



UNIVERSITI PUTRA MALAYSIA

***OPTICAL BIOSENSOR BASED ON IMMOBILIZED COPPER SENSITIVE
OPERON REPRESSOR PROTEIN FOR DETECTION OF COPPER IONS
IN WATER***

HASSAN ISMAIL

FBSB 2016 2



**OPTICAL BIOSENSOR BASED ON IMMOBILIZED COPPER SENSITIVE
OPERON REPRESSOR PROTEIN FOR DETECTION OF COPPER IONS
IN WATER**

By

HASSAN ISMAIL

**Thesis Submitted to School of Graduate Studies,Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

May 2016

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DEDICATION

I dedicated the entire work to my lovely parents and my late brother Abubakar Hassan.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

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May 2016

Chairman : Abu Bakar B. Salleh, PhD
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Water pollution, from heavy metals, embodies a latent risk for both terrestrial and aquatic organisms. As such, continuous, simple and sensitive detection tools for toxic metals have been a great challenge of the existing methods used in monitoring of these metals. In this case, biosensors stand worthwhile for constant monitoring of metals in polluted areas. Here a new biosensor based on a tapered Multi-Mode Fiber (MMF) coated with CsoR_{GZ} protein from *Geobacillus zalihae* (CsoR_{GZ}) as a bioreceptor is presented. The optical property of the coated layer changes once it was subjected to copper, resulting in an increase, at the UV-region (240 nm), of the absorption of evanescent waves. This increase of absorption is proportional to the concentration of copper added. The biosensor displayed a continuous response over the range of 5 - 40 μM copper. The limit of detection and limit of quantification were 5 μM and 40 μM copper respectively, with the sensitivity of 0.045 μM^{-1} based on 20 μm sensor. The biosensor showed a fast response time of 19.8 s at room temperature and pH of 7.0. The biosensor retained its selectivity and did not respond to equivalent additions of cobalt (II) and Nickel (II). This work also revealed, for the first time, the prospect of remote, selective and sensitive monitoring of copper ions in water, from a distance of 50 m, where a continuous response in the range of 5 - 40 μM copper was obtained with no significant difference ($p > 0.06$) with the non-remote one.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

BIOSENSOR OPTIKAL BERASASKAN PROTEIN PENGAWALSELIAAN KUPRUM PEGUN UNTUK PENGESANAN ION KUPRUM DALAM AIR

Oleh

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Pencemaran air, dari logam berat, mempunyai risiko yang berbahaya untuk kedua-dua organisma daratan dan akuatik. Untuk mencipta alat pengesan logam toksik yang berterusan, mudah dan sensitif menjadi cabaran yang amat besar. Dalam kes ini, biosensor sangat sesuai digunakan untuk pemantauan berterusan logam di kawasan tercemar. Di sini biosensor baru berdasarkan fibre tirus pelbagai mod (MMF) disalut dengan CsoR protein sebagai bioreseptor dicipta. Ciri-ciri optik lapisan yang bersalut berubah apabila kuprum ditambah, menyebabkan peningkatan penyerapan gelombang di kawasan UV (240 nm). Peningkatan penyerapan gelombang adalah berkandar langsung dengan kepekatan kuprum yang ditambah. Biosensor memaparkan tindak balas yang berterusan di sepanjang julat 5 - 40 μM kuprum. Had pengesanan adalah 5 μM tembaga dengan kepekaan 0.045 μM^{-1} . Biosensor itu menunjukkan masa tindak balas yang cepat iaitu 19.8 s pada suhu bilik dan pH 7.0. Biosensor berupaya mengekalkan pemilihan dan tidak bertindak balas terhadap penambahan kobalt (II) dan nikel (II). Kerja ini juga mendedahkan, buat kali pertama, prospek pemantauan jarak jauh, yang terpilih dan sensitif ke atas ion kuprum di dalam air, dari jarak 50 m, di mana tindak balas yang berterusan dalam lingkungan 5 μM kepada 40 μM kuprum telah diperolehi dengan tiada perbezaan yang signifikan ($p > 0.06$) dibanding dengan yang dipantau pada jarak dekat.

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

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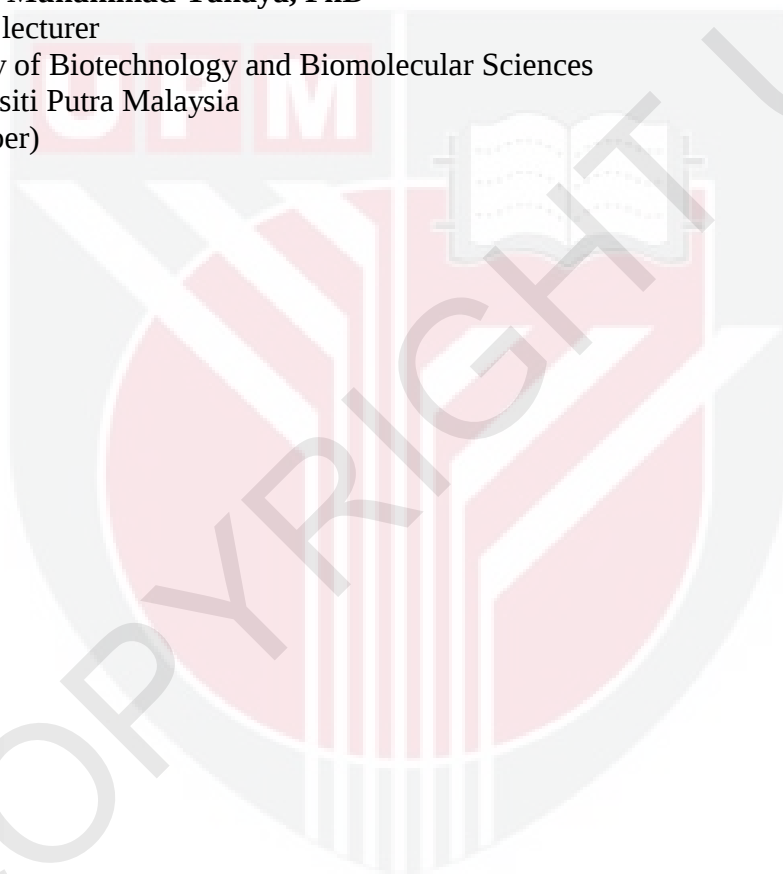
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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AChE	Acetylcholinesterase
AES	Atomic Emission Spectroscopy
APTES	Aminopropyl triethoxysilane
BSA	Bovine Serum Albumin
CATV	Cable Television
DDVP	Dimethyl-2,2-dichlorovinyl phosphate
EDTA	Ethylene diamine tetraacetic acid
EPA	Environmental Protection Agency
HCL	Hydrochloric acid
ICP-MS	Inductively coupled plasma mass spectrometry
IPTG	Isopropyl thiogalactoside
ITC	Isothermal Titration Calorimetry
IUPAC	International Union of Pure and Applied Chemistry
kDa	Kilo Dalton
MMF	Multi-Mode Fibre
MOPS	Morpholine propane sulfonic acid
MWCO	Molecular Weight Cut Off
NA	Numerical Aperture
pI	Isoelectric point
RDA	Recommended Daily Allowance
ROS	Reactive Oxygen species
RT	Room Temperature

SMF

Single-Mode Fibre

WD

Wilson's disease



CHAPTER 1

INTRODUCTION

Environmental pollution from toxic metals is a global phenomenon coupled with industrial developments. Heavy metals mostly constitute the most toxic pollutants in the environment, and also in other areas such as in food industry and medicine. Some heavy metals like Cadmium (Cd), and Lead (Pb) pose a great threat due to the toxicity and extensive industrial application. There are several methods used for detection of heavy metals such as electrochemical or spectroscopic; such as atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES) and inductively coupled plasma mass spectrometry (ICP-MS) etc. (Lia et al., 2006, Askari et al., 1983). These techniques, however, are either expensive or lack sensitivity. Furthermore, the techniques cannot account for the real time concentrations of heavy metals that are accessible to the aquatic organisms. Therefore, it is highly desirable to develop simpler approaches of monitoring pollution caused by heavy metals. Sensitive biosensors employing metal binding proteins, such as methallothioneins (SmtA) or regulator proteins (MerR), combined to a remarkably sensitive capacitive transducer were recently labelled to access heavy metals (Bontidean et al., 2004).

1.1 Problem statement

Heavy metals bioaccumulate higher in the upper trophic levels making organism in the upper trophic more prone to the toxicity (Ilangovan et al., 2006, Krawczyk et al., 2000).

The conventional approaches employed in the monitoring of heavy metals using spectrophotometry, chromatography, mass spectrometry techniques involve sophisticated and expensive equipment, vastly skilled personnel and they are generally laborious (Chouteau et al., 2005). The total amount of heavy metals detected by such means might not always relate to toxicity of these samples as the original bioaccessibility of the metal ions cannot be accounted for. Thus, there is need for the cheaper and simpler methods that can be used to monitor heavy metals.

Biosensor, being a promising tool in this regard, is an investigative device, which translates a biological response into a measurable signal. Several patterns of biosensors have been defined previously for detection of heavy metals. Extensive varieties of bioreceptor and transducer set-up have been applied for the construction of biosensors (Amine et al., 2006).

Enzymes are more commonly employed as bioreceptor in construction of biosensors. Although enzyme-based biosensors enjoy relatively high degree of substrate specificity, their use in biosensors design may be hindered due the laborious, longer

and expensive purification steps, coupled, sometimes, with the need to have coenzyme/cofactor to produce detectable response. Microorganisms provide an ideal alternative (Marzuki et al., 2012). The ability of the cells to uptake and therefore detect great amount of chemicals from the various enzymes and co-factors that co-exist in the cells; conversely, affects the selectivity.

Metalloproteins are another category of the bioreceptor used for the integration of the heavy metal sensors and their specificity for metal binding make them more attractive tool (Corbisier et al., 1999). For developing heavy metal biosensors, different metalloproteins/peptides have been employed (Cherian et al., 2003, Chow et al., 2005). The good selectivity shown by the metalloproteins even though in more multifaceted natural environments such as in blood or marine water especially when integrated to a seemly transducer has made it a very good bioreceptor that may provide an alternative to the existing measuring techniques of metal ions concentrations (Barondeau et al., 2002).

1.2 General objective of the study

To develop an optical biosensor based on CsoR_{Gz} protein as bioreceptor that will be used for selective detection of copper ions in water.

1.2.1 Specific objectives

1. To characterize the bioreceptor CsoR_{Gz} protein for its binding potential to copper ions.
2. To immobilize the CsoR_{Gz} protein on optical fiber and integrate the biosensor.
3. To assess the performance of the biosensor for the detection of copper ions in water.

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