UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF A CAPACITANCE-BASED BIOSENSOR FOR THE DETERMINATION OF HISTAMINE CONCENTRATION

HELMI WASOH @ MOHAMAD ISA

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By

HELMI WASOH @ MOHAMAD ISA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

March 2009

Dedicated to,
teachers and family, for their endless support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the degree of Doctor of Philosophy

DEVELOPMENT OF A CAPACITANCE-BASED BIOSENSOR FOR THE DETERMINATION OF HISTAMINE CONCENTRATION

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HELMI WASOH @ MOHAMAD ISA

March 2009

Chair : Abu Bakar Salleh, PhD

Faculty : Institute of Biocience

Histamine level has been suggested as a rapid fish spoilage indicator. Besides that, a biosensor device with low cost, simple and portable is an advantage for fish freshness monitoring. Therefore, a histamine biosensor device was developed using the enzyme diamine oxidase (DAO) and a capacitance (*C*) concept. A capacitor with two plates system gives linear relationship between frequency (*f*) and histamine concentration. It has been proven that the relationship between *f*, dielectric constant (*k*) and histamine could be used to develop a histamine biosensor. The behavior of histamine reaction can be monitored in the presence of free enzyme in solution. Based on this relationship, immobilized enzyme electrode was developed by immobilizing 1.0 mg DAO/L in the ratio of DAO:polymer= 1:1. Good sensor response could be observed when the shape of the electrode was in a row and the electrode material was made from copper, with a distance between electrode, *d*=2.5 mm. By using this device, a linear range of histamine from 20 to 100 ppm was obtained (R^2=0.9897) with good correlation between biosensor method and HPLC (R^2 = 0.9998). For the determination of

histamine in prawn tissues, the recovery obtained was 98.13% after spiking with 150 ppm histamine and 98.79% after spiking with 200 ppm histamine (average RSD, 1.16-5.67%).

To complete the device, electronic reader was developed comprises PIC (peripheral integrated circuit) microcontroller, LCD (light crystal display), capacitor-resistor circuit and *computer-programming* in C code (installed in PIC). By using astable operation of capacitor-resistor circuit, a difference sensor response was generated from difference histamine concentration. During histamine reaction, the physicochemical changes was converted into electrical signal and translated in histamine concentration as part per million (ppm). PIC is very useful in modifying the writing process of *computer-programming* due to its capability and easily reprogrammed.

For the basic test (without enzyme), the device (with electronic reader) gave different (*f*) values for histamine (50-300 ppm, RSD≤2.01%). After the reader was developed successfully, enzyme electrode was designed with the dimension of copper material at d=2.0 mm and area, A=2.5x10 mm. By immobilizing enzyme onto this electrode, the device showed linear response to histamine concentrations (25 to 100 ppm, R^2=0.998, RSD≤2.74%). The biosensor response was still 80% of the initial value after 10 days of storage (4^{o}C). The histamine biosensor exhibited reproducibility characteristic with RSD value equals to 8.88% (n=4). This device can be used up to 30 times without a major change in sensor response (11.76±3.41 Hz/s). Experiment with prawn tissues shows that the performance of histamine biosensor device is comparable to HPLC with R^2=0.9895. The histamine biosensor is a promising device for on site screening of fish freshness.

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

PEMBANGUNAN BIOPENDERIA BERASASKAN-KAPASITAN UNTUK PENENTUAN KEPEKATAN HISTAMIN

Oleh

HELMI WASOH @ MOHAMAD ISA

Mac 2009

Pengerusi : Abu Bakar Salleh, PhD

Fakulti : Institut Biosains

Tahap histamin telah dicadangkan sebagai petunjuk pantas kerosakan ikan. Dengan itu, biopenderia kos murah, ringkas dan mudah alih merupakan kelebihan bagi mengesan ikan segar. Jadi, biopenderia histamin telah dibangunkan sebagai alat petunjuk kesegaran dibantu oleh enzim diamine oksidase (DAO) dan konsep fizik, *kapasitan* (*C*). Kapasitor dengan sistem dua plat memberikan respon linear antara frekuensi (*f*) dan kepekatan histamin. Ianya menjadi petunjuk awal bahawa hubungan antara *f*, pemalar dielektrik (*k*) dan histamin boleh digunakan untuk membina biopenderia histamin ini. Kelakuan tindak balas histamin didapati boleh dikaji dengan menggunakan DAO bebas dalam larutan. Berdasarkan kemampuan ini, elektrod berenzim pegun dibina menggunakan 1.0 mg DAO/L dan nisbah DAO:polimer=1:1. Respon yang baik diperolehi bila elektrod kuprum dibina memanjang dan jarak plat, *d*=2.5 mm. Alat ini memberikan julat linear dari 20-100 ppm histamin dengan R^2=0.9897. Korelasi yang baik diperolehi bagi biopenderia dibandingkan dengan kaedah konvensioanal HPLC (R^2=0.9998). Analisis dengan tisu udang menunjukkan perolehan-semula mampu dicapai pada 98.13%

selepas ditambah 150 ppm histamin dan 98.79% selepas ditambah 200 ppm histamin (purata RSD, 1.16-5.67%).

Bagi melengkapkan pembinaan alat ini, pencatat elektronik dibina, mengandungi PIC (peripheral integrated circuit) kawalan-mikro, LCD (light crystal display), dan *program-komputer* (kod C yang di muat dalam PIC). Dengan menggunakan operasi astable litar kapasitor-perintang, respon biopenderia yang berlainan dijana daripada kepekatan histamin berbeza. Semasa tindakbalas, perubahan fizikokimia larutan ditukar ke isyarat elektrik dan diterjemah kepada kepekatan histamin, bahagian-per-juta (ppm). PIC berguna untuk mengubahsuai penulisan *program-komputer* berdasarkan kemampuannya yang mudah deprogram semula.

Bagi ujikaji awal (tanpa enzim), biopenderia histamin (dengan pencatat elektronik) memberikan bacaan (*f*) berbeza untuk histamin (50-300pm, RSD≤2.01%). Selepas pencatat berjaya dibina, elektrod direkabentuk menggunakan plat kuprum, d=2.0 mm dan keluasan, A=2.5x10 mm. Penggunaan electrod berenzim pegun memberikan respon linear bagi histamin antara 25 ke 100 ppm (R^2=0.998, RSD≤2.74%). Nilai respon biopenderia didapati masih melebihi 80% berbanding nilai asal selepas 10 hari penyimpanan dalam keadaan sejuk (4^{o}C). Biopenderia histamin mempamerkan ciri kebolehhasilan dengan nilai RSD=8.88% (n=4). Elektrod yang sama ini juga boleh diulang pengunaannya sebanyak 30 kali tanpa perubahan besar pada respon penderia (11.76±3.41 Hz/s). Analisis dengan tisu udang menunjukkan prestasi analisis alat ini adalah setanding dengan kaedah HPLC (R^2=0.9895). Biopenderia histamin ini berpotensi untuk menjadi alat yang sesuai di lapangan untuk mengesan kesegaran ikan.

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I certify that a Thesis Examination Committee has met on 19th March 2009 to conduct the final examination of Helmi Wasoh @ Mohamad Isa on his thesis entitled "Development of a Capacitance-based Biosensor for Determination of Histamine" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

Members of the Examination Committee were as follows:

Nor Aripin Shamaan, PhD.
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Kaida Khalid, Ph.D.
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mariana Nor Shamsudin, Ph.D.
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Musa Ahmad, Ph.D.
Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

_____
BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra malaysia

Date: 21 Mei 2009

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

Abu Bakar Salleh, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Main supervisor)

Fatimah Abu Bakar, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Lee Yook Heng, PhD
Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(Member)

_____
HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 8 June 2009

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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(**HELMI WASOH @ MOHAMAD ISA**)

Date: 23 June 2009

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