GOLD-BASED METAL-ORGANIC FRAMEWORK CONTAINING COPPER OR NICKEL FOR SIMULTANEOUS ELECTROCHEMICAL DETECTION OF DOPAMINE AND URIC ACID

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2023

FS 2023 29

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Chair : Professor ChM. Janet Lim Hong Ngee, PhD
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The rapid and efficient detection of small biomolecules in human body fluids is a critical step in developing effective electrode materials. Metal-organic frameworks (MOFs) have gained significant attention from researchers due to their unique properties, such as remarkable surface area and tunable pore size.

An Au@Cu-metal-organic frameworks (Au@Cu-MOFs) composite material has been successfully synthesized via one-pot solvothermal method. The composite material was modified onto a screen-printed carbon electrode (SPCE) to serve as an electrocatalyst for the oxidation of dopamine (DA) and uric acid (UA). The inclusion of gold nanoparticles (Au NPs) on the octahedral crystal shape of Cu-MOFs has dramatically improved the catalytic performance of the developed sensor. The electrochemical performance of the proposed sensor based on Au@Cu-MOFs toward simultaneous detection of DA and UA shows an extended linear range from 1 μM to 1000 μM, with good sensitivity and remarkably lower detection limits (LOD) of 2.12 μA μM^{-1} cm^{-2} and 0.06 μM for DA, as well as 1.76 μA μM^{-1} cm^{-2} and 0.08 μM for UA.

Another composite material of MOF by using different metal center (Au@Ni-MOF) was developed for comparison. The composite material of Au@Ni-MOF was prepared via two-step approach. Firstly, Ni-MOF was synthesized using an *in-situ* growth strategy, which involved the growth of $[Ni_3(BTC)_2]$ n (H_3BTC = trimeric acid) with coordinatively unsaturated Ni(II) sites. Then, the as-synthesized Ni-MOF powder was mixed with the Au NPs solution to produce the Au@Ni-MOF hybrid material. This sensor demonstrated LODs of approximately 0.027 and 0.028 μM for the simultaneous determination of DA and UA (S/N = 3) over a wide linear range of 0.5 μM to 1 mM, with an excellent sensitivity of 1.43 μA μM^{-1} cm^{-2} and 1.35 μA μM^{-1} cm^{-2}, respectively. The application was further

investigated in human serum and urine for the simultaneous determination of DA and UA, which also showed exceptionally high recovery in the 93.8% to 105.0% range with a lower RSD of less than 3%.

In conclusion, there is a great need for rapid, efficient, and highly sensitive detection of small biomolecules by improving novel MOF-based electrode materials in medical research. Therefore, we evaluated the analytical performance of Au@M-MOF electrodes (M = Cu and Ni) and successfully applied them to determine simultaneous DA and UA in human urine and serum samples.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KERANGKA LOGAM-ORGANIK BERASASKAN EMAS YANG MENGANDUNGI TEMBAGA ATAU NIKEL UNTUK PENGESANAN ELEKTROKIMIA SERENTAK DOPAMIN DAN ASID URIK

Oleh

ZHOU FENG

September 2023

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Pengesanan yang cepat dan efisien bagi biomolekul kecil dalam cecair badan manusia adalah langkah penting dalam pembangunan bahan elektrod yang berkesan. Struktur logam-organik (MOFs) telah mendapat perhatian ketara daripada para penyelidik kerana sifat uniknya, seperti luas permukaan yang menakjubkan dan saiz liang boleh laras.

Bahan komposit Au@Cu-struktur logam-organik (Au@Cu-MOFs) telah berjaya disintesis melalui kaedah solvoterma satu periuk. Bahan komposit telah diubahsuai ke atas Elektrod Karbon Bercetak Skrin (SPCE) yang berfungsi sebagai elektrokatalis yang cemerlang untuk pengoksidaan dopamin (DA) dan asid urik (UA). Kemasukan nanopartikel emas (Au NPs) pada bentuk kristal oktahedral Cu-MOFs telah secara dramatik meningkatkan prestasi katalitik sensor yang dibangunkan. Prestasi elektrokimia sensor yang dicadangkan berasaskan Au@Cu-MOFs terhadap pengesanan serentak DA dan UA menunjukkan julat linear yang luas dari 1 μM sehingga 1000 μM, dengan kepekaan yang baik dan had pengesanan yang sangat rendah (LOD) iaitu 2.12 $\mu A\ \mu M^{-1}\ cm^{-2}$ dan 0.06 μM untuk DA, serta 1.76 $\mu A\ \mu M^{-1}\ cm^{-2}$ dan 0.08 μM untuk UA.

Satu lagi bahan komposit MOF dengan menggunakan pusat logam yang berbeza (Au@Ni-MOF) telah dibangunkan untuk perbandingan. Bahan komposit Au@Ni-MOF telah disediakan melalui pendekatan dua langkah. Pertama, Ni-MOF telah disintesis dengan menggunakan strategi tumbesaran in-situ, yang melibatkan pertumbuhan $[Ni_3(BTC)_2]$ n (H_3BTC = asid trimera) secara berkoordinat tapak nikel (II) yang tidak tepu. Kemudian, serbuk Ni-MOF yang telah disintesis dicampurkan dengan larutan Au NPs untuk menghasilkan bahan hibrid Au@Ni-MOF. Sensor ini telah menunjukkan LODs kira-kira bernilai 0.027 dan 0.028 μM untuk penentuan serentak DA dan UA (S/N = 3) dalam julat linear

yang luas dari 0.5 μM sehingga 1 mM, dengan kepekaan yang cemerlang iaitu masing-masing dengan nilai 1.43 μA μM^{-1} cm^{-2} dan 1.35 μA μM^{-1} cm^{-2}. Aplikasi sensor telah dikaji lebih lanjut di dalam serum dan air kencing manusia untuk penentuan serentak DA dan UA, yang juga menunjukkan kadar pemulihan yang sangat tinggi iaitu dalam julat 93.8% hingga 105.0% dengan sisihan piawai relatif (RSD) lebih rendah daripada 3%.

Kesimpulannya, pengesanan yang cepat, efisien, dan sangat sensitif bagi biomolekul kecil adalah sangat diperlukan dengan menambahbaik bahan elektrod berasakan MOF yang novél dalam penyelidikan perubatan. Oleh itu, kami telah menilai prestasi analitikal elektrod Au@M-MOF (M = Cu dan Ni) dan berjaya menggunakannya untuk menentukan DA dan UA secara serentak dalam sampel air kencing dan serum manusia.

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my deepest gratitude to all those who have helped me in the completion of this thesis.

First and foremost, I would like to express my heartfelt thanks to my supervisor Professor Dr. Lim Hong Ngee for her invaluable guidance, unwavering support, and encouragement throughout this journey. Her expert knowledge and keen insights have been instrumental in shaping my research direction and refining my work. I am truly grateful for her constant encouragement, insightful feedback, and unwavering support throughout my doctoral studies.

I am also deeply grateful to my co-supervisors, committee members, and other faculty members for their invaluable guidance, feedback, and support. Their expertise and insight have been invaluable in shaping my research and in helping me to develop the necessary skills and knowledge to be successful in my field.

I would like to express my sincere appreciation to Dr. Izwaharyanie and Dr. Gowthaman for the invaluable guidance and support throughout my Ph.D. career. Their extensive knowledge, patience, and dedication have helped me tremendously in completing this thesis.

I would also like to extend my gratitude to my colleagues Si Lin, Hanusha, Amali, Leng, and Jolyn. Their friendship, support, and encouragement have been a source of inspiration and motivation for me. I am grateful for the many insightful discussions and shared experiences we have had during our time together.

Finally, I would like to express my heartfelt gratitude to my family and friends for their unwavering support, encouragement, and love throughout my studies. Their belief in me has been a constant source of motivation and strength, and I could not have done this without them.

Thank you all for your unwavering support and encouragement throughout my doctoral studies.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 14 March 2024

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LIST OF ABBREVIATIONS

AA	Ascorbic Acid
a-CSF	artificial cerebrospinal fluid
AMP	Amperometry
AuNPs	Gold Nanoparticles
BET	Brunauer-Emmett-Teller
BTC	Benzene tricarboxylate
CA	Chronoamperometry
CCDC	Cambridge Crystallographic Data Center
CV	Cyclic Voltammetry
DA	Dopamine
DC	Drop-coating
DMF	N, N-dimethylformamide
DPV	Differential Pulse Voltammetry
ED	Electrodeposition
EDX	Energy-Dispersive X-ray spectroscopy
EIS	Electrochemical Impedance Spectroscopy
E_{pa}	The oxidation peak potential
E_{pc}	The cathodic peak potential
FESEM	Field Emission Scanning Electron Microscopy
FT-IR	FourierTransform Infrared Spectrometry
GCE	Glassy Carbon Electrode
HRTEM	High-Resolution Transmission Electron Microscopy
I_{pa}	The oxidation peaks current

LOD	Limit Of Detection
MOF	Metal-organic Framework
PBS	Phosphate-Buffered Saline
SPCE	Screen-Printed Carbon Electrode
SWV	Square Wave Voltammetry
UA	Uric Acid
XRD	X-Ray powder Diffractometer

CHAPTER 1

INTRODUCTION

1.1 Background and Motivations

Neurotransmitters are chemical messengers that facilitate communication between neurons and other cells in the nervous system, enabling the transmission of signals and information (Ayano, 2016; Hampel & Lau, 2020; L. Nguyen et al., 2001). Neurotransmitters are typically stored within tiny sacs known as vesicles within the axon terminals of neurons. When an action potential reaches the axon terminal, these vesicles are stimulated to fuse with the cell membrane and release their contents into the synapse, the small gap between the axon terminal and the target cell (Ayano, 2016). More than one hundred molecules have been identified as neurotransmitters, including acetylcholine, dopamine, serotonin, glutamate, glycine, γ-amino-butyric acid, and enkephalin (Ayano, 2016; Hampel & Lau, 2020). Neurotransmitters play a crucial role in the human brain and maintaining homeostasis in the body, which can significantly impact mood, sleep, concentration, and weight (Ayano, 2016; Mittal et al., 2017). Several factors can disrupt neurotransmitter levels, including stress, poor diet, exposure to neurotoxins, genetic predisposition, use of prescription and recreational drugs, and consumption of alcohol and caffeine. These factors can cause neurotransmitter levels to fall outside the optimal range (Ayano, 2016).

Additionally, among these neurotransmitters, dopamine (DA) is a prevalent neurotransmitter that is produced in various regions of the brain, including the substantia nigra, ventral tegmental area, and hypothalamus in the human body (Narvaes & de Almeida, 2014). It plays a crucial role in the functioning of the central nervous, endocrine, and cardiovascular systems (Q. He et al., 2018). It involves various behavioral functions, including movement, motivation, reward processing, and emotional arousal (Ayano, 2016; Hemandez & Hoebel, 1988; Sarkar et al., 2010; Schultz, 2007; Shohamy & Adcock, 2010). DA plays an essential role in the brain's reward processing system, which is responsible for reinforcing behaviors with a hedonic component and is deemed beneficial (Arias-Carrión et al., 2010). Evidence suggests that receiving rewards can lead to an increase in DA activity, such as eating or exercising(Arias-Carrión et al., 2010; Schultz, 2007). Addictive drugs, such as cocaine and amphetamines, can be highly addictive because they potently activate the brain's reward system (Gardner, 2005). Additionally, DA is involved in several neurological and psychiatric disorders, such as Parkinson's disease, a neurodegenerative disorder resulting from decreased levels of dopamine in the nigrostriatal and mesolimbic pathways (Ayano, 2016; Hampel & Lau, 2020; Mittal et al., 2017). Other disorders that involve DA dysfunction include schizophrenia and addiction, which are caused by elevated levels of DA in the human brain (Ayano, 2016; Sarkar et al., 2010).

Uric acid (UA) is a natural waste product in the human body. It is one of the essential nitrogen compounds with the chemical formula $C_5H_4N_4O_3$ (7, 9-dihydro-1H-purine-2,6,8(3H)-trione) and a molecular weight of 168 daltons. It can be produced when purines are broken down in certain foods and the body's tissues (Hafez et al., 2017; Jin et al., 2012). Furthermore, plasma urate levels of UA in humans are typically high, ranging from 3.5 to 7.5 mg/dL (200-450 mol/L), which is higher than levels observed in other mammals. UA is typically dissolved in the blood and is mainly excreted through the kidneys in urine (Hafez et al., 2017). Hyperuricemia is defined as either the body producing an excess of UA or the kidneys being unable to eliminate it effectively, resulting in the accumulation of UA in the body and leading to adverse health conditions (Jin et al., 2012). Recent studies have reported that hyperuricemia can cause crystals to form in joints and tissues, leading to health conditions such as gout, nephrolithiasis, and chronic nephropathy (Grassi et al., 2013). Physiologically, several factors can increase uric acid levels in the body, including hypertension, metabolic syndrome, abdominal obesity, endothelial dysfunction, inflammation, subclinical atherosclerosis, and an increased risk of cardiovascular events (De Oliveira & Burini, 2012; Gagliardi et al., 2009).

Although DA and UA are produced in different parts of the body and have distinct functions, some studies have suggested that UA may play a role in regulating intrarenal vascular reactivity to DA in the body (Sulikowska et al., 2008). Additionally, there are some conditions in which high levels of UA may lead to changes in DA function in the human body. For example, research has indicated that high levels of serum UA are related with a considerably decreased incidence of Parkinson's disease. UA has been shown to slow the DA auto-oxidation rate in caudate and substantia nigra homogenates of Parkinson's patients (Andreadou et al., 2009; Dănău et al., 2022). Therefore, it has been performed using modified electrodes to detect DA and UA simultaneously. But DA and UA have very similar oxidation potentials, leading to a challenging task for their electrochemical detection (Sajid et al., 2016). In conclusion, the necessity to build simple, selective, and sensitive electrochemical sensing devices must be explored and prioritized in the future.

1.2 Problem Statements

The simultaneous detection of several electroactive analytes in mixed solutions is crucial for the early detection of disease diagnosis to monitor the human health. Among various electroactive analytes in human physiological fluids, UA and DA play important functions in the metabolic processes of the human body (Krishnan et al., 2020; Qu et al., 2021a; F. Xu et al., 2023). Hence, it is imperative to develop an analytical method with sensitivity for the simultaneous determination of UA and DA to facilitate further analytical applications. This method will be valuable for early diagnostics of abnormal UA and DA concentrations, serving as an indicator of potential disease development in the human body.

Various analytical techniques are being used to detect DA and UA which include capillary electrophoresis (Pormsila et al., 2009a; Y. Zhao et al., 2011a), high performance liquid chromatography (HPLC) (Hubbard et al., 2010; Ross, 1994), fluorescence spectroscopy (Azmi et al., 2015a), chemiluminescence (Amjadi et al., 2014), colorimetry (Liu et al., 2013), optical sensing (Grabowska et al., 2008; Kamal Eddin & Wing Fen, 2020), and enzymatic sensor (Forzani et al., 1997; Yu et al., 2011). Although these methods have their own advantages and limitations, they are not suitable for the rapid detection of DA and UA in clinical blood serum and urine samples. For instance, HPLC offers high sensitivity and selectivity, but requires time-consuming preliminary treatment of analyte prior to use, which is not suitable for prompt clinical determination. Similarly, fluorescence spectroscopy has low sensitivity and instability, which limits its application for realistic clinical monitoring. Electrochemical analysis has the distinct advantage, including excellent specificity, high sensitivity, and no complex prerequisite treatments for monitoring (X. Li et al., 2017; S. Lu et al., 2020b; Qiu et al., 2019a; Wu et al., 2012; D. Zhao et al., 2016). Besides, voltametric analysis, an electrochemical technique, offers the added advantage of convenient on-site analysis.

In addition, given the proximity of their oxidation potential, the oxidation potential for UA and DA on bare electrodes, such as SPCE, may overlap with each other. The overlapping of the oxidation potentials of UA and DA may cause insufficient peak separation, resulting in reduced sensitivity of electrochemical analysis. Therefore, to address this issue, we have applied several materials to the SPCE surface, combining the advantages of metal-organic framework and metal nanoparticles to achieve well-separated peaks for UA and DA. These materials have gained significant attention due to their unique catalytic properties (Müller et al., 2011). The reaction mechanism involves the target analytes (DA and UA) being adsorbed onto the Au@MOF composite surface, followed by the oxidation of DA and UA by the AuNPs. The AuNPs act as catalysts by providing a localized surface plasmon resonance effect, which enhances the oxidation reaction rate (Cittadini et al., 2014; Nano et al., 2017). The AuNPs play a critical role in catalyzing the oxidation of DA and UA, while the MOF framework provides a high surface area and stable support for the AuNPs (Nano et al., 2017). Hence, the proposed composite materials allow for distinct peaks for UA and DA with outstanding sensitivity.

However, AuNPs are relatively expensive to produce and are used as the starting material for Au@MOF synthesis (Lung et al., 2007). AuNPs can be unstable under certain conditions, such as changes in temperature, pH, or ionic strength (Jimenez-Ruiz et al., 2015; C. Y. Lin et al., 2010). In addition, AuNPs can aggregate or clump together under certain conditions, which can affect their properties and potentially limit their effectiveness in specific applications (Yang et al., 2011). Some factors that can lead to nanoparticle aggregation include electrostatic interactions, Van der Waals forces, high salt concentrations, and high temperatures (Y. M. Chen et al., 2008; Dutta et al., 2016; C. Yang et al., 2011). Various techniques can prevent or reduce AuNP aggregation, such as adding stabilizing agents, functionalizing the nanoparticles with specific molecules, or controlling the conditions under which the nanoparticles are used (Alexandridis, 2011; Y. Yang et al., 2007).

1.3 Research and Objectives

The primary aim of this study is to create a fast, reliable, straightforward, discriminative, and responsive electrochemical sensing system using Au@MOF to detect critical biomolecules individually and simultaneously in the human body. The research's specific objectives are outlined below:

i. To synthesize Au@Cu-MOF and Au@Ni-MOF composites using electrochemical and chemical methods.

ii. To characterize the synthesized Au@MOFs, with a particular focus on the Au@Cu-MOF and Au@Ni-MOF composites, using a range of analytical techniques.

iii. To evaluate the electrochemical sensing performance for detecting DA and UA, individually and simultaneously.

iv. To assess the selectivity, stability, reproducibility, repeatability, and anti-interference ability of the electrochemical sensors based on Au@Cu-MOF and Au@Ni-MOF composites for DA and UA detection.

1.4 Scope and Limitation

Electrochemical sensors are widely utilized in a variety of applications, such as environmental monitoring, medical diagnostics, and industrial process control, as a result of their many benefits. Our developed electrochemical sensors have several distinct features that make them suitable for multiple applications:

i. High sensitivity: The Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE electrochemical sensors can be highly sensitive and able to detect DA and UA at low concentrations.

ii. Selectivity: The design of Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE electrochemical sensors ensures high selectivity for detecting DA and UA even in complex matrices.

iii. Fast response time: The electrochemical sensors utilizing Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE offer quick response times, allowing for real-time measurements of DA and UA detection.

iv. Portable and easy to use: It is feasible to design mobile and user-friendly electrochemical sensing devices using Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE technology.

v. Low power consumption: Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE-based electrochemical sensing devices have low power consumption requirements.

vi. Wide range of applications: With the ability to detect a broad range of DA and UA, electrochemical sensing devices based on Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE have diverse applications in medical diagnostics.

The scope of study in electrochemical sensors is broad and encompasses various aspects related to the design, fabrication, and application of sensors that utilize electrochemical principles for detection and analysis. Here are some key areas within the scope of study in electrochemical sensors:

i. Sensor design and fabrication: Au@Cu-metal organic framework (Au@Cu-MOF) is successfully synthesized and modified a screen-printed carbon electrode (SPCE) to synthsize Au@Cu-MOF/SPCE sensor. A novel conductive Ni-based MOF decorated with AuNPs (Au@Ni-MOF) was developed and used as a drop-casted thin film electrode to obtain Au@Ni-MOF/SPCE.

ii. Electrochemical techniques: Understanding and utilizing different electrochemical techniques such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), and Electrochemical impedance spectroscopy (EIS) methods for sensor characterization.

iii. Materials for Sensing: Exploring and synthesizing novel materials (Au@MOF) for use in sensor construction.

iv. Sensor Performance Optimization: Studying factors affecting sensor performance, such as sensitivity, selectivity, stability, and anti-interference.

v. Applications: These sensors can be detected target analytes in human serum and urine samples, which can be used in medical diagnostics.

Electrochemical sensors are commonly employed to quantify various biological and chemical analytes, but they have some limitations that must be acknowledged. Here are some of the primary rules for electrochemical sensors:

i. Selectivity and interference: Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE-based electrochemical sensing devices can only detect two target analytes in complex sample matrices where interference from other substances may occur.

ii. Sensitivity and detection limits: Electrochemical sensors utilizing Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE technology may have limitations in terms of sensitivity, particularly for trace-level detection. Achieving low detection limits is crucial for applications such as environmental monitoring and medical diagnostics.

iii. Stability and long-term performance: The stability of Au@Cu-MOF and Au@Ni-MOF materials over time can be a concern, leading to sensor

degradation and drift in performance. Long-term use may result in fouling or biofouling of electrodes, affecting the sensor's reliability.

iv. Reproducibility and standardization: Ensuring the reproducibility of sensor fabrication and performance across different batches and laboratories is a challenge. Lack of standardized methods for sensor characterization and testing can hinder comparisons between studies.

v. Environmental factors: Electrochemical sensors that utilize Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE technology may be susceptible to variations in output due to environmental factors such as temperature and humidity. These factors can significantly impact the performance of the sensors, potentially leading to inaccurate readings and reduced sensitivity.

vi. Miniaturization and integration challenges: Electrochemical sensing devices based on Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE technology may have limited miniaturizing sensors for portable and wearable applications while maintaining performance can be technically challenging. Integrating sensors with other technologies in a seamless and cost-effective manner poses additional challenges.

vii. Sample preparation: The sample preparation required for electrochemical sensing devices utilizing Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE technology may be complex, leading to a time-consuming and costly process.

viii. Cost and accessibility: The screen-printed carbon electrode (SPCE) can be expensive and is not reusable, leading to increased costs for electrochemical sensing devices based on Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE technology. Accessibility to advanced equipment and expertise may restrict the implementation of electrochemical sensors in certain regions.

In conclusion, our electrochemical sensors, based on Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE technology, have several limitations when selecting sensors for specific applications or designing experiments.

1.5 Thesis Outlines

This thesis comprises five chapters, each providing unique information on the research topic. The chapters include an introduction, a literature review, methodology, results and discussion, a conclusion, and recommendations for future research. The specific details of each chapter are as follows:

Chapter One serves as the introduction to the thesis and comprises six sections. It begins with an overview of the background and motivation for the study, followed by a detailed introduction to the Au@MOF electrochemical sensors,

which are the focus of the research. In the third section, the research objectives are presented. The chapter concludes with a discussion of the scope and limitations of the study in the fifth section.

Chapter Two provides a detailed literature review of previous techniques based on electrochemical sensors, highlighting this research's structure, and working principle. The importance of small biomolecules such as dopamine and uric acid is introduced and reviewed. The chapter further examines previous studies on developing MOF materials and gold nanoparticles and explains electrochemical technology and voltammetric sensors. These are then compared to prior works. The chapter then describes and reviews the mechanisms for electrochemical detection of small biomolecules using Au@MOF materials and selective and simultaneous electrochemical detection of small biomolecules using Au@MOF materials separately.

Chapter Three describes the materials and methods used to develop the dopamine and uric acid sensors. The synthesis of AuNPs, Cu-MOF, and Ni-MOF is presented, along with the characterization of the composites using various instruments, including field emission scanning electron microscopy (FESEM), high-resolution transmission electron microscopy (HRTEM), Brunauer-Emmett-Teller (BET) method, X-ray powder diffractometer (XRD) measurement, and Fourier transform infrared spectrometry (FT-IR). The chapter also includes information on the electrochemical measurements used for quantitative analysis and the electrochemical performance of dopamine and uric acid. Additionally, the optimisation procedures, calculation of the limit of detection (LOD), and detection sensitivity of Au@MOF for DA and UA are described in this chapter.

Chapter Four provides a comprehensive analysis of the results obtained from the experiments conducted in this study. The chapter begins by discussing the characterization of the synthesized AuNPs, Cu-MOF, and Ni-MOF composites using techniques such as FESEM, HRTEM, BET, XRD, and FT-IR. The results showed that the composites have a high surface area and excellent stability, making them suitable for electrochemical sensors for DA and UA.

In Chapter Five, the research study's conclusion is illustrated by summarising the survey results and making recommendations for additional research initiatives.

In conclusion, our electrochemical sensors, based on Au@Cu-MOF/SPCE and Au@Ni-MOF/SPCE technology, have several limitations when selecting sensors for specific applications or designing experiments.

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