UNIVERSITI PUTRA MALAYSIA

OPTICAL SENSING OF URIC ACID USING URICASE AS BIO-RECEPTOR

LIEW JIN YEE

FBSB 2015 57

OPTICAL SENSING OF URIC ACID USING URICASE AS BIO-RECEPTOR

LIEW JIN YEE

DEPARTMENT OF BIOCHEMISTRY
FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
UNIVERSITI PUTRA MALAYSIA

2015

OPTICAL SENSING OF URIC ACID USING URICASE AS BIO-RECEPTOR

LIEW JIN YEE

161111

Thesis Submitted in Partial Fulfillment of the Requirement
For the course BCH 4999 (Project) in the Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

June 2015

PENGESAHAN

Dengan ini adalah disahkan bahawa laporan yang bertajuk " Penderiaan Optik Asid Urik Menggunakan Urikase Sebagai Bio-reseptor" telah disiapkan serta dikemukakan kepada Jabatan Biokimia, Fakulti Bioteknologi dan Sains Biomolekul, Universiti Putra Malaysia oleh Liew Jin Yee (161111) sebagai syarat untuk kursus BCH 4999 (Projek).

Disahkan oleh:

…….. Tarikh: ……..……

Prof. Dato' Dr. Abu Bakar Salleh
Penyelia Projek

…….. Tarikh: ……..……

Prof. Dato' Dr. Abu Bakar Salleh
Ketua
Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

ABSTRACT

Purines are natural substances found in body's cells and in virtually all foods. When purine derivatives undergo metabolic breakdown, uric acid is produced. Abnormally high concentration of uric acid in the body can lead to diseases such as gout and uric acid stone formation. Thus, uric acid determination serves as a marker and plays a very important role in diagnosis. For clinical diagnosis and monitoring purposes, biosensor has been introduced as it is a fast and reliable analytical device. Demands for the current trend of biosensor development keep on increasing due to the advancement in technology especially the applications of biosensors using optical detection. In the present study, uricase from *Arthrobacter globiformis* was used as a bio-receptor and integrated to optical fibre for uric acid determination. Preliminary experiments of uricase immobilization techniques on glass slide were conducted before proceeding on optic fibre. Glass activation produces more reactive hydroxyl groups on the glass surface and utilization of both chitosan and glutaraldehyde as cross-linker demonstrated a better response towards uric acid. The immobilization techniques and optimum enzyme concentration obtained were then employed and proceeded on a tapered optical fibre. Uricase-immobilized tapered optic fibre with waist diameter of 80 µm and waist length of 20 mm was shown to have a positive relationship between absorbance value and concentration of uric acid range from 1.0 mM to 2.0 mM. From the results obtained, optical sensing of uric acid was possible and successful by using uricase as bio-receptor.

ABSTRAK

Asid urik terbentuk daripada pemecahan purin yang terdapat dalam kebanyakan tisu manusia dan makanan. Paras asid urik yang luar biasa tingginya dalam badan boleh menyebabkan penyakit seperti gout dan penjadian/pembentukan batu karang. Oleh sebab itu, pengesanan paras asid urik adalah sangat penting sebagai penanda terutamanya dalam sektor perubatan. Untuk tujuan ini, biosensor telah diperkenalkan sebagai alat pengesanan kerana penggunanya adalah sangat cepat dan tepat. Permintaan terhadap penciptaan biosensor kian tinggi di pasaran disebabkan oleh kemajuan teknologi yang cangih terutamanya pengesanan optik. Dalam kajian ini, uricase dari *Arthrobacter globiformis* dipilih sebagai bio-reseptor dan diintegrasikan dengan gentian optik untuk mengesan paras asid urik. Eksperimen awal melibatkan teknik immobilasi enzim di atas kaca slaid telah dijalankan. Pengaktifan kaca untuk menghasilkan kumpulan hidroksil yang aktik atas permukaan dan penggunana chitosan dan glutaraldehyde sebagai pemaut silang menunjukkan respond yang lebih baik terhadap asid urik. Teknik immobilasi dan kepekatan enzim optimum yang diperolehi telah diggunakan dan dialihkan ke atas gentian optik. Urikase terimmobilasi pada gentian optik dengan diameter 80µm dan panjangan 20mm telah terbukti mempunyai hubungan positif antara nilai keserapan dan kepekatan asid urik dari kadar 1mM ke 2mM. Berdasarkan keputusan yang diperolehi, pengesanan optik asid urik adalah tidak mustahil dan berjaya dicapai dengan menggunakan urikase sebagai bio-reseptor.

ACKNOWLEDGEMENT

First and foremost, I would like to extend my deepest gratitude to my supervisor, Prof. Dato' Dr. Abu Bakar Salleh for his valuable guidance and advice. His patience, encouragement and understanding helped me in all time of research and writing of this thesis. I would also like to extend my appreciation to Dr. Amir for his comments and kindness during my research.

Furthermore, I would like to thank all the seniors and members of Enzyme & Microbial Technology Research Centre, EMTech (140 and 209) especially Arilla, Zarir and Hamza for the assistance, support and sharing of experience and knowledge throughout the study. My warmest gratitude also goes to Akmar and Yasmin from engineering laboratory.

Last but not least, I would like to thank my family members especially my parents and friends especially Elisa, Emay, Haeida, Kai Lee, Mun Hong, Wyatt and Weini for their loving support, companion and encouragement throughout this period. This research project would not be possible without helps of the particular that mentioned above. Thank you very much!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
°C	Degree celsius
µl	Microlitre
µm	Micrometer
Ag	Silver
Cm	Centimetre
$C_5H_4N_4O_3$	Uric acid
CO_2	Carbon dioxide
et al.,	And friends
HCl	Hydrogen chloride
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
HRP	Horseradish peroxidase
M	Molar
mg/dL	Milligram per decilitre
mg/ml	Milligram per millilitre
mm	Millimetre
mM	Millimolar
MMF	Multi-mode fibre
NaOH	Sodium hydroxide
NH_3+	Positive ammonium group
nm	Nanometer
OH	Hydroxyl group
O_2	Oxygen
PC	Personal computer

SMF	Single-mode fibre
U/ml	Unit per millilitre
UV	Ultraviolet
VIS-NIR	Visual light – near infrared
ZnO	Zinc oxide

CHAPTER 1

INTRODUCTION

Purines which consist of a pyrimidine ring fused to an imidazole ring are natural substances found in body's cells and in virtually all foods. When purine derivatives undergo metabolic breakdown, uric acid (2,6,8-trihydroxypurine) is produced (Schrenkhammer & Wolfbeis, 2008). Detection and quantification of uric acid plays a very important role as abnormally high concentrations of uric acid (hyperuricemia) are linked to diseases such as gout, cardiovascular disease, hypertension and arthritis (Devi & Pundir, 2014). Hence, uric acid determination serves as a marker and is important in diagnosis and for monitoring a large number of diseases (Misra *et al*., 2013).

Several different methods of uric acid detection are available. The most common one is based on the use of the enzyme uricase. Uricase, also known as urate oxidase, is absent in humans and catalyses the oxidation of uric acid to produce hydrogen peroxide, allantoin and carbon dioxide (Zhao *et al*., 2008).

Biosensor is an analytical device that consists of a bio-receptor and a transducer. Uricase is selected as a bio-receptor because enzymes are well-known as a biological sensing materials due to their specificity and bio-recognition capability (Malhotra & Chaubey, 2003) while optical transducer is selected to convert the recognition event into a measureable signal based on optical techniques (Choi, 2004).

Different types of uricase-based biosensor such as polyaniline biosensor, amperometirc biosensor and optical biosensor are available for the determination of uric acid (Arslan, 2008; Erden & Kılıç, 2013; Kan *et al*., 2004; Kuswandi *et al*., 2008; Schrenkhammer & Wolfbeis, 2008). However, the operation and application of certain biosensors require long sample preparation time causing certain amounts of uric acid may also be oxidized, require laboratory environment for working, lack of stability, sensitivity and low reproducibility. Thus, demands for the current trend of biosensor development keep on increasing due to the advancement in technology.

The application of biosensors using optical detection has developed greatly as it performs remote, rapid and in-line determination of various analytes in a range of

application fields (Choi, 2004). It has indeed captured the attention of many scientists due to its greater sensitivity, higher efficiency and simpler to use.

In this study, uricase from *Arthrobacter globiformis* was integrated as a bio-receptor with an optical transducer for determination of uric acid. This is a preliminary study for future creation of a more sophisticated, versatile and sensitive enzyme-based optic fibre biosensor.

The objectives of this study include:

1. To access the effectiveness of uricase as the bio-receptor for uric acid.
2. To explore methods for optical sensing of uric acid using uricase as bio-receptor.

REFERENCES

Altun, G. D., & Cetinus, S. A. (2007). Immobilization of pepsin on chitosan beads. *Food Chemistry*, *100*(3), 964–971.

Arslan, F. (2008). An amperometric biosensor for uric acid determination prepared from uricase immobilized in polyaniline-polypyrrole film. *Sensors*, *8*(9), 5492–5500.

Azmi, N. E., Ramli, N. I., Abdullah, J., Abdul Hamid, M. A., Sidek, H., Abd Rahman, S., Ariffin, N., & Yusof, N. A. (2014). A simple and sensitive fluorescence based biosensor for the determination of uric acid using H_2O_2-sensitive quantum dots/dual enzymes. *Biosensors & Bioelectronics*, *67*, 129–133.

Ballesta-Claver, J., Rodríguez-Gómez, R., & Capitán-Vallvey, L. F. (2013). Disposable biosensor based on cathodic electrochemiluminescence of tris(2,2-bipyridine)ruthenium(II) for uric acid determination. *Analytica Chimica Acta*, *770*, 153–160.

Batumalay, M., Harith, Z., Rafaie, H. A., Ahmad, F., Khasanah, M., Harun, S. W., Nor, R. M., & Ahmad, H. (2014). Tapered plastic optical fiber coated with ZnO nanostructures for the measurement of uric acid concentrations and changes in relative humidity. *Sensors and Actuators A: Physical*, *210*, 190–196.

Batumalay, M., Harun, S. W., Ahmad, F., Nor, R. M., Zulkepely, N.R. & Ahmad, H. (2014). Tapered plastic optical fiber coated with graphene for uric acid detection. *IEEE Sensors Journal*, *14*(5), 1704-1709.

Borisov, S. M., & Wolfbeis, O. S. (2008). Optical biosensors. *Chemical Reviews*, *108*(2), 423–461.

Brena, B., González-Pombo, P., & Batista-Viera, F. (2013). Immobilization of enzymes: a literature survey. In Guisan, J. M. (Ed.), *Methods in Biotechnology : Immobilization of Enzymes and Cells Second Edition* (pp. 15-31). Totowa, NJ: Humana Press.

Bunganaen, Y., & Lamb, D. W. (2005). An optical fibre technique for measuring optical absorption by chromophores in the presence of scattering particles. *Journal of Physics: Conference Series*, *15*, 67-73.

Cahyaningrum, S. E., & Sianita, M. M. (2014). Immobilization of pepsin onto chitosan silica nanobeads with glutaraldehyde as crosslink agent. *Bulletin of Chemical Reaction Engineering & Catalysis*, *9*(3), 263–269.

Chaudhari, R. D., Joshi, A. B., & Srivastava, R. (2012). Uric acid biosensor based on chemiluminescence detection using a nano-micro hybrid matrix. *Sensors and Actuators B: Chemical*, *173*, 882–889.

Chauhan, N., Kumar, A., & Pundir, C. S. (2014). Construction of an uricase nanoparticles modified au electrode for amperometric determination of uric Acid. *Applied Biochemistry and Biotechnology*, *174*(4), 1683–1694.

Chauhan, N., Preeti, Pinky, & Pundir, C. S. (2014). Covalent immobilization of uricase inside a plastic vial for uric acid determination in serum and urine. *Analytical Sciences : the International Journal of the Japan Society for Analytical Chemistry*, *30*(4), 501-506.

Choi, M. M. F. (2004). Progress in Enzyme-Based Biosensors Using Optical Transducers. *Microchimica Acta*, *148*(3-4), 107–132.

Devi, R., & Pundir, C. S. (2014). Construction and application of an amperometric uric acid biosensor based on covalent immobilization of uricase on iron oxide nanoparticles/chitosan-g-polyaniline composite film electrodeposited on Pt electrode. *Sensors and Actuators B: Chemical*, *193*, 608–615.

Domagk, G. F., & Schlicke, H. H. (1968). A colorimetric method using uricase and peroxidase for the determination of uric acid. *Analytical Biochemistry*, *22*(2), 219–224.

Dong, L. C., Wang, G., Xiao, Y., Xu, Y., Zhou, X., Jiang, H., & Luo, Q. (2011). Immobilization of glucose oxidase on a novel crosslinked chitosan support grafted with L-lysine spacers. *Chemical & Biochemical Engineering Quarterly*, *25*(3), 395–402.

Edgar181, (2013). Chemical structure of glutaraldehyde. [OnlineImage]. Retrieved May 9, 2015, from http://commons.wikimedia.org/wiki/File:Glutaraldehyde_structure.png.

Erden, P. E., & Kılıç, E. (2013). A review of enzymatic uric acid biosensors based on amperometric detection. *Talanta*, *107*, 312–323.

European Patent Organization, (2009). Chemical reaction of phenol, 4-aminoantipyrine, hydrogen peroxide and peroxidase. [OnlineImage]. Retrived May 9, 2015, from https://data.epo.org/publication-server/rest/v1.0/publication-dates/19851023/patents/EP0158796NWA2/document.html#disclaimer.

Harun, S. W., Lim, K. S., Tio, C. K., Dimyati, K., & Ahmad, H. (2013). Theoretical analysis and fabrication of tapered fiber. *Optik - International Journal for Light and Electron Optics*, *124*(6), 538–543.

Janissen, R., Oberbarnscheidt, L., & Oesterhelt, F. (2009). Optimized straight forward procedure for covalent surface immobilization of different biomolecules for single molecule applications. *Colloids and Surfaces. B, Biointerfaces*, *71*(2), 200–207.

Jorge, P. A. S., Caldas, P., Rosa, C. C., Oliva, A. G., & Santos, J. L. (2004). Optical fiber probes for fluorescence based oxygen sensing. *Sensors and Actuators B: Chemical*, *103*(1-2), 290–299.

Kahraman, M. V., Bayramoglu, G., Kayaman-Apohan, N., & Gungor, A. (2007). α-Amylase immobilization on functionalized glass beads by covalent attachment. *Food Chemistry*, *104*(4), 1385–1392.

Kan, J., Pan, X., & Chen, C. (2004). Polyaniline-uricase biosensor prepared with template process. *Biosensors & Bioelectronics*, *19*(12), 1635–1640.

Kimica Corporation, (2009). Sturtuce of chitosan. [OnlineImage]. Retrived May 9, 2015, from http://www.kimica.jp/eng-chitosan-chemi-2.htm.

Koyun, A., Ahlatcioglu, E., & İpek, Y. K. (2012). Biosensors and their principles. In Kara S. (Ed.), *A Roadmap of Biomedical Engineers and Milestones* (pp. 115-142). Rijeka, Croatia: InTech.

Krajewska, B. (2004). Application of chitin- and chitosan-based materials for enzyme immobilizations: a review. *Enzyme and Microbial Technology*, *35*(2-3), 126–139.

Kumar, V., Misra, N., Paul, J., Dhanawade, B. R., & Varshney, L. (2014). Uricase-immobilization on radiation grafted polymer support for detection of uric acid using Ag-nanoparticle based optical biosensor. *Polymer*, *55*(11), 2652–2660.

Kundu, T., Sai, V. V. R., Dutta, R., Titas, S., Kumar, P. & Mukherjee, S. (2010). Development of evanescent wave absorbance-based fibre-optic biosensor. *Pramana*, *75*(6), 1099–1113.

Kuswandi, B., Andres, R., & Narayanaswamy, R. (2001). Optical fibre biosensors based on immobilised enzymes. *Analyst*, *126*(8), 1469–1491.

Kuswandi, B., Ratnasari, I., Hermanto, D., & Gani, A. A. (2008). Optical Chemical Sensors and Biosensor for Detection of Uric Acid in Clinical Samples [PowerPointSlides]. Retrieved October 20, 2014, from http://www.docstoc.com/docs/133003157/Optical-Chemical-Sensors-and-Biosensor-for-Detection-of-Uric-Acid-in-Clinical-Samples.

Lee, Y. H., & Mutharasan, R. (1995). Chapter 6 Biosensors. In Wilson, J. S. (Ed.), *Sensor Technology Handbook* (pp. 161–180). Massachusetts, US: Newnes.

Leung, A., Shankar, P. M., & Mutharasan, R. (2007). A review of fiber-optic biosensors. *Sensors and Actuators B: Chemical*, *125*(2), 688–703.

Longtin, (2013). Interactions between charged amine groups and silanol sites. [OnlineImage]. Retrived May 9, 2015, from http://www.nanotechnologies.qc.ca projects/nanopatterning/nanopatterning.

Malhotra, B. D., & Chaubey, A. (2003). Biosensors for clinical diagnostics industry. *Sensors and Actuators B: Chemical*, *91*(1-3), 117–127.

Migneault, I., Dartiguenave, C., Bertrand, M. J., & Waldron, K. C. (2004). Glutaraldehyde : behavior in aqueous solution , reaction with proteins , and application to enzyme crosslinking. *BioTechniques*, *37*(5), 790-802.

Misra, N., Kumar, V., Borde, L., & Varshney, L. (2013). Localized surface plasmon resonance-optical sensors based on radiolytically synthesized silver nanoparticles for estimation of uric acid. *Sensors and Actuators B: Chemical*, *178*, 371–378.

Mundaca-Uribe, R., Bustos-Ramírez, F., Zaror-Zaror, C., Aranda-Bustos, M., Neira-Hinojosa, J., & Peña-Farfal, C. (2014). Development of a bienzymatic amperometric biosensor to determine uric acid in human serum, based on mesoporous silica (MCM-41) for enzyme immobilization. *Sensors and Actuators B: Chemical*, *195*, 58–62.

Mutua, F. N., Lin, P., Koech, J. K., & Wang, Y. (2012). Surface Modification of Hollow Glass Microspheres. *Material Sciences and Applications*, *3*, 856–860.

Neurotiker, (2007). Structure of uric acid. [OnlineImage]. Retrieved May 9, 2015, from http://commons.wikimedia.org/wiki/File:Harnsäure_Ketoform.svg.

Schrenkhammer, P., & Wolfbeis, O. S. (2008). Fully reversible optical biosensors for uric acid using oxygen transduction. *Biosensors & Bioelectronics*, *24*(4), 994-999.

Sharma, S. K., Sehgal, N., & Kumar, A. (2003). Biomolecules for development of biosensors and their applications. *Current Applied Physics*, *3*(2-3), 307–316.

Shriver-Lake, L. C., Donner, B., Edelstein, R., Bhatia, S. K., & Ligler, F. S. (1997). Antibody immobilization using heterobifunctional crosslinkers. *Biosensors and Bioelectronics*, *12*(11), 1101–1106.

Tedeschi, L., Domenici, C., Ahluwalia, A., Baldini, F., & Mencaglia, A. (2003). Antibody immobilisation on fibre optic TIRF sensors. *Biosensors and Bioelectronics*, *19*(2), 85–93.

Tian, Y., Wang, W., Wu, N., Zou, X., & Wang, X. (2011). Tapered optical fiber sensor for label-free detection of biomolecules. *Sensors*, *11*(4), 3780–3790.

Torres, M. A., Vieira, R. S., Beppu, M. M., Arruda, E. J., & Santana, C. C. (2007). Production of chemically modified chitosan microspheres by a spraying and coagulation method. *Materials Research*, *10*, 347–352.

Vojinović, V., Azevedo, A. M., Martins, V. C. B ., Cabral, J. M. S., Gibson, T. D., & Fonseca, L. P. (2004). Assay of H2O2 by HRP catalysed co-oxidation of phenol-4-sulphonic acid and 4-aminoantipyrine: characterisation and optimisation. *Journal of Molecular Catalysis B: Enzymatic*, *28*(2-3), 129–135.

Waechter, H., Litman, J., Cheung, A. H., Barnes, J. A., & Loock, H. P. (2010). Chemical sensing using fiber cavity ring-down spectroscopy. *Sensors (Basel, Switzerland)*, *10*(3), 1716–1742.

Wathoni, N., Hasanah, A. N., Gozali, D., Wahyuni, Y., & Fauziah, L. L. (2014). Determination of uric acid level by polyaniline and poly (allylamine): based biosensor. *Journal of Advanced Pharmaceutical Technology & Research*, *5*(1), 13–16.

Zhang, Y., Wen, G., Zhou, Y., Shuang, S., Dong, C., & Choi, M. M. F. (2007). Development and analytical application of an uric acid biosensor using an uricase-immobilized eggshell membrane. *Biosensors & Bioelectronics*, *22*(8), 1791–1797.

Zhao, Y., Yang, X., Lu, W., Liao, H., & Liao, F. (2009). Uricase based methods for determination of uric acid in serum. *Microchimica Acta*, *164*(1-2), 1–6.