UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF MINIATURIZED TAPERED FIBER OPTICS DIAMINE OXIDASE-BASED SENSOR FOR HISTAMINE DETECTION

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DEVELOPMENT OF MINIATURIZED TAPERED FIBER OPTICS DIAMINE OXIDASE-BASED SENSOR FOR HISTAMINE DETECTION

By

HAMZA BOKO USMAN

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirements for the Degree of Master of Science

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Dedicated to,

my beloved wife Sameerat, to my children Jaafar and Rahmat

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

DEVELOPMENT OF MINIATURIZED TAPERED FIBER OPTICS DIAMINE OXIDASE-BASED SENSOR FOR HISTAMINE DETECTION

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May, 2015

Chairman: Abu Bakar Salleh, PhD
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Estimating the level of histamine in fish and fish products is very important because of their implication in fish poisoning in human; hence, ascertaining histamine levels in the afore-mentioned serves as a chemical index for spoilage. Factors such as expensive instrumentation, time consumption, size and weight are some of the problems with conventional analytical methods of detection. Tapered fiber optics-enzyme based sensors are prospective candidates towards resolving these quagmires due to their portability and simplicity vis-à-vis currently available detectors. Taking into cognizance immobilization as the backbone of any biosensor, this work, reports a technique to immobilize an ordered multilayer of diamine oxidase (DAO) by means of chemical cross-linking on the biconical tapered fiber surface step-wisely alternating between chitosan, glutaraldehyde (GA) and the enzyme. The optimum parameters for the fabricated biosensor included 160 mg/ml DAO, 0.5% chitosan, 2.5% GA, pH 7.0, and tapered fiber surface of waist diameter 12 µm and length 20 mm. A spectrophotometric signal resulted from horseradish peroxidase catalyzed reduction of H_2O_2, a secondary product of the oxidative deamination of histamine monitored at 450 nm in 0.1 M phosphate buffer (pH 7.0 and room temperature). Atomic force microscopy (AFM), scanning electron microscope (SEM) and spectrophotometric technique confirmed the functionality of the biosensor. The biosensor showed a response and recovery time of 14 sec, a linear response range up to 1.5 mM, a good sensitivity of 0.64 mM^{-1} with detection and quantification limits towards histamine of 0.086 mM (15.8 ppm) and 0.204 mM (37.7 ppm) and a linear response range of 0-1.5 mM. The sensor showed an excellent anti-interferents property towards the common interferents' agents of <5%, with good recovery performance towards varying concentration of histamine ranging from 95.6 to 103.6% (RSD <5%). It showed operational stability to up to 40 repeated analyses without significant loss of sensitivity. The developed miniaturized biosensor has a good potential for use in quantitative measurement of histamine in seafood.

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

PEMBANGUNAN DIAMINE OXIDASE FIBER OPTIK BERSAIZ KECIL TIRUS BERASASKAN SENSOR UNTUK PENGESANAN HISTAMINE

Oleh

USMAN BOKO HAMZA

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Penganggaran tahap histamin dalam ikan dan produk ikan adalah sangat penting kerana implikasinya terhadap keracunan ikan pada manusia; oleh itu, penentuan tahap histamin adalah penting untuk mengetahui tahap kerosakan indeks kimia. Antara masalah bagi analisis pengesanan konvensional adalah punca masalah disebabkan oleh faktor seperti peralatan mahal, penggunaan masa, saiz dan berat badan. Maka, pengesan yang berasaskan gentian optik tirus-enzim telah digunakan untuk menyelesaikan masalah tersebut oleh kerana kebolehan pengesan yang mudah alih dan sederhana untuk digunakan pada masa kini. Menyedari ia sebagai tulang belakang kepada mana-mana biosensor, projek ini melaporkan teknik untuk memegunkan satu lopisan tersusun dengan mengarahkan diamine oxidase (DAO) dengan cara kimia silang pada permukaan gentian tirus yang dwikon secara selingan antara kitosan, glutaraldehid (GA) dan enzim. Parameter optimum untuk biosensor yang direka adalan dengan menggunakan 160 mg/ml DAO, 0.5% kitosan, 2.5% GA, pH 7.0, dan permukaan gentian tirus yang mempunyai diameter 12 μm dan panjang 20 mm. Isyarat spektrofotometri dihasilkan oleh horseradish peroxidase yang menjadi pemangkin pengurangan H_2O_2, produk sekunder oleh pengoksidaan deaminasi histamin yang telah dipantau pada 450 nm dalam 0.1 M larutan penimbal fosfat (pH 7.0 dan suhu bilik). Daya mikroskop atom (AFM), mikroskop elektron imbasan (SEM) dan teknik spektrofotometri telah mengesahkan keupayaan biosensor itu. Hasil keputusan biosensor telah menunjukkan tindak balas masa selama 14 saat, dan peningkatan linear sehingga 1.5 mM, kepekaan yang baik iaitu 0.64 mM^{-1} dengan pengesanan dan had kuantifikasi histamin sebanyak 0.086 mM (15.8 ppm) dan 0.204 mM (37.7 ppm) dan tindak balas linear sebanyak 0-1.5 mM. Sensor ini telah menunjukkan kejayaan anti-interferen terhadap ejen yang biasa iaitu <5%, dengan peningkatan prestasi terhadap perbezaan kepekatan histamine di antara 95.6 sehingga 103.6% (RSD <5%). Ini menunjukkan kestabilan operasi meningkat dalam 40 kali analisis tanpa kehilangan tahap sensitiviti dengan ketara. Pembangunan biosensor mini ini telah menunjukkan potensi yang baik untuk digunakan dalam penentuan kuantitatif sebatian histamine makanan lau.

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I certify that a Thesis Examination Committee has met on 26th May 2015 to conduct the final examination of (student's name) on his (her) thesis entitled "development of miniaturized tapered fiber optics diamine oxidase-based sensor for histamine detection" 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 Master of Science Degree in Biochemistry.

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LIST OF ABBREVIATIONS

AO	Amine oxidase
BA	Biogenic amine
Cad	Cadaverine
DAO	Diamine oxidase
DD	Degree of deacetylation
GA	Glutaraldehyde
GOD	Glucose oxidase
HMT	Histamine-N-methyl transferase
IUPAC	International union of Pure and applied chemistry
KDA	kilo Dalton
LbL	Layer by layer
LOD	Limit of detection
LOQ	limit of quantification
MMF	Multi-mode fiber
ODA	O-Dianisidine
PEGDGE	Poly(ethylene glycol) diglycidyl ether
PEM	Polyelectrolyte multilayer
POD	Peroxidase
PPM	Part per million
PSAO	Pea-Seedling amine oxidase
PUO	Putrescence oxidase
Put	Putrescine
SAM	Self-assembly multilayer
SPR	Surface Plasmon resonance
TFOB	Tapered Fiber Optic Biosensor

CHAPTER 1

INTRODUCTION

1.1 Rationale and motivation

Histamine is a strong bioactive compound that can exert many responses within the body. These compounds apart from their biochemical significance to the biological system, they have been linked with fish poisoning (scombrotoxicosis, scombroid fish poisoning or histamine fish poisoning). An illness originally referred to as "scombroid poisoning", due its association with fish, in the families' Scombridae (such as mackerel and tuna) and Scomberesocidae (such as saury) although other non-scombroid species such as mahi-mahi, sardines, pilchards, anchovies, herring, marlin and bluefish also were implicated (Steiner, Meier, Duerkop, & Wolfbeis, 2010). Histamine poisoning is associated with higher level of histamine in fish (Keow et al., 2007). Histamine was observed to accumulate in tissues of fish and other seafood when spoilage commenced during storage because of inadequate refrigeration or preservation (Chemnitius & Bilitewski, 1996). Despite successes to unravel the recommended level as chemical indicator for safety or otherwise for the consumption of fish and it related products, with regard histamine toxicity, however, the big issue is how to unravel a simple and robust method to detect and determine these levels, with the global demand of fish and aquaculture products is constantly increasing. Current data for fish and aquaculture showed a total annual supply of 148, 154 and 158 million tons in 2010, 2011 and 2012, respectively, with increasing estimates for 2013 (FAO., 2014). Fish and fish products, cheese, meat and other variety of food had been widely documented to contain high level of biogenic amines preventing the consumption of spoiled food would be better than curing the disease.

In the past, several conventional technique such as derivatisation with fluorescent reagents, chromatographic methods of separation, electrophoresis, immunochemical methods, titration and lots more have been proposed as possible options (Tombelli & Mascini, 1998). However, these methods tends to disallow an easily and continuous monitoring process, because these instruments are costly, slow, needs well-trained personnel, require sample pre-treatment and also because of their physical characteristics such as size weight and fragility (Mello & Kubota, 2002).

The goal of biosensor research is to develop sensors capable of addressing these lapses, due to need for real time quantitative and/or qualitative measurement in the field and so far, it remains the best alternative to ease the routine analysis of fish and other seafood. Therefore, the design of a biosensor device capable of eliminating the lapses, and also maintaining the precision and accuracy of laboratory analysis is of paramount importance (Monk & Walt, 2004). Biosensor technology has attracted researchers due to their characteristic easy-to-use, accurate, fast, inexpensive, less sample pre-treatment, potential for miniaturization, simple and portable equipped platform for construction and high sensitivity and specificity in recognizing their analytes (Mello & Kubota, 2002).

Leland C Clark Jr is celebrated as the father of biosensor since he first coined the 'biosensor concept' in his published definitive paper on the oxygen electrode in 1956 (Chauhan, Vibhuti, & Singh, 2004). Clark's idea was realized when the first commercialized glucose biosensor was re-launch in 1975 (first launch 1973) by the Yellow Springs Instrument Company (Ohio). This is followed by a lactate analyzer (LA 640) device in 1976, by La Roche, Switzerland (Chaubey & Malhotra, 2002). Over the past decades, different devices evolved under the name 'biosensor', utilizing different platforms and biosensing receptors (biocatalytic, bioaffinity and hybrid receptors). Biosensor application has evolved from the area of laboratory testing to commercialization. They have gained wider application namely in the field of medical diagnostics, food safety, homeland security, industrial process control, battle field and environmental monitoring (Lee, 2008). In 2008, the global market for commercialized biosensors stands at about $7 billion, with medical diagnostics (e.g., glucose biosensors and pregnancy test strips) being dominant and this is estimated to reach US$12 billion by the year 2015 (Bahadır & Sezgintürk, 2015; Lee, 2008). Bayer, Roche Diagnostics, and LifeScan occupies the apex of competing mediated biosensors companies with a combined percentage sell of about 85% of the world market for biosensor.

There are many efforts geared towards developing a biosensor for biogenic amine detection, and with respect to the solid support. Few mentions amongst several of these works by these researchers are amperometry employing a screen printed electrode (SPE) (Chemnitius & Bilitewski, 1996; Keow et al., 2012; Keow et al., 2007; Wimmerová & Macholán, 1999). Amperometry utilizing Pt electrode (Carelli, Centonze, Palermo, Quinto, & Rotunno, 2007; Carsol & Mascini, 1999; Karube, Satoh, Araki, Suzuki, & Yamada, 1980; Tombelli & Mascini, 1998), and utilizing a control pore-glass electrode (Yao, Satomura, & Wasa, 1992), and a capacitive sensor utilizing copper electrode (Wasoh et al., 2012). However, in any biotechnological research, the commercialization process is a stepwise gradual improvement from existing system until is made available to the final users in a portable and simplified form. Therefore, looking into the prospect of having a miniaturized diamine oxidase-based biosensor would be a step towards achieving this goal. The advantages offered by miniaturized biosensor include the requirement of small quantity of biorecognition element for biosensor development, portability, and thus a prospect for a miniaturized and disposable type of biosensor.

To the best of our knowledge, among the biogenic amine biosensors fabricated so far, no much effort geared towards fabricating a tapered fiber optic biosensor for DAO. Herein we propose the use of tapered fiber optics (taper) for this research work, due to its advantages over the use of other transduction techniques. These advantages include compactness, high sensitivity, affordability, fast response time, requirement for small volume of enzyme, lightweight and compatibility with optoelectronic devices (Baldini, Brenci, Chiavaioli, Giannetti, & Trono, 2012).

1.2 Objectives

Therefore, the objectives of these studies are:

i. To immobilize diamine oxidase on chitosan and cross-linked with bifunctional agent (GA).
ii. To modify a multi-mode fiber (MMF) through tapering.
iii. To fabricate a biosensor; through coating the enzyme multilayer, by alternating between chitosan, GA and DAO onto the tapered fiber.
iv. To optimize and characterize the designed biosensor.

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