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UPNM

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ELECTROCHEMICAL APTASENSOR BASED POLYPYRROLE-IRON OXYHYDROXIDE NANOCOMPOSITE FOR DETECTION OF DIMETHYL METHYLPHOSPHONATE (DMMP)

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Thesis submitted to the Centre for Graduate Studies, Universiti Pertahanan Nasional
Malaysia, in fulfilment of the requirements for the Master Degree of Chemistry

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ABSTRACT

Chemical warfare agents (CWAs) such as nerve agents, blister agents, blood agents and incapacitating agents were used by various military and terrorist groups in several conflicts in previous decades, prompting the need to construct and improve existing sensor systems for detecting these compounds. Many methods have been used to detect these substances, including traditional methods involving large instruments (gas chromatography, liquid chromatography and ion mobility spectrometry). Since more human resources are required to handle the equipment and it is heavy to move around the place, these procedures come at a high cost. Furthermore, there is a biosensor that can give on-site detection that is rapid, portable, low-cost and selective. However, due to the obvious extreme toxicity of CWAs, dimethyl methylphosphonate (DMMP) has been used to simulate their effects due to their almost identical molecular structures. The present biosensors, on the other hand, only use sensing materials that are either sensitive or selective to DMMP. In an electrochemical sensor, there are no sensing materials that combine the traits of being very sensitive and selective towards DMMP. This work was carried out in order to build and improve the existing biosensor for detecting DMMP. The polypyrrole nanoparticles (PPy NPs) and polypyrrole-iron oxyhydroxide (PFFs) nanocomposite were successfully synthesised using a chemical oxidative polymerization method with two different sonication periods (one and three hours). One-hour sonication produced finer nanoparticles than three hours of sonication. The enlargement of the sharp peak of –NH stretching at 3217 cm^{-1} confirmed the polymerisation of the pyrrole monomer, whereas the disappearance of the N-H band and the appearance of a new –OH sharp band at roughly 3500 cm^{-1} indicated the interaction between iron oxyhydroxide (FeOOH) and PPy NPs. FESEM pictures demonstrated that the 5 wt% FeOOH nanocomposites had iron metals that were well distributed in the PPy NPs, as opposed to the 10 wt % FeOOH nanocomposites, which displayed particle aggregation. The obtained PPy NPs and PFFs nanocomposite sizes were between 50 and 70 nm and 110 and 160 nm, according to TEM examination. Using cyclic voltammetry, the best sensing materials for the electrochemical inquiry were found to be one hour sonication of PPy NPs (36%) and 5 wt% PFFs (22%) nanocomposites (CV). The successful synthesised PPy NPs and PFFs nanocomposite as sensing materials were determined as the initial objective. The aptasensor made from PPy NPs and PFFs are optimised under two conditions: aptamer concentration and incubation times. 1 μM of aptamer incubated for 1 hour is optimal for the PPy NPs aptasensor, while 5 μM of aptamer incubated for the same 1-hour time is optimal for the PFFs aptasensor. Newly developed electrochemical sensors based on aptamer functionalized PPy NPs and PFFs were also evaluated for sensitivity and selectivity. The PPy NPs sensor has a higher sensitivity of LOD 3.576 ppm than the PFFs nanocomposites sensor LOD of 5.802 ppm. The finer the particles, the greater the sensor's electron transfers, resulting in a rise in peak current. Furthermore, when other analytes (methanol, DCM, acetonitrile, and hexane) were present, the presence of aptamer integration of PPy NPs and PFFs nanocomposites sensors improved the sensor's selectivity towards the target analyte DMMP.

ABSTRAK

Ejen perang kimia (CWA) seperti agen saraf, agen lepuh, agen darah dan agen yang melumpuhkan telah digunakan oleh pelbagai kumpulan tentera dan pengganas dalam beberapa konflik dalam dekad sebelumnya, menyebabkan keperluan untuk membina dan menambah baik sistem sensor sedia ada untuk mengesan sebatian ini. Banyak kaedah telah digunakan untuk mengesan bahan ini, termasuk kaedah tradisional yang melibatkan instrumen besar (kromatografi gas, kromatografi cecair dan spektrometri mobiliti ion). Memandangkan lebih banyak sumber manusia diperlukan untuk mengendalikan peralatan dan ia adalah berat untuk bergerak di sekitar tempat itu, prosedur ini memerlukan kos yang tinggi. Tambahan pula, terdapat biosensor yang boleh memberikan pengesanan di tapak yang pantas, mudah alih, kos rendah dan terpilih. Disebabkan ketoksikan melampau CWA yang jelas, dimetil metilfosfonat (DMMP) telah digunakan untuk mensimulasikan kesannya kerana struktur molekulnya yang hampir sama. Biosensor sekarang, sebaliknya, hanya menggunakan bahan penderiaan yang sama ada sensitif atau selektif kepada DMMP. Dalam penderia elektrokimia, tiada bahan penderiaan yang menggabungkan ciri-ciri menjadi sangat sensitif dan selektif terhadap DMMP. Kerja ini dijalankan untuk membina dan menambah baik biosensor sedia ada untuk mengesan DMMP. Nanopartikel polipirol (PPy NPs) dan nanokomposit polipirol-ferum oksihidroksida (PFFs) telah berjaya disintesis menggunakan kaedah pempolimeran oksidatif kimia dengan dua tempoh sonikasi berbeza (satu dan tiga jam). Sonikasi selama satu jam menghasilkan nanozarah yang lebih halus daripada tiga jam sonikasi. Pembesaran puncak tajam regangan –NH pada 3217 cm-1 mengesahkan pempolimeran monomer pirol, manakala kehilangan jalur NH dan penampilan jalur tajam –OH baharu pada kira-kira 3500 cm-1 menunjukkan interaksi antara oksihidroksida besi (FeOOH) dan PPy NPs. Gambar FESEM menunjukkan bahawa nanokomposit FeOOH 5% berat mempunyai logam besi yang diedarkan dengan baik dalam PPy NPs, berbanding dengan nanokomposit FeOOH 10 wt%, yang memaparkan pengagregatan zarah. Saiz nanokomposit PPy NPs dan PFFs yang diperoleh adalah antara 50 dan 70 nm dan 110 dan 160 nm, mengikut peperiksaan TEM. Menggunakan voltammetri kitaran, bahan penderiaan terbaik untuk siasatan elektrokimia didapati adalah sonikasi satu jam PPy NPs (36%) dan 5% berat PFFs (22%) nanokomposit (CV). Kejayaan mensintesis PPy NPs dan PFFs nanokomposit sebagai bahan penderiaan ditentukan sebagai objektif awal. Aptasensor yang diperbuat daripada PPy NPs dan PFFs dioptimumkan di bawah dua keadaan: kepekatan aptamer dan masa inkubasi. 1 µM aptamer yang diinkubasi selama 1 jam adalah optimum untuk aptasensor PPy NPs, manakala 5 µM aptamer yang diinkubasi untuk masa 1 jam yang sama adalah optimum untuk aptasensor PFFs. Sensor elektrokimia yang baru dibangunkan berdasarkan aptamer berfungsi PPy NPs dan PFFs juga dinilai untuk kepekaan dan selektiviti. Sensor PPy NPs mempunyai kepekaan yang lebih tinggi iaitu LOD 3.576 ppm lebih rendah daripada sensor nanokomposit PFFs sebanyak 5.802 ppm. Lebih halus zarah, lebih besar pemindahan elektron sensor, mengakibatkan kenaikan arus puncak. Tambahan pula, apabila analit lain (metanol, DCM, asetonitril, dan heksana) hadir, kehadiran penyepaduan aptamer PPy NPs dan sensor nanokomposit PFFs meningkatkan selektiviti sensor terhadap DMMP analit sasaran.

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APPROVAL

I certify that an Examination Committee has met on 23rd March 2022 to conduct the final examination of Farah Nabila Binti Diauudin on her master degree thesis entitled 'Electrochemical Aptasensor Based Polypyrrole-Iron Oxyhydroxide Nanocomposite for Detection of Dimethyl Methylphosphonate (DMMP)'. The committee recommends that the student be awarded the Master Degree in Science (Chemistry).

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APPROVAL

This thesis was submitted to the Senate of Universiti Pertahanan Nasional Malaysia and has been accepted as fulfilment of the requirements for the **Master Degree of Chemistry**. The members of the Supervisory Committee were as follows.

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Date of birth : 24th February 1995

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Academic session : January 2019

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TABLE OF CONTENTS

CHAPTER

1 INTRODUCTION

2 LITERATURE REVIEW

3 METHODOLOGY

4 RESULTS AND DISCUSSIONS

5 CONCLUSION AND FUTURE WORKS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

CWAs	Chemical warfare agents
Ops	Organophosphorus
CNS	Central nervous system
RCAs	Riot-control agents
AChE	Acetylcholinesterase
ACh	Acetylcholine
GA	Tabun
GB	Sarin
GD	Soman
GF	Cyclosarin
VX	O-ethyl S-diisopropylaminomethyl methylphosphonothiolate
VR	S-[-(diethylamino)ethyl] O-(2-methylpropyl) methylphosphonothioate
CVX	O-Butyl S-[2-(diethylamino)ethyl] methylphosphonothioate
SAW	Surface acoustic wave
MEMS	Microelectromechanical systems
VOCs	Volatile organic compounds
PANI	Polyaniline
PPy	Polypyrrole
PTs	Polythiophenes
DMMP	Dimethyl methylphosphonate
DCP	Diethyl chlorophosphate
DFP	Diisopropyl fluorophosphate

DMNP	Dimethyl p-nitrophenyl phosphate
GC	Gas chromatography
LC	Liquid chromatography
AED	Atomic emission detection
QCM	Quartz crystal microbalance
OCP	Organic conducting polymer
Fe_2O_3	Iron oxide
TiO_2	Titanium oxide
ZnO	Zinc oxide
PPy NPs	Polypyrrole nanoparticles
PFFs	Polypyrrole-iron oxyhydroxide
FTIR	Fourier Transform Infrared Spectroscopy
FESEM	Field Emission Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
SPCE	Screen-printed carbon electrode
$[Fe(CN)_6]^{3-}$	Ferricyanide
CV	Cyclic voltammetry
DPV	Differential pulse voltammetry
MeOH	Methanol
DCM	Dichloromethane
SnO_2	Tin (II) oxide
WO_x	Tungsten oxide
Pd	Palladium
Pt	Platinum
PPy/TiO_2	Polypyrrole/titanium oxide

NO_2	Nitrogen dioxide
mV/s	Millivolt per unit seconds
Ppm	Part per million
HCL	Hydrochloric acid
NH_3	Ammonia
PEDOT	Poly (3,4 ethylenedioxythiophene)
PTCPTA	Polythiophene copolymer
PEDOT-SSA	3,4-ethylenedioxythiophene- sulphosalicylic acid
OPH	Organophosphorus hydrolase
BuChE	Butyrylcholinesterase
ELISA	Immunosorbent assays
DETP	Diethyl thiophosphate
DEDTP	Diethyl dithiophosphate
DEP	Diethyl phosphate
IMS	Ion mobility spectrometry
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
UV-VIS	Ultraviolet-visible
AuNPs	Gold nanoparticles
ATChI	Acetylthiocholine iodide
Ppb	Part per billion
LOD	Limit of detection
FeOOH	Iron oxyhydroxide

CHAPTER 1

INTRODUCTION

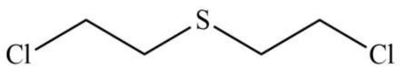
1.1 Background of the Research

Chemical warfare agents (CWAs) are substances that have been developed with the intent of incapacitating a target and can be employed as weapons in a war scenario (Ganesan et al., 2010). This chemical weapon has the potential to induce momentary incapacitation, long-term health damage, and death. Gases, liquids, and solids all include in the classes of CWAs. They are distinguished by their method of action and are referred to be fast-acting substances because of their principal effect (Pacsial-Ong and Aguilar, 2013). This type of chemical agents has been used as a weapon since the ancient time. The Peloponnesian War became one of the first war utilized the chemical agents as a weapon by burning the mixture of sulphur, coal and pith towards their enemies (Gillespie, 2011). Boeotians and their allies attacked Delium using those chemical agents by directing the smoke and flame into the village through a hollowed-out log (Sidell, 1998).

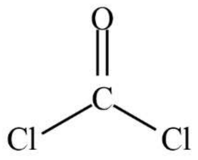
However, the CWAs became increasingly well-known during World Wars I and II (Ganesan et al., 2010). During World War I, vesicants, or blistering agents, were among the first chemicals to be used as lethal or tactical weapons. The most extensively used mustard agents, often known as sulphur mustard or mustard gas (Balalimoud and Balali, 2009). Mustard gas was first used in World War I in July 1917, in Ypres, Belgium, during the Battle of Flanders (the French name for mustard gas is Ypérite). It was then used in Ethiopia in 1918 and again in 1936. The mustard gas was the most widely used as chemical warfare agent during World War II, with many countries producing and stockpiling it. The gas remains as the most frequently used chemical warfare agent worldwide (Szinicz, 2005). The mustard gas was used more recently in the Egypt-Yemen War (1963–1967) and the Iraq-Iran war in 1984 (Koutsospyros et al., 2006).

These CWAs have unique qualities and belong to different groups of chemicals with distinct physicochemical, physiological, and chemical properties (Guide, 2000). As a result, the CWAs are categorized in a variety of ways including persistent or non-persistent agents based on their volatility. The faster the agent evaporates and disperses, the more volatile it is. Non-persistent agents for example chlorine, phosgene, and hydrogen cyanide are more volatile, whereas persistent agents, such as sulphur mustard and VX are less volatile (Black, 2016). Organophosphorus (OPs), organosulfur, organofluorine compounds and arsenicals can be classified according to their chemical structure (Bittencourt et al., 2019).

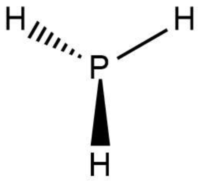
In general, types of CWAs are classified as blister, choking, blood, riot-control and nerve agents (D'Agostino et al., 2008). Blister agents including sulphur mustard and phosgene are found in oily compounds that react when inhaled or come into contact. Then, choking agents can induce alveoli to discharge fluid into the lungs in a continuous flow that leading victims to drown for instance phosphine gas and chlorine (Hurst, 2015). Hoenig, (Hoenig, 2007) claimed blood agents commonly cyanide-containing compounds such as hydrogen cyanide poison enzyme cytochrome oxidase, which prevents the body's cells from using oxygen. As a result, these chemicals inhibit the normal oxygen flow from the blood to the body's tissues. All body tissues, particularly those of the central nervous system (CNS), are affected quickly by a shortage of oxygen. Apparently, riot-control agents (RCAs) such as tear gas (2-chlorobenzalmalononitrile) and pepper spray (capsaicin) tend to inflict discomfort to anyone who has exposed or unprotected eyes, skin, or respiratory regions in order to regulate their activity (Fry, 2009). Figure 1.1 shows the chemical structure for sulphur mustard, phosgene, phosphine gas, hydrogen cyanide, 2-chlorobenzalmalononitrile and capsaicin.



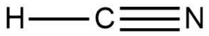
Sulphur mustard



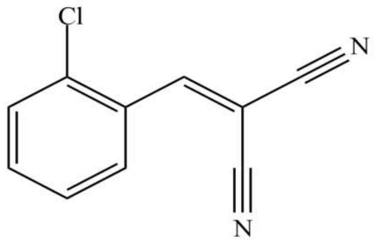
Phosgene



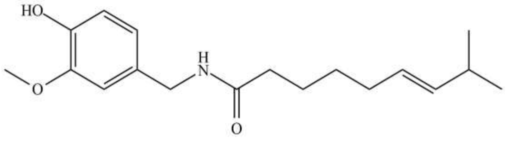
Phosphine gas



Hydrogen cyanide



2-chlorobenzalmalononitrile

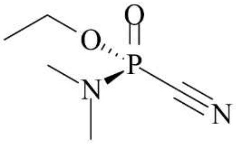


Capsaicin

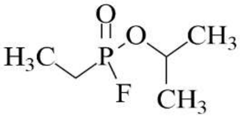
Figure 1.1: The chemical structure for sulphur mustard, phosgene, phosphine gas, hydrogen cyanide, 2-chlorobenzalmalononitrile and capsaicin.

Among all CWAs, nerve agents are recognized as the most dangerous and well-known chemical weapons. The nerve agents containing organophosphate compounds are very effective irreversible inhibitors of the acetylcholinesterase (AChE) enzyme, which hydrolyzes acetylcholine (ACh) (Zoltani, 2020). Overstimulation of muscarinic and nicotinic receptors occurs when ACh accumulates in the synaptic cleft. This cholinergic hyperactivity can have an impact on all organ systems. Hypersalivation, increased lung secretions, sweating, diarrhoea and respiratory arrest are all hazardous symptoms in humans due to the hyperactivity (Stojiljković et al., 2020). In general, the nerve agents are classified into two groups: G-series and V-series. The G-series is in liquid form and has a mild odour, commonly, spicy or fruity. The G-series gaseous compounds include tabun (GA), sarin (GB), soman (GD), and cyclosarin (GF) (Council, 2003).

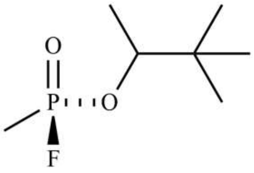
Meanwhile, the V-series gas inhibitors efficacy against AChE is remarkable and they are significantly more stable in biological systems than the G-agents. This condition results in persistent toxicologically relevant agent concentrations that make the exposure of the V-series gas causes a delayed development of clinical symptoms and necessitates long-term antidotes therapy (Wille et al., 2012). The gas in the V-series is an amber-coloured liquid and odourless. O-ethyl S-diisopropylaminomethyl methylphosphonothiolate (VX), S-[-(diethylamino)ethyl] O-(2-methylpropyl) methylphosphonothioate (VR) and O-Butyl S-[2-(diethylamino)ethyl] methylphosphonothioate (CVX) are all instances of the V-series gases (Stewart, 2006). Figure 1.2 depicts chemical structures of tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), VX, VR and CVX.



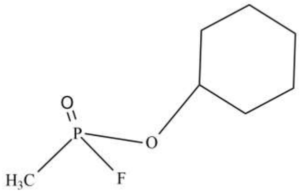
Tabun (GA)



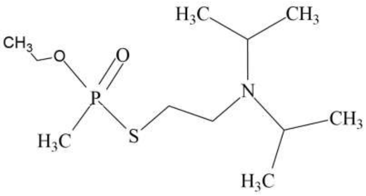
Sarin (GB)



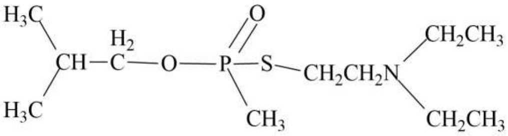
Soman (GD)



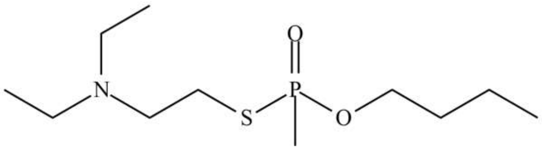
Cyclosarin (GF)



VX



VR



CVX

Figure 1.2: Chemical structures of tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), VX, VR and CVX

Sarin gas has been selected as one of the CWAs that need to be focused in this project as it is easily made by combining isopropyl alcohol with two widely available halogenated methyl-phosphonates. When these ingredients are combined, an exothermic reaction occurs thus, resulting in a production of sarin and hydrochloric acid. The output is tainted (l-10 percent sarin) and stockpiling the chemical necessitates a hazardous distillation process (Volans, 1996). On March 20 1995, 15 Tokyo subway stations were contaminated with a noxious chemical that was subsequently confirmed as a diluted version of sarin gas. During the Monday morning rush hour, five subway commuter vehicles were impacted. Terrorists carried diluted sarin solution in plastic bags into the subway trains and inserted a sharpened umbrella tip into these bags at the same time. There were 12 fatalities and 5,500 people who were ill due to the tragedy (Okumura et al., 1998).

The desire to construct a high sensitivity and selectivity chemical sensor for CWAs has become mandatory as sarin was spread in the eastern outskirts of Damascus on a night on 2013, killing 1400 civilians and badly injuring hundreds more (Rosman et al., 2014). The deceased was claimed to have displayed miosis (contraction of the pupils), other indications of cholinergic crisis and died within 24 hours after probable exposure (John et al., 2018). Chemicapacitor, surface acoustic wave (SAW), microelectromechanical systems (MEMS), chemiresistive, and electrochemical sensors are among the chemical sensors currently developed. The chemicapacitor is a sensor that detects volatile organic compounds (VOCs) with minimal readout electronic power, at low cost, and with the ability to detect target molecules selectively (Blue and Uttamchandani, 2016). SAW sensor has high thermal stability, possible wireless integration and able to sense the small gas molecules to larger bio-analytes (Mujahid and Dickert, 2017). For the MEMS sensor, it manages to provide fast response and high selectivity towards the target molecules applicable to the gas sensing applications (Gatty, 2015).