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DEVELOPMENT OF NON-INVASIVE QUANTUM DOTS-ENZYME BASED BIOSENSOR FOR URIC ACID DETECTION

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By

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DEVELOPMENT OF NON-INVASIVE QUANTUM DOTS-ENZYME BASED BIOSENSOR FOR URIC ACID DETECTION

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November 2017

Chairman : Jaafar Abdullah, PhD
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Uric acid is the primary end product of purine metabolic pathway which is generated by the enzyme xanthine oxidase. Higher uric acid occurrence in body can lead to some disorders such as gout, arthritis, cardiovascular disease, kidney disease and hyperuricemia. Instead of causing disease at higher concentration, abnormally low uric acid level will also lead to multiple sclerosis. Non-invasive based biosensor provides an alternative method to detect uric acid in human metabolite without drawing blood or puncturing the skin. Non-invasive method allows painless procedure and very useful for people with problems in collecting blood samples such as hemophiliacs, neonates, elderly people, and disabled people. This research focus on the development of non-invasive quantum dots (QDs)-enzyme biosensor for uric acid detection. Two types of biosensor were developed namely CdS QDs/enzymes/sol-gel based biosensor and manganese doped CdS QDs/enzymes/sol-gel based biosensor. The developed biosensor presents a novel and convenient technique using sol-gel encapsulated QDs-enzyme in 96 well plates. The system consists of uricase/horseradish peroxidase (HRP) enzymes and QDs which is used as fluorescence indicator to reveal fluorescence property of the system resulting from enzymatic reaction of uricase/HRP in the presence of uric acid. Upon addition of uric acid to the hybrid uricase/HRP-CdS QDs, it will be oxidized to yield allaintoin, CO_2 and H_2O_2. The produced H_2O_2 is able to quench the QDs fluorescence intensity which is proportional to the uric acid concentration. The developed biosensor could detect up to 96 samples per assay within 20 minutes with linear concentration range of 0.06–2.00 mM, limit of detection (LOD) of 0.05 mM and 0.03–1.00 mM, limit of detection (LOD) of 0.02 mM for undoped and doped QDs, respectively. Mn doped MPA-CdS biosensor exhibits longer stokes shift hence less noise and interference from foreign substances in uric acid detection compared to undoped biosensor. This observation proved the higher sensitivity was obtained with doped QDs biosensor compared to undoped QDs biosensor. The proposed method also shown its potential for the

determination of uric acid concentration in urine samples and provides a promising tool for clinical diagnosis.

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

PEMBANGUNAN BIOSENSOR BUKAN INVASIF BERASASKAN TITIK KUANTUM-ENZIM UNTUK PENGESANAN ASID URIK

Oleh

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Asid urik merupakan produk akhir utama bagi laluan metabolik purine yang dihasilkan oleh enzim *xanthine oxidase*. Kehadiran asid urik yang lebih tinggi dalam tubuh boleh menyebabkan beberapa gangguan seperti gout, arthritis, penyakit kardiovaskular, penyakit buah pinggang dan hyperurikemia. Selain daripada menyebabkan penyakit pada kepekatan yang lebih tinggi, paras asid urik yang tidak normal juga akan membawa kepada penyakit sclerosis berbilang. Biosensor berasaskan kaedah bukan invasif menjadi alternatif untuk mengesan asid urik dalam metabolit manusia tanpa pengeluaran darah atau tusukan kulit. Kaedah bukan invasif melibatkan prosedur yang tidak menyakitkan dan sangat berguna untuk orang yang menghadapi masalah untuk mendapatkan sampel darah seperti hemophiliacs, bayi yang baru lahir, orang tua, dan orang kurang upaya Kajian ini memberikan tumpuan terhadap pembangunan biosensor bukan invasif berasaskan titik kuantum (QDs)-enzim bagi pengesanan asid urik. Dua jenis biosensor telah dibangunkan iaitu biosensor berasaskan CdS QDs/enzim/sol-gel dan biosensor berasaskan mangan dopan CdS QDs/enzim/sol-gel. Biosensor yang dibangunkan mempunyai ciri novel dan teknik pengesanan yang mudah menggunakan sistem QDs/enzim/sol-gel dipegun dalam 96 plat leper. Sistem ini mengandungi enzim uricase/horseradish peroksidase (HRP) dan QDs yang digunakan sebagai penunjuk pendarfluor untuk mempamerkan ciri pendarflour sistem yang terhasil dari tindak balas enzim uricase/HRP dengan kehadiran asid urik. Dengan penambahan asid urik dalam sistem hibrid uricase/HRP-CdS QDs, ia akan dioksidakan kepada allaintoin, karbon dioksida dan hidrogen peroksida. Hidrogen peroksida yang terhasil akan menyebabkan kesan pelindapkejutan terhadap keamatan pendarfluor yang berkadar langsung dengan kepekatan asid urik. Biosensor yang dibangunkan dapat mengesan 96 sampel serentak dalam masa 20 minit dengan julat kepekatan linear 0.06–2.00 mM, had pengesanan (LOD) 0.05 mM and 0.03–1.00 mM, had pengesanan (LOD) 0.02 mM untuk sistem tanpa dopan dan mangan dopan QDs,

masing-masing. Mangan dopan MPA-CdS biosensor memberikan stokes shift yang lebih panjang dan ini mengurangkan kesan gangguan pendarfluor dan mengalami kurang gangguan daripada bahan asing dalam pengesanan asid urik berbanding dengan biosensor tanpa dopan. Pemerhatian ini membuktikan kepekaan yang lebih tinggi diperolehi dengan mangan dopan biosensor berbanding biosensor tanpa dopan. Kaedah yang dibangunkan menunjukkan potensi untuk mengesan asid urik dalam air kencing dan menyediakan kaedah yang menyakinkan untuk diagnosis klinikal.

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I certify that a Thesis Examination Committee has met on 29 November 2017 to conduct the final examination of Nur Ellina binti Azmi on her thesis entitled "Development of Non-Invasive Quantum Dots- Enzyme based Biosensor for Uric Acid 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

APTMS	(3-Aminopropyl)trimethoxysilane
DNA	Deoxyribonucleic acid
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
GPTMS	(3-Glycidoxypropyl)methyldiethoxysilane
HPLC	High Performance Liquid Chromatography
HRP	Horseradish Peroxidase
LOD	Limit of detection
MPA	Mercaptopropionic acid
NHS	N-Hydroxysulfosuccinimide
PBS	Phosphate Buffer Solution
POCT	Point Of Care Testing
QDs	Quantum Dots

CHAPTER 1

INTRODUCTION

1.1 Background of study

Nowadays, technological advances and globalisation has led to a new medical solution for rapid monitoring or screening of public healthcare by developing point of care testing (POCT). POCT refer to testing at or near to the patient which typically involved blood and urine testing. The goal of POCT is to collect the specimen and obtain accurate results in a very short period of time at or near the location of the patient. POCT test results can lead to the change in the care of the patient in terms of providing a faster turnaround time than testing performed in the central laboratory (Shaw, 2016). Usually, the developed POCT will use transportable, portable, and handheld instruments. Examples of POCT are diagnostic kit based on self-monitoring system which represent the largest commercial product for POCT (John, 2010).

The diagnostic kit system provides information on patient health condition which helps the clinician to enable appropriate treatment for patient. A further fascination of diagnostic kit is that it generally requires less sample volume than the tests performed in the central laboratory. The prompt diagnosis of critical health changes could enable the prevention of serious or lethal events. Furthermore the immediate availability of the results from diagnostic kit can be linked to patient management to facilitate movement of individual patients through the system faster or to allow for handling of more patients in a lessened time frame (Price, 2001).

The development of diagnostic kit usually involves the use of biological or chemical system for rapid detection such as biosensors and chemical sensors as platform technology for diagnosis applications. Biosensors are becoming increasingly important and practical tools in the development of diagnostic kits. There are many types of diagnostic kit based on biosensor platform that has been established which include monitoring of glucose (Lai et al., 2016; Pakapongpan & Poo-arporn, 2017), cholesterol (Dey & Raj, 2010; Sekretaryova et al., 2014) and colorectal (Raji et al., 2015; Soler et al., 2016), respectively. Despite numerous advantages of biosensors have been offered, current advances in micro- and nanotechnologies have led to the development of biosensors that capable of performing complex molecular assays and provide more sensitive diagnostic kit system. A variety of new strategies have been explored using nanotechnology based biosensors for healthcare applications.

Nanotechnology application has played an important role in the development of biosensors. Selectivity and sensitivity of the biosensors can be improved by incorporation of nanomaterials in the fabrication. In current technology, usage of nanomaterials or matrices with dimensions ranging in the scale from 1 to 100 nm, could improve the sensitivity of diagnostic biosensor due to the effects such as the

quantum size, surface area enhancement and unique optical properties (Zhang et al., 2010). Few examples of nanomaterials used in the biosensors development are gold nanoparticles, carbon nanotube, magnetic nanoparticles and quantum dots (QDs). Several methods have been explored for the preparation of water soluble QDs for the use in biological studies. These water based QDs have significant advantage over traditional fluorescence dyes including better stability, stronger fluorescence intensity and narrow emission wavelength (Medintz et al., 2005). In this study, the development of biosensors based on quantum dots-enzymes hybrid system for the determination of uric acid in urine samples has been explored.

1.2 Problem statement

Uric acid ($C_5H_4N_4O_3$) is the primary end product of purine metabolic pathway which is generated by the enzyme xanthine oxidase. Uric acid production and metabolism are complex processes comprising various factors that regulate hepatic production, as well as renal and gut secretion of this compound. The endogenous production of uric acid is mostly from the liver, intestines and other tissues like muscles, kidneys and the vascular endothelium (Maiuolo et al., 2016). The exogenous pool varies significantly with diet and animal proteins contribute significantly to this purine pool. Uric acid can always be found in high purine content food such as liver, anchovies, mackerel, scallops and beer. Figure 1 shows uric acid structure which comprise heterocyclic compound of carbon, nitrogen, oxygen and hydrogen.

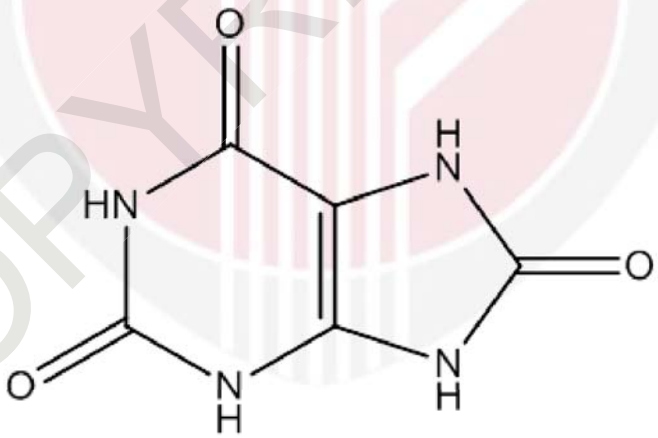

Figure 1.1 : Uric acid structure

It is important to determine uric acid level in human body fluid in order to diagnose diseases caused by disorders of purine catabolism. Usually, normal uric acid level in serum is in the range of 0.13– 0.46 mM (2.18–7.7 mg/dL) and 1.4– 4.4 mM (25–74 mg/dL) (Retna Raj & Ohsaka, 2003) in urinary excretion. Higher uric acid occurrence in body can lead to some disorders such as gout, arthritis, cardiovascular disease, kidney disease and hyperuricemia (Kanyong et al., 2012). Instead of causing disease

at higher concentration, abnormally low uric acid level will also lead to multiple sclerosis (Misra et al., 2013). Thus it is very important to determine uric acid level in human body fluid to monitoring healthcare conditions.

The non-invasive concept has been introduced more than 30 years ago. Nevertheless, it can be said that most of the non-invasive technologies are still in their early stages of development. Many non-invasive technologies have been described in the literature (Guilbault et al., 1995; So et al., 2012; Soni & Jha, 2015), and there is an increasing interest in this research work recently. Urine sampling generally involves a simple and non-invasive method that allows painless procedure is particularly useful for people with problems in collecting blood samples such as hemophiliacs, neonates, elderly people, and disabled (Guilbault et al., 1995). In this study, biosensor based on non-invasive method for uric acid detection using human's urine samples have been explored.

First method for uric acid analysis was developed in 1894 by using phosphotungstate complexes (Matos et al., 2000). Grown with technologies advancement, several others method have been reported such as spectrophotometry (Huang et al., 2004), electrochemical techniques (Retna Raj & Ohsaka, 2003), flow-injection chemiluminescence (Hong & Huang, 2003), high performance liquid chromatographic (HLPC) (Zuo et al., 2015) and fluorescence method (Galbán et al., 2015). However, some of these methods have issues associated with selectivity and sensitivity.

For electrochemical technique, amperometric detection usually will facing interference problem especially from ascorbic acid which oxidize at potential close to uric acid result in overlapping of the signal responses. The use of high potential value in amperometric measurement also lead to signal interference in the uric acid detection system (Huang et al., 2004). Potentiometric sensor reported to be more selective than amperometric but have limitation in term of ion sensitive electrode that only allowed charged molecules to be detected.

Standard method using HPLC analysis offer good sensitivity but this technique will involve high technical skill personnel. The HPLC analysis also involve laborious procedure for sample extraction steps, high end equipment and time consuming especially on the lengthy of the retention time for specific standard assay (Raoof et al., 2012). For assay kit based on fluorescence-enzyme system, usually used conventional fluorescent dye that is not stable in solution form. The assay requires reagents preparation, incubation of the reagents with analyte and the reagent must be promptly used once being dissolved. Thus, this study focusing on development of biosensor detection system using nanocrystal semiconductor quantum dots (QDs) nanomaterial as fluorescence indicator which offer rapid, stable and selective for uric acid detection.

1.3 Objectives of the study

The main objective of the study is to develop biosensor for rapid detection of uric acid in urine through non-invasive technique. QDs-enzyme conjugates have been explored as fluorescence indicator in order to develop biosensor system for uric acid detection in urine sample. The specific objectives of the research are summarized and listed as follows:

I. To characterize water soluble mercaptopropionic acid (MPA)-CdS QDs and manganese (Mn) doped MPA capped CdS QDs.

II. To develop and evaluate assay system based on enzyme-QDs for the determination of uric acid.

III. To develop and evaluate biosensor based on enzyme-MPA CdS QDs and enzyme-Mn Doped MPA-CdS QDs for the determination of uric acid.

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