
UNIVERSITI PUTRA MALAYSIA

# IMMOBILIZATION OF ANTI-17 BETA ESTRADIOL ANTIBODY ON OPTICAL FIBER SURFACE

LEE MAY YING

FBSB 2015 106

# IMMOBILIZATION OF ANTI-17 BETA ESTRADIOL ANTIBODY ON OPTICAL FIBER SURFACE

By

LEE MAY YING

162957

Dissertation submitted in partial fulfillment of the requirement for the course of BMY 4999 Project in the Department of Microbiology,
Faculty of Biotechnology and Biomolecular Sciences,
University of Putra Malaysia.

2015

**PENGESAHAN**

Dengan ini adalah disahkan bahawa laporan projek bertajuk "**Immobilization of anti-17 beta estradiol antibody on optical fiber surface**" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Lee May Ying sebagai syarat untuk kursus BMY 4999 projek.

Disahkan oleh, Tarikh:

……. …….....

Dr. Asilah Binti Ahmad Tajudin
Penyelia Projek,
Jabatan Mikrobiologi,
Fakulti Bioteknologi dan Sains Biomolekul,
Universiti Putra Malaysia.

Disahkan oleh, Tarikh:

……. …….....

Prof. Madya Dr. Muhajir Hamid
Ketua Jabatan Mikrobiologi,
Fakulti Bioteknologi dan Sains Biomolekul,
Universiti Putra Malaysia.

## ACKNOWLEDGEMENTS

First, I would like to express my deepest gratitude to my supervisor, Dr. Asilah Binti Ahmad Tajudin. Her caring, unlimited supports and valuable advices are always motivated me to move on during hard time.

I also want to thank Mr. Zarir who is always provided useful ideas for me to improve the methods of the project. Moreover, I also would like to acknowledge Dr. Nizam from the Faculty of Science, UPM. His teaching and critical comments based on the project also elevate my understanding about optical fiber which is a thing that I never learn before.

Besides that, the companionship from my lab partners, Amy, C.R.A and Amirah is also very important to encourage me to accomplish the project.

And last but not least, special thanks to my family, all of the friends, staffs and lecturers from Faculty of Biotechnology and Science Biomolecules, UPM as every single of them is willing to provide their help from time to time and also endless moral support which pushed me to move forward at every single moment.

## TABLE OF CONTENTS


| | | Page |
|---|---|---|
| **ACKNOWLEDGEMENTS** | | **i** |
| **TABLE OF CONTENTS** | | **ii-iii** |
| **LIST OF TABLES** | | **iv** |
| **LIST OF FIGURES** | | **v-vi** |
| **LIST OF ABBREVIATIONS** | | **vii** |
| **ABSTRACT** | | **viii** |
| **ABSTRAK** | | **ix** |
| **CHAPTERS** | | |
| **1.** | **INTRODUCTION** | 1-2 |
| **2.** | **LITERATURE REVIEW** | 3 |
| | 2.1 Immobilization of biomolecules | 3 |
| | 2.1.1 Antibody immobilization | 4-5 |
| | 2.2 Immobilization methods | 6 |
| | 2.3 Simple adsorption | 7 |
| | 2.4 Chemical adsorption | 8 |
| | 2.4.1 Employment of silane and crosslinker | 8 |
| | 2.4.2 Recruitment of Protein A | 9 |
| | 2.4.3 Antibody fragments as probes | 10 |
| | 2.5 Optical fiber | 10-11 |
| | 2.6 Biosensor | 12 |
| | 2.6.1 Applications of antibody-based biosensor | 13-14 |
| **3.** | **MATERIALS & METHODOLOGY** | 15 |
| | 3.1 Preparation of optical fiber | 15 |
| | 3.2 List of chemical reagents | 16 |
| | 3.3 Physical adsorption | 17 |
| | 3.4 Chemical adsorption | 18 |
| | 3.5 Bradford assay | 20 |
| | 3.6 BSA as the blocking reagent after antibody immobilization | 21 |
| | 3.7 Selection of the optimum parameter for BSA immobilization | 21 |
| | 3.7.1 Concentration of BSA | 21 |
| | 3.7.2 Coupling time | 21 |
| | 3.7.3 Washing reagent | 22 |
| | 3.8 Microscopic observations | 22 |
| | 3.9 Analysis from Optical Spectrum Analyzer (OSA) | 23 |

| | | |
|---|---|---|
| **4.** | **RESULTS & DISCUSSION** | 24 |
| | 4.1 Result of microscopic observation | 24 |
| | 4.2 Physical adsorption result | 25-27 |
| | 4.3 Chemical adsorption result | 27-30 |
| | 4.4 BSA immobilization result | 31-32 |
| | 4.5 Optimization of parameters for BSA immobilization | 33 |
| | 4.5.1 Results of optimization of BSA concentration | 32-33 |
| | 4.5.2 Results of optimization of BSA coupling time | 34-35 |
| | 4.5.3 Results of optimization of washing reagent | 35-36 |
| **5.** | **CONCLUSION AND RECOMMENDATIONS** | 37 |
| **6.** | **BIBLIOGRAPHY** | 38-40 |
| **7.** | **APPENDICES** | 41-45 |

## LIST OF TABLES


| Table | | Page |
|---|---|---|
| 1. | Evaluation of BSA concentrations. | 34 |
| 2. | Evaluation of coupling times for BSA. | 35 |
| 3. | Evaluation of washing reagents. | 36 |
| 4. | Preparation of BSA solutions for the calibration curve. | 41 |

## LIST OF FIGURES


| Figure | | Page |
|---|---|---|
| 1. | The schematic diagram of an antibody molecule. | 4 |
| 2. | The schematic presentation of immobilization of antibodies on the surface of solid support. | 5 |
| 3. | Two types of immobilization methods. | 6 |
| 4. | Immobilization of antibodies on solid surface by simple adsorption. | 7 |
| 5. | The chemical reactions along chemical adsorption. | 9 |
| 6. | The total internal reflection (TIR) occurs at the boundary between core and cladding materials of optical fiber. | 11 |
| 7. | The schematic presentation of biosensor. | 12 |
| 8. | Optical fiber. | 15 |
| 9. | Optical fiber stripes were soaking in acetone. | 15 |
| 10. | Optical fiber placed in an optical fiber holder. | 17 |
| 11. | Pipetting of APTES onto the optical fiber core surface. | 19 |
| 12. | The incubation of optical fiber. | 19 |
| 13. | Washing of optical fiber after APTES and GTA coating. | 19 |
| 14. | Optical spectrum analyzer (OSA). | 23 |
| 15. | The uncoated optical fiber. | 24 |
| 16. | The GTA activated optical fiber. | 24 |
| 17. | Graph of antibody immobilization by physical adsorption. | 26 |
| 18. | Graph of antibody immobilization by chemical adsorption. | 30 |
| 19. | Graph of BSA adsorption. | 31 |
| 20. | BSA standard curve. | 42 |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| APTES | 3-Aminopropyl-triethoxysilane |
| BSA | Bovine serum albumin |
| CHO | Aldehyde group |
| CN | Amide bond |
| EDC | Endocrine disrupting compound |
| GMBS | N-Succinimidyl 4-maleimidobutyrate ester |
| GTA | Glutaraldehyde |
| HCl | Hydrochloric acid |
| $H_2SO_4$ | Sulphuric acid |
| IUPAC | International Union of Pure and Applied Chemistry |
| MDS | Mercaptomethyldimethylethoxysilane |
| MTS | 3-Mercaptopropyltrimethoxysilane |
| $NH_2$ | Amino group |
| NaOH | Sodium hydroxide |
| OH | Hydroxyl group |
| OPF | Optical fiber |
| OSA | Optical spectrum analyzer |
| PBS | Phosphate buffered saline |
| PBST | Phosphate buffered saline with 0.05 % Tween 20 |
| SH | Sulfhydryl group |
| $SiO_2$ | Silica oxide |
| TIR | Total internal reflection |
| $^{\circ}C$ | Degree Celsius |
| dB | Decibel |
| µl | Microliter |

| | |
|---|---|
| µg | Microgram |
| M | Molar |
| nm | Nanometer |
| v | Volume |
| w | Weight |

## ABSTRACT

Antibody immobilization onto solid surface has been researched and studied exclusively for biosensor developments and immunoassay applications. In the project, two types of immobilization methods: physical and chemical adsorptions were carried out on optical fiber surface to study the immobilization level measured by Optical Spectrum Analyzer (OSA). The chemical adsorption using the crosslinker, glutaraldehyde (GTA) which allows stronger strength of binding between anti-17 beta estradiol antibody and optical fiber surfaces, showed significantly high antibody immobilization level compared to physical adsorption. The weaker bond of attraction, van der Waals force is one of the limitations of the antibody immobilization by physical adsorption. The chemical reactions between the hydroxyl groups (-OH) on optical fiber surfaces and the silane chemical, 3-aminopropyl-triethoxysilane (APTES) before antibody immobilization were also studied. APTES was useful to modify the surface of optical fiber by chemical reaction to provide binding sites for GTA to activate the surface. Amino groups (-$NH_2$) of antibody could eventually bind with the aldehyde groups (-CHO) of GTA to form amide bond which is a stronger linkage. In addition, the coating of GTA on optical fiber surface was also evaluated by trinocular microscope to investigate the differences between the bare optical fiber and GTA activated optical fiber.

## ABSTRAK

Antibodi boleh bergerak ke permukaan pepejal dan situasi ini telah dikaji secara eksklusif untuk perkembangan biosensor dan aplikasi immunoassay. Dalam projek ini, dua jenis kaedah telah digunakan: penjerapan secara fizikal dan kimia telah dijalankan ke atas permukaan gentian optik dan tahap bergerak akan diukur oleh Optical Spectrum Analyzer (OSA). Penjerapan kimia menggunakan crosslinker iaitu glutaraldehid (GTA) yang membolehkan kekuatan yang lebih kukuh untuk mengikat antara anti-17 beta estradiol antibodi dan serat optik permukaan. Penjerapan kimia juga menunjukkan tahap antibodi bergerak ketara tinggi berbanding dengan penjerapan fizikal. Ikatan lemah tarikan oleh van der Waals adalah salah satu batasan untuk menjerap antibodi oleh penjerapan fizikal. Reaksi kimia antara kumpulan hidroksil (-OH) pada permukaan gentian optik dan kimia silana, 3-aminopropyl-triethoxysilane (APTES) sebelum antibodi penjerapan juga telah dikaji. APTES berguna untuk mengubah suai permukaan gentian optik oleh tindak balas kimia untuk menyediakan kawasan kepada GTA untuk mengaktifkan permukaan tersebut. Kumpulan amino ($-NH_2$) antibodi akhirnya boleh mengikat dengan kumpulan aldehid (-CHO) daripada GTA untuk membentuk ikatan amida yang lebih kukuh. Di samping itu, lapisan glutaraldehid pada permukaan gentian optik juga telah dinilai oleh mikroskop trinocular untuk memerhatikan perbezaan antara gentian optik yang biasa dan gentian optic yang diaktifkan oleh GTA.

# CHAPTER 1

## INTRODUCTION

The 17 beta estradiol which is the natural endocrine disrupting compound (EDC), a hormone, precisely environmental estrogen was widespread and imperilled the lives of organisms in the environment starting these few years due to bad pollution (Long et al., 2013; Ruan et al., 2008). The worse impacts are including the unbalance of aquatic ecosystem, changes of male characteristic among aquatic organisms as well as toxicity to human beings (Kang et al., 2001).

To detect the harmful 17 beta estradiol in the environment, antibody is the suitable bio-recognition protein due to strong binding affinity as well as the excellent specificity to the target (Saerens et al., 2008). Moreover, antibody has the binding region that is only specific to the unique part of the 17 beta estradiol. Basically, the structure of all antibodies are almost identical and significantly used for the detection of target elements by their hypervariable regions and their reactive amino group ($-NH_2$) can also match with the chemical groups of target substances. When the movement of the antibody is restricted and fully attached to the surface of a solid support for the development of biosensor, the attachment process is termed immobilization (Elnashar, 2011).

In this project, optical fiber is utilised as a solid support for the immobilization of anti-17 beta estradiol antibody on its surface. Optical fibers are made of a cylindrical core and a surrounding cladding material

and the design was purposely used for propagation of light with minimal loss (Leung et al., 2007).

Nowadays, antibodies are immobilized on the optical fiber by several ways, either by improved methods or conventional techniques to design a suitable biosensor for the detection of toxic substances found in the environment. Yet, the immobilization techniques have been never standardised even though the process for antibody attachment on optical fiber surface is straightforward (Tedeschi et al., 2003). By referring to the related articles, some techniques are modified for the optimization of immobilization results, in the project, the objectives are:-

1. To immobilize anti-17 beta estradiol antibody on optical fiber surface by physical and chemical methods.
2. To investigate the results of immobilization of anti-17 beta estradiol antibody after using physical and chemical methods.

## BIBLIOGRAPHY


Baniukevic, J., Kirlyte, J., Ramanavicius, A. and Ramanaviciene, A. 2012. Comparison of oriented and random antibody immobilization techiniques on the efficiency of immunosensor. *Procedia Engineering* 47: 837-840.

Bao, H. M., Chen, Q. W., Zhang, L. Y. and Chen, G. 2011. Immobilization of trypsin in the layer-by-layer coating of graphene oxide and chitosan on in-channel glass fiber for microfluidic proteolysis. *Analyst* 136: 5190-5196.

Bartos, A. and Leszczynska, J. 2010. Immobilized antibodies for biosensoric detection of protein food allergens in grocery products. *Food Chemistry and Biotechnology* 74: 75-84.

Bhatia, S. K., Shriver-Lake, L. C., Prior, K. J., Georger, J. H., Calvert, J. M., Bredehorst, R. and Ligler, F. S. 1989. Use of thiol-terminal silanes and heterobifunctional crosslikers for immobilization of antibodies on silica surface. *Analytical Biochemistry* 178: 408-413.

Contesini, F. J., de Alencar Figueira, J., Kawaguti, H. Y., de Barros Fernandes, P. C., de Oliveira Carvalho, P., da Graca Nascimento, M. and Sato, H. H. 2013. Potential applications of carbohydrases immobilization in the food industry. *International Journal of Molecular Sciences* 14: 1335-1369.

Davis, C. C. Fiber optic technology and its role in the information revolution. Retrieved May 1, 2014, from http: //www.ece.umd.edu/~davis/optfib.html.

Kang, I. J., Yokota, H., Oshima, Y., Tsuruda, Y., Yamaguchi, T., Maeda, M., Imada, N., Tadokoro, H. and Honjo, T. 2001. Effect of 17 beta estradiol on the reproduction of Japanese medaka (*Oryzias latipes*). *Chemosphere* 47: 71-80.

Elnashar, M. M. 2011. The art of immobilization using biopolymers, biomaterials and nanobiotechnology. *Biotechnology of Biopolymer* 1: 3-25.

Kausaite, A., Dijk, M., Castrop, J., Ramanaviviene, A., Baltrus, J. P. and Acaite, J. 2007. Surface plasmon resonance label-free monitoring of antibody antigen interactions in real time. *Biochemistry and Molecular Biology Education* 35: 57-63.

Kausaite, A., Ramanaviviene, A, Kirlyte, J. and Ramanavicius, A. 2010. Comparative study of random and oriented antibody immobilization techniques on the binding capacity of immunosensors. *Analytical Chemistry* 82: 6401-6408.

Kumarasamy, V., Wahab, A. H., Chua, S. K., Hassan, Z., Chem, Y. K., Mohamad, M. and Chua, K. B. 2007. Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. *Journal of Virological Methods* 140: 75-79.

Lee, J. E., Seo, J. H., Kim, C. S., Kwon, Y. K., Ha, J. H., Choi, S. S. and Cha, H. J. 2013. A comparative study on antibody immobilization strategies onto solid surface. *Korean Journal of Chemical Engineering* 30: 1934-1938.

Leung, A., Shankar, P. M. and Mutharasanm R. 2007. A review of fiber-optic biosensors. *Sensors and Actuators* 125: 688-703.

Long, F., Zhu, A. and Shi, H. C. 2013. Recent advances in optical biosensors for environmental monitoring and early warning. *Sensors* 13: 13928-13948.

Luppa, P. B., Sokoll, L. J. and Chan, D. W. 2001. Immunosensors-principles and applications to clinical chemistry. *Clinica Chimica Acta* 314: 1-26.

Makaraviciute, A. and Ramanaviciene, A. 2013. Site-directed antibody immobilization techniques for immunosensors. *Biosensors and Bioelectronics* 50: 460-471.

McBride W. J. H. 2009. Evaluation of dengue NS1 test kits for the diagnosis of dengue fever. *Diagnostic Microbiology and Infectious Disease* 64: 31-36.

Migneault, I., Dartiguenave, C., Bertrand, M.. J. and Waldron, K. C. 2004. Glutaraldehyde: behavior in aqeous solution, reaction with proteins, and application to enzyme crosslinking. *Biotechniques* 37: 790-802.

Qian, W. P., Yao, D. F., Yu, F., Xu, B., Zhou, R., Bao, X. and Lu, Z. H. 2000. Immobilization of antibodies on ultraflat polystyrene surfaces. *Clinical Chemistry* 46: 1456-1463.

Rodriguez-Mozaz, S., Marco, M. P., Lopez de Alda, M. J. and Barcelo, D. 2004. Biosensors for environmental monitoring of endocrine disruptors: a review article. *Analysis of Bioanalytical Chemistry* 378: 588-598.

Ruan, A., Zong, F. J., Zhao, Y., Liu, C. X. and Chen, J. 2013. Effects 17 beta estradiol pollution on water microbial methane oxidation activity. *Environmental Toxicology and Chemistry* 33: 768-775.

Ruan, Y. L., Foo, T. C., Warren-Smith, S., Hoffmann, P., Moore, R. C., Ebendorff-Heidepeiwm, H. and Monro, T. M. 2008. Antibody immobilization within glass microstructured fibers: a route to sensitive and selective biosensors. *Optics Express* 16: 18514-18523.

Saerens, D., Huang, L., Bonroy, K. and Muyldermans, S. 2008. Antibody fragments as probe in biosensor development. *Sensors* 8: 4669-4686.

Saleh, B. E. A and Teich, M. C. (1991). Fiber optics. In *Fundamentals of Photonics*, eds. J.W. Goodma, pp 272-309. NewYork. John Wiley and Sons, Ltd.

Sassolas, A., Prieto-Simon, B. and Marty, J. L. 2012. Biosensors for pesticide detection: new trends. *American Journal of Analytical Chemistry* 3: 210-232.

Schramm, W., Paek, S. H. and Voss, G. 1993. Strategies for the immobilization of antibodies. *Immunomethods* 3: 93-102.

Scouten, W. H., Luong, J. H. and Brown, R. S. 1995. Enzyme or ptotein immobilization techniques for applications in biosensor design. *Tibtech* 13: 178-185.

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

Shriver-Lake, L. C., Donner, B., Edelstein, R., Breslin, K., Bhatia, S. K. and Ligler, F. S. 1997. Antibody immobilization using heterobifunctional linkers. *Biosensors and Bioelectronics* 12: 1101-1106.

Singh, R. K., Tiwari, M. K., Singh, R. and Lee, J. K. 2013. From protein engineering to immobilization: promising strategies for the upgrade of industrial enzymes. *International Journal of Molecular Sciences* 14: 1232-1277.

Sun, D. D., Guo, T., Ran, Y., Huang, Y. Y and Guan, B. O. 2014. In-situ DNA hybridization detection with a reflective microfiber grating biosensor. *Biosensors and Bioelectronics* 61: 541-546.

Tsai, W. C. and Pai, P. J. R. 2009. Surface plasmon resonance-based immunosensor with oriented immobilized antibody fragments on a mixed self-assembled monolayer for the determination of staphylococcal enterotoxin B. *Microchim Acta* 1: 1007-1013.

Tedeschi, L., Domenici, C., Ahluwalia, A., Baldini, F. and Mencaglia, A. 2003. Antibody immobilization on fiber optic TIRF sensors. *Sensors and Bioelectronic* 19: 85-93.