ACETAMINOPHEN, DOPAMINE, AND ASCORBIC ACID DETECTION USING FLUORINATED PYRAZOLONE DERIVATIVE-MODIFIED MULTIWALLED CARBON NANOTUBES ELECTROCHEMICAL SENSOR

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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THESIS PRESENTED TO QUALIFY FOR A DOCTOR OF PHILOSOPHY

FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2025

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ACKNOWLEDGEMENT

Firstly, I praise Almighty Allah for the sanctification and guidance in providing me with this challenging opportunity to complete my Ph.D. venture. Through the Ph.D. journey, many people supported and encouraged me to make it possible to complete my Ph.D. endeavor. I would like to express my deepest gratitude to my main supervisor, Dr. Mohamad Syahrizal Ahmad for the supervision, guidance, endless and priceless support, motivation, enthusiasm, immense knowledge, and patience throughout this research. I also want to thank my co-supervisor, Prof. Dr. Illyas Md Isa, for his help and advice while I am doing my work. I felt privileged and fortunate to be a Ph.D. student.

I am greatly indebted to Ministry of Higher Education (MOHE) for providing scholarship SLAB, Universiti Pendidikan Sultan Idris (UPSI), Universiti Teknologi MARA (UiTM) for given me this golden opportunity to pursue Ph.D., Faculty of Science and Mathematics (FSM, UPSI), Faculty of Applied Sciences (FSG, UiTM), and to all technicians and laboratory assistants that have been involved throughout the completion of this thesis. Special thanks also to all my friends who have contributed either directly or indirectly to this research. With support, encouragement, and constructive feedback, I can achieve this goal.

Nevertheless, I like to acknowledge the support and backing of my husband, Mohamed Azwan Ramly, and my children (late Nurul Damia Izazi, Nurul Anis Izazi, Nurul Aliya Izazi, Nurul Athirah Izazi, and Muhammad Ayyash Yusuf Izazi) for their understanding, encouragement, and prayers throughout this journey. I am also endlessly grateful to my mother, Siti Sarah Talib, my brothers, my sisters, and their respective families. Finally, I want to dedicate my work to my late father, Mohamad Mahbob Abd. Moksin for the feeling of love and affection I always have during my lifetime.

ABSTRACT

This study aims to develop modified multiwalled carbon nanotubes paste electrodes based on pyrazolone derivative ligand for detecting acetaminophen (APAP), dopamine (DOP), and ascorbic acid (AA). 1-Phenyl-3-methyl-4-benzoyl-5-pyrazolone (HPMBP) has been synthesized by mixing of 1-phenyl-3-methyl-5-pyrazolone (PMP) and benzoyl chloride. Second ligand, 1-phenyl-3-methyl-4-(2-fluorobenzoyl)-5-pyrazolone (HPMoFBP) and third ligand, 1-phenyl-3-methyl-4-(3-fluorobenzoyl)-5-pyrazolone (HPMmFBP) have been prepared by mixing of PMP with 2-fluorobenzoyl chloride and 3-fluorobenzoyl chloride separately. To produce the sensor, these ligands are mixed with multiwalled carbon nanotubes and placed in a Teflon tube. The carbon-13 nuclear magnetic resonance (^{13}C NMR) spectrum of HPMBP exhibited chemical shift of 119.20 ppm and 132.01 ppm at the C2" and C3" position, respectively. The presence of the fluorine atom deshielded the C2" of HPMoFBP and C3" of HPMmFBP to 159.91 ppm and 160.99 ppm, respectively. Under optimum conditions, the HPMBP/MWCNT/CPE (ΔE_S=5 mV, a=60 mV, f=180 Hz) detected APAP in 0.1 M PBS (pH 6.4) in the range of 1.0 µM to 1.0 mM with the detection limit (LOD) of 0.245 µM. The HPMoFBP/MWCNT/CPE (ΔE_S=1 mV, a=150 mV, f=60 Hz) in 0.1 M PBS (pH 8.0) and HPMmFBP/MWCNT/CPE (ΔE_S=6 mV, a=150 mV, f=150 Hz) in 0.1 M PBS (pH 7.0) showed a same linear range between 1.0 µM to 1.0 mM and LOD=0.1 µM for detection of DOP and AA, respectively. The sensors performances were not affected by some organic and inorganic substances. The sensors exhibited excellent recoveries between 90% and 105.3%, respectively. Conclusively, the developed sensors based on pyrazolone derivative ligands demonstrated outstanding sensing performance with high selectivity and sensitivity towards the detection of APAP, DOP, and AA. By implication, the developed sensors can be used as an alternative method for the determination of APAP, DOP, and AA in pharmaceutical formulations and human urine.

PENGESANAN ASETAMINOFEN, DOPAMIN, DAN ASID ASKORBIK MENGGUNAKAN SENSOR ELEKTROKIMIA KARBON NANOTIUB BERBILANG DINDING TERUBAHSUAI-TERBITAN PIRAZOLONA BERFLUORIN

ABSTRAK

Kajian ini bertujuan membangunkan elektrod pes karbon nanotiub berbilang dinding terubahsuai berasaskan ligan terbitan pirazolona bagi mengesan asetaminofen (APAP), dopamin (DOP), dan asid askorbik (AA). 1-Fenil-3-metil-4-benzoil-5-pirazolona (HPMBP) telah disintesis dengan mencampurkan 1-fenil-3-metil-5-pirazolona (PMP) dan benzoil klorida. Ligan kedua, 1-fenil-3-metil-4-(2-fluorobenzoil)-5-pirazolona (HPMoFBP) dan ligan ketiga, 1-fenil-3-metil-4-(3-fluorobenzoil)-5-pirazolona (HPMmFBP) telah disediakan dengan mencampurkan PMP dengan 2-fluorobenzoil klorida dan 3-fluorobenzoil klorida secara berasingan. Bagi menghasilkan sensor, ligan-ligan ini dicampurkan dengan karbon nanotiub berbilang dinding dan dimasukkan ke dalam tiub Teflon. Spektrum resonans magnet nukleus karbon-13 (^{13}C NMR) HPMBP menunjukkan anjakan kimia pada 119.20 ppm dan 132.01 ppm pada posisi C2" dan C3", masing-masing. Kehadiran atom fluorin telah mengurangkan perlindungan C2" HPMoFBP dan C3" HPMmFBP kepada 159.91 ppm dan 160.99 ppm, masing-masing. Pada keadaan optimum, HPMBP/MWCNT/CPE (ΔE_S=5 mV, a=60 mV, f=180 Hz) mengesan APAP dalam 0.1 M PBS (pH 6.4) dalam julat 1.0 µM hingga 1.0 mM dengan had pengesanan (LOD) sebanyak 0.245 µM. HPMoFBP/MWCNT/CPE (ΔE_S=1 mV, a=150 mV, f=60 Hz) dalam 0.1 M PBS (pH 8.0) dan HPMmFBP/MWCNT/CPE (ΔE_S=6 mV, a=150 mV, f=150 Hz) dalam 0.1 M PBS (pH 7.0) menunjukkan julat linear yang sama antara 1.0 µM hingga 1.0 mM dan LOD=0.1 µM untuk pengesanan DOP dan AA, masing-masing. Prestasi sensor-sensor tidak terjejas oleh beberapa bahan organik dan tak organik. Sensor-sensor menunjukkan pemulihan yang sangat baik antara 90% dan 105.3%, masing-masing. Kesimpulannya, sensor-sensor yang dibangunkan berdasarkan ligan terbitan pirazolona menunjukkan prestasi pengesanan yang luar biasa dengan kebolehpilihan dan kepekaan yang tinggi terhadap pengesanan APAP, DOP, dan AA. Implikasinya, sensor-sensor yang dibangunkan boleh digunakan sebagai kaedah alternatif untuk penentuan APAP, DOP, dan AA dalam formulasi farmaseutikal dan air kencing manusia.

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LIST OF ABBREVIATIONS

Ag/AgCl	Silver-silver chloride
CPE	Carbon paste electrode
CNT	Carbon nanotubes
CV	Cyclic voltammetry
$CDCl_3$	Deuterated chloroform
DHA	Dehydroascorbic acid
DPV	Differential pulse voltammetry
EC	Electrochemical sensors
EDX	Energy dispersive X-ray
EIS	Electrochemical impedance spectroscopy
FESEM	Field emission electron microscopy
FTIR	Fourier transform infrared spectroscopy
GCE	Glassy carbon electrode
GU	Guanine
HPMpFP	1-phenyl-3-methyl-4-(4-fluorobenzoyl)-5-pyrazolone
KCl	Potassium chloride
$K_4Fe[CN]_6$	Potassium ferrocyanide
LOD	Limit of detection
LOQ	Limit of quantification
MWCNT	Multiwalled carbon nanotubes
NaOH	Sodium hydroxide

NCDs	Non-communicable diseases
NMR	Nuclear magnetic resonance
PMP	1-phenyl-3-methyl-5-pyrazolone
HPMBP	1-phenyl-3-methyl-4-benzoyl-5-pyrazolone
HPMoFBP	1-phenyl-3-methyl-4-(2-fluorobenzoyl)-5-pyrazolone
HPMmFBP	1-phenyl-3-methyl-4-(3-fluorobenzoyl)-5-pyrazolone
RSD	Relative standard deviation
SWV	Square wave voltammetry
TEM	Transmission electron microscopy
XRD	X-ray diffraction

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CHAPTER 1

INTRODUCTION

1.1 Overview

The global public health crisis has become a top priority for a plethora of actors involved in academic, professional research, and policies (Kehr et al. 2023; Wenham et al., 2023). According to the United Nations, the urban population will reach 6.252 billion by 2050, with an urbanization rate of 67.2%, as a result of global urbanization, modernity, and exponential human population expansion. Rapid urbanization has resulted in urban disease, which has triggered many social and environmental concerns, as well as chronic diseases such as cardiovascular disease, obesity, Alzheimer's disease, diabetes, and cancer (Lawaniya et al., 2023; Stephens, 2017; Waqar et al., 2023; Zhang et al., 2023). Chronic diseases are non-communicable diseases (NCDs) caused by a mix of genetic, physiological, environmental, and behavioral factors and they have emerged as a major public health problem with the prolongation of human life (Medeni, Topcu, Bozdağ, & Medeni, 2025). According to current chronic illness statistics, 41 million people die each year, accounting for approximately 71.0% of all deaths worldwide, with

85.0% occurring in low- and middle-income countries (Afshari et al., 2023). The inclusion of death rates from the NCDs has brought this issue to the forefront. Cardiovascular disease (CVD), generally known as heart disease, is the world's leading cause of death, accounting for an estimated 17.7 million deaths in 2015. CVD is classified into four types: coronary artery disease, cerebrovascular disease, peripheral artery disease, and aortic atherosclerosis (Lopez, Ballard, & Jan, 2023). The cardiovascular system relies heavily on dopamine, a neurotransmitter. It directly affects the mammalian heart. These implications may include greater contraction force, higher heart rate, and coronary arteries constriction (Neumann, Hofmann, Dhein, & Gergs, 2023).

There are numerous medications used to treat NCDs. Drugs particular to these diseases present various unmanageable adverse effect, such as long-term use of dopaminergic drugs for CVD treatment has been linked to a variety of side effects, including daytime sleepiness and sleep attacks, impulse control disorder, addiction, and augmentations (de Almeida et al., 2014). The concentrations of these chemicals in blood, plasma, serum, and urine have a substantial impact on human health and vary depending to a variety of conditions such as disease, therapy, and nutrition. Changes in concentration can activate different metabolic pathways, potentially leading to a variety disorders and symptoms (Mansouri, Azadi, Drebadami, & Nakhaee, 2024). As a result, it is required to develop sensors capable of detecting an excessive quantity of dangerous analytes associated with such illnesses.

Hazardous or harmful analytes include heavy metals, ions, perfluorooctane sulfonate (toxic pollutant), peroxide value, enzymes (PI-PLC, esterase, and β-

galactosidase enzyme), bacteria (*E. coli*, *S. typhimurium*, and *L. monocytogens*), virus (SARS-CoV-2), C-reactive protein, mutagenic products (malondialdehyde), urinary protein, and pesticides (chlorpyrifos, thiacloprid, organophosphate, and imidacloprid). They represent a significant threat to human survival. Therefore, it is vital to monitor and maintain human health (Sonwal et al., 2024; Kumar et al., 2022). The ability to identify disease-associated molecules is essential not only for accurate diagnosis but also for therapeutic research and development. Furthermore, the development of smart sensors for public health, environmental monitoring, security, and industries worldwide would be extremely beneficial to people (Jadoon et al., 2021).

Sensing is a significant tool in today's world, being utilized extensively in the medical field, agrifood, home, agricultural applications, and much more (Hirata et al., 2023; Huang et al., 2023; Mahajan et al., 2023; Pundi & Chang, 2023). Sensor development has advanced significantly in response to the requirement to address critical challenges in these areas (Tovar-Lopez, 2023). According to Fazio et al. (2021), the Electrochemical Sensor Market was valued at USD 6.19 billion in 2020 and is predicted to grow to USD 11.83 billion by 2026.

Over the last decade, a variety of functional sensors and integrated sensing devices have been used in everyday life. Sensors have been used to detect a variety of physical parameters, chemical reactions, and biological interactions. These sensors can detect changes in the detection target and convert them into thermal, optical, electrical, or other readily detectable signals for transmission, processing, and storage. An ideal sensor should have the following characteristics: quick response time, high stability, low detection limit, excellent selectivity, high sensitivity, and durability. The need for

high sensitivity, fast reaction, and durability, as well as the variety of detection targets and the complexity of the detection environment, have prompted the renewal and development of sensing materials (Hu et al., 2023; Wu et al., 2023).

Electrochemical sensors are the most commonly utilized form of sensor in health care and clinical services, particularly in multiplex assays, because of their low cost, high susceptibility, and diminutive (Yunus et al., 2023). In today's sensor technology, ensuring selectivity is the most important aspect of target analysis. The development of analytical methods with high sensitivity and selectivity for traces of targets, such as biomolecules, metal ions, and small organic molecules, without time-consuming and expensive methods is critical for early disease diagnosis and treatment (Karadurmus, Kaya, Cetinkaya, & Ozkan, 2023).

1.2 Electrochemical Sensor

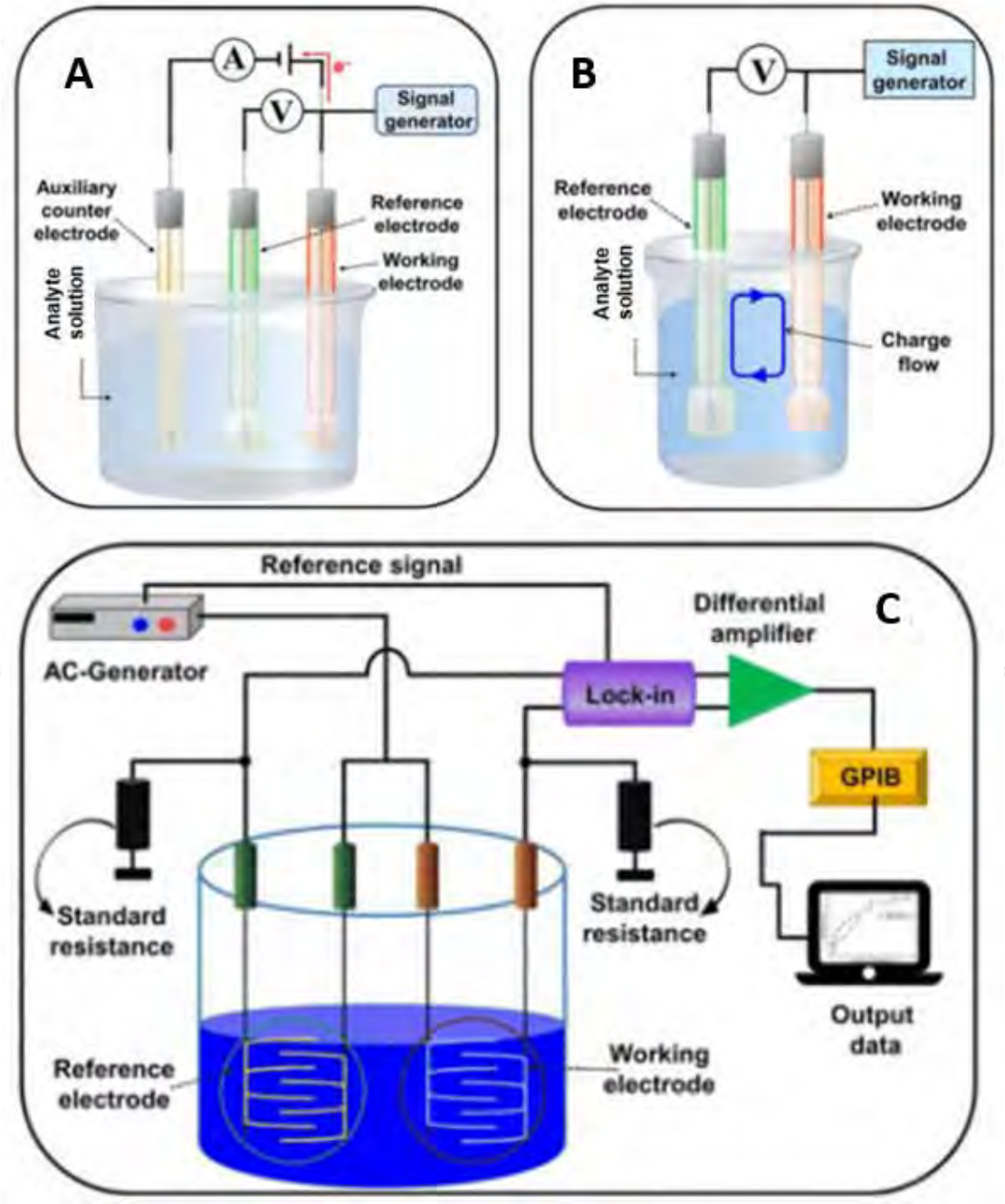
Electrochemical sensors are an important type of sensor that uses electrodes to transduce electrical impulses for analyte testing. Material science, physical chemistry, solid state physics, analytical chemistry, electrical engineering, biochemistry, statistical analysis, and device fabrication are among the many subjects covered by electrochemical sensors (Madhura, Devi, & Ramaraj, 2021). Nanomaterials have a significant impact on researchers, notably in the field of electrochemical sensors, due to their large surface area. Nanomaterials can be utilized to develop electrochemical sensors that can detect drugs/biomolecules and a variety of analytes. Electrochemical sensors are widely utilized in the oil industries, food, biomedical, and environmental

applications, as well as agriculture, due to their convenient solution (Kumar, Sharma, Sharma, & Garg, 2023). In agriculture, for example, modern farmers use electrochemical sensors to get critical information about soil fertility. By identifying ions in the soil, electrochemical sensors may determine soil quality depending on pH and nutrient levels.

The popularity of electrochemical sensing stems from two main advantages: low theoretical detection limits caused by differences in Faradaic and non-Faradaic currents, and variability of reporting signals such as electrochemical impedance, overall power output, current, or voltage (Baranwal et al., 2022). Electrochemical sensors detect electrochemical reactions, which typically involve a reduction-oxidation reaction between an electrode and an analyte. They work by transferring charges between two phases: the sample (liquid or solid) and the electrode, both of which are undergoing chemical changes. The sensing process consists of reactions at the electrode or the conveyed charge, which can be chemically changed to enable sensing (Murray, 1989). There are three types of sensors: potentiometric, amperometric, and conductometric (Figure 1.1). The potentiometric sensors rely on potential shifts between the indicator and reference electrodes. The amperometric sensors respond to current variations in analyte concentration during the oxidation-reduction process. The amount of charge carriers variations affects conductometric sensors. They are based on electrolyte conductivity measurements (Siddeeg et al., 2020).

Figure 1.1

Schematic diagram of (A) amperometric/voltammetric, (B) potentiometric, and (C) conductometric sensors



Adapted from Curulli (2021)

Electrochemical sensors have eliminated slow preparation and the need for expensive chemicals. They provide inexpensive analytical tools (Wang, Xu, Zhang, & Li, 2008). Thus, as low-cost, portable, and easy-to-use analytical tools, electrochemical sensors outperformed conventional analytical instruments such as gas chromatography coupled with mass spectrometry and high-performance liquid chromatography (Sadak, 2023). Nowadays, electrochemical sensors are becoming more precise, selective, and sensitive. Numerous characterization methodologies are extensively used to record the electrochemical response and determine the various amounts of electro-active analytes in both qualitative and quantitative terms, such as cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV), and pulse amperometry (PA) (Bagyalakshmi, Sivakami, Pal, Sarankumar, & Mahendran, 2022).

1.3 Fabrication of Electrochemical Sensors

Electroanalytical techniques are gaining a great deal of attention for sensing analytes due to their remarkable qualities such as portability, cost-effectiveness, and instrumental simplicity. Sluggish surface dynamics of conventional electrodes have a considerable impact on their sensitivity and selectivity (Kumunda, Adekunle, Mamba, Hlongwa, & Nkambule, 2021). Analytes on conventional electrodes typically produce a broad peak, with no peak appearing at lower concentrations. The unmodified electrodes' peak broadness makes it difficult to resolve the peaks of analytes with relatively close oxidation or reduction potentials. Conventional electrodes have limitations when dealing with these types of analytes, making them less appealing for

commercialization. To proceed with the reaction at fast rates, a large applied voltage is required, especially if the slow electrode reaction of analytes occurs on the bare conventional electrode. This reaction significantly exceeds their formal redox potential (Feng et al., 2021; Tajik et al., 2020).

Kinetically inhibited electrode reactions necessitate the use of an appropriate electrocatalyst that capable of speeding up the electrochemical reaction while lowering the redox potential. It is clear that electrode material is vital in the development of high-performance electrochemical sensors (Asadian, Galkhani, & Sahrokhian, 2019). Sensitivity and selectivity are two critical components in the development of electrochemical sensors. These properties are typically strengthened by modifying the electrode's surface to increase the affinity of the target analytes. Most typically, recognition compounds are immobilized on the electrode surface to boost the target compound's selectivity.

Bard and Itaya presented surface electrode modifications in 1978. Since then, the researchers have attempted to improve the surface kinetics of the electrodes. To develop an ultrasensitive surface with better selectivity, the rise of nanoscience and nanotechnology has piqued the interest of many scientists around the world in incorporating nanomaterials into electrode fabrication (Rathee et al., 2021). This is evidenced by the large number of articles in recent years describing the use of nanomaterials as electrode modifiers for electrochemical sensing.

The use of nanomaterials and nanotechnology in the construction of electrochemical sensors has recently received a lot of attention among researchers. Nanomaterials are an incredible class of materials that includes a wide range of examples with dimensions ranging from 1 to 100 nm. Their expanding applications are due to their outstanding magnetic, electrical, optical, chemical, mechanical, and catalytic properties, which differ significantly from their bulk counterparts. Recently, more attention has been placed on the synthesis or development of dimensions- and shape-directed nanomaterials in order to achieve not only ultrasensitive but selective electrochemical sensors.

Nano-scaled materials with highly regulated physicochemical properties, charge, size, surface area, and shape may now be developed via several synthetic techniques (Baig, Kammakakan, & Falath, 2021). The major advantages of using nanomaterials in electrode modification include improved surface kinetics, acceleration of electrochemical reactions due to the increased electroactive surface area, and improved analyte adsorption on the electrode surface, which aids in trace level quantification. Furthermore, nanomaterials provide both a stable support and active sites for functionalization, which improves electrode selectivity (Baig, Sajid, & Saleh, 2019).

Nanomaterials can be categorized into several types based on their characteristics, size, and shape. Three of the most well-known categories are semiconductor nanoparticles (such as cerium-based nanoparticles), metal nanoparticles (such as gold nanoparticles), and carbon-based nanomaterials.

1.4 Carbon Nanomaterials

Carbon nanomaterials are a promising material for improving the performance of electrochemical sensors. The last 20 years have seen a surge in scientific production of carbon materials (Gonzalez-Garcia, 2018). Carbon nanomaterials should be stressed as extremely versatile materials since they have remarkable fundamental features such as mechanical, chemical, electrical, and thermal, which are suitable for a wide range of applications (Schroeder et al., 2018).

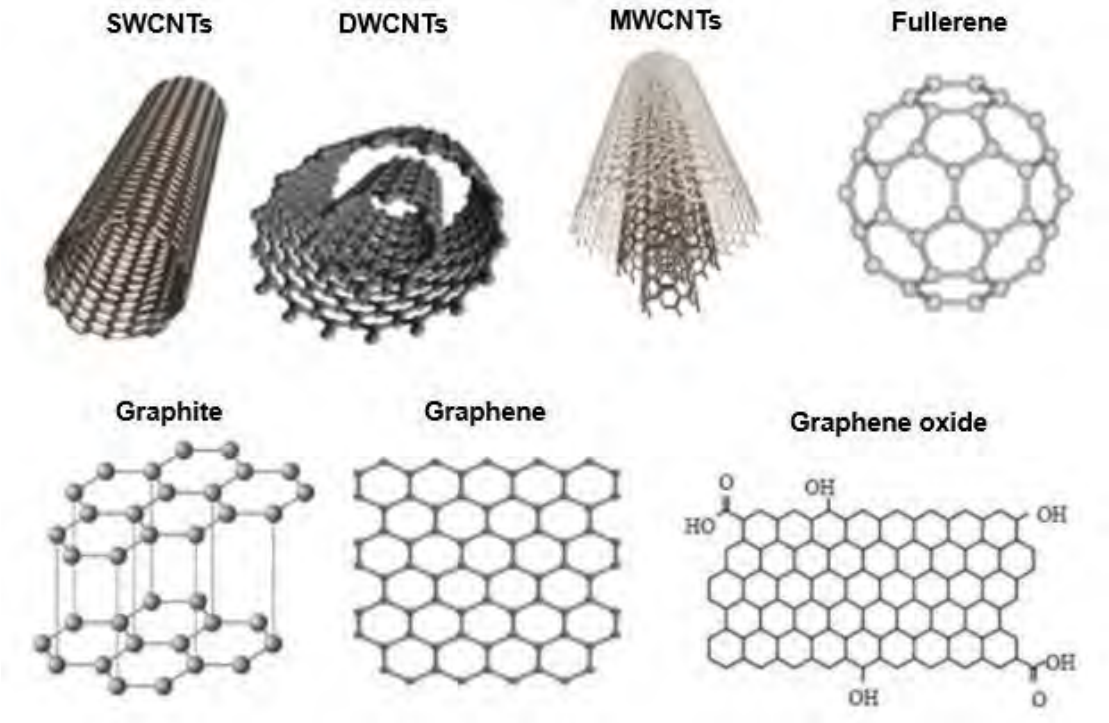
Carbon is a widely utilized material in electrochemistry, with uses ranging from energy conversion, energy storage, metals production, and electroanalysis. Carbon-based electrodes come in a variety of sizes, ranging from submicron to square meters. Carbon has a reasonably plentiful surface chemistry, a low background current, and inert electrochemical properties. It can be customized with various functions and affinities to improve its sensitivity and selectivity.

Carbon-based nanomaterials electrochemical sensors are used to detect various analytes or targets while also boosting high electron-transfer kinetics in proteins. Carbon has an electron configuration of $1s^2$ $2s^2$ $2p^2$. Carbon can exist in several allotropes. Carbon allotropes are accessible in a variety of crystallographic structures, ranging from simple graphite-like to complicated. Carbon nanotubes, fullerene, diamond, graphene, and graphite are well-known allotropes of carbon. Every carbon allotrope has unique chemical and physical features. The relative density of the surface's edge and basal planes distinguishes these carbon allotropes and determines electrochemical reactivity on the electrode surface.

The remarkable sensitivity of diverse carbon allotropes (Figure 1.2), as previously stated, makes them appropriate for the development of electrochemical sensors. Carbon-based materials have several advantages as a working electrode, including low ohmic resistance, stability, reproducibility, and ease of manufacture (Bagyalakshmi et al., 2022; Power, Gorey, Chandra, & Chapman, 2018). CNT-based sensors have received a lot of attention because of their large specific surface area, ability to reduce electrode fouling, excellent electrochemical properties, provision of accelerated electrochemical reaction rates, high tensile strength, good mechanical and chemical stability, and high electrical conductivity (Sajid, Baig, & Alhooshani, 2019).

Figure 1.2

Different structures of carbon nanomaterials. SWCNTs: single-walled carbon nanotubes; DWCNTs: double-walled carbon nanotubes; MWCNTs: multi-walled carbon nanotubes



Adapted from Azizi-Lalabadi, Hashemi, Feng, and Jafari (2020)

Ijima discovered CNTs in 1991. Since then, researchers have been given new opportunities in the physics and chemistry of carbon. Many investigations on CNTs applications have been done due to their outstanding electronic conductivity, large specific surface area, nanoscale size, and novel hollow-tube structure. As a result, there is an increased interest in developing carbon nanomaterials for industrial applications (Chen, Wei, & Xie, 2021; Novoselov et al., 2004). CNTs are a type of graphitic sheets that has been rolled into closed concentric cylinders, displaying a peculiar arrangement of sp^2-hybridized carbon atoms with lengths of micrometers and width of nanometers (Liu et al., 2014; Ghoreishi et al., 2012).

Because they are electrochemically active and act as efficient channels for electrons, the expanding family of CNT, particularly multi-walled carbon nanotubes (MWCNT) has been used to improve the sensitivity of electrochemical sensors in the determination of various analytes (dos Santos Neto et al., 2023; Gibi, Liu, Barton, Anandan, & Wu, 2023; Kumar et al., 2023; Nardi et al., 2023; Tümay et al., 2021; Ghoreishi et al., 2012). MWCNT is a superior type of carbon that contains multiple graphitic layers that were organized as concentric cylinders. MWCNTs can be formed as a bamboo-like structure, a herringbone pattern, or a hollow tube. They perform better in terms of redox process rates. MWCNT is an ideal electrode material for electrochemical reasons due to its high electrical and metallic conductivities (1.85×10^3 S/cm), optical activity, mechanical strength, thermal stability, and outstanding structural arrangements and electronic properties (Bibi et al., 2023; Settu, Lai, & Liao, 2021). Postolovi and Stanić (2023) found that modifying the electrode surface with MWCNT increases electroactive surface area and improves

electrical communication inside the film, leading to a low detection limit and great sensitivity.

1.5 Carbon Paste Electrode

Mercury-based electrodes such as thin mercury film, dropping mercury electrode (DME), and hanging mercury drop electrode (HMDE), have been widely employed as working electrodes. These mercury devices have several advantages, including the capacity to obtain pure surfaces, the ability to form amalgams, a large cathodic potential range, a high hydrogen overpotential, and great sensitivity and reproducibility. However, its restricted modification capabilities, a small anodic range (limited by mercury oxidation), and mercury toxicity made its usage environmentally undesirable. Several attempts have been made to replace it with a new mercury-free and reliable electrode (Meena, 2023; Ariño, Serrano, Díaz-Cruz, & Esteban, 2017).

Despite their sensitivity issues, carbon paste electrodes (CPE) are a viable alternative to mercury electrodes. CPEs are carbon-based materials made from a suitable pasting liquid and carbon powder. This study focuses on the role of the CPE in electrochemical sensing. Because of the ambiguous electron transfer kinetics and low cost, many CPE-based electrochemical sensors have been developed for pharmaceuticals, food additives, environmental contaminants, and different biomolecules (Rejithamol & Beena, 2022).

Carbon paste is one of the substrates that can undergo a variety of biological and chemical modifications. CPE as ion-selective electrodes have various advantages, including ease of fabrication, low ohmic resistance, a consistent analytical response, reproducibility, and chemical inertness (Wijaya et al., 2023). CPE's diverse modifiers, modification procedures, and methodologies have led to the development of applications in a variety of industrial and scientific fields. They are used as sensors for environmental control, fuel cells, thin film production technology, photochemistry, electrocatalysis, and electroanalysis (Hilali, Mohammadi, Amine, Zine, & Errachid, 2020; Nasir, 2018). CPE allows for the analysis of a wide variety of analytes. However, using unmodified CPE results in low sensitivity (high detection limit) and low selectivity (interference concerns). To address these concerns, surface modification of CPE was considered, including electro-deposition, drop-casting, and bulk modification (Mohammed, Hassan, Mahmoud, Farghali, & Hassouna, 2023).

Gibi et al. (2023) and Postolović and Stanić (2023) found that the amount of modifier in the paste is determined by the type of modifier and its potential to form more active sites on the modified paste's surface. These characteristics are significant because they effectively boost the rate of electrochemical processes and accelerate electron transfer rates. In CPE, nanoparticles were used to modify the working electrode surface. They exhibit properties that are distinct from macroscopic objects and microscopic particles due to their nanoscale particle size and the distinctive effects of their large surfaces, such as macroscopic quantum tunnelling effect, quantum size effect, volume effect, and surface effect. These properties are incomparable to other conventional materials, hence nanoparticles are widely used in electroanalysis (Fu, Lu, & Lai, 2021). Nanomaterials can be classified into two types: carbon-based

nanomaterials (graphene, fullerene, SWCNT, and MWCNT) and non-carbon-based nanomaterials (organic polymers, indium tin oxide (ITO), nanowire, silica nanoparticle, and metallic nanoparticle) (Cho, Kim, & Park, 2020; Jadon, Jain, Sharma, & Singh, 2016).

1.6 Analytes of Interest

Electrochemical techniques are based on the chemical response of an analyte of interest to electrical stimulation. It refers to the loss or gain of electrons (oxidation and reduction) that a material experiences during the electrical process. These redox reactions can reveal chemical state, kinetics and reaction processes, analyte concentration, and other features of a species' behaviour in solution. Electroanalytical methods make use of electrochemical techniques combined with electrochemical sensors to identify analytes of interest. Electrochemical sensors work by reacting with analytes of interest to produce an electrical signal proportional to their concentration in the sample (Manasa & Rout, 2023; Islam, Reza, Jeong, & Lee, 2016).

Unquestionably, the interest analytes: acetaminophen, dopamine, and ascorbic acid were the driving factors for this work. Acetaminophen, dopamine, and ascorbic acid are electrochemically active substances that can be studied using electrochemical techniques. As a result, the following subsections will provide a brief summary of recent developments in detecting those targeted analytes.

1.6.1 Multi-walled Carbon Nanotube (MWCNT) and Its Application in Electroanalysis of Acetaminophen

Ghadimi, Tehrani, Ali, Mohamed, and Ab Ghani (2013) developed an electrode modified with a composite film of poly (4-vinylpyridine) (P4VP) and MWCNT (P4VP/MWCNT GCE) for the voltammetric detection of acetaminophen in a phosphate buffer solution at pH 7 (Figure 1.3). The voltammogram obtained using the CV technique demonstrates a quasi-reversible electrode process (the reverse peak is less than the forward peak). The large redox couple peaks of acetaminophen could be attributable to the slow rate of electron transfer.

Hegde, Vishwanatha, and Nandibewoor (2024) fabricated a glassy carbon electrode with a suspension of CuO nanoparticles (CuONPs) and MWCNTs. CuONPs-MWCNTs/GCE, a modified electrode, displayed improved anodic peak current by inserting the anionic surfactant sodium dodecyl sulphate in phosphate buffer solution at a physiological pH of 7.4. Using DPV, the fabricated electrode possessed a linear dynamic range of 9 to 160 nM acetaminophen concentration. According to the calibration plot, the computed detection limit was 5.06 nM and the quantification limit was 16.88 nM.

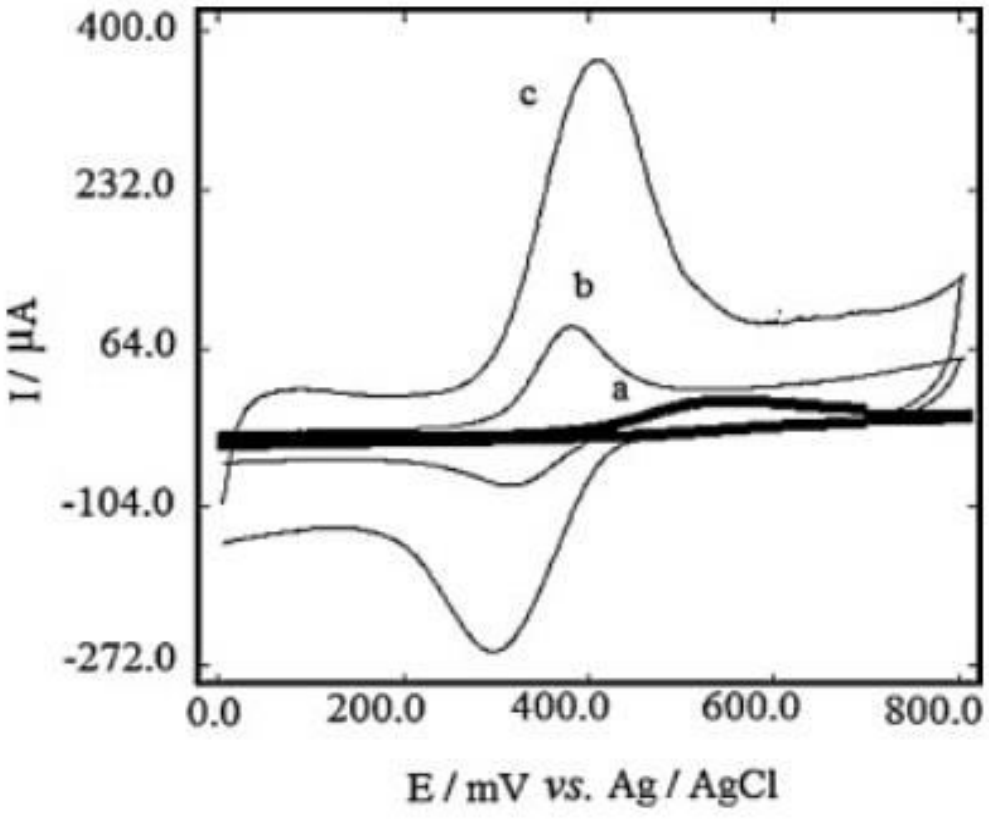
Azin and Pourghobadi (2021) investigated the electrochemical behavior of acetaminophen utilizing the CV method at the MWCNT/TiO_2/CILE. The MWCNT/TiO_2/CILE was also used to detect acetaminophen in real samples using the SWV approach. The proposed nanocomposite demonstrated excellent electrocatalytic activity, resulting in acetaminophen electrochemical oxidation in phosphate buffer

solution (pH 6.0). SWV results showed a linear range of 0.01–30 μM and detection limits of 0.003 μM for the modified electrode under optimal conditions.

The composite modified electrode, MXene@PDA/NH_2-MWCNTs, has electrocatalytic activity, good conductivity, large specific surface area, and hierarchical structure. It has a good linear response from 5.0 nM to 10.0 μM and 10.0 μM to 60.0 μM, with a low detection limit of 1.0 nM (S/N = 3). Furthermore, the MXene@PDA/NH_2-MWCNTs electrode has high stability, selectivity, and reproducibility (Chen et al., 2021).

Figure 1.3

Cyclic voltammograms of 0.1 mM acetaminophen at the (a) bare GCE, (b) MWCNT, and (c) P4VP/MWCNT GCE in 0.1 M phosphate buffer (pH 7) at a scan rate 20 mV s^{-1}



Adapted from Ghadimi et al. (2013)

1.6.2 Multi-walled Carbon Nanotube (MWCNT) and Its Application in Electroanalysis of Dopamine

A practical, cost-effective, accurate, and simple electrochemical sensor based on Co_2SnO_4/MWCNTs modified glassy carbon electrode has been developed for the dopamine detection (Manikanta et al., 2023). The fabricated sensor demonstrated excellent reproducibility, a wide linear range (50–350 nM), satisfactory selectivity, superb stability, and a very low detection limit of 10.3 nM. Furthermore, the Co_2SnO_4@MWCNTs/GCE electrode's reliability was established by real-time analysis of human urine samples and dopamine hydrochloride injections, indicating a favorable recovery percentage.

To improve electron transfer and electrocatalytic activity in dopamine detection, Zhou et al. (2023) presented glycerol-based carbon dots (CDs) generated via liquid dielectric barrier discharge (DBD) microplasma. A composite carbon nanomaterial electrode of CDs/MWCNTs was produced, taking advantage of MWCNTs' superior electrical conductivity. As a dopamine biosensor, the interaction and electron exchange between MWCNTs and dopamine can be improved to the π-π stacking force, permitting sensitive electrochemical detection of dopamine. The sensor performs well in detecting dopamine, with a linear range of 2.0–100 μM, a limit of detection (LOD) of 11.08 nM (S/N = 3), and a sensitivity of 29020 μA cm^{-2} mM^{-1}. The proposed electrode identified dopamine levels in human serum samples with a satisfactory recovery rate and selectivity.

Shenbagam, Sivasankari, Boobalan, Uma Sivakami, and Kannagi (2023) developed amperometric sensors for detecting dopamine at 0.1 M KNO_3 (pH 7). The modified graphite composite electrode (GCE) has a suitable amount of chromium placed on the surface of MWCNT/RTIL/GCE, followed by electrodeposition of hexacyanoferrate (CrHCF). The modified electrode detects partial discharges well, with a linear range of 0.1 μM to 4.0 μM and a detection limit of 0.0121 nm (signal-to-noise ratio = 3).

In another study, Radha, Kumar, Chappanda, and Aggarwal (2023) used a Zr-NDI-based metal–organic frameworks (MOF) and its composite Zr-NDI/MWCNT to detect dopamine using fluorescence and electrochemical methods in an aqueous phosphate buffer. The limits of detection for MOF and Zr-NDI/MWCNT were 2 M and 0.6 M, respectively, using fluorescence microscopy and differential pulse voltammetry. The sensor has great selectivity and sensitivity in the presence of the most frequent interfering analytes, such as uric acid and ascorbic acid. The sensor also displayed outstanding stability, with over 95% retention in recyclability tests and a high recovery rate (99–105%) in the presence of an actual urine sample.

1.6.3 Multi-walled Carbon Nanotube (MWCNT) and Its Application in Electroanalysis of Ascorbic Acid

Motsaathebe and Fayemi (2022) reported their study on the electrochemical detection of ascorbic acid in oranges utilizing screen-print carbon electrodes (SPCEs) made of a nanocomposite of antimony oxide nanoparticle and carbon nanotube (MWCNT-AONP). In comparison to other manufactured electrodes, the electrode modified by the nanocomposite demonstrated electrocatalytic response towards ascorbic acid and increased electron transfer. Compared to the bare electrode, the current response at the electrode modified by the nanocomposite was four times higher. regression value R^2 = 0.985 was obtained when utilizing SWV to detect ascorbic acid. The sensitivity and limit of detection (LOD) at the nanocomposite modified electrode were 0.3663 [ascorbic acid]/μM and 140 nM, respectively, with linearity from 0.16–0.64 μM.

For the sensitive detection of ascorbic acid, Huang et al. (2019) created a novel electrochemical sensor based on a zinc porphyrin dye (YD2-o-C8, also known as YD) functionalized MWCNTs hybrid. YD inner electroactivity was stimulated on a modified glass carbon electrode (GCE) in an aqueous solution using a cationic surfactant of tetraoctylammonium bromide (TOAB). Under optimal circumstances, the sensor showed a linear response to ascorbic acid in the concentration range of 18.72 μM to 1.85 mM, a low limit of determination (0.18 μM), and a high sensitivity of 19.16 μA mM^{-1}. Additionally, the TOAB/YD/MWCNT/GCE ascorbic acid reaction in an aqueous solution demonstrated exceptional stability and reproducibility. For vitamin C tablets, the recommended detection recoveries varied from 97.1% to 105.2%, indicating the precision and potency of ascorbic acid determination in actual samples.

The developed electrode had a notable impact on the electrochemical response signal of ascorbic acid detection, as shown by a series of electrochemical tests using the modified electrode NiO@$LaMnO_3$/MWCNTs. With a linear regression equation of $I_{(\mu A)} = 0.5902C$ (μmol/L) + 1.4407 ($R^2 = 0.9909$), and a detection limit of 2.41 μmol/L (S/N = 3), the current response increases linearly in the range of 2.60–36.44 μmol/L. Moreover, the electrochemical sensor NiO@$LaMnO_3$/MWCNTs/GCE showed good selectivity, stability, repeatability, and can be applied to the analysis of ascorbic acid content in actual beverages. By combining the aminated NiO@$LaMnO_3$ p-p homotype heterojunction with carboxylated MWCNTs via electrostatic self-assembly, the NiO@$LaMnO_3$/MWCNTs core–shell p-p homotype heterojunction was fabricated. This heterojunction served as the electrode material for the electrochemical sensor that was used to detect ascorbic acid (Zhong et al., 2023).

A built-in microelectrode called the gelatin-multiwalled carbon nanotubes/carbon fiber microelectrode (gelatin-MWCNTs/CFME) was used to monitor the neurochemical molecule utilizing the protein-pre-treated method (Deng et al., 2022). The microelectrode was used to detect neurochemical compounds in a human blood sample with consistent sensitivity and free from protein interference. Gelatin-MWCNTs shown excellent biocompatibility. To preserve the biological activity of the biorecognition element and guarantee the stability of the test results, it is essential to have strong biocompatibility. Gelatin-MWCNTs are an excellent choice for in vivo electrochemical sensing applications because of the improved properties and increased biocompatibility of CNTs. Because gelatin and gelatin-based composites are inexpensive, environmentally friendly, and have outstanding biocompatibility, they are perfect for creating biosensors. Guan, Huang, and Li (2022) and Rajkumar,

Thirumalraj, Chen, and Chen (2017) both mention that gelatin's good film-forming characteristic allows it to immobilize biorecognition components without compromising the sensitivity and stability of biosensors.

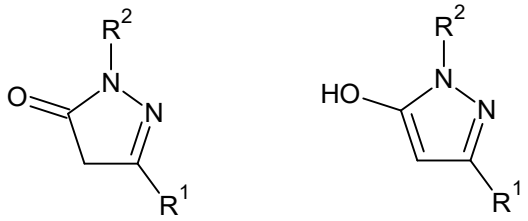
1.7 Pyrazolone-based Ligands

The chemistry has a large number of heterocyclic compounds. The cyclic organic compounds with at least one hetero atom are known as heterocyclic compounds. The three heteroatoms that are most common are nitrogen, sulphur, and oxygen. Over the past 20 years, the discipline of organic and medicinal chemistry, as well as the pharmaceutical industry, have paid close attention to nitrogen-containing heterocyclic molecules because of their diverse range of properties. One of the most important and distinctive classes of applied organic chemistry is nitrogen-based heterocyclic chemistry, where a large amount of research is devoted to the development of novel compounds and composites.

Because of their many uses and distinguishing characteristics, pyrazolones have been the subject of much research (Zhao et al., 2020). One of the most significant heterocyclic compounds is pyrazolones, which are lactam rings with five members that have a nitrogen-nitrogen bond and an additional ketonic group (C=O). Although they can also be found in the enol form, pyrazolones are primarily found in the keto form (Figure 1.4).

Figure 1.4

Keto and enol form of pyrazolone

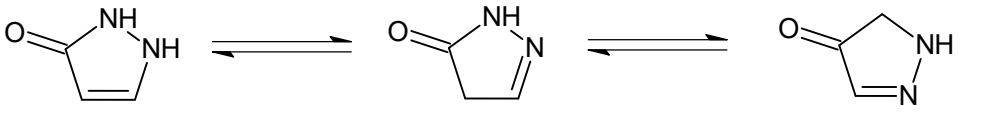


Keto form Enol form

Oxo derivatives of pyrazolines are known as pyrazolones. These compounds have an aromatic quality because their nucleus contains two double bonds. The pyrazolones are categorized as follows: 3-pyrazolone (also called 3-pyrazoline-5-one), 5-pyrazolone (also called 2-pyrazoline-5-one), and 4-pyrazolone (also called 2-pyrazoline-4-one) (Figure 1.5).

Figure 1.5

Classification of pyrazolones



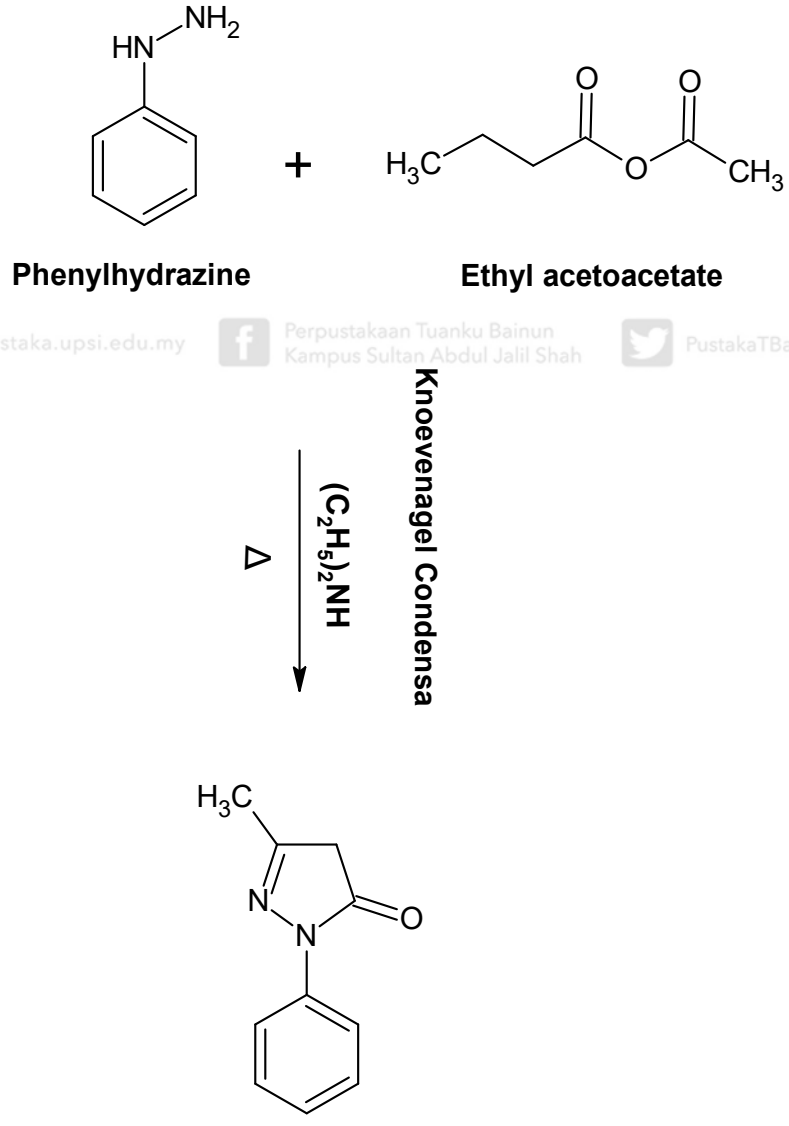
3-pyrazolin-5-one 2-pyrazolin-5-one 2-pyrazolin-4-one

Chemical synthesis is one way to produce pyrazolone compounds; 1-phenyl-3-methyl-5-pyrazolone (PMP) is one such compound that is commonly used as the primary precursor model in pyrazolone research due to its availability and need for a

straightforward preparation process (Zhang, Hu, Wang, Zhao, & Chen, 2023). The first pyrazolone was successfully produced fourteen decades ago in 1883 by German chemist Ludwig Knorr via a condensation process between phenyl hydrazine and ethyl acetoacetate (Figure 1.6) (Mustafa et al., 2022).

Figure 1.6

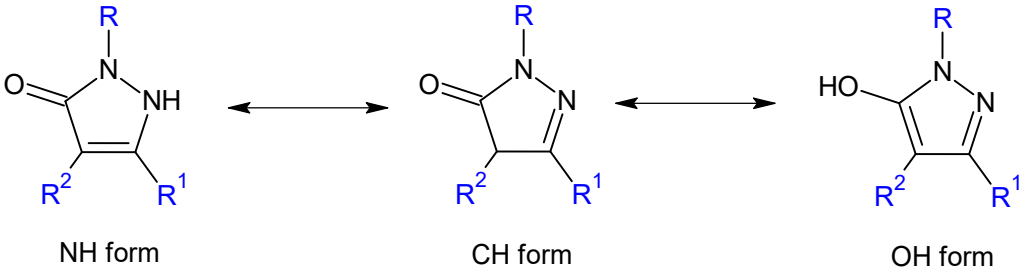
Synthesis route of 1-phenyl-3-methyl-5-pyrazolone



Because of the three distinct isomeric forms (CH, NH, OH) resulting from lactam-lactim tautomerism, imine-enamine tautomerism, and keto-enol tautomerism (Figure 1.7) (Pettinari & Pettinari 2005; Nishihama, Hirai, & Komasawa, 2001), these compounds are structurally intriguing. Because changes in structure result in changes in attributes, these phenomena may have an impact on targets' biological activities, their responsiveness, and the synthetic processes in which they participate (Zainul et al., 2022; Adhikari, Singh, Sharma, & Arora, 2021; Kijun & Turel, 2017).

Figure 1.7

Isomeric forms of 3-pyrazolin-5-one



Many drugs, vitamins, antibiotics, natural substances like alkaloids, and medicinally active biomolecules contain pyrazolone and its derivatives, which are highly promising heterocyclic scaffolds (Li, Yu, & Tu, 2021; Shaabani, Nazeri, & Afshari, 2019; Cherukupalli et al., 2018). The use of pyrazolone-based ligands as pigments for dyes, functional materials, biological applications, catalysis, and sensors is growing in popularity. Due to their important pharmacological properties and biological activities in medicinal chemistry, such as their lipid-lowering, antihyperglycemic, anti-proliferative, analgesic, antitumor, antimicrobial, antioxidant,

CNS activity, anti-inflammatory, anti-tubercular, antiviral, and enzyme inhibitory properties, they are drawing increased attention (Mustafa et al., 2022; Zhao et al., 2020).

The synthesis of functionalized pyrazolone structures has become more and more popular in synthetic chemistry, due to its remarkable biological properties. An endless number of works have been reported in this regard (Barad, Chaudari, Roy, Butcher, & Jadeja, 2024; Ali et al., 2022; Becerra, Abonia, & Castillo, 2022). The development of a novel electrochemical sensor based on pyrazolone skeleton is crucial given the significance of pyrazolones and their derivatives in synthetic and medicinal chemistry (Xu & Qian, 2019).

Li et al. (1997) identified pyrazolones, particularly 4-acylpyrazolone derivatives, as a distinctive family of β-diketones that have a pyrazole fused to a chelating arm. Because of their enormous biological potential (anti-bacterial, anti-tumour, anti-oxidant, anti-pyretic, anti-infective, protective neurons, analgesic), electrical, photochromic, fluorescence probe, and catalytic activities, the chemistry of acylpyrazolone complexes has attracted a lot of attention in the last few decades (Travadi, Jadeja, & Butcher, 2024). Significant differences in the ligand's solubility, steric, and electronic characteristics are brought about by the type of substituent in the pyrazolone ring's fourth position, which has an impact on the ligand's chemical and biological activities. The acylpyrazolones' enol form OH is suited for electrochemical sensing applications since it is mildly acidic (Ghoneim et al., 2008; Li, Li, & Li 2007; Xu et al. 2005). The electronic characteristics of the ligands are improved by electron-withdrawing substituents such fluorine in 1,3-*β*-diketone pyrazolones (Dalal et al., 2022; De Sa et al., 2000). Zong, Wei, Yan, and Fan (2018) state that 4-benzoyl-3-

methyl-1-phenyl-2-pyrazoline-5-one and its derivatives are widely used for desensitization and sterilization as well as for determining the amounts of trace rare earth elements in steel and nonferrous metals, nitrate, nitrite, and iodine.

Since acetaminophen, dopamine, and ascorbic acid are found in physiological fluids, it is critical to monitor their amounts when analysing and diagnosing illnesses. There have been published methods for detecting acetaminophen, dopamine, and ascorbic acid. Nevertheless, there hasn't been any information published about electrochemical sensors containing 4-acylpyrazolone scaffolds that respond to such analytes. This study discusses the qualities of the developed ligands that improve the multiwalled carbon nanotubes' ability to operate as sensors and their potential uses.

1.8 Scope of Study

In this study, 1-phenyl-3-methyl-5 pyrazolone (PMP) was used as the starting material to develop 4-acyl-5-pyrazolone ligands. Due to its inexpensive cost and the extensive use of C-acylated compounds as extractants in separation science, PMP has been selected as the starting material. Additionally, three different forms of aroyl chlorides (benzoyl chloride, 2-fluorobenzoyl chloride, and 3-fluorobenzoyl chloride) were used as source materials. The synthetic ligands' aromaticity enables them to absorb onto the CNT surfaces via π-π force, resulting in the formation of the composite film. Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (^{1}H NMR, ^{13}C NMR) were used to characterize the ligands in order to confirm their structure.

Acetaminophen, dopamine, ascorbic acid, and potassium ferrocyanide ($K_4Fe[CN]_6$) were the analytes used in the preliminary investigation of all ligands using CV, which is frequently employed for preliminary analysis of electrochemically active systems. It provides useful details on the analytes, modifier, electrode, and reaction process, as well as reduction and oxidation potentials.

Acetaminophen, dopamine, and ascorbic acid were the three analytes of interest that were determined using an electrochemical analysis, which is widely acknowledged as a potent technique for analytes determinations because of its simple operation, inexpensive cost, and speedy analysis. The methods relied on SWV and CV. Carbon-based electrodes, or carbon paste were used as the working electrode in this investigation. An electrode based on carbon has been selected as a reliable working electrode due to its capacity to form strong covalent bonds with various materials for modification. Furthermore, because of its special qualities such as its wide cathodic and anodic functions, pleasant surface renewal ability, and low background current, carbon paste electrode (CPE) was selected for the electrochemical sensor fabrication.

1.9 Problem Statement

Acetaminophen (APAP), also referred to as paracetamol, is an organic compound that is frequently used in pharmaceutical products for pain management (Chetana et al., 2023). At typical therapeutic dosages, APAP rapidly conjugates with glutathione and is mostly metabolized in the liver using first-order kinetics, creating cysteine and mercapturic acid conjugates. However, APAP overdose (consumption of a single dose

more than 7 g in an adult and 150 mg/kg in a child) can result in the accumulation of toxic metabolites, causing severe and occasionally fatal hepatotoxicity and nephrotoxicity (Magerusan, Pogacean, & Pruneanu, 2022).

Hospital waste, manufacturing facilities, consumer use, and disposal all contribute to the ongoing introduction of APAP into the aquatic environment (Tasic et al., 2021). The prevalence of APAP in the environment is due to inadequate removal during wastewater treatment and poor medication disposal; hence, these compounds may persist in treated effluent and infiltrate surface waterways. According to the literature, the concentration of paracetamol in natural waters around the world varies greatly depending on factors such as local usage patterns, wastewater treatment effectiveness, and environmental conditions. Concentrations ranging from 1–100 μg/L were found in wastewaters from Korea, Spain, and the Western Balkans (Miglione et al., 2024).

It has been established that low concentrations of APAP can have detrimental effects on aquatic creatures, including acute and chronic damages, as well as reproductive damages and inhibition of cell growth in human cells (Pollap et al., 2020). Negative long-term impacts could result from a high transformation rate and continuous pollutant infiltration into the ecosystem (Montaseri & Forbes, 2018).

As a result, sensitive analytical methods are needed to identify APAP in various biological and environmental samples. Since APAP is an electroactive compound, electrochemical analysis techniques can be applied to its examination. For the purpose of determining APAP, various researchers have developed modified electrodes, such as

CZTS/MoS_2/CNT (Chetana et al., 2023), Gd_2ZnMnO_6/ZnO/Ionic liquid/CPE (Javad Tavakoli et al., 2023), CPE/NiZ/GO (Porada et al., 2023), CdO/MCPE (Manjunatha et al., 2023), MWCNT/GO/Poly (Thr)/GCE (Prasad et al., 2023), ND@Dy_2O_3-IL/CPE (Ghadirinataj et al., 2023), and Stv-CPE (Gharous et al., 2023).

Dopamine (DOP) is one of the most important neurotransmitters. Under physiological conditions, normal DOP concentrations in brain and body fluids range between 10 and 1000 nM (Liu & Liu, 2021). DOP plays a direct or indirect role in almost every physiological function, including neuronal handling, synaptic activities, Parkinson's disease, and mental health issues such as depression, anxiety, stress, Alzheimer's disease, and schizophrenia (Franco et al., 2021). According to the WHO mental health survey conducted in August 2020 in 130 countries, COVID-19 has had a considerable detrimental influence on mental health (Masood et al., 2022). DOP has been linked to depression and anxiety, although the underlying mechanisms remain unclear (Šik Novak et al., 2022). Because abnormal amounts of those neurotransmitters can induce a variety of behavioral and physiological problems, it is critical to assess DOP concentration in human metabolic fluid (Lakard et al., 2021).

Vitamin C, or ascorbic acid (AA), is a nutrient that plays a role in a number of physiological and biochemical reaction mechanisms, including blood vessel maintenance, metal ion absorption, collagen synthesis acceleration, and adrenal gland release. Additionally, AA's ability to scavenge free radicals benefits human tissue by maintaining the equilibrium between oxidation and reduction. It is also essential for the growth of the metabolic functions of the human body, such as cell differentiation, collagen synthesis, anti-inflammatory steroid synthesis, and amino acid metabolism. In

human plasma, the concentration of AA usually falls between 33 μM to 111 μM. Serious conditions like scurvy, cancer, Alzheimer's, Parkinson's, and cardiovascular illnesses are brought on by AA concentrations below the clinical limit. However, too much AA leads to diarrhea, kidney stones, and stomach cramps (Gautam, Verma, Ram, Singh, & Sarkar, 2024; Darabi et al., 2023; Dhara & Debiprosad, 2019; Kong et al., 2023). Consequently, it is essential to find AA under ideal circumstances. For the sensitive and selective current detection of AA, a variety of methods, including electroanalytical analysis, have already been reported (Anwar et al., 2023). However, it can be difficult to evaluate AA by direct electrooxidation using common electrodes such glassy carbon electrode (GCE), Pt, Au, and Hg due to the fouling effect that oxidation exerts on the electrode surface. Accordingly, the problem that arose might be fixed by electrochemically altering the electrode surface (Skrovankova et al., 2015).

Bulky, intricate, and costly chromatography and spectroscopy setups, such as gas chromatography, high-performance liquid chromatography, flame absorption spectrometry, electrothermal atomic absorption spectrometry, and inductively coupled plasma-optical emission spectrometry, are typically used in the analytical methods for the determination of APAP, DOP, and AA (Brombach et al., 2015; Zhou et al., 2013). Some drawbacks limit the scope of study. Despite the fact that these analytical methods have advantages like being suitable for a wide range of compounds and having a high sensitivity with a lower limit of detection, they must be handled by qualified operators, and are not suitable for on-site monitoring due to the complex analytical procedures and multistep sample preparation. Additionally, they require additional extraction and separation techniques and are time-consuming.

Because of its many benefits, including its ease of integration into point-of-care systems, selectiveness, sensitivity, and demonstrated cost-effectiveness, electrochemical sensors offer an alluring substitute for the analysis of dopamine, ascorbic acid, and acetaminophen in order to mitigate these drawbacks (Mujawar et al., 2020).

1.10 Significant of the Study

Common approaches for determining the concentration of identified analytes rely heavily on chromatography and spectroscopic techniques. However, due to the elaborate and sophisticated apparatus, these methods are difficult and unsuitable for in-situ measurements. Regardless, electrochemical methods have raised interest rates. This is due to electrochemical technologies' ease of fabrication and manipulation, low cost, rapid reaction and non-destructive analysis, strong selectivity over a wide range of concentrations, and relatively low detection limits.

The electrode material has a significant impact on both the analytical functional performance of CV and SWV. Carbon-based electrodes have been a great choice for use as a working electrode due to the benefits they provide, such as the ability to scan to more negative potentials than platinum or gold, as well as good anodic potential windows. Furthermore, they have exceptional electrochemical stability across a wide range of potentials while having minimal electrical resistance. Because of their hydrophobic nature or other affinities for the electrode, a substantial number of the various reaction products (unwanted samples and contaminants from the aqueous

solution) typically accumulate on the bare electrode surface. It decreases reproduction and slows the kinetic electron transfer mechanism. To overcome the aforementioned limitations, electrode surface modification has become one of the fastest expanding fields of research in electrochemistry.

Ligands produced from oxygen and nitrogen-containing compounds play an essential role as electrode modifiers. These ligands have a diversity of binding sites, resulting in increased coordination, kinetics, and stability. Because of their unique electron transferability and π-conjugation system of the 4-acylpyrazolone ligand, these series of compounds can be used as a chelating agent and modulator in electrochemistry. The advantages of multi-walled carbon nanotubes with 4-acylpyrazolone ligands for life science research as easy, accurate, and sensitive targeted analytes detectors would be advantageous.

Based on the points raised above, this work produced new modified carbon-based electrodes that use ligands formed from the combination of PMP and benzoyl chloride as electrode modifiers to determine specific analytes utilizing electrochemical methodologies. The novelty of this study can be described as follows:

a) New modified electrode using 4-acylpyrazolone ligands as a modifier to improve detection performance by at least 50% over the unmodified electrode (by comparing electrochemical performance indicators such as peak current (I_p) from cyclic voltammetry (CV), charge transfer resistance (R_{ct}) from electrochemical impedance spectroscopy (EIS), and limit of detection (LOD)

and sensitivity from square wave voltammetry (SWV)) for the determination of selected analytes in pharmaceutical formulations and human urine samples.

b) Fabrication of a novel 4-acylpyrazolone ligand-multi-walled carbon nanotube-modified carbon paste electrode with high sensitivity, outstanding reproducibility, and stability for the detection of acetaminophen, dopamine, and ascorbic acid.

From a thorough search of the relevant literature, there has been no report on the use of fluorinated ligand derivatives of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone-modified carbon nanotubes for their end-use performance as an electrochemical sensing of bioanalytes such as acetaminophen, dopamine, and ascorbic acid. As a result, this study is significant since it suggests a new alternative for determining the aforementioned compounds. Furthermore, it is a particularly convenient instrument that does not require any complicated operations. This approach consumed less power and was plainly less expensive than the other alternatives.

1.11 Objectives of the Study

This research intends to develop pyrazolone-based electrode modifiers for quick determination of specified analytes, with the following objectives:

1. To synthesize and characterize 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (HPMBP), 1-phenyl-3-methyl-4-(2-fluorobenzoyl)-5-pyrazolone (HPMoFBP), and 1-phenyl-3-methyl-4-(3-fluorobenzoyl)-5-pyrazolone (HPMmFBP) using morphological and spectroscopic techniques such as field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), energy dispersive X-ray spectroscopy (EDX), and X-ray diffraction spectroscopy (XRD).
2. To fabricate and investigate multiwalled carbon nanotube (MWCNT)/carbon paste electrode (CPE) modified with HPMBP, HPMoFBP, and HPMmFBP using electrochemical techniques.
3. To evaluate the performance of the fabricated MWCNT/CPE modified with HPMBP, HPMoFBP, and HPMmFBP towards acetaminophen, dopamine, and ascorbic acid, respectively.
4. To investigate the application of the fabricated MWCNT/CPE modified with HPMBP, HPMoFBP, and HPMmFBP for real samples determination.

1.12 Thesis Outline

This thesis contains six chapters. The first chapter provides a summary of the research, the problem statement to be addressed, the research aims, and the study's significance. Chapter 2 presents a literature review of the evaluation of conventional techniques, electroanalytical techniques, carbon-based material for modified electrodes, electrode modifiers, and the details of selected analytes.

Chapter 3 describes the materials and detailed research methodology used in the synthesis and characterization of the ligands 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (HPMBP), 1-phenyl-3-methyl-4-(2-fluorobenzoyl)-5-pyrazolone (HPMoFBP), and 1-phenyl-3-methyl-4-(3-fluorobenzoyl)-5-pyrazolone (HPMmFBP), as well as their electroanalytical studies towards acetaminophen (APAP), dopamine (DOP) and ascorbic acid (AA), respectively.

Chapter 4 describes the features of the ligands as determined by ^{1}H NMR, ^{13}C NMR, FTIR, EDX, XRD, FESEM and TEM. Chapter 5 describes the findings of modifying a carbon paste electrode (CPE) with multi-walled carbon nanotubes (MWCNTs) and the synthetic ligands HPMBP, HPMoFBP, and HPMmFBP to fabricate HPMBP/MWCNT/CPE, HPMoFBP/MWCNT/CPE, and HPMmFBP/MWCNT/CPE for APAP, DOP, and AA detection, respectively. CV, SWV, and EIS were used to investigate the electrochemical activity of the modified electrodes in the detection of analytes. The experimental parameters used in this investigation were pulse size, step size, frequency, pH value of the supporting

electrolyte, and percentage of modifiers. The developed electrodes were used to analyse APAP, DOP, and AA levels in pharmaceutical formulations and urine samples.

Finally, Chapter 6 of this thesis summarizes the study's overall outcomes. Several suggestions for improving the research results are presented. In the final section of this chapter, potential applications of synthesized acylpyrazolone as a material for electrode modification for future study are discussed.