UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF ELECTROCHEMICAL BIOSENSOR SYSTEMS FOR CHLORPYRIFOS PESTICIDE DETECTION

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Environmental monitoring systems are of great interest and highly significant in our life since every day we are exposed to many and various dangerous and fatal contaminants. Organophosphate pesticides such as Chlorpyrifos are widely used in agriculture to eliminate plant destroying pests. However, these pesticides may affect the environmental equilibrium unless continuous monitoring on their presence in water, soil, and agricultural products is carried out to protect human health and other living organisms.

One of the approaches that can be adopted is to develop an on-line monitoring system. The widely used electronic and computer technology can simplify the development process of acquiring and monitoring system. In addition, the invention of biosensors to detect biologically-based materials such as Chlorpyrifos pesticide in the agricultural sector, glucose level in blood, heavy elements in the drinking water etc., have attracted interest.

This research is divided into two parts: development of computer-based instrument and development of biosensor. Both systems are integrated to form a real monitoring device for the detection of Chlorpyrifos pesticide concentration. In the development of computer-based instrument, a serial interfacing board that can be linked to the computer to acquire data from the various developed biosensors was developed. The developed board includes signal conditioning, analog-to-digital (A/D) and universal asynchronous receiver and transmitter (UART) circuits. Visual Designer software was used to control the data flow process and at the same time displaying the biosensor responses in real time, and the data can be stored in hard disk.

For the biosensor development, two amperometric based biosensor electrodes and capacitance-based biosensor were developed. In the amperometric based biosensor electrodes, two different patterns and substrate electrodes (copper and gold) were developed. Both structures can produce similar responses where the electrical signal (in this case voltage) variation is proportional to the pesticide concentration. Also, they can be linked to the computer via the PC-based serial interfacing board. For the capacitance-based biosensor, detection of Chlorpyrifos pesticide was carried out by monitoring the variation of dielectric (mixed pesticide and antibody) between two parallel plates. An oscillator circuit was used to detect the response by measuring the generated signal frequency. It has been shown that the measured frequency is inversely proportional to the pesticide concentration. Therefore, from the results, the developed biosensors are able to detect the presence of the pesticide. Also, the evaluation and comparison between the results of these systems show that the capacitance-based biosensor is the most effective structure. The construction of that structure is relatively simple.

Analysis of previously developed biosensors has been carried out as references. They are a pH glass electrode and a fibre optical biosensor.

The remarkable significant findings and contribution of the author in this research. can be represented in the design of a potentiostat electronic circuit that provides a bias voltage to the electrode systems inserted in a sample of the Chlorpyrifos pesticide. This generates an electrochemical reaction where an electric current proportional to the concentration of the Chlorpyrifos pesticide sample is provided, Secondly, the design of capacitance-based biosensor that relates frequency readings with Chlorpyrifos pesticide concentrations. Finally, the integration of both the various biosensor systems and the PC-based serial board where a real data acquisition system was established to detect the Chlorpyrifos pesticide

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

PEMBANGUNAN SISTEM BERASASKAN BIOSENSOR UNTUK PENGESANAN PESTISID CHLORPYRIFOS

Oleh

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Pestisid organofosfat seperti chlorpyrifos digunakan secara meluas dalam sektor pertanian unfuk menghapuskan perosak tanaman. Namun pestisid ini boleh menyebabkan ketidakseimbangan persekitaran kecuali jika pemautauan berterusan dibuat bagi memastikan kandungan pestisid di dalam air, tanah dan penghasilan pertanian tidak memudaratkan manusia dan kehidupan lain. Satu cara yang boleh digunakan ialah dengan membangunkan sistem pemantauan. Sistem pengawasan persekitaran adalah sangat penting dan berkait rapat dengan kehidupan kita yang sentiasa terdedah kepada berbagai bahan cemar dan berbahaya.

Penggunaan teknologi komputer dan elektronik dapat membantu memudahkan proses pemantauan. Tambahan pula, rekaan biosensor untuk mengesan bahan berasaskan biologikal seperti pestisid chlorpyrifos dalam sektor pertanian, kandungan glukosa dalam darah, bahan kimia dalam air minuman dan seumpamanya amat menarik minat. Penyelidikan ini dibahagikan kepada dua bahagian: pembangunan instrumentasi berasaskan komputer dan pembangunan struktur biosensor khusus untuk mengesan pestisid chlorpyrifos.

Dalam pembangunan instrumentasi berasaskan komputer, litar antaramuka yang boleh disambungkan kepada komputer untuk mendapatkan data dari berbagai jenis biosensor telah dibangunkan. Litar yang dibangunkan termasuk keadaan isyarat, penukar analog kepada digital dan litar penerima dan penghantar. Perisian "Visual Designer" telah digunakan untuk mengawal pengaliran data dan pada masa yang sama mempamerkan isyarat biosensor yang diperolehi. Data yang diperolehi boleh juga disimpan dalam cakera keras. Bagi struktur biosensor, elektrod biosensor amperometrik dan biosensor berasaskan kapasitan telah dibangunkan. Dalam biosensor elektrod amperometrik, dua jenis corak dan substrat elektrod (kuprum dan emas) berbeza telah dibangunkan. Kedua-dua struktur boleh menghasilkan tindakbalas yang hampir sama di mana perbezaan isyarat elektrik (voltan) adalah berkadaran dengan kandungan pestisid. Ia juga boleh disambungkan kepada komputer melalui kad antara muka. Bagi biosensor berasaskan kapasitans pengesanan pestisid chlorpyrifos telah dijalankan dengan memerhatikan perbezaan nilai dwielektrik (campuran pestisid dan antibodi) antara dua plat selari. Litar pengayun digunakan untuk mengesan tindakbalas dengan mengukur isyarat frekuensi yang dihasilkan. Didapati isyarat frekuensi adalah berkadar songsang dengan kandungan pestisid. Oleh itu, dari keputusan yang diperolehi, biosensor yang telah direka boleh mengesan kehadiran pestisid. Dari keputusan yang diperolehi, didapati biosensor berasaskan kapasitans adalah struktur lebih efektif dan pembinaannya adalah sangat mudah. Analisis biosensor terdahulu telah dijadikan sebagai rujukan. Ia adalah elektrod kaca pH dan biosensor optik fibre. Akhir kata, instrumentasi berasaskan komputer dan biosensor telah digabungkan untuk membentuk sistem pemantauan secara lansung. Isyarat biosensor didapati boleh dipaparkan pada skrin komputer menandakan litar antaramuka dan perisian kawalan telah berjaya dibangunkan.

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LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

AC	Alternating current
ADC	Analogue-to-Digital Converter
AP	Alkaline Phosphatase
ASV	anodic stripping voltammetry
BOD	Biological Oxygen Demand
CPU	Central Processing Unit
DA	Data Acquisition
DAC	Digital-to-Analogue Converter
DC	Direct Current
DCE	Data Communication Equipment
DTE	Data Terminal Equipment
DTNB	DITHIO-bis(2-NITROBENZOIC ACID)
ELISA	Enzyme Linked Immunosorbant Assay
EPROM	Erasable Programmable Read Only Memory
FET	Field Effect Transistors
FPXRF	Field Portable X-ray Fluorescence
GEMs	Genetically Engineered Microorganisms
HRP	Horse Radish Peroxidase
IC	integrated circuit
INTR	Interrupt
I/O	Input/Output
ISFET	ion-selective field effective transistor
LED	light emitting diode
MARDI	Malaysia Agricultural Research and Development Institute
ODAM	OZO Diversified Automation Machine
OPCs	Organophosphorus Compounds
OPH	Organophosphate Hydrolase
PCB	Printed Circuit Board
PC	Personal Computer
PLC	Programmable Logic Controller
ppb	part per billion
ppm	part per million
REDOX	Reduction and Oxidation
RTD	Resistance-Temperature Detector
RxD	Receiving
SAMs	Self Assembled Monolayers
SHE	Standard Hydrogen Electrode
SPR	Surface Plasmon Resonance
TBR	Transmitter Buffer Register
TIRF	Total Internal Reflection Fluorescence
TTL	Transistor-Transistor logic
TxD	Transmission
UART	Universal Asynchronous Receiver-Transmitter
UiTM	Universiti Technology Mara Malaysia

CHAPTER ONE

INTRODUCTION

1.1 Overview of the Environment Monitoring Approaches

During the three decades ago, there was a strong demand to monitor hazardous compounds in life environment as well as to determine thousands of compounds in various areas like clinical chemistry (determining of disease markers and drugs, etc.), food contaminant assessment quality control, and others [1]. The sensitive and selective determination of a large number of compounds is of great relevance for scientific research as well as for several branches of industry, e.g., for process control in the chemical and food industries [2]. Also in the field of health care it is indispensable for the diagnosis of diseases [1]. The frequent outbreaks of food poisoning serve to underline the need for enhanced monitoring of the food processing industry through disposable, operator friendly microbial contamination detection systems [2]. Such thing has given rise to increased effort in science and technology all over the world to develop simple, rapid, sensitive and inexpensive methods of monitoring and analysis. For instance, during the last decade, the breakout of the industrial revolution in Malaysia has resulted in very dangerous various contaminated environments. As the case in the developed countries, Malaysia shows a growing need and a major concern about safety and quality of food supply. For this purpose, in all over the Malaysian peninsula states different specialised institutions have grown to meet the requirements of creating free pollutants environment.

High selectivity, in trace analysis, has been gained by considerable progress in analytical instrumentation, as is reflected by modern gas chromatography, high-pressure liquid chromatography, mass spectrometry, and atomic absorption spectroscopy. However, these powerful instrumental techniques are only used in specialized laboratories. They are not suited to on-line operation. One of the approaches that can be adopted is to develop on-line monitoring system. The wide used of electronic and computer technology can simplify the development process of the system. As an addition, the invention of an electrochemical biosensor to detect biological based materials such as Chlorpyrifos pesticide in the agricultural sector, glucose level in blood, heavy elements in the drinking water etc., have attracted many interests. Moreover, the simplicity of electrochemical biosensor construction and not being limited to the laboratory based systems, proved to replace these heavy and expensive systems. Therefore, the development of electrochemical biosensors, which are highly selective and easy to handle, is thus a key solution in analysis.

1.2 Organophosphate Pesticides

Although Organophosphate pesticides such as Chlorpyrifos pesticide presents a high toxicity that may represent a serious risk for the equilibrium to the environment including the industrial production, agricultural and domestic use, they are considered to be the most important chemicals being utilized extensively in agricultural sectors. Consequently, monitoring these chemicals in water, soil, and food products is highly needed to protect human health and living organisms. A lot of efforts and researches have been oriented and exerted to monitor these pesticides based on the principle of enzyme inactivation [3].

1.3 Definition of Electrochemical Biosensors

Electrochemical biosensors are analytical tools combining biochemical reaction component with a physical transducer (Figure 1.1); the biological sensing element can be an enzyme, antibody, DNA sequence, or event microorganism. The biochemical component serves to selectively catalyse a reaction or facilitates a binding event. The selectivity of the biochemical recognition event allows for the operation of biosensors in a complex sample matrix. The transducer converts the biochemical event into a measurable signal, thus providing the means for detecting it [5]. Measurable events range from spectral changes, which are due to production or consumption of an enzymatic reaction's product/substrate, to mass change upon biochemical complexity.

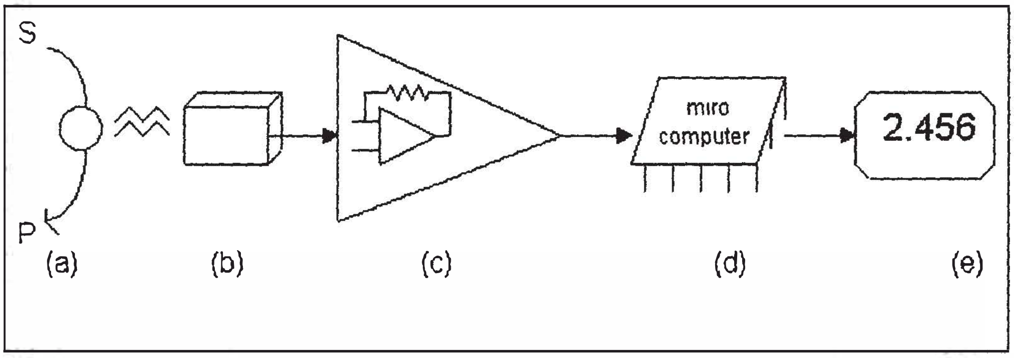

Figure 1.1: Basic Operation of the Electrochemical Biosensor Principle

In Figure 1.1, (*S*) a sample illustrates any substance such as pesticides, blood etc., that needed to be detected. (*a*) Biocatalyst - converts the analyte into product. See Figure 1.2. (*b*) Transducer - detects the occurrence of the reaction and converts it into an electrical signal. (*c*) Amplifier - amplifies the usually small signal to a useable level. (*d*) Microcomputer - signal is digitized and stored for further