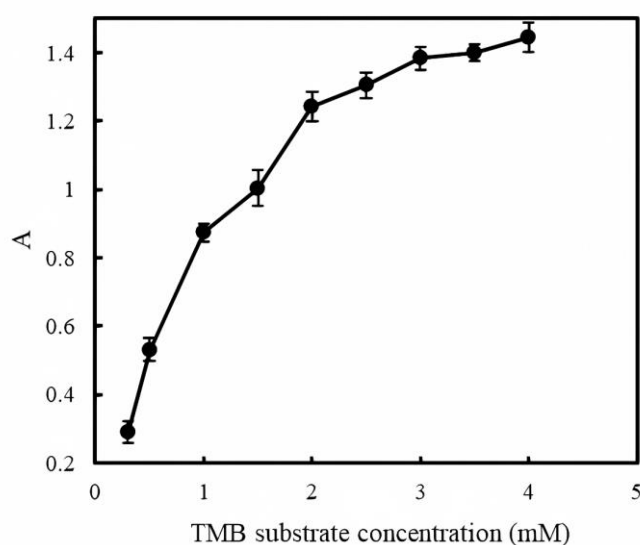


Figure 5.14: The changes of the catalytic activity (absorbance) with respect to the TMB concentration during catalysis. Reaction conditions: pH= 5.0; reaction time=5 min; GO/Au-PtNPs volume=6 μ L; TMB concentration=0.3, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 mM; H_2O_2 concentration=0.02 M; λ =652 nm

5.5 Analysis of the catalase-like kinetics of GO/Au-PtNPs

To further analyze the catalase-like activity of GO/Au-PtNPs, steady-state kinetics were studied, and a series of initial reaction rates were calculated via the Michaelis–Menten equation 3.3 (Wang et al., 2022; Chen et al., 2021b).

As shown in Figure 5.15, the initial catalytic velocity of GO/Au-PtNPs increases initially and then stabilizes with increasing substrate concentration. This conforms to the Michaelis–Menten law and proves that the catalytic activity of GO/Au-PtNPs follows the Michaelis–Menten equation. Two apparent mechanical parameters, K_m and V_{max} , were obtained by double reciprocal linear fitting. As shown in Table 5.1, the K_m of GO/Au-PtNPs with TMB as the substrate is 2.1, which is a smaller value compared with the existing reports (Zhang et al., 2020; Fang et al., 2020; Zhen et al., 2020; Jiang et al., 2021; Jiang et al., 2022). The smaller value of K_m means that the catalysts have a smaller affinity

for the substrate. These findings indicate that the binding affinity between GO/Au-PtNPs and TMB is high and that the concentration of TMB needed by GO/Au-PtNPs is low when the maximum catalytic effect is achieved.

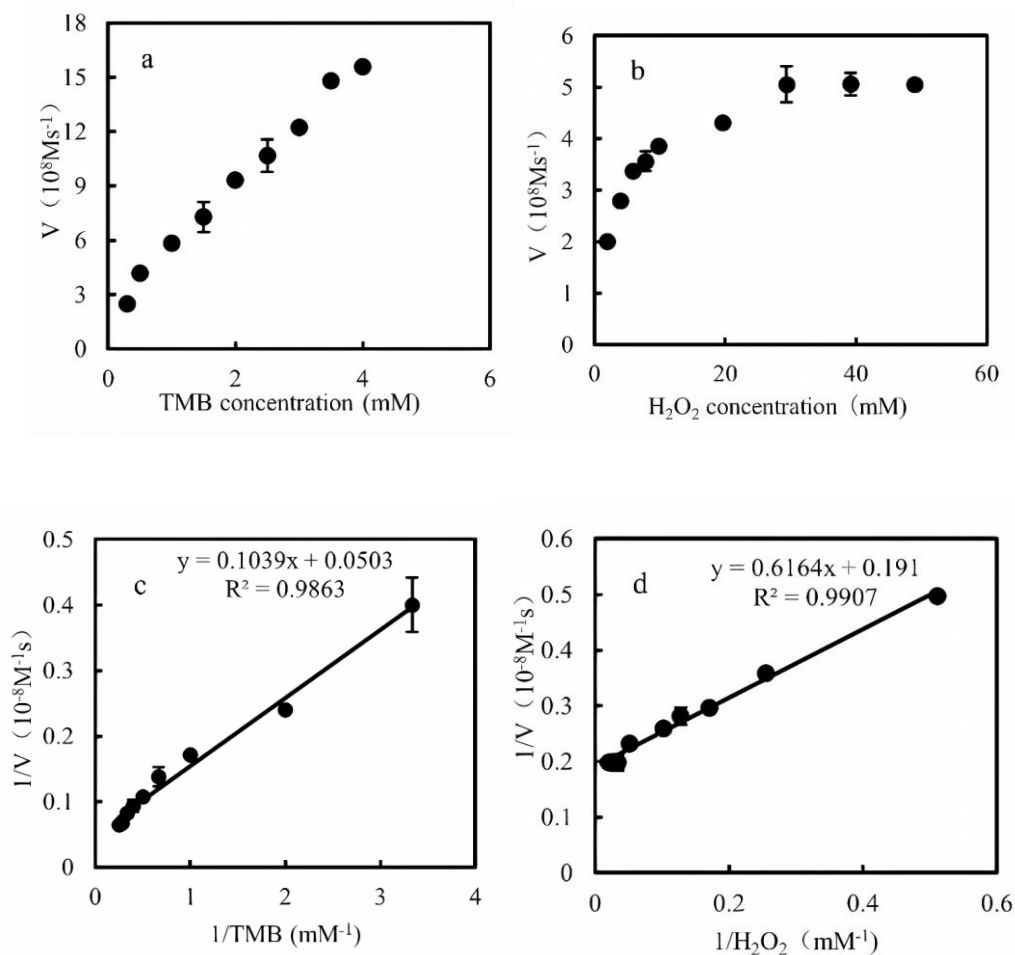


Figure 5.15: (a) Catalytic kinetic curves for different TMB concentrations. Reaction conditions: pH= 5.0; reaction time=1-5 min; GO/Au-PtNPs volume=6 μL ; TMB concentration=0.3, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 mM; H_2O_2 concentration=0.02 M; λ =652 nm; (b) Catalytic kinetic curves for different H_2O_2 concentrations. Reaction conditions: pH= 5.0; reaction time=5 min; the number of GO/Au-PtNPs=6 μL ; TMB concentration=0.5 mM; H_2O_2 concentration=0.002, 0.004, 0.006, 0.01, 0.02, 0.03, 0.04, 0.05 M; λ =652 nm; (c) and (d) Double inverse curves of (a) and (b), respectively

Table 5.1: Comparison of apparent Michaelis constant (K_m) and maximum reaction rate (V_{max}) between GO/Au-Pt NPS and other catalases

Catalyst	Substrate	$K_m(\text{mM})$	$V_{max}(10^{-8}\text{M}\cdot\text{s}^{-1})$	References
GO/Au-PtNPs	TMB	2.1	19.9	This study
	H ₂ O ₂	3.2	5.2	
Au-PtNCs-GMP	TMB	6.8	253.8	(Zhang et al., 2020b)
	H ₂ O ₂	\	\	
Co-ferrocene metal-organic framework	TMB	29.7	27.6	(Fang et al., 2020)
	H ₂ O ₂	\	\	
Iridium oxide nanoparticles	TMB	179.0	19.3	(Zhen et al., 2020)
	H ₂ O ₂	\	\	
Cy-AuNCs	TMB	1.9	212.3	(Jiang et al., 2021)
	H ₂ O ₂	2.8	15.8	
GSH-AgNPs	TMB	4.2	247.0	(Jiang et al., 2022)
	H ₂ O ₂	58.6	21.5	

5.6 The principle of the colorimetric sensing detection method

As shown in Figure 5.16, the GO/Au-PtNPs composites have peroxidase-like properties that catalyze the decomposition of H₂O₂ to generate hydroxyl radicals ($\cdot\text{OH}$). We assessed the formation of $\cdot\text{OH}$ by incorporating the fluorescent probe terephthalic acid (TA) into H₂O₂+GO/Au-PtNPs. The TA molecule readily reacts with $\cdot\text{OH}$ to form the

strongly fluorescent product 2-hydroxyterephthalic acid (TAOH), which emits a distinctive fluorescence at approximately 435 nm. The results showed that the fluorescence intensity of the experimental system gradually increased with increasing amounts of GO/Au-PtNPs nanocomposites, indicating that the greater the number of nanocomposites was, the more $\cdot\text{OH}$ was generated.

The colorimetric sensor constructed in this study relies on modulating the catalytic properties of Ag^+ and Hg^{2+} on the peroxidase-like peroxidase catalytic properties of GO/Au-PtNPs composites to detect these two metals. First, in the H_2O_2 -TMB system, when Ag^+ and Hg^{2+} are not present, the GO/Au-PtNPs composites can catalyze the production of $\cdot\text{OH}$ from H_2O_2 , and $\cdot\text{OH}$ can oxidize TMB to oxTMB, which changes the system from colorless to blue. The absorbance value of the system at a wavelength of 652 nm was measured to be A^0 (as shown in Figure 5.17). When Ag^+ and Hg^{2+} are contained in the system, Ag^+ can react with Pt^0 on the surface of graphene oxide in a specific redox reaction to form $\text{Ag}@ \text{PtNPs}$, and Hg^{2+} can react with Au^0 and Pt^0 in an amalgam reaction to form an amalgam (Sun et al., 2017). This means that the electronic state of the active sites can be changed through metal interactions. The addition of Ag^+ and Hg^{2+} reduces access to Pt^0 and Au^0 , masks the active sites of AuNPs and PtNPs, and leads to the poor affinity between the composite and the substrate TMB (Zhang et al., 2020b). As a result, the presence of these metal ions inhibits the catalytic activity of the composite and leads to a decrease in the absorbance. At this time, the absorbance value decreased to $A_{\text{Hg}^{2+} + \text{Ag}^+}$. The decrease in the absorbance value $\Delta A_{\text{Hg}^{2+} + \text{Ag}^+}$ caused by the combination of Ag^+ and Hg^{2+} can be found using equation 5.1. However, the presence of ethylenediamine tetraacetic acid (EDTA) in the system chelates Hg^{2+} , which can mask Hg^{2+} ions. This allows the absorbance of A_{Ag^+} (when only Ag^+ exists in the system) to be determined, and

the change in the absorbance of the system caused by the presence of Ag^+ alone can be obtained from equations 5.2 Furthermore, equation 5.3 can be used to calculate $\Delta A_{\text{Hg}^{2+}}$ and a standard curve of the concentrations of $\Delta A_{\text{Hg}^{2+}}$ and Hg^{2+} can be established so that the quantitative detection of Hg^{2+} can be achieved. Based on this mechanism, Ag^+ and Hg^{2+} can be simultaneously detected in the system.

$$\Delta A_{\text{Hg}^{2+} + \text{Ag}^+} = A_0 - A_{\text{Hg}^{2+} + \text{Ag}^+} \quad \text{Equation 5.1}$$

$$\Delta A_{\text{Ag}^+} = A_0 - A_{\text{Ag}^+} \quad \text{Equation 5.2}$$

$$\Delta A_{\text{Hg}^{2+}} = \Delta A_{\text{Hg}^{2+} + \text{Ag}^+} - \Delta A_{\text{Ag}^+} \quad \text{Equation 5.3}$$

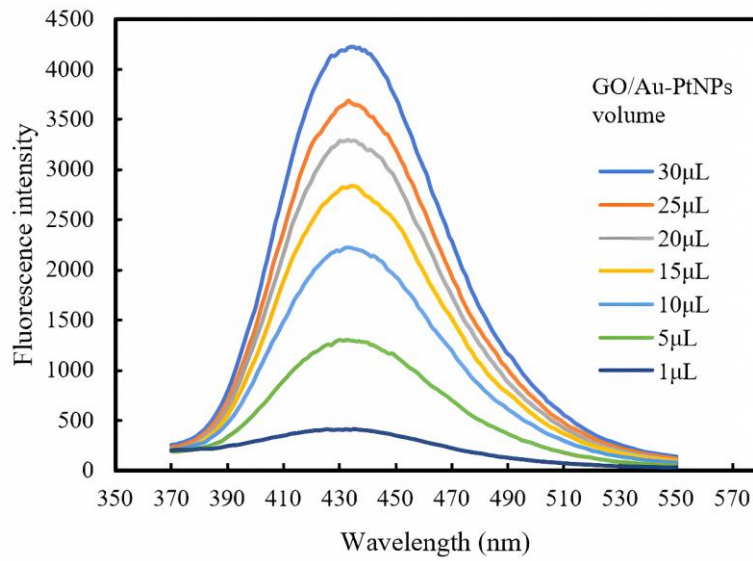


Figure 5.16: Effect of different volumes of GO/Au-PtNPs on the fluorescence intensity after oxidation of benzene dicarboxylic acid. 200 μL of benzene dicarboxylic acid (0.35 mM), 20 μL of H_2O_2 (30 mM), and pH=4 buffer solution were added to 1000 μL , the reaction time was 5 min, the reaction temperature was 40 $^\circ\text{C}$, the excitation wavelength was 316 nm, and the emission wavelength was 435 nm

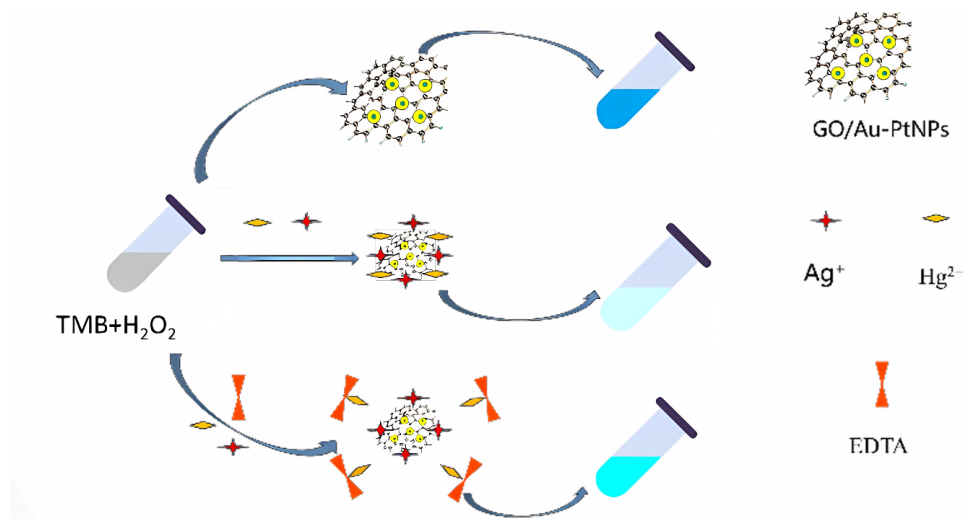


Figure 5.17: Schematic diagram for the detection of Hg²⁺ and Ag⁺

5.7 Detection of Hg²⁺ and Ag⁺

To explore the detection range and sensitivity of the colorimetric sensor for Hg²⁺ and Ag⁺, a series of standard solutions with different concentrations of Hg²⁺ and Ag⁺ were added to the reaction system, and the change in the absorbance value of the system was determined. As shown in Figure 5.18a and Figure 5.19a, the color of the system becomes increasingly lighter as the concentrations of Hg²⁺ and Ag⁺ gradually increase. Figure 5.18 b and Figure 5.19b show scatter plots of the absorbance after the addition of different concentrations of Hg²⁺ and Ag⁺ solutions with a logarithmic distribution. Therefore, a logarithmic fit was made to the horizontal coordinates, and a standard curve with a vertical coordinate of ΔA and a horizontal coordinate of $-\log c$ was established, as shown in Figure 5.18c and Figure 5.19c. The detection range of Hg²⁺ was 1–10000 nM, the detection limit was 1 nM, and the correlation coefficient of linear fitting was 0.9971. The detection range of Ag⁺ was up to 50–100000 nM, the detection limit was 50 nM, and the linear fit correlation coefficient was 0.9943.

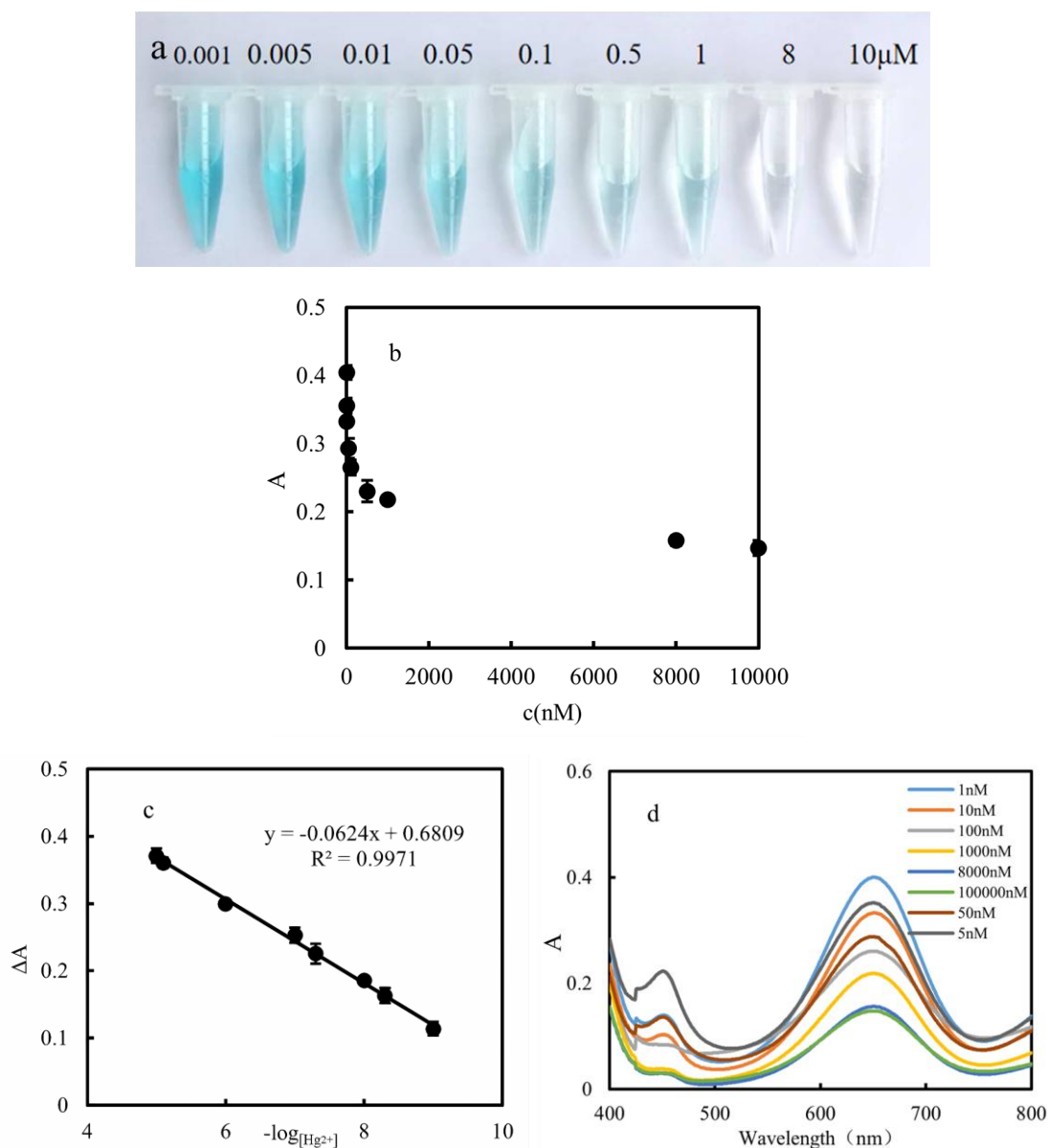


Figure 5.18: Detection range of Hg^{2+} solution at various concentrations (0.001, .002, 0.01, 0.05, 0.1, 0.5, 1, 8, and 10 μM) by a colorimetric sensor: (a) Photos of the color change in the system solution at different concentrations of Hg^{2+} ; (b) The relationship between the absorbance and Hg^{2+} concentration; (c) Linear fitting curve between the absorbance difference and $-\log[\text{Hg}^{2+}]$; (d) UV-VIS spectra of different Hg^{2+} concentration reaction conditions: pH= 5.0; Reaction time=5 min; GO/Au-PtNPs 6 μL ; TMB concentration=0.5 mM; H_2O_2 concentration=0.02 M; $\lambda=652$ nm

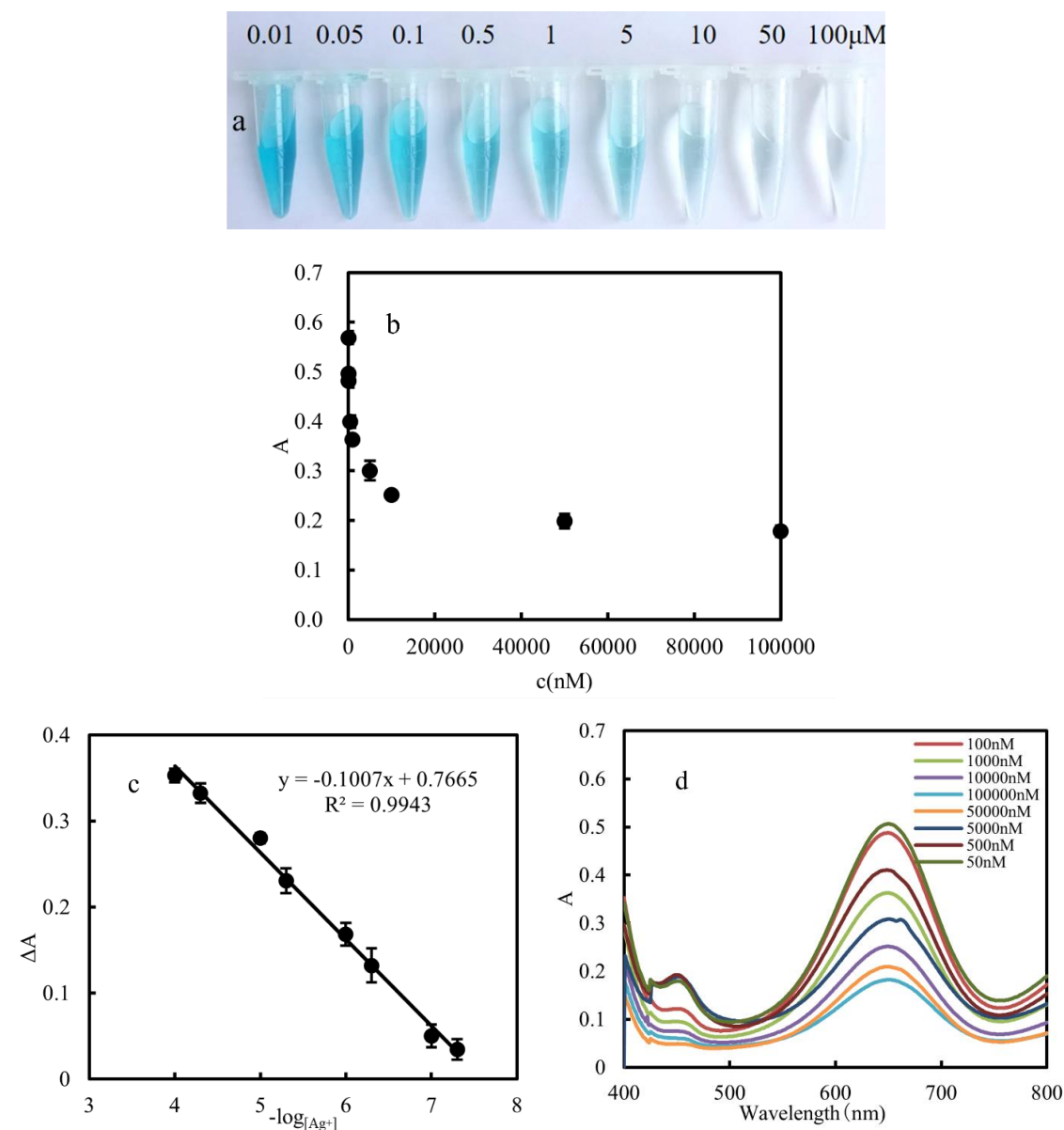


Figure 5.19: Detection range of the colorimetric sensor for Ag^+ solution at different concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 μM) (a) Physical photos of the color change in the system solution at different concentrations of Ag^+ ; (b) The relationship between the absorbance and Ag^+ concentration; (c) Linear fitting curve of the difference in absorbance to $\log[\text{Ag}^+]$; (d) UV-VIS spectra of different Ag^+ concentrations. Reaction conditions: pH= 5.0; reaction time=5 min; total number of GO/Au-PtNPs=6 μL ; TMB concentration=0.5 mM; H_2O_2 concentration=0.02 M; λ =652 nm

5.8 Simultaneous detection of Hg^{2+} and Ag^+ in actual samples

EDTA was employed in samples containing both Hg^{2+} and Ag^+ ions or in actual samples with complex ions of unknown content to detect the presence of each ion since both have similar reactions with GO/Au-PtNPs. Under the same detection conditions, the absorbance in the presence of EDTA in the system was greater than that in the absence of EDTA, which was due to the chelating effect of EDTA on Hg^{2+} . Substituting the measured absorbance into equations (4) and (5), the calculation results are shown in Table 5.2 and Figure 5.20 campus lake water sample, tap water, and serum were added to different concentrations of Ag^+ (10 nM, 100 nM, 1000 nM) and Hg^{2+} (10 nM, 100 nM, 1000 nM).

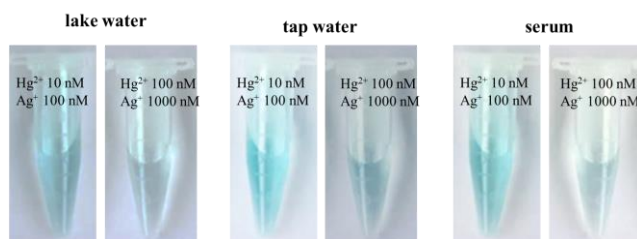


Figure 5. 20: Photos of the color change in real samples

Table 5.2: Detection in real samples

Water sample type	Sample number	Addition amount (nM)	Measured amount (nM)	Addition amount (nM)	Measured amount (nM)	Recovery(%)	
		Hg^{2+}	Ag^+	Hg^{2+}	Ag^+	Hg^{2+}	Ag^+
lake water	1	10	100	10±6	99.9±6	97.22±6.32	98.6±5.7
	2	100	1000	103±1	992±5	103.13±6.47	99.24±4.67

Table 5.2: continued

tap water	1	10	100	9±4	100±5	92.49± 3.99	100.07 ±4.58
	2	100	1000	96±3	997±3	95.71± 3.53	96.96± 3.4
serum	1	10	100	10±1	98±4	97.22± 6.33	97.81± 4.47
	2	100	1000	104±2	999±3.5	104.29 ±2.24	99.96± 3.47

The detected concentrations of Hg^{2+} and Ag^+ are similar to the standard addition concentration, and the recovery rate is between 92% and 104%, which is within an acceptable range (90%~110%). This shows that this detection method has high accuracy and reproducibility and can visualize Hg^{2+} and Ag^+ at the same time. As shown in Table 5.3, due to the improved catalytic performance of GO/Au-PtNPs, the sensitivity to the target ions was significantly improved and the GO/Au-PtNPs colourimetric sensor had a wide range of detection capabilities and lower detection limits for Hg^{2+} and Ag^+ compared to other detection methods; moreover, compared with existing reports, the detection limit is at the upper-middle level, which can realize simultaneous visual detection of Hg^{2+} and Ag^+ .

Table 5.3: Comparison of the Detection Limits of Hg^{2+} and Ag^+ by Different Methods

Material	Method	Examination Area		Detection Limit		References
		Hg^{2+}/nM	Ag^+/nM	Hg^{2+}/nM	Ag^+/nM	
GO/Au-PtNPs	colorimetry	1-10000	10-100000	1	10	This study
CdZnTe QDs	fluorescence method	\	500-4000	\	170	(Cui et al., 2021)

Table 5.3: continued

NIDEA	fluorescence method	0-7900	\	20.9	\	(Su et al., 2022)
GSH-MnO ₂	colorimetry	\	10-800	\	4.23	(He et al., 2018)
NFCD	fluorescence method	\	0-45000	\	15	(Guo et al., 2021)
Au/Ag NCs	colorimetry	10-10000	\	4.8	\	(Bi et al., 2021)
Chl-AgNPs	colorimetry	2700-60000	\	2700	\	(Yılmaz et al., 2021)
BiL-Eu-MOF	fluorescence method	\	0-600000	\	2100	(Ameen et al., 2022)
GO/PtNPs	colorimetry	\	50-1000000	\	50	Chapter 4

5.9 Analysis of specific evaluation results

There could be many interfering ions in the actual sample, and good specificity is the basis for the colorimetric sensor to be applied to actual sample detection. To explore the specificity of the colorimetric sensor and maintain the other variables unchanged, 10 μL of Ni^{2+} , Cu^{2+} , Cd^{2+} , Al^{3+} , Mg^{2+} , Na^+ , K^+ , or Zn^{2+} metal solution at a concentration of 1uM were individually added to the system. As shown in Figure 5.20, when ions such as Ni^{2+} , Cu^{2+} , Cd^{2+} , Al^{3+} , Mg^{2+} , Na^+ , K^+ , and Zn^{2+} are present in the system, H_2O_2 can still oxidize TMB to oxTMB, which causes the solution system to change from colorless to blue, and the absorbance does not change much when compared with that of the blank sample. Ag^+ and Hg^{2+} can specifically inhibit the catalase-like catalytic activity of GO/Au-PtNPs, which decreases the color of the system and the absorbance. This is because when Ag^+ and Hg^{2+} exist in the system, Ag^+ and Hg^{2+} will be adsorbed on the surface of the GO/Au-PtNPs, covering up the active site (Chen et al., 2015), which will inhibit the

catalytic activity of the composites and lead to a decrease in absorbance. The presence of Ag^+ and Hg^{2+} in the system can inhibit the oxidation of TMB to oxTMB (Qu et al., 2023), and the color of the system will not change obviously. Thus, the colorimetric sensor has good selectivity and specificity for Ag^+ and Hg^{2+} .

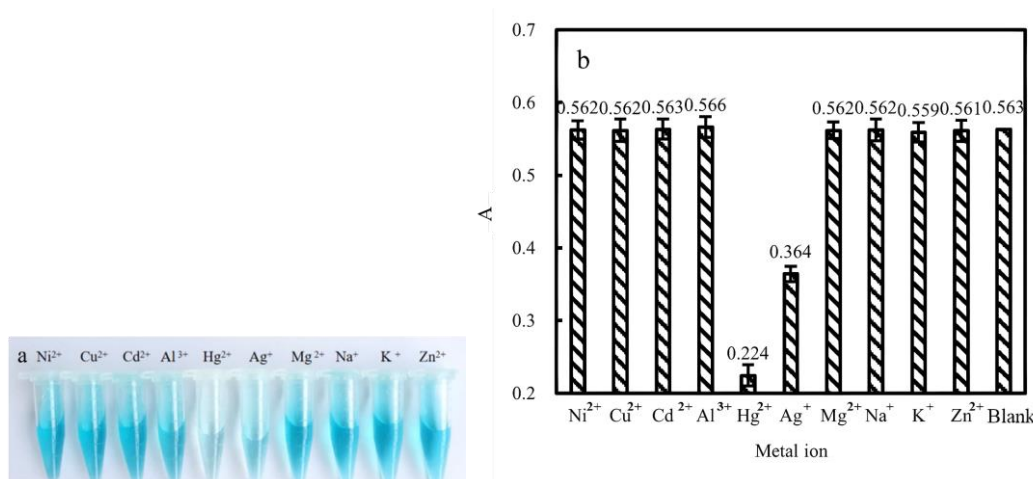


Figure 5.21: Specific detection of the colorimetric sensor. (a) Physical maps of specific tests; (b) Changes in absorbance values for specific tests. Reaction conditions: pH= 5.0; reaction time=5 min; total number of GO/Au-PtNPs=6 μL ; TMB concentration=0.5 mM; H_2O_2 concentration=0.02 M; λ =652 nm; and total concentration of heavy metal ions=1 mM

5.10 Chapter Summary

GO/Au-PtNPs composites with similar catalyst properties were synthesized and their preparation methods were optimized in the chapter. The best catalytic performance of GO/Au-PtNPs composites was obtained when the GO solution concentration was 0.5 mg/mL, the ratio of Au/Pt was 1:1, the pH of the system was 8.5, and the hydrothermal condition was controlled at 180 $^{\circ}\text{C}$ for 10 hours. The TEM, Raman, EDS, and FT-IR characterization revealed that the Au-Pt nanoparticles were loaded on the GO surface in a

flower-like structure with a 1:1 Au: Pt ratio, where the lattice of Au nanoparticles was 0.226 nm and that of Pt nanoparticles was 0.218 nm.

Then, the detection conditions of this colorimetric sensor were optimized. When the pH = 5, the composite volume was 6 μ L, the concentration of TMB substrate was 0.5 mM, the concentration of H₂O₂ was 0.02 M, and the reaction time was 5 min, the absorbance of the detection system reached 0.531, which could satisfy the requirements of Ag⁺ and Hg²⁺ detection. Meanwhile, the catalytic-like kinetic results of the composites showed that the K_m was 2.066 mM with TMB as the substrate and 3.227 mM with H₂O₂ as the substrate, which indicated that the GO/Au-PtNPs had a better affinity for TMB.

Finally, the specificity of the GO/Au-PtNPs composites in metal ion detection was explored, and Ag⁺ and Hg²⁺ were detected in real water samples. The synthesized GO/Au-PtNPs composites showed high selectivity for Ag⁺ and Hg²⁺ in various metals. The method was able to detect Hg²⁺ in the range of 1-10,000 nM with a detection limit of 1 nM, and Ag⁺ in the range of 50-100,000 nM with a detection limit of 50 nM, which is wider in range and lower in detection limit than other detection methods.

CHAPTER 6

A PAPER-BASED COLORIMETRIC SENSOR BASED ON GRAPHENE OXIDE/PLATINUM COMPOSITE FOR SIMULTANEOUS DETECTION OF Hg²⁺ AND Ag⁺

6.1 Overview

Chapter 6 describes a paper-based colorimetric sensor based on a graphene oxide/platinum composite for simultaneous detection of Hg²⁺ and Ag⁺. Based on the research basis of Chapter 5, a paper-based colorimetric sensor for Hg²⁺ and Ag⁺ was successfully developed, which enables rapid on-site accurate detection of Hg²⁺ and Ag⁺. Therefore, the main contents of this chapter include the preparation and characterization of the composite and paper-based colorimetric sensors, the optimization of the detection conditions of the paper-based colorimetric sensor, and the analysis of the detection limit, sensitivity, detection range, and specificity of this paper-based colorimetric sensor for Ag⁺ and Hg²⁺.

6.2 Characterization of GO/Pt@AuNPs composites

TEM, Raman, and FT-IR were used to obtain detailed characterisation information of GO/PtNPs.

6.2.1 Transmission electron microscopy (TEM)

TEM can provide information about the size, shape, dimensions, and lattice of materials. The structure and morphology of the graphene oxide and graphene oxide/Pt composites were characterized using a JEOL-JEM2100F transmission electron microscope.

As shown in Fig. 6.1(a), the distribution of graphene oxide is relatively flat, with noticeable wrinkles and curls in some regions. As shown in Fig. 6.1(B), the dispersion of Pt nanoparticles on the graphene oxide surface is good overall. As shown in Fig. 6.1(B), all the transparent graphene oxide (GO) was uniformly decorated by nanoscale Pt@AuNPs with minimal aggregation, indicating strong interactions between the graphene carriers and the particles. Highly dispersed metal nanoparticles and carriers with large surface areas have advantages in terms of catalytic activity and sensor sensitivity. The high-resolution image of the GO/Pt@AuNPs composite in Figure 6.1(C) shows the ordered lattice fringes of the Pt nanoparticles and Au nanoparticles. The lattice stripe spacings are 0.203 nm and 0.25 nm for Pt and Au, which correspond to the Pt(111) and Au(111) crystal planes, respectively (Taurino et al., 2015; Zuber et al., 2016).

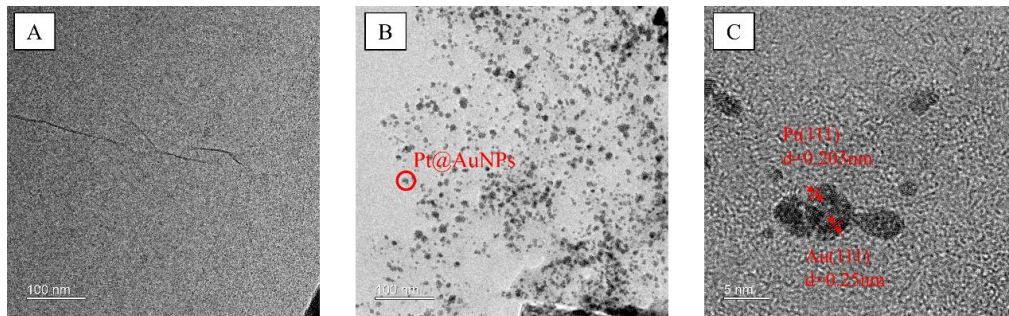


Figure 6.1: (A) TEM image of GO; (B) TEM images of GO/Pt@AuNPs composites; (C) High-Resolution Transmission Electron Microscope (HRTEM) image of GO/Pt@AuNPs composites

6.2.2 Raman spectrum analysis

Raman spectroscopy can provide structure information on graphene materials. GO and GO/Pt@AuNPs were characterized using a LabRAM HR confocal micro-Raman spectrometer. As shown in Fig. 6.2, both the graphene oxide and GO/Pt@AuNPs composites exhibit characteristic peaks in the D and G bands. The G peak is the primary characteristic peak of graphene and results from the in-plane vibration of sp^2 carbon atoms,

occurring near 1580 cm^{-1} . The D peak is typically regarded as a disordered vibrational peak of graphene caused by lattice vibrations away from the center of the Brillouin zone. It is utilized to characterize structural defects or edges in graphene samples that occur near 1320 cm^{-1} (Qu et al., 2023). The I_D/I_G ratio of the graphene oxide-loaded Pt@AuNPs increased from 1.14 to 1.27. This increase may be attributed to the partial reduction of graphene oxide, leading to the deoxygenation of many sp^3 hybridized carbon atoms and the formation of new sp^2 hybridized regions. The reorganized sp^2 regions were smaller than those of graphene oxide, resulting in smaller average sp^2 region sizes and a greater number of reduced graphene. This increase was reflected in the Raman plot as the I_D/I_G increased (Nayak et al., 2015). Thus, graphene oxide (GO) was partially reduced during the preparation of GO/Pt@AuNPs.

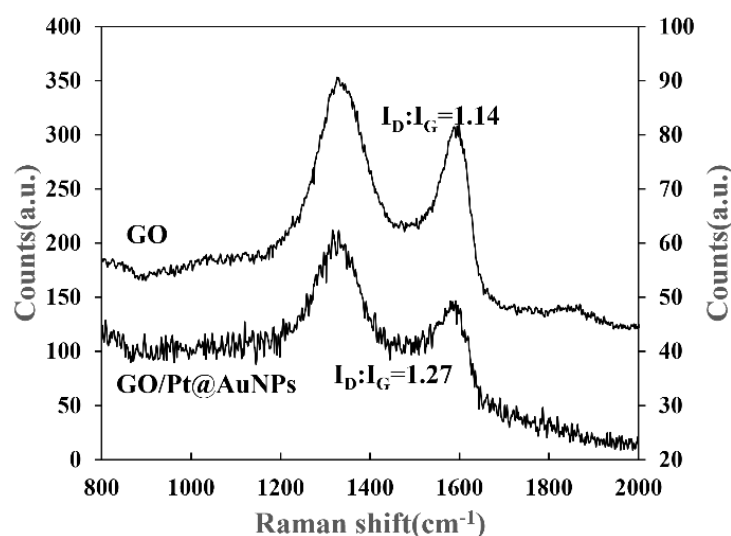


Figure 6.2: Raman characterization of the GO/Pt@AuNPs composites

6.2.3 FT-IR analysis

Fourier transform infrared spectroscopy (FT-IR) is a powerful technique used to analyse the molecular structure and chemical composition of substances. It serves as an effective method for detection and is also widely employed for qualitative and quantitative

analysis of the substances under investigation. To analyse the changes in the surface functional groups of GO before and after material synthesis, FT-IR spectroscopy of GO and GO/Pt@AuNPs was carried out using a Nicolet iS5 Fourier transform infrared spectrometer. As shown in Fig. 6.3, in the infrared spectra of GO, 3471 cm^{-1} corresponds to the O-H stretching vibration peak, 1732 cm^{-1} corresponds to the C=O stretching vibration peak, 1614 cm^{-1} corresponds to the C-OH bending vibration peak, and 1226 cm^{-1} corresponds to the carbon-oxygen bonding stretching vibration peak. The disappearance of the C=O stretching vibration peak at 1732 cm^{-1} for GO/Pt@AuNPs compared to that for GO may be due to the reduction reaction occurring during the synthesis of the composites (Qu et al., 2023).

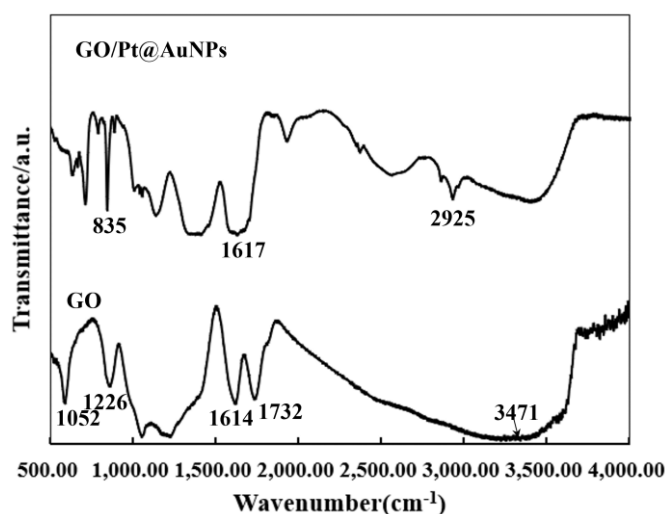


Figure 6.3: Infrared characterization of GO and the GO/Pt@AuNPs composites

6.3 Analysis of the catalase-like kinetics of GO/Pt@AuNPs

Given that GO/Pt@AuNPs function as catalysts for the decomposition of H_2O_2 , the principles and methods of enzyme kinetics were employed. The commonly used form of the Michaelis–Menten equation was employed (Equation 3.3) (Qu et al., 2023).

Typical Michaelis–Menten curves for GO/Pt@AuNPs are shown in Fig. 6.4. The apparent kinetic parameters and other simulated enzyme materials are shown in Table 6.1. The K_m values of the GO/Pt@AuNPs composites with TMB and H_2O_2 as substrates were 0.705 and 1.546, respectively. Compared with those of the other simulated enzyme materials, the GO/Pt@AuNPs have relatively small K_m values for H_2O_2 and TMB. In addition, the K_m value of H_2O_2 for GO/Pt@AuNPs was lower than that of the biological enzyme horseradish peroxidase (HRP). The data suggest that H_2O_2 and GO/Pt@AuNPs are strongly related.

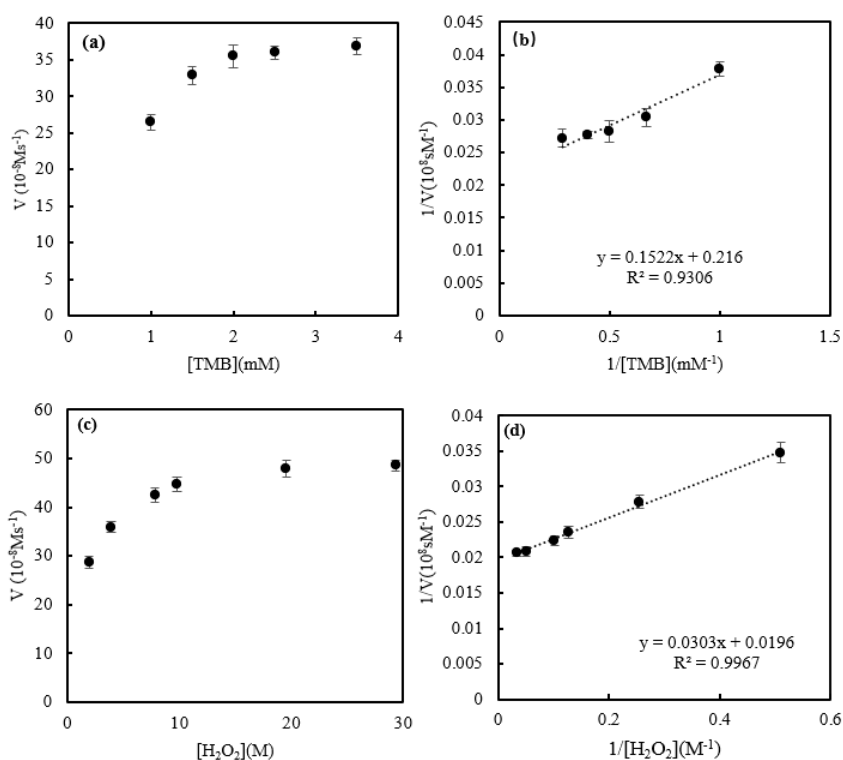


Figure 6.4: Steady-state kinetic experiments of GO/Pt@AuNPs. The Michaelis-Menten plots (a) and Lineweaver-Burk plots (b) of catalytic activity of GO/Pt@AuNPs when the substrate is Tetramethylbenzidine (The constant concentrations of H_2O_2 and GO/Pt@AuNPs were $0.02 \text{ mol} \cdot \text{L}^{-1}$ and $1.42 \mu\text{g/mL}$, respectively). The Michaelis-Menten plots (c) and Lineweaver-Burk plots (d) of catalytic activity of GO/Pt@AuNPs when the substrate is H_2O_2 (The constant concentrations of Tetramethylbenzidine and GO/Pt@AuNPs were $0.5 \text{ mmol} \cdot \text{L}^{-1}$ and $1.42 \mu\text{g/mL}$, respectively)

Table 6.1: Comparison of apparent Michaelis constant (K_m) and maximum reaction rate (V_{max}) between GO/Au-Pt NPS and other catalases

Catalyst	Substrate	$K_m(\text{mM})$	$V_{max}(10^{-8}\text{M}\cdot\text{s}^{-1})$	References
GO/Pt@AuNPs	TMB	0.705	4.63×	This study
	H ₂ O ₂	1.546	51.02	
GO/PtNPs	TMB	0.041	16.23	(Qu et al., 2023)
	H ₂ O ₂	111.56	31.25	
Co-ferrocene metal-organic framework	TMB	29.7	27.6	(Fang et al., 2020)
	H ₂ O ₂	\	\	
Iridium oxide nanoparticles	TMB	179.0	19.3	(Zhen et al., 2020)
	H ₂ O ₂	\	\	
Cy-AuNCs	TMB	1.9	212.3	(Jiang et al., 2021)
	H ₂ O ₂	2.8	15.8	
GSH-AgNPs	TMB	4.2	247.0	(Jiang et al., 2022)
	H ₂ O ₂	58.6	21.5	
HRP	TMB	0.434	10	(Dutta et al., 2012)
	H ₂ O ₂	3.70	8.71	

6.4 Optimization of detection conditions for the paper-based colorimetric sensor

The colour of the paper-based colorimetric sensor affects the accuracy, sensitivity and detection limit of the constructed colorimetric sensor, which was optimized in terms of the type of paper base, composite concentration, TMB concentration, hydrogen peroxide concentration, pH, and reaction time in order to obtain a suitable colour.

6.4.1 Effect of paper base type in the system

Under the same conditions, the test paper type is different, and the uniformity of the colour development is different, resulting in a large deviation in the grey value. As shown in Fig. 6.5, A4 printing paper with cellulose film showed uneven color development and poor absorption. Quantitative filter paper (QTFP) is the most uniform and has the best colour development effect. Therefore, quantitative filter paper was selected as the paper base.

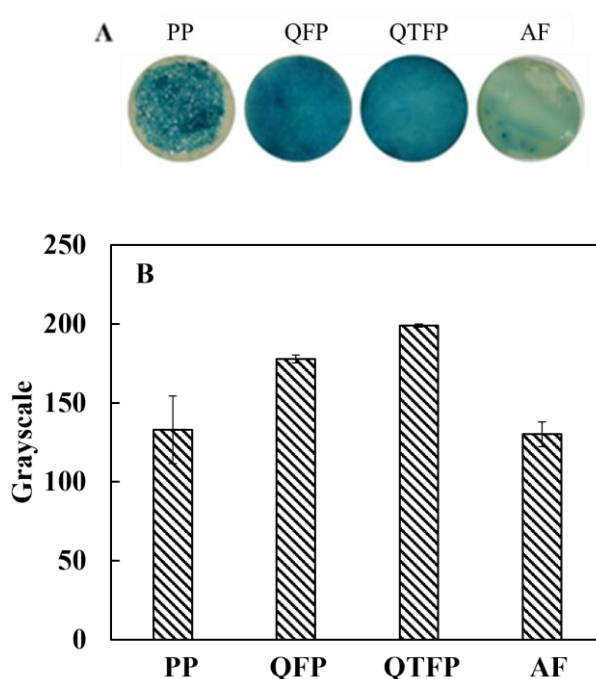


Figure 6.5: Effect of different paper base materials on the colour of paper-based colorimetric sensors (a) Colours rendering photographs of test results of different paper-based materials; (b) The grayscale value of paper-based sensor responds to different paper base materials. The concentration of GO/Pt@AuNPs, Tetramethylbenzidine and H_2O_2 was $168.531 \mu\text{g/mL}$, 8 mmol/L and 10 mol/L , respectively.

6.4.2 Effect of reaction time

The reaction time significantly affects both the color change of the colorimetric sensor and the detection speed of the sensor. As shown in Fig. 6.6, the change in color of

the colorimetric sensor and the gray value of the paper base within the range of 0.5-5 min for the color development time are not significant. This indicates that the GO/Pt@AuNPs composite exhibits an efficient peroxidase-like property, enabling rapid catalysis of the color development reaction to reach equilibrium within 1 min. Therefore, we chose a 1 min color development time as a parameter for subsequent experiments.

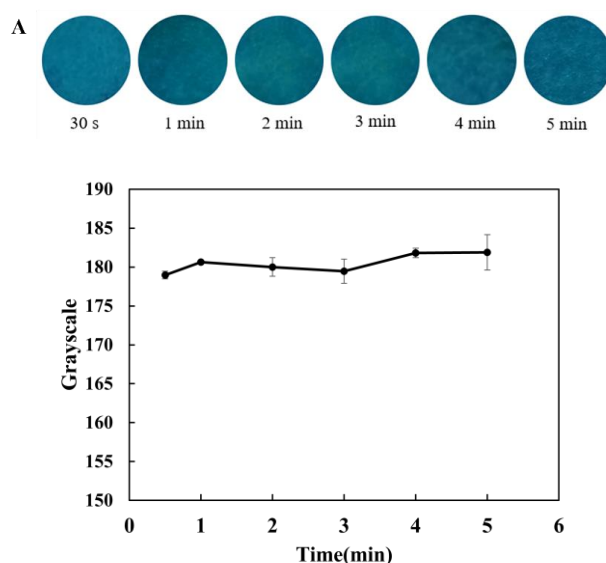


Figure 6.6: Determination of colour change (a) and grey value (b) of paper-based sensors after different reaction times. The concentration of GO/Pt@AuNPs, Tetramethylbenzidine and H_2O_2 was 168.531 $\mu\text{g/mL}$, 8 mmol/L and 8 mol/L, respectively, pH=4.5.

6.4.3 Effect of the composite material concentration

As shown in Fig. 6.7, the color and gray values of the paper-based sensors exhibited a pattern of increase and then levelling off as the concentration of the GO/Pt@AuNPs composites increased. The best catalytic performance was observed when the concentration of the composite was 67.413 $\mu\text{g/mL}$. This is because the number of catalytically active sites increases with increasing concentration of the GO/Pt@AuNPs composite. This leads to an increase in the decomposition rate of H_2O_2 and the yield

of $\cdot\text{OH}$. An increased concentration of $\cdot\text{OH}$ leads to more $\cdot\text{OH}$ reacting with TMB, deepening the color of the paper base (Zhao et al., 2016). When the concentration reached $67.413 \mu\text{g/mL}$, the grey value basically stabilized. At this time, the concentration of GO/Pt@AuNPs is not a constraint to limit this catalytic reaction. So the grey value no longer changes when the concentration increases. The limiting factor for the grey value to continue to increase is the concentration of hydrogen peroxide and TMB in the system. Therefore the absorbance reaches a plateau period and does not continue to increase and the reaction is as in equation 6.1. Therefore, a concentration of $67.413 \mu\text{g/mL}$ was selected for the subsequent experiments for the GO/Pt@AuNPs composite.

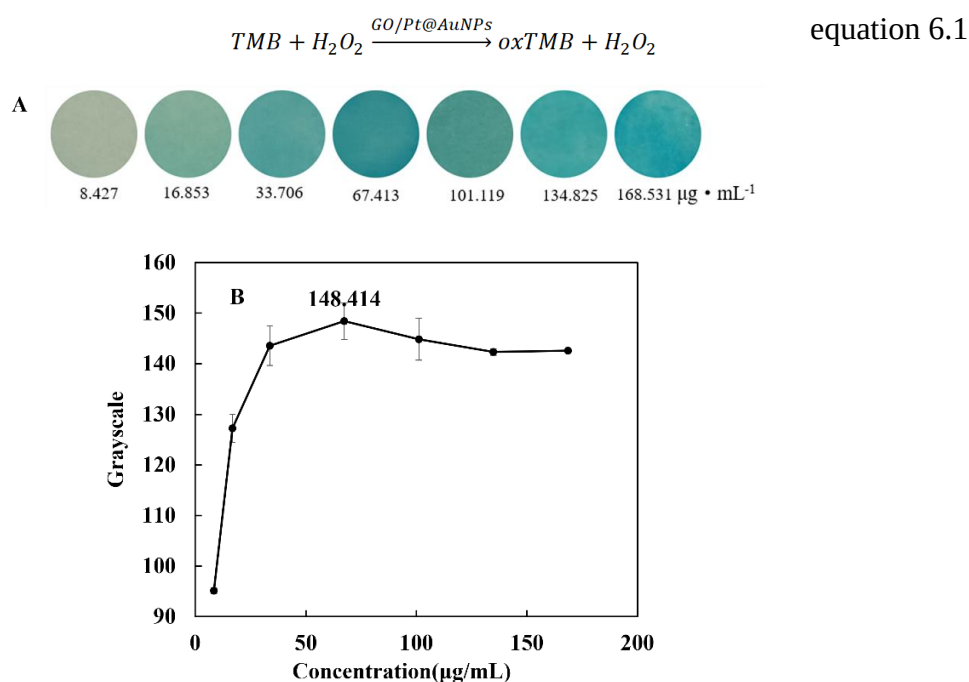


Figure 6.7: Effect of different composite concentrations on colour change (a) and grey value (b) of paper-based sensors. The concentration of Tetramethylbenzidine and H_2O_2 was 8 mmol/L and 8 mol/L, respectively. pH=4.5, reaction time is 1 min.

6.4.4 Effect of H_2O_2 concentration

The GO/Pt@AuNPs composites exhibited peroxidase-like enzymatic activity. The concentration of H_2O_2 directly affects the catalytic reaction and color change of the system.

As shown in Figure 6.8, with the gradual increase in H_2O_2 concentration, the gray value exhibited a trend of increasing and then leveling off. With increasing H_2O_2 concentration, more $\cdot\text{OH}$ can be produced per unit time, which accelerates the oxidation of TMB to blue (Zhang et al., 2022b). When the concentration of H_2O_2 reached 8 mol/L, the color of the paper base changed significantly. The composite material exhibited a more stable catalytic effect, with a gray value of 144.036. Therefore, we used an H_2O_2 concentration of 8 mol/L as the experimental condition for the subsequent study.

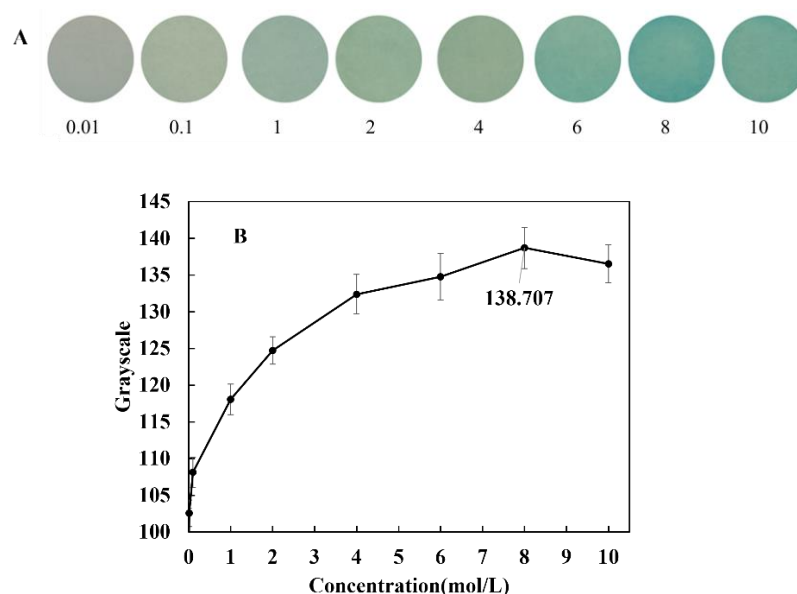


Figure 6.8: Effect of different H_2O_2 concentration on colour change (a) and grey value (b) of paper-based sensors. The concentration of GO/Pt@AuNPs and Tetramethylbenzidine was 67.413 $\mu\text{g/mL}$ and 8 mmol/L, respectively. pH=4.5, reaction time is 1 min.

6.4.5 Effect of the TMB substrate concentration

The GO/Pt@AuNPs nanocomposites exhibited peroxidase-like activity that promoted the decomposition of H_2O_2 into hydroxyl radicals ($\cdot\text{OH}$). This reaction further leads to the oxidation of the substrate 3,3',5,5'-tetramethylbenzidine (TMB) to form oxidized TMB (oxTMB), resulting in a blue colouration of the system. As shown in Fig. 6.9, the color and grayscale values of the paper-based colorimetric sensor gradually

increased and then remained essentially unchanged as the concentration of TMB gradually increased. This phenomenon occurs because a low concentration of TMB is the limiting factor for the chromogenic reaction in the entire catalytic system (Zhao et al., 2021b). When the concentration of TMB reaches 6 mmol/L, the change in colour and grey value of the paper base slows down. This indicates that at this point, the TMB concentration is no longer the limiting factor for the catalytic reaction. Therefore, we selected a TMB concentration of 6 mmol/L as the experimental condition for subsequent experiments.

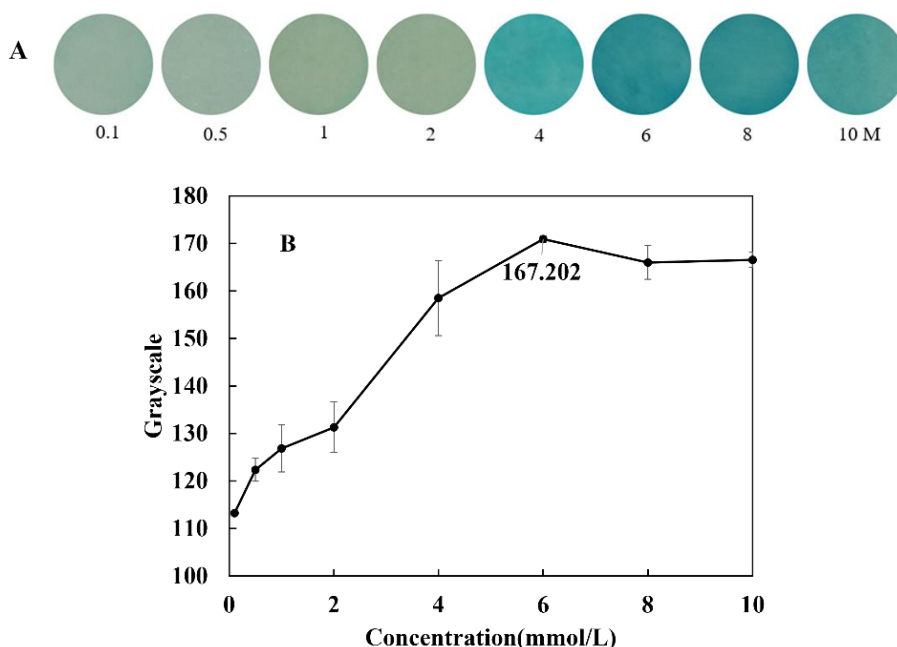


Figure 6.9: Effect of different Tetramethylbenzidine concentration on colour change (a) and grey value (b) of paper-based sensors. The concentration of GO/Pt@AuNPs, and H_2O_2 was 67.413 $\mu\text{g/mL}$ and 8 mol/L, respectively. pH=4.5, reaction time is 1 min.

6.4.6 Effect of pH in the system

Citric acid buffer was used to adjust the pH in the color development system to investigate the impact of pH variations on the color development process. As shown in Fig. 6.10, the color and gray values of the paper base exhibited a trend of increasing and then decreasing with increasing pH, reaching their maximum values at pH=4. The

GO/Pt@AuNPs composites exhibited higher catalytic activity in weakly acidic solutions than in neutral and alkaline environments. This is mainly due to the fact that upon oxidation TMB produces a blue quinone imine intermediate. In a strongly acidic environment, this intermediate is further protonated to form a stable yellow compound, 3,3',5,5'-tetramethyl-2-(1H)-benzimidazolone. The blue quinone imine form is more stable under weak acidic conditions, thus giving the system a blue colour. Thus, the hydrogen ion (H^+) indirectly determines the final colour change by affecting the protonation state of the intermediate (Qu et al., 2023; Zhao et al., 2016). Therefore, a reaction system with a pH of 4 was chosen as the experimental condition for the subsequent study.

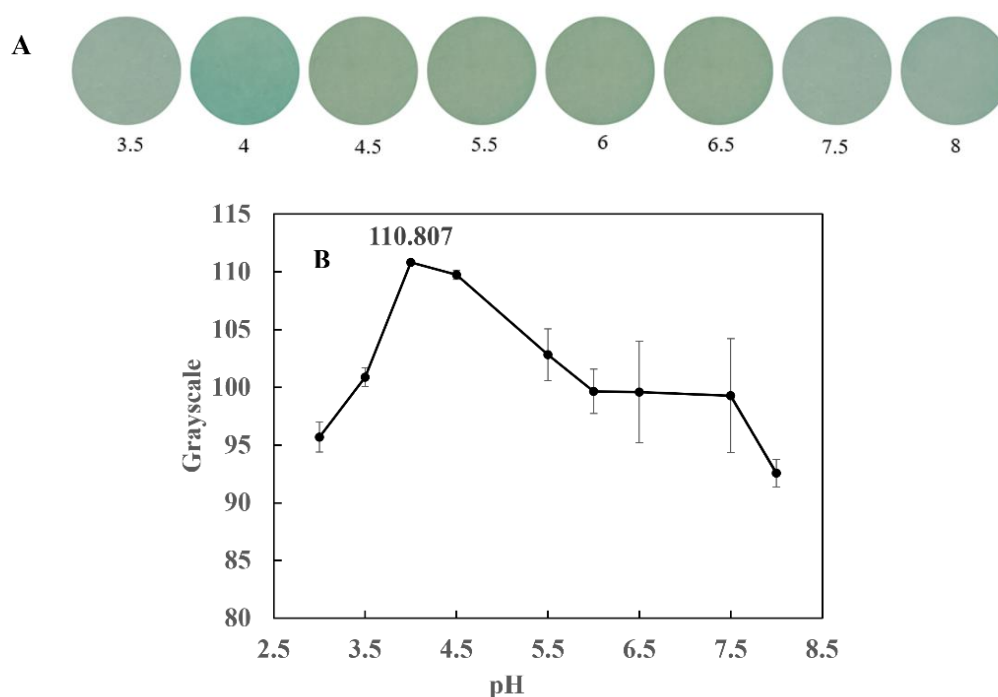


Figure 6.10: Effect of pH on colour change (a) and grey value (b) of paper-based sensors. The concentration of GO/Pt@AuNPs, Tetramethylbenzidine and H_2O_2 was 67.413 $\mu g/mL$, 6 mmol/L and 8 mol/L, respectively. Reaction time is 1 min.

6.5 Characterization of paper-based colorimetric sensors

Two characterisation methods, SEM and EDS, were used to analyse the changes in the surface structure and elemental composition of the paper base before and after the deposition of GO/Pt@AuNPs composites.

6.5.1 Analysis by scanning electron microscopy

From the scanning electron microscopy (SEM) image of the paper base in Fig. 6.11 (A), it can be observed that the qualitative filter paper base of the undoped GO/Pt@AuNPs composite displays a fibrous structure and possesses a porous internal structure, which is beneficial for liquid absorption. This provides favourable conditions for the successful preparation and application of paper-based colorimetric sensors. The GO/Pt@AuNPs composites were attached to the surface of the paper base, and most of them appeared as particles, as shown in Fig. 6.11 (B). This indicates that the GO/Pt@AuNPs were successfully composited on the paper base.

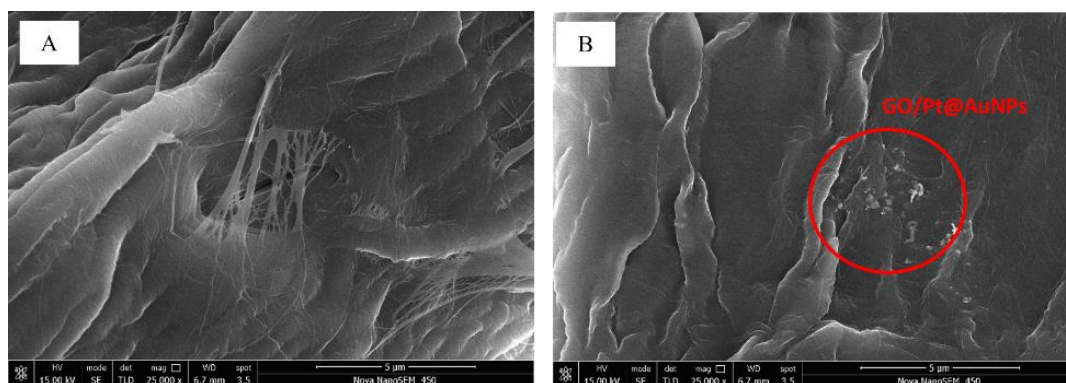


Figure 6.11: SEM images of the GO/Pt@AuNPs composite paper-based colorimetric sensor (A) Deposited GO/Pt@AuNPs composite before the paper base, (B) Deposited GO/Pt@AuNPs composite after the paper base

6.5.2 X-ray energy spectroscopy analysis

To evaluate the variances in the paper matrix before and after composite application, the GO/Pt@AuNPs were characterized by energy dispersive spectroscopy (EDS) using a JSM-7500F cold field emission electron microscope. As shown in Fig. 6.12, before the deposition of GO/Pt@AuNP composites, the paper matrix contained only carbon (C) and oxygen (O). The paper matrix after the deposition of the GO/Pt@AuNPs composites contained C, O, Pt, and Au, where C and O were derived from the paper matrix and GO and Pt and Au were derived from GO/Pt@AuNPs. The atomic percentage of Pt is approximately 0.11, and that of Au is approximately 0.26, which confirms that the GO/Pt@AuNPs composites were successfully deposited on the paper matrix.

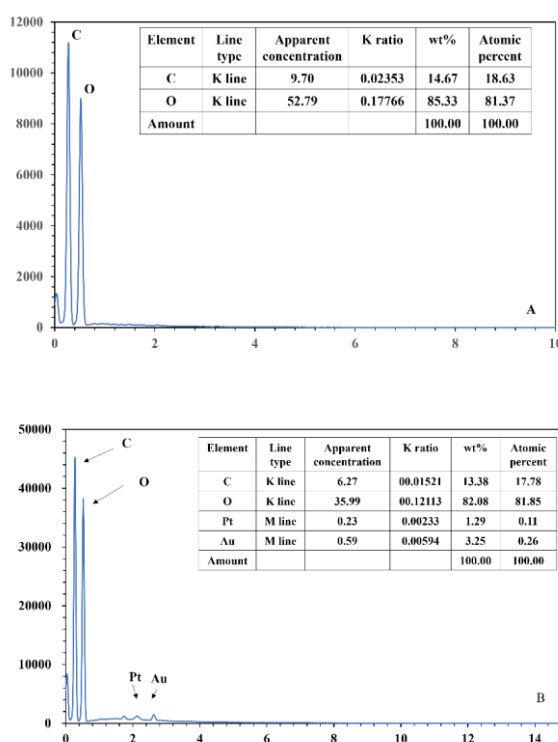


Figure 6.12: EDS spectra of the GO/Pt@AuNPs composite paper-based colorimetric sensor (A) Paper base before depositing the GO/Pt@AuNPs composite; (B) paper base after depositing the GO/Pt@AuNPs composite

6.6 Detection principles

The detection principle is illustrated in Figure 6.13. The synthesized composites of graphene oxide (GO)/platinum-coated gold nanoparticles (Pt@AuNPs) demonstrate peroxidase-like properties, facilitating the catalysis of the oxidation reaction between the hydrogen peroxide (H_2O_2) substrate 3,3',5,5'-tetramethylbenzidine (TMB) and the oxidized TMB (oxTMB) by the hydroxyl group (-OH) generated from H_2O_2 . This reaction is visually perceived as a transition in color from colorless to blue on the paper substrate, and the grayscale value is quantified as GO. When both Ag^+ and Hg^{2+} ions are simultaneously present in the system, it has been observed that both Ag^+ and Hg^{2+} ions can undergo adsorption onto the surface of GO/Pt@AuNPs. The process of adsorption results in redox reactions involving Pt^0 and Au^0 , leading to alterations in the electronic state of the active site as a consequence of metal interactions. Among the various elements, Hg^{2+} can form alloys with Pt^0 and Au^0 in composites, leading to the creation of mercury-gold alloys (Zhang et al., 2020b). Additionally, Ag^+ can produce bimetallic nanoparticles, specifically silver-platinum nanoparticles (Ag@PtNPs) and silver-gold nanoparticles (Ag@AuNPs), through the integration of Pt^0 and Au^0 onto the surface of graphene oxide (Qu et al., 2023; Chen et al., 2016). This phenomenon leads to a decrease in the affinity between the composites of GO/Pt@AuNPs and the TMB substrate, consequently impeding their catalytic capability. Consequently, the intensity of the color on the paper substrate decreased, leading to a decrease in the recorded gray value. The magnitude of the decrease in gray value, referred to as $\Delta G_{\text{Hg}^{2+} + \text{Ag}^+}$, is influenced by the presence of both Ag^+ and Hg^{2+} ions. Equation 6.1 can be utilized to compute the numerical value of $\Delta G_{\text{Hg}^{2+} + \text{Ag}^+}$. However, in the presence of ethylenediaminetetraacetic acid (EDTA), Hg^{2+} can form a structurally stable metal chelate, thereby concealing the existence of Hg^{2+} (Mohammadi et

al., 2021). Therefore, the gray value (G_{Ag^+}) of the paper base can be determined exclusively in the presence of Ag^+ in the system. The change in the absorbance ΔG_{Ag^+} of the system resulting from the presence of Ag^+ alone can be determined using equation 6.2. By establishing a standard curve of ΔG_{Ag^+} as a function of the concentration of Ag^+ , it is possible to achieve quantitative detection of Ag^+ . To determine $\Delta G_{Hg^{2+}}$, equation 6.3 can be utilized, and a standard curve relating $\Delta G_{Hg^{2+}}$ to the Hg^{2+} concentration can be constructed. The aforementioned method enables the precise measurement of Hg^{2+} concentration. With this mechanism, simultaneous, visual, real-time, rapid, on-site detection of Ag^+ and Hg^{2+} can be achieved in the system.

$$\Delta G_{Hg^{2+}+Ag^+} = G_0 - G_{Hg^{2+}+Ag^+} \quad \text{Equation 6.1}$$

$$\Delta G_{Ag^+} = G_0 - G_{Ag^+} \quad \text{Equation 6.2}$$

$$\Delta G_{Hg^{2+}} = \Delta G_{Hg^{2+}+Ag^+} - \Delta G_{Ag^+} \quad \text{Equation 6.3}$$

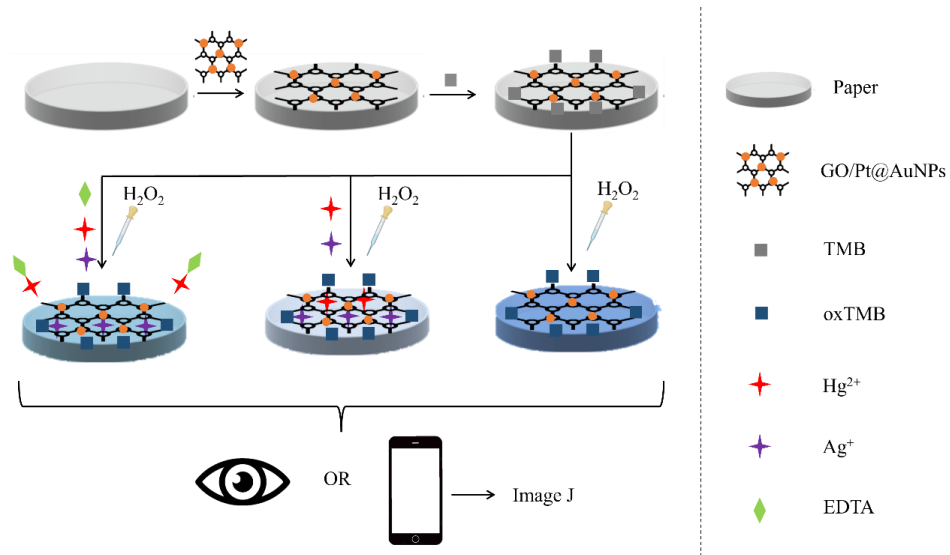


Figure 6.13: Schematic illustrations of Hg^{2+} and Ag^+ detection

6.7 Detection of Hg^{2+} and Ag^+ in real samples

Analytical validation is a crucial aspect of any newly developed method for determining target chemicals in sample matrices. Therefore, the linear range, limit of detection (LOD), limit of quantification (LOQ), accuracy, and selectivity for the determination of Hg^{2+} and Ag^+ were investigated. The LOD is the ability to reliably distinguish the smallest concentration or amount of a substance from a blank (no substance to be measured) signal at a given level of confidence. It can be obtained by calculation using Equation 6.4. LOQ is the lowest concentration or amount that can be quantitatively analysed with appropriate precision and accuracy at a given level of confidence. The linear ranges of Hg^{2+} and Ag^+ were determined by plotting calibration curves on the paper substrate of this colorimetric sensor with varying concentrations of Hg^{2+} ranging from 5×10^{-8} to 1×10^{-5} mol/L and Ag^+ ranging from 2.5×10^{-6} to 1×10^{-4} mol/L. The gray values were quantified using ImageJ software. As depicted in Figures 6.14 and 6.15, the color of the paper base and the measured grayscale values decreased with increasing concentrations of Hg^{2+} and Ag^+ . The linear equations of $\Delta G_{\text{Hg}^{2+}}$ versus $-\log C_{\text{Hg}^{2+}}$ ($y = -12.046x + 92.91$) and ΔG_{Ag^+} versus $-\log C_{\text{Ag}^+}$ ($y = -32.256x + 186.29$) were plotted to determine the concentrations of Hg^{2+} and Ag^+ present in the unknown samples. As shown in Fig. 6.14(C) and Fig. 6.15(C), the paper-based colorimetric sensor demonstrates a good linear range for both Hg^{2+} and Ag^+ . The detection range for Hg^{2+} was 5×10^{-8} to 1×10^{-5} mol/L, with a correlation coefficient of 0.9925 (R^2), while the detection range for Ag^+ was 2.5×10^{-6} to 1×10^{-4} mol/L, with a correlation coefficient of 0.9923 (R^2). The LOD was calculated by determining the lowest concentration of the analyte on the paper base and calculating the standard deviation ($n = 6$). When the lowest concentration of the analyte was deposited on the paper base, the LOD was obtained by setting the concentration to 10 times the standard

deviation, which resulted in a change in the colour intensity of the paper base. The LOD of the smartphone paper-based sensor was 5×10^{-8} mol/L for the determination of Hg^{2+} and 5×10^{-7} mol/L for the determination of Ag^+ . The LOQs of this paper-based colorimetric sensor were 5×10^{-8} mol/L for Hg^{2+} and 2.5×10^{-6} mol/L for Ag^+ . In comparison with the currently reported colorimetric sensors, this colorimetric sensor not only enables the simultaneous detection of both Hg^{2+} and Ag^+ ions but also has a lower detection limit and a wider detection range (Table 6.2).

$$\text{LOD} = 10\sigma/S$$

Equation 6.4

σ (sigma) is the standard deviation of the blank response, which reflects the amount of background noise; S is the slope of the response versus concentration curve for the substance to be measured, i.e. the slope of the calibration curve.

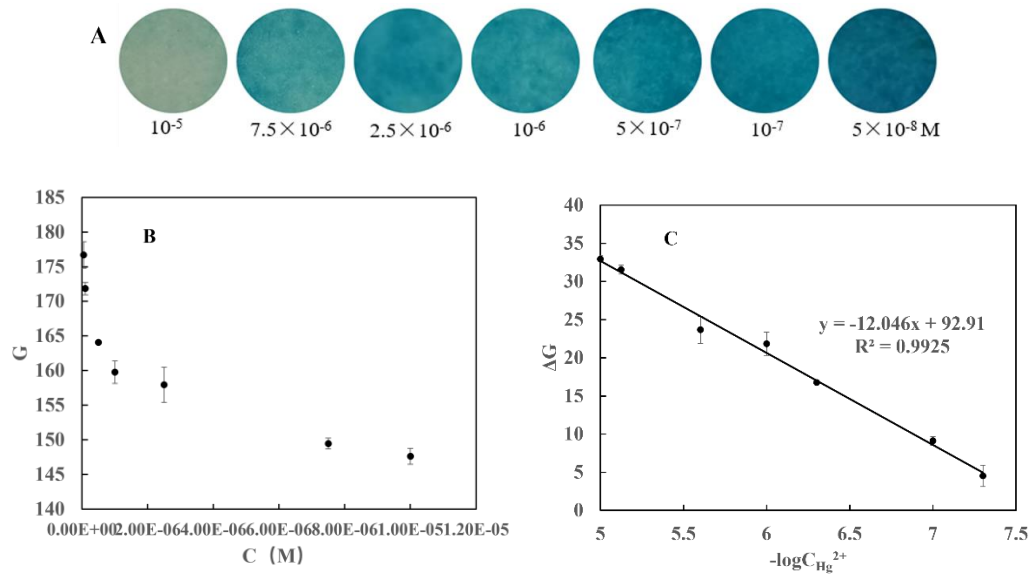


Figure 6.14: Sensitivity of the method for detecting Hg^{2+} . Paper-based colorimetric detection involves the colors (a), detection range (b), and calibration plots of gray value variations (ΔG) (c) in relation to the concentrations of added Hg^{2+} . Linear equation: $y = -12.046x + 92.91$. All error bars represent the standard deviation for the three measurements.

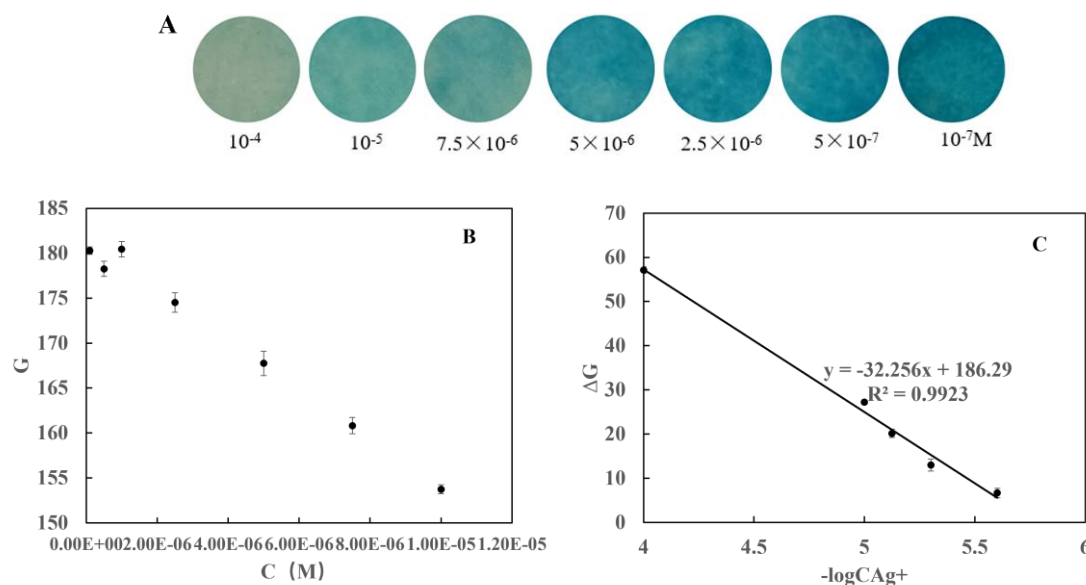


Figure 6.15: Sensitivity of the method for detecting Ag^+ . Paper-based colorimetric detection involves the colors (a), detection range (b), and calibration plots of gray value variations (ΔG) (c) in relation to the concentrations of added Ag^+ . Linear equation: $y = -32.256x + 186.29$. All error bars represent the standard deviation for the three measurements.

To assess the feasibility of this colorimetric sensor, it was validated using the standard addition method. The accuracy and selectivity of the sensor were determined by calculating the recoveries of different concentrations of Hg^{2+} and Ag^+ deposited on the paper substrate from real samples (lake water, tap water, and serum). Relative recoveries were calculated as the ratio of the concentration of the analyte found (determined using a linear least square equation) to the amount of analyte added to the paper substrate. As shown in Table 6.3, the relative recoveries ranged from 91.2% ~ 96.2%, which is within an acceptable range (90%~110%), its reliability in determining Hg^{2+} and Ag^+ in complex samples. In addition, the successful recoveries demonstrated the selectivity of the smartphone paper-based sensor for determining Hg^{2+} and Ag^+ in complex sample matrices.

Table 6.2: Performance comparison of several silver ion and mercury ion sensors

Core materials	Detection ion	Test method	Detection scope (μM)	Detection limit (μM)	Reference
GO/Pt@AuNPs	Hg^{2+}	Paper-based color comparison sensor	0.05~10	0.05	This study
	Ag^+		2.5~100	2.5	
SOFFS	Hg^{2+}	Colorimetric fluorescence sensor	0.001~1	0.001	(Liu et al.,2019)
	Ag^+		\	\	
AgNPs	Hg^{2+}	Colorimetric sensor	1~100	1.25	(Aminu et al.,2021)
	Ag^+		\	\	
Ag DENPs	Hg^{2+}	Colorimetric sensor	100~500(ppb)	5.88	(Gürbüz et al.,2019)
	Ag^+		\	\	
DAC-Tu	Hg^{2+}	Cellulose colorimetric sensor	\	\	(Wang et al.,2020)
	Ag^+		10000~1	1	
Bulk Ion-selective Optodes	Ag^+	Colorimetric sensor	1.92-500	1.92	(Phichi et al.,2020)
	Hg^{2+}		5.74×10^{-4} - 5.00×10^{-2}	5.74×10^{-4} - $\backslash$	
Silatrane	Ag^+	Colorimetric sensor	0.141-1	0.141	(Singh et al.,2019)
	Hg^{2+}		\	\	
GO/PtNPs	Ag^+	Colorimetric sensor	0.5~1000	0.5	(Qu et al.,2023)
	Hg^{2+}		\	\	

Table 6.3: Actual sample testing

Sample	Detection ion		Standard addition(M)	Testing value ($\times 10^{-7}M$)	Recovery (%)	Standard Deviation
lake water	Hg^{2+}	1	10^{-7}	0.912 ± 0.01	91.2 ± 0.98	2.15%
		2	10^{-6}	9.55 ± 0.12	95.5 ± 1.18	
		3	10^{-5}	93.3 ± 0.31	93.3 ± 0.31	
	Ag^{+}	1	10^{-5}	91.6 ± 0.03	91.6 ± 0.03	2.56%
		2	5×10^{-5}	460 ± 5.05	92 ± 0.95	
		3	5×10^{-4}	483 ± 3.69	96.22 ± 0.74	
tap water	Hg^{2+}	1	10^{-7}	0.912 ± 0.01	91.2 ± 0.92	3.36%
		2	10^{-6}	9.55 ± 0.32	95.5 ± 3.21	
		3	10^{-5}	0.933 ± 0.02	93.3 ± 1.95	
	Ag^{+}	1	10^{-5}	91.6 ± 0.21	91.6 ± 0.21	0.44%
		2	5×10^{-5}	460 ± 4.62	92 ± 0.92	
		3	5×10^{-4}	483 ± 3.12	96.22 ± 0.62	
serum	Hg^{2+}	1	10^{-7}	0.912 ± 0.01	91.2 ± 0.83	5.50%
		2	10^{-6}	9.55 ± 0.19	95.5 ± 1.87	
		3	10^{-5}	93.3 ± 0.03	93.3 ± 0.03	
	Ag^{+}	1	10^{-5}	91.6 ± 0.02	91.6 ± 0.02	5.52%
		2	5×10^{-5}	460 ± 3.12	92 ± 0.62	
		3	5×10^{-4}	483 ± 4.09	96.22 ± 0.82	

6.8 Specificity evaluation of the colorimetric sensing method

The presence of many interfering ions in real samples necessitates a high level of specificity in the colorimetric sensor to facilitate its use in detecting real samples. To investigate the specificity of the colorimetric sensor, various ions, including Hg^{2+} , Ag^+ , Ni^{2+} , Cr^{3+} , Zn^{2+} , Cu^{2+} , Cd^{2+} , Na^+ , K^+ , and Al^{3+} , were introduced into the system while maintaining the other variables constant. Changes in color intensity and grayscale values were studied after these chemicals were added to the paper base. As shown in Fig. 6.16, there was no significant change in the gray values of the other metal ions, while the gray values of Hg^{2+} and Ag^+ were significantly lower. This phenomenon occurs due to the interaction of Hg^{2+} and Ag^+ with Pt and Au in the composites (Qu et al., 2023; Zhang et al., 2020a; Chen et al., 2016), resulting in the inhibition of the catalytic activity of the GO/Pt@AuNPs composites. As a result, the colour on the paper substrate becomes lighter, and the measured grey value decreases. Therefore, the smartphone-based paper-based colorimetric sensor was selective for detecting Hg^{2+} and Ag^+ in lake water, tap water, and serum samples.

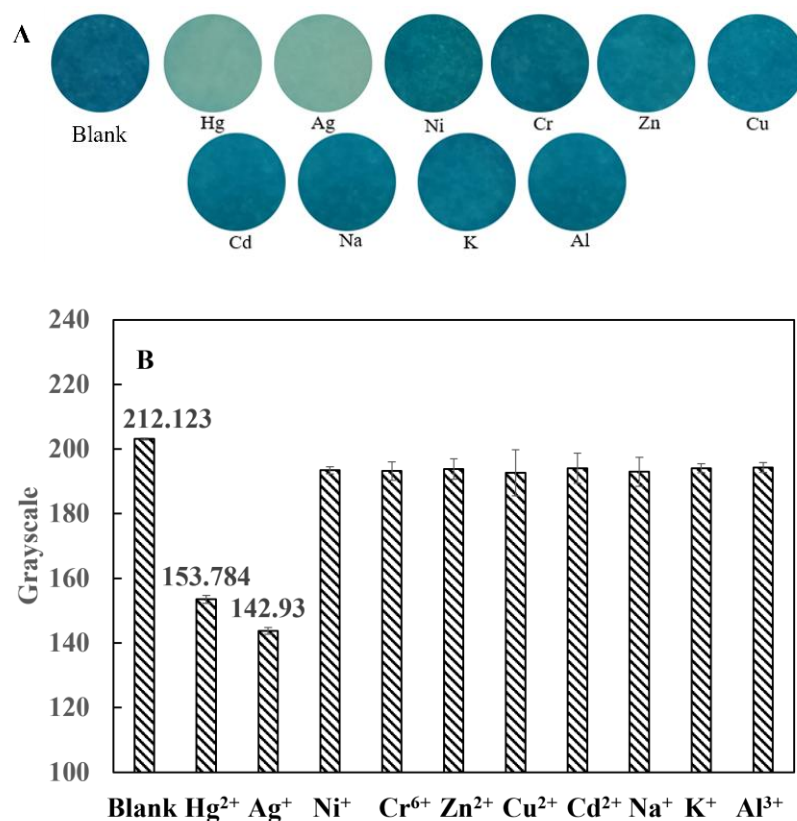


Figure 6.16: Selectivity of the method for Hg²⁺ and Ag⁺ simultaneous detection. (a) Colors of paper-based colorimetric tests treated with different substances. (b) The change in grayscale value of the method responds to Hg²⁺, Ag⁺ and other competitive substances. The concentration of competitive substances was 3×10^{-5} mol/L, while the concentration of Hg²⁺ and Ag⁺ was 1×10^{-5} mol/L.

6.9 Chapter summary

This paper-based colorimetric sensor can be used for the determination of Hg²⁺ and Ag⁺ in various practical samples. The quantitative analysis of Hg²⁺ and Ag⁺ is typically conducted using complex and expensive instruments such as Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), which are bulky and not suitable for on-site sample analysis. In addition, the method does not rely on expensive instruments, and the operating procedures are simple. This means that even

nonexperts can carry out the analyses. The paper-based colorimetric sensor also exhibited good selectivity and specificity for Hg^{2+} and Ag^+ . It is free from interference from other metals and is suitable for practical sample detection. The method can detect Hg^{2+} and Ag^+ in real samples within 1 min with LOQs as low as 5×10^{-8} mol/L for Hg^{2+} and 2.5×10^{-6} mol/L for Ag^+ . The paper-based colorimetric sensor is user friendly, economical, and portable and can be utilized for analysing environmental samples. The newly developed method is easily accessible, lightweight, portable, and flexible and can be used for chemical analysis at the sample source. The method can be used for the determination of Hg^{2+} in various environmental, biological, and vegetable samples.

CHAPTER 7

CONCLUSION AND RECOMMENDATIONS

7.1 Concluding remarks of this study

In this thesis, a series of new rapid response, high sensitivity and high specificity heavy metal colorimetric sensors have been established based on the application of GO. Firstly, GO/PtNPs with efficient catalytic enzyme-like properties were synthesized using graphene oxide as a carrier, and GO in GO/PtNPs was partially reduced. The size of PtNPs was in the range of 2-3 nm. The unique faceted-centred cubic structure of precious metal Pt appeared as characteristic peaks in the XRD characterization, which indicated that Pt nanoparticles were loaded on the surface of graphene oxide. The coupling effect between Pt and Ag^+ was utilised to achieve accurate, rapid and intuitive detection of silver ions. Under the optimal conditions, the detection time of the method was 5 min, the detection range of Ag^+ was 0.5 ~ 1000 μM , the detection limit was 0.5 μM , and the color could be distinguished by the naked eye.

Using the synergistic catalytic property between Pt and Au, GO/Au-PtNPs composites with higher catalytic efficiency were synthesized. The graphene oxide surface is loaded with two types of nanoparticles, where spherical gold nanoparticles are located in the middle and platinum nanoparticles are dispersed on the outside of the gold nanoparticles. And the simultaneous detection of silver and mercury ions was achieved. Under the optimal detection conditions, the colorimetric sensor can detect Hg^{2+} in the range of 1 ~ 10000 nM with a detection limit of 1 nM, and Ag^+ in the range of 50 ~ 100000 nM with a detection limit of 50 nM, which is lower than the detection limits of the

previously reported colorimetric sensors for Hg^{2+} and Ag^+ , with higher sensitivity and wider detection range.

Finally, based on the application of nanomaterials, a new type of heavy metal colorimetric sensor with fast response, high sensitivity, and high specificity was established by combining the solid-phase carrier of test strips for the instantaneous on-site detection of heavy metals in response to the practical difficulties such as the inability to carry a large-scale instrument, the long-time of detection, and the complexity of the detection procedure. GO/Pt@AuNPs were found to be immobilised on the paper-based surface by characterising the paper-based colorimetric sensor. The detection limits of the sensor were 0.05 and 2.5 μM for Ag^+ and Hg^{2+} , respectively, and the detection ranges were 0.05 ~ 10 μM and 2.5 ~ 100 μM , respectively.

In this study, a colorimetric sensor designed using graphene oxide nanocomposites is able to visually detect heavy metal content in the environment through colour changes, providing a fast, simple and cost-effective means of environmental monitoring. This not only helps timely detection and management of heavy metal pollution in industrial wastewater, but also enhances food safety monitoring and safeguards public drinking water, which is of great significance for environmental protection and human health. With technological advancement, it is expected that its sensitivity and selectivity will be enhanced in the future, multi-parameter detection will be realised, and portable detection equipment will be developed. The application of this technology will help to advance the goals of sustainable development, particularly by playing a positive role in clean water sources, responsible production and the promotion of technological innovation.

7.2 Recommendations for future work

With the rapid development of society and the economy, pocket-sized heavy metal sensors will play an increasingly important role in Industry 4.0. Therefore, developing portable heavy metal sensors with low cost, high accuracy and good intelligence based on new devices, materials and mechanisms to overcome the limitations of current application scenarios has become a key scientific issue. To this end, this thesis provides a certain experimental basis and application research for the development of portable heavy metal sensors based on novel nanomaterial construction, but subsequent research needs to be carried out and further explored in the following aspects:

i. Currently, most nanoenzymes still suffer from insufficient specificity and catalytic activity compared to natural enzymes and are not sufficiently persistent; therefore, the development of novel nanoenzymes with excellent catalytic properties, high specificity, and persistence is essential to improve the detection performance of paper-based sensors.

ii. Nanomaterial inkjet printing technology and artificial intelligence image recognition technology were combined to achieve mass production of paper-based sensors for on-site rapid detection of environmental pollutants, food additives, and disease markers to lay the foundation for a better move towards large-scale market applications to serve resource-poor regions.

iii. Finally, design and preparation of multifunctionalized flexible visualized sensor arrays with excellent performance, in-depth investigation of the integrated synthesis mechanism of nanosensitive materials and devices, and data processing of the arrays using high-order data processing algorithms to construct high-performance, visualized portable and wearable heavy metal sensors.

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APPENDICES

Appendix A: Journal Publications

1. **Qu, Y.**, Yakub, I., Bains, R., Hu, W., & Wang, L. (2023). Ag⁺ colorimetric sensor based on graphene oxide/nano-platinum composite. *Journal of Analytical Science and Technology*, 14(1), 35.
2. **Qu, Y.**, Yakub, I., Bains, R., Guo, M., & Luo, Y. (2023). Simultaneous Ag⁺ and Hg²⁺ colorimetric sensors based on graphene oxide/Au-Pt nanoparticles. *International Journal of Environmental Analytical Chemistry*, 1-23.
3. **Qu, Y.**, Yakub, I., Bains, R. (2024). A paper-based colorimetric sensor based on graphene oxide/platinum-gold nanocomposite for simultaneous detection of Hg²⁺ and Ag⁺. *Water, Air, & Soil Pollution*. (Under Review)
4. **Qu, Y.**, Bai, Y., Wang, Y., Wang, L., Lin, L. (2023). A colorimetric test paper for detecting Hg²⁺ and Ag⁺ and its preparation method and application. *Qilu Institute of Technology*. 202311655491.9

Examples of calculations of the Michaels-Menton equation

Method for calculation of initial reaction rate: Absorbance data were back-calculated to concentration by the Beer–Lambert Law using a molar absorption coefficient of 39000 $\text{M}^{-1} \text{cm}^{-1}$ for TMB-derived oxidation products. Apparent steady-state reaction rates at different concentrations of substrate were obtained by calculating the slopes of initial absorbance changes with time (Absorbance determination was performed in 5 min at the frequency of one time per minute), as shown in following Table S1.

Table S1. An illustration for calculation of initial reaction rate

A (Average)	Time (s)	The concentration of TMB-derived oxidation products (M^{-1}) ($c=A/\epsilon b$, where $\epsilon=39000 \text{ M}^{-1}\text{cm}^{-1}$, $b=1 \text{ cm}$)	Rate ($\text{M}^{-1}\text{s}^{-1}$)
0.021	60	5.4E-07	2.62 E-08
0.078	120	2.0E-06	
0.129	180	3.31E-06	
0.196	240	5.02E-06	
0.269	300	6.90E-06	

Raw data for optimising the detection conditions of the GO/Au-PtNPs colorimetric sensor

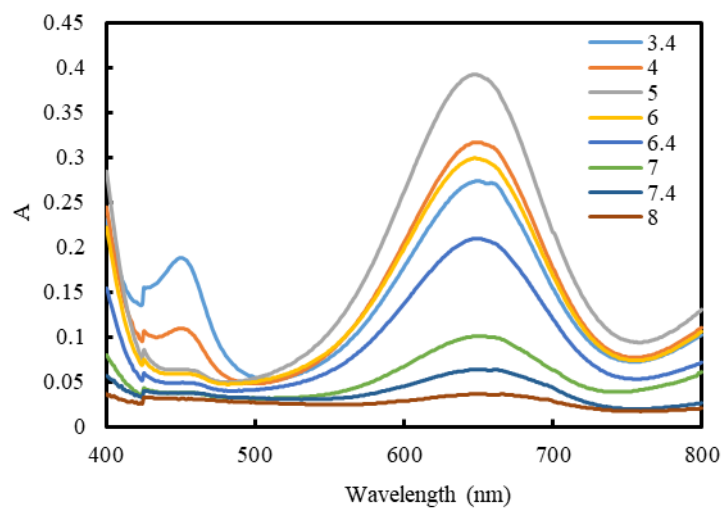


Figure S1 Uv-vis spectra of pH optimization in catalytic process

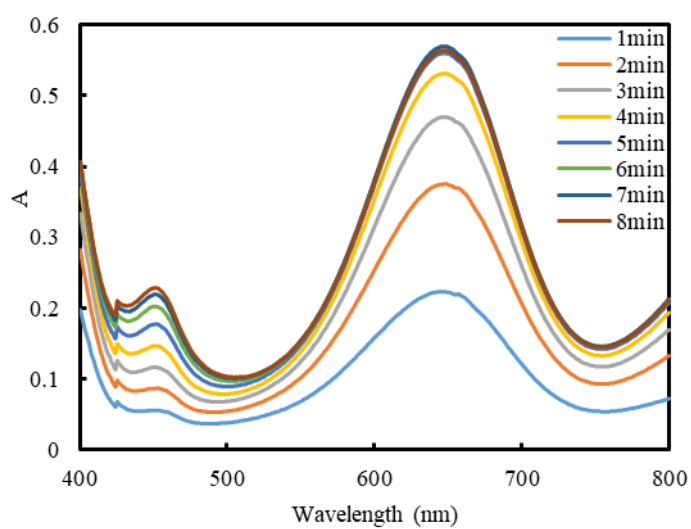


Figure S2 Optimization of the reaction time during the catalytic process by UV-VIS spectroscopy

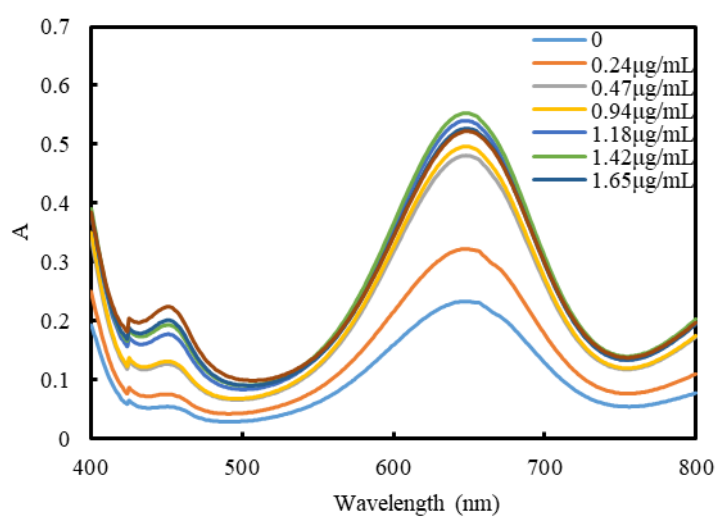


Figure S3 Uv-vis spectrogram of GO/Au-PtNPs concentration optimization during catalysis

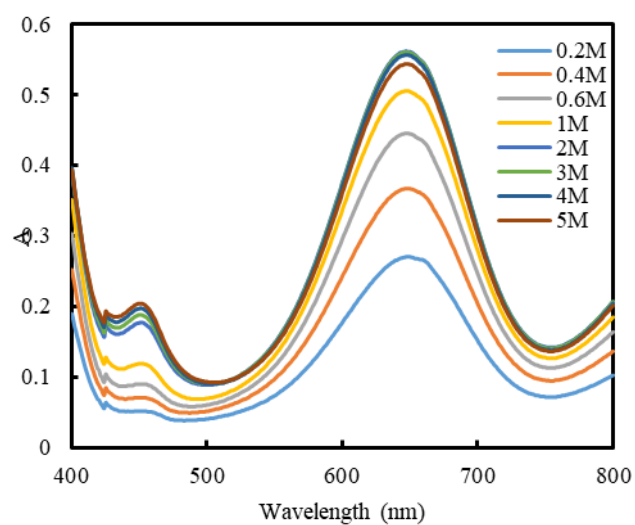


Figure S4 Uv-vis spectra optimized for H₂O₂ concentration during catalysis

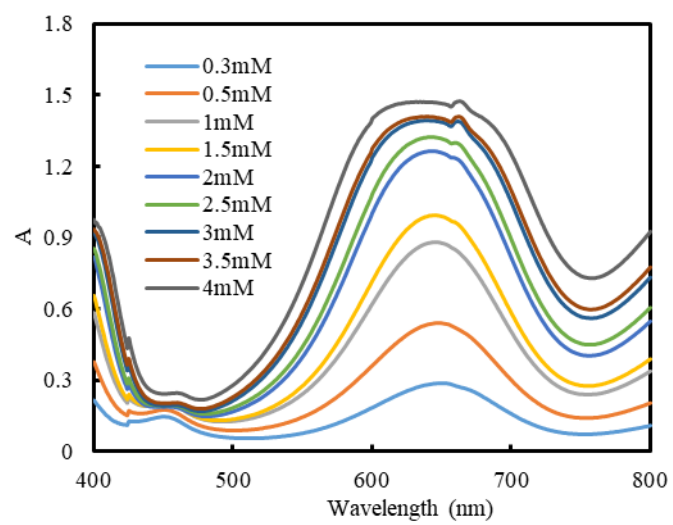


Figure S5 Uv-vis spectra optimized for TMB concentration during catalysis