CONTENTS

Page

Acknowledgement
Abstract in Thai
Abstract in English
List of Tables i
List of Figures j
List of Abbreviations
List of Symbols
List of Glossary
Chapter 1 Introduction
1.1 Historical Background
1.2 Research Objectives
Chapter 2 Literature Review
2.1 Organophosphate pesticides
2.2 Toxicity of OPs
2.3 Effect of OPs
2.4 OPs lights reserved e
2.5 Detection of OPs
2.6 Determination of OPs using mass spectroscopic techniques
2.7 Biosensors for OPs screening 10
2.8 Nanozymes used in OPs biosensing 14
2.9 Iron oxide nanozymes in OPs biosensing

Chapter 3 Experimental	18
3.1 Equipments and Chemicals	18
3.2 Experiments	20
3.3 Detection of OPs by mixed iron oxide NPs based colorimetric assay	21
3.4 Detection of OPs in the spiked real sample by Gas Chrometography-	23
Mass Spectrometry (GC-MS)	
Chapter 4 Results and Discussion	24
4.1 Peroxidase like-activity of iron oxide NPs	24
4.2 Catalytic performance of mixed nanozyme under the optimum condition	26
4.3 Detection of OPs using optimum	29
4.4 Determination of OPs in the spiked sample by the GC-MS	33
Chapter 5 Conclusions	36
References	37
Appendix	42
Curriculum Vitae	43
ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright [©] by Chiang Mai University All rights reserved	

LIST OF TABLES

	10
Table 2.1 Methods of immobilization of the biological component in biosensors	12
Table 2.2 Different types of nanoparticles used in colorimetric assays	16
-0161912	
Table 4.1 % Recovery and RSD for the detection of OPs in spiked real sample	32
using proposed colorimetric assay	
Table 4.2 Data for detection of OP standards in spiked real sample using GC-MS	35
Table 4.3 Comparison of the proposed method for detection of OPs with the	35
standard GC-MS method	
CHERNIN BROTT	
ลิสสิทธิ์มหาวิทยาลัยเชียงใหม่	

Copyright[©] by Chiang Mai University All rights reserved

LIST OF FIGURES

Figure 2.1 General structure of OPs	3
Figure 2.2 Main types of organophosphorus pesticides (R is usually methyl	4
or ethyl group and the leaving group X is aliphatic, homocyclic	
or heterocyclic)	
Figure 2.3 Chemical structure of some OPs compounds	4
Figure 2.4 Mechanism of OP toxicity	5
Figure 2.5 Key reactions occurring between OP and AChE	5
Figure 2.6 Structure of dimethoate	7
Figure 2.7 Struucture of profenofos	7
Figure 2.8 Diagram of a sector mass spectrometer	8
Figure 2.9 Schematic of a typical bench top GC-MS system	9
Figure 2.10 The principle of biosensor system	10
Figure 2.11 Principle of working of acetylcholinesterase (AChE) based	14
biosensor for OPs detection	
Figure 2.12 Dual enzyme like activities (catalase and peroxidase mimics)	16
of iron oxide NPs	
ลิขสิทธิมหาวิทยาลัยเชียงไหม	
Figure 3.1 Oxidation reaction of TMB	20
Figure 3.2 The formations of H ₂ O ₂ from catalyses of AchE and ChOx	22
and oxidized TMB catalyzed by NPs	
Figure 4.1 The color formation of oxidized TMB	24
Figure 4.2 UV-Vis spectra of oxidized TMB obtained from the oxidation	25
reaction with presence of each iron oxide NPs	

Figure 4.3 UV-VIS spectra of oxidized TMB obtained from the different ratios	26
of mixed iron oxide NPs	
Figure 4.4 The absorbance of the catalytic reaction of iron oxide NPs	27
undertaken in different pH	
Figure 4.5 Concentration dependence of peroxidase-like activity of mixed iron	28
oxide NPs	
Figure 4.6 Effect of TMB concentration on color formation	29
Figure 4.7 Dose-response curve for dimethoate detection using the mixed	30
iron oxide NPs based colorimetric assay	
Figure 4.8 Dose-response curve for profenofos detection using the mixed	30
iron oxide NPs based on colorimetric assay	
Figure 4.9 Standard calibration curve of dimethoate	31
Figure 4.10 Standard calibration curve of profenofos	31
Figure 4.11 Typical chromatograms of the pesticide standards (1.0 μ M)	33
Figure 4.12 Typical chromatograms of the sample spiked with mixed pesticides	34
standard at 2.0 µM.	
Figure 4.13 Typical chromatograms of the sample with no addition of OPs	34
MALINERSI	
UNIVE	

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

LIST OF ABBREVIATION

ABTS	2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
AChE	Acetylcholinesterase
ACh	Acetylcholine
BChE	Butyrylcholinesterase
СНО	Choline oxidase
CNTs	Carbon nanotubes
DMSO	Dimethyl sulfoxide
EI S.	Electron impact ionization
γ-Fe ₂ O ₃	Iron III Oxide
Fe ₃ O ₄	Mixed Iron (II, III) Oxide
FI SOP	Fiber Introduction
FTIR	Fourier Transform Infrared Spectroscopy
GABA	γ-aminobutyric acid
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
HRP	Horseradish peroxidase
HPLC	High Performance Liquid Chromatography
HS–SPME	Headspace solid-phase micro extraction
H_2O_2	Hydrogen peroxide
HRPCopyright	Horseradish enzyme
IC AII r	Ion chromatography
I.D	Internal diameter

ICP	Inductively Coupled Plasma
LOD	Limit of detection
LOQ	Limit of quantification
LC	Liquid Chromatography
MS	Mass spectrometry
m/z	Mass-charge
MPNs	Magnetic Nanoparticles
MS	Mass spectrometry
MSPD	Matrix solid-phase dispersion
Min 🔨	Minutes
NaOAC- HOAC	Sodium Acetate Ethyl Acetate Buffer
NMR	Nuclear magnetic resonance
NPs	Nanoparticles
O ₂	Oxygen
OP	Organophosphate
OPD	O-Phenylenediamine hydrochloride
OPs	Organophosphorus pesticides
ОРН	Organophosphorus hydrolase
PBS	Phosphate Buffered Saline
ppb	Part per billion
ppt	Part per trillion
RSD dansi	Relative standard deviations
RT Copyright	Retention time
SDME	Single drop micro extraction
SPME	Solid phase micro extraction
ТАТР	Triacetate Triperoxide
TMB	3, 3, 5, 5-tetramethylbenzidine
TMBDI	3, 3', 5, 5'-tetramethylbenzidine diimine
V ₂ O ₅	Vanadium oxide
WHO	World Health Organization
H ₂ O	Water

LIST OF SYMBOLS

Co_3O_4	Cobalt (II, III) oxide
°C	Degree Celsius
eV	Electron volt
e.g.	exempli gratia
i.e.	exempli gratia
etc.	et cetera
et al.	et alia
F	Fluorine
γ	Gamma
OHº	Hydroxyl radical
%	Percent
Au	Gold
Р	Phosphorus atom
P=O	Phosphoric bond
P=S	Thiophosphoric bond
Μ	Molar
M2/M4	Muscarinic receptors
m	Metre
mm Copyr	Millimetre by Chiang Mai University
μΑΙΙ	Microights reserved
μΜ	Micromolar
μL	Microlitre
μg	Microgram
mg	Milligram
mg/L	Milligram per Litre



LIST OF GLOSSARY

Artificial Enzyme

Assay

A2

The phospholipases (A1, A2, C and D) are a complex and crucially important group of enzymes that hydrolyse phospholipids, releasing a variety of products depending on the site of hydrolysis. Phospholipases A2 (PLA2) refer to the enzymes that cleave the sn-2 position of phospholipids to generate the corresponding fatty acid and lysophospholipid. PLA2 enzymes has its origins in the abundant digestive enzymes of the pancreas and a wide variety of snake, insect and arachinid venoms.

The term artificial enzyme was coined by Ronald Breslow in 1970. An artificial enzyme is a synthetic, organic molecule or ion that recreate some function of an enzyme and or a small molecule complex that models the molecular structure, spectroscopic properties, or reactivity of an enzyme.

An assay is an investigative (analytic) procedure in laboratory, medicine, pharmacology, environmental biology and molecular biology for qualitatively assessing or quantitatively measuring the presence, amount, or functional activity of a target entity (the analyte). The analyte can be a drug, a biochemical substance, or a cell in an organism or organic sample. Colorimetry Colorimetry or colorimetry is a technique "used to determine the concentration of colored compounds in solution. Colorimetric analysis is the method of determining the concentration of a chemical element or chemical compound in a solution with the aid of color reagents. It is applicable both to inorganic and organic compounds and may be use with or without enzymatic stage.

> Horseradish Peroxidase (HRP) is an enzyme found in the roots of horseradish, is used extensively in biochemistry applications primarily for its ability to amplify a weak signal and increase detectability of a target molecule. It is a metalloenzyme with many isoforms, of which the most studied type is C. It is an all alpha-helical protein which binds heme as a redox cofactor.

> JMP is the data analysis tool created by Statistical Analysis System (SAS), USA, in 1989 to empower scientists and engineers to explore data visually. It can be used for data acquisition, cleanup, visualization, basic analysis, designing an experiments and for statistical modeling.

Chiang Mai University

M2/M4 are acetylcholine receptors (AChR) that bind acetylcholine and transmit its signal. Muscarinic AChRs are named after the agonists muscarine. They are G-protein coupled receptors (GPCRs) that mediate a slow metabolic response second messenger cascades. Muscarinic via receptors are characterized through their interaction with muscarine, water-soluble toxin derived from the mushroom Amanita

HRP

JMP

Copyright[©]

M2/M4 muscarinic receptors

Nanomaterial

ລີບສີກຣິ່ນກ Copyright[©] All rig

peripheral sympathetic nervous system through its binding to muscarinic AChRs, resulting in convulsions and even death. There are five subtypes of muscarinic AChRs based on pharmacological activity, M1-M5. All five are found in the CNS, while M1-M4 are also found in various tissues. M1 AChRs are common in secretory glands, M2 AChRs are found in cardiac tissue, M3 AChRs are found in smooth muscles and in secretion glands. M2 and M4 inhibit adenylate cyclase, thereby decreasing the production of the second messenger cAMP. The activation of the M2 receptor in the heart is important for closing calcium channels in order to reduce the force and rate of contraction.

muscaria that causes substantial activation of the

Nanomaterials are chemical substances or materials that are manufactured and used at a very small scale (down to 10,000 times smaller than the diameter of a human hair). Nanomaterials are developed to exhibit novel characteristics (such as increased strength, chemical reactivity or conductivity) compared to the same material without nanoscale features. Hundreds of products containing nanomaterials are already in use. Examples are batteries, coatings, anti-bacterial clothing etc.

Nanozymes

Nanozymes are nanomaterials with enzyme like characteristics. Nano materials shares certain similarities with natural enzymes such as overall size, shape and surface charge which enable them to mimic them. Properties of nanozymes are dependent on several factors including size, shape and morphology, surface coating and modification, composition, activators and inhibitors as well as forming hybrids.

Peroxidase Enzymes

Group of enzymes that catalyzes the oxidation of a substrates by reducing peroxide to water. These enzymes are often located in peroxisomes. For many of these enzymes the optimal substrate is hydrogen peroxide, but others are more active with organic hydroperoxides such lipid as Peroxidases peroxides. can contain a heme cofactor in their active sites and or alternately redox-active cysteine or selenocysteine residues. Examples of peroxidase are horseradish peroxidase (HRP), cytochrome c and glutathione. While the exact mechanisms have yet to be determined, peroxidases are known to play a part in increasing a plant's defenses against pathogens.

^{™B}ลิขสิทธิ์มห Copyright[©]เ All rig 3, 3', 5, 5'-Tetramethylbenzidine (TMB) is a noncarcinogenic substitute for benzidine. The substrate is a white crystal powder which produces a soluble end product that is pale blue in color and can be read spectrophotometrically at 370 or 620-650 nm. The TMB reaction may be stopped with 2M H_2SO_4 (resulting in a yellow color), and read at 450 nm. TMB is degraded by sunlight and by fluorescent lights.

Standard Addition

The method of standard addition is a type of quantitative analysis approach often used

in analytical chemistry whereby the standard is added directly to the aliquots of analyzed sample. This method is used in situations where sample matrix also contributes to the analytical signal, a situation known as the matrix effect, thus making it impossible to compare the analytical signal between sample and standard using the traditional calibration curve approach.

Sample spiking

Technique that is used to evaluate the performance of an analytical procedure when testing a specific sample (matrix) type. A spiked sample is generated by adding a known amount (a spike) of analyte to a sample, testing the spiked sample, and determining if the amount that have been added has been recovered.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

TRAC MAI