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CHEMICAL ATTACHMENT OF MONOCLONAL ANTIBODY
ON GOLD SURFACE


Miss Sutawan Buchatip

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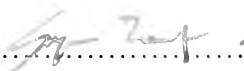
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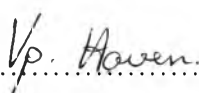
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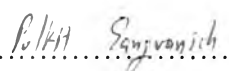
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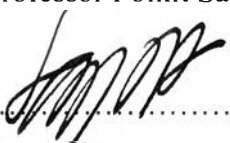

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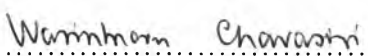
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ได้พัฒนา 5 เมกะเฮิร์ทซ์ ควอทซ์คริสตัลไมโครบาลานซ์ (คิวซีเอ็ม) เซนเซอร์เพื่อการตรวจวัดเชื้อแบคทีเรีย *Vibrio harveyi* (*V. harveyi*) ซึ่งเป็นสาเหตุของโรคเรืองแสงในกุ้ง โดยการติดโมโนโคลนอลแอนติบอดี (เอ็มเอบี) ที่มีความจำเพาะกับเชื้อ *V. harveyi* บนควอตซ์คริสตัลที่เคลือบด้วยทองผ่านวิธีการที่ประกอบด้วย 3 ขั้นตอน ขั้นหนึ่งเป็นการเตรียมโมโนเลเยอร์ที่จัดเรียงตัวได้เองของสารประกอบอัลเคนไทออลที่มีปลายข้างหนึ่งเป็นหมู่คาร์บอกซิลบนพื้นผิวควอตซ์คริสตัลที่เคลือบด้วยทอง ขั้นที่สองเป็นการกระตุ้นหมู่คาร์บอกซิลที่เตรียมได้ด้วย เอ็น-ไฮดรอกซีซัคซินิไมด์ (เอ็นเอชเอส) และ 1-เอทิล-3-(3-ไดเมทิลอะมิโนโพรพิล)คาร์โบไดอิมิด (อีดีซีไอ) และขั้นที่สามเป็นการติดเอ็มเอบีเข้ากับหมู่คาร์บอกซิลที่ถูกกระตุ้น ติดตามการเกิดปฏิกิริยาในแต่ละขั้นตอนด้วย รีเฟลกชัน-แอมบอร์พชั่น อินฟราเรดสเปกโทรสโกปี การวัดมุมสัมผัสของน้ำและคิวซีเอ็ม จากการศึกษาพบว่าคิวซีเอ็มอิมมูโนเซนเซอร์ที่พัฒนาขึ้นสามารถใช้ในการตรวจวัด *V. harveyi* ได้ โดยมีช่วงการตรวจวัดที่ 10^3 - 10^7 เซลล์ต่อมิลลิลิตร และแสดงปฏิกิริยาข้ามกับเชื้อ *V. vulnificus* ได้เล็กน้อย แต่ไม่จำเพาะเจาะจงกับกับเชื้อ *V. parahaemolyticus* และยังพบว่าการควบคุมความหนาแน่นของเอ็มเอบีซึ่งเป็นหมู่ที่ว่องไวต่อการจับแบคทีเรียโดยใช้โมโนเลเยอร์ที่จัดเรียงตัวได้เองของสารประกอบอัลเคนไทออลแบบผสมและ 1 เปรอร์เซ็นต์ของบีเอสเอเป็นบล็อกกิงรีเอเจนต์สามารถเพิ่มประสิทธิภาพในการจับกับแบคทีเรียเป้าหมายได้อย่างมีนัยสำคัญ

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SUTAWAN BUCHATIP: CHEMICAL ATTACHMENT OF
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A 5 MHz quartz crystal mibrobalance (QCM) sensor was developed for detection of *Vibrio harveyi*, a bacteria causing luminous in shrimp. Immobilization of monoclonal antibody (MAb) against *V. harveyi* onto gold electrode of quartz crystal involved a three-step procedure. The procedure includes: (1) a formation of self-assembled monolayer (SAM) of carboxyl-terminated alkanethiol, (2) an activation of carboxyl groups by *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI), and (3) an attachment of the MAb to the activated carboxyl groups. The stepwise chemical modification of the gold-coated substrate was characterized by reflection-absorption infrared spectroscopy, water contact angle measurements and QCM. It has been demonstrated that the developed QCM-based immunosensor can be used for the detection of *V. harveyi* with a working range of 10^3 - 10^7 CFU/mL, a slight cross reactivity to *V. vulnificus*, and no specificity to *V. parahaemolyticus*. Controlling the density of MAb which is the active binding site of bacteria by the use of mixed SAMs of alkanethiols and 1%BSA as a blocking reagent can significantly improve the binding efficiency of the targeted bacteria.

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CONTENTS

	Page
ABSTRACT IN THAI.....	iv
ABSTRACT IN ENGLISH.....	v
ACKNOWLEDGEMENTS.....	vi
LIST OF FIGURES.....	x
LIST OF TABLES.....	xiii
LIST OF ABBREVIATION.....	xv
CHAPTER I INTRODUCTION.....	1
1.1 Statement of Problem.....	1
1.2 Objectives.....	2
1.3 Scope of Investigation.....	2
CHAPTER II THEORY AND LITERATURE REVIEW.....	3
2.1 Luminous Vibriosis in Shrimp.....	3
2.2 Biosensors.....	6
2.2.1 Quartz Crystal Microbalance (QCM).....	8
2.3 Self-assembled Monolayer.....	10
2.3.1 The n-Alkanethiolate SAM.....	12
2.3.2 Characteristic.....	13
2.4 Antibody-antigen.....	15
2.4.1 Monoclonal Antibody.....	18
2.4.2 Advantages of Monoclonal Antibodies.....	19
2.5 Identification of Components of a Mixture of Proteins.....	20
2.5.1 Matrix-assisted laser desorption ionization (MALDI).....	21
2.5.2 Electrospray ionization (ESI) method.....	22

	Page
2.6 Characterization Techniques.....	22
2.6.1 Quartz Crystal Microbalance.....	22
2.6.2 Contact Angle Measurements.....	23
2.6.3 Reflection-Absorption Infrared Spectroscopy.....	24
 CHAPTER III EXPERIMENTAL.....	 26
3.1 Materials.....	26
3.2 Equipments.....	27
3.2.1 Reflection-Absorption Infrared Spectroscopy (RAIRS).....	27
3.2.2 Contact Angle Measurements.....	27
3.2.3 Quartz Crystal Microbalance (QCM).....	27
3.2.4 Matrix-assisted Laser Desorption Ionization (MALDI).....	28
3.3 Methods.....	28
3.3.1 Self-assembly Monolayer (SAM) Formation of Carboxyl-terminated Thiol.....	28
3.3.2 Activation of Carboxyl Groups of SAM-modified Substrates.....	29
3.3.3 Immobilization of Monoclonal Antibody (MAb) against <i>Vibrio harveyi</i> on NHS-modified Substrate..	29
3.3.4 Determination of Bacteria Binding.....	29
3.3.5 Regeneration of MAb-immobilized Quartz Crystal..	30
 CHAPTER IV RESULTS AND DISCUSSION.....	 31
4.1 Self-assembly Monolayer (SAM) Formation of Carboxyl- terminated Alkanethiol onto Gold Surface.....	32
4.2 Activation of Carboxyl Groups of SAM-modified Substrates.....	36
4.3 Immobilization of Monoclonal Antibody against <i>Vibrio harveyi</i>	38

	Page
4.4 Determination of Bacteria Binding.....	40
4.4.1 Variables Affecting Bacteria Binding.....	40
4.4.2 Confirmation of Monoclonal Antibody Immobilization and Bacteria Binding.....	45
4.4.3 Efficiency of the Immunosensor.....	46
CHAPTER V CONCLUSION AND SUGGESTION.....	50
REFERENCES.....	52
APPENDIX.....	56
VITAE.....	63

LIST OF FIGURES

Figure	Page
2.1 Recognition-perturbation-transduction scheme followed by analysis.....	7
2.2 General concept of quartz crystal microbalance.....	9
2.3 Self-assembly of amphiphilic adsorbates onto a solid surface.....	10
2.4 Schematic view of the forces in a self-assembled monolayer.....	13
2.5 Structure of SAMs of alkanethiols on Au(111) from two different perspectives : (a) top view, where the open circles represent gold atoms in a hexagonal close-packed arrangement, and the shaded circles represent alkanethiolate adsorbates (the darker shaded circles highlight the hexagonal ($\sqrt{3} \times \sqrt{3}$)R30° overlayer structure), and (b) side view, where the adsorbates are packed 5Å apart with their alkyl chains tilted 30° from the surface normal in a trans-extended conformation.....	14
2.6 The basis structure of antibody.....	16
2.7 Most antibody molecules contain light and heavy chains Each comprises one variable domain and different numbers of constant domains. The combination of 2 light + 2 heavy chains is a higher order building block. Immunoglobulins of different classes show different states of oligomerization, with additional chains where necessary serving as linkers.....	17
2.8 Matrix-assisted laser desorption ionization (MALDI) source	21
2.9 Schematic representation of the Young's equation.....	23
2.10 Schematic representation of wettability.....	24
2.11 Reflection-absorption infrared spectroscopy.....	25
4.1 Schematic diagram showing the procedure for the preparation of a <i>Vibrio harveyi</i> immunosensor.....	31
4.2 Structures of three thiol compounds.....	32

Figure	Page
4.3 Frequency shift due to SAM formation (Δf_s) and water contact angle of SAM obtained from 10 mM MPA as a function of time.....	33
4.4 Frequency shift due to SAM formation (Δf_s) of alkanethiol as a function of concentration.....	34
4.5 Water contact angle of SAM of alkanethiol as a function of thiol concentration.	35
4.6 RAIRS spectra of carboxyl-terminated monolayers on gold-coated substrates.	35
4.7 Mechanism of the activation of surface carboxyl groups by NHS/EDCI system.....	36
4.8 Water contact angle and frequency shift due to the activation (Δf_a) of the MPA-modified substrate as a function of immersion time using 15:45 mM of NHS/ EDCI.	37
4.9 Water contact angle and frequency shift due to the activation (Δf_a) of the MPA-modified substrate as a function of NHS/EDCI concentration using 30 min immersion time.....	37
4.10 MALDI-TOF spectrum of monoclonal antibody against <i>V. harveyi</i>	38
4.11 Frequency shift due to the MAb immobilization (Δf_i) on the NHS-modified substrate as a function of immobilization time using 0.1 mg/mL MAb.....	39
4.12 Frequency shift due to the MAb immobilization (Δf_i) as a function of MAb concentration using the immobilization time of 15h.....	40
4.13 Frequency shift due to the <i>V. harveyi</i> binding (Δf_b) with the MAb-immobilized substrate after the treatment with blocking reagents. The concentration of <i>V. harveyi</i> used was 10^5 CFU/mL	41
4.14 Frequency shift due to the <i>V. harveyi</i> binding (Δf_b) with the MAb-immobilized substrate as a function of MAb concentration used in the immobilization step after the treatment with 1%BSA.....	42

Figure	Page
4.15 Schematic diagram showing the MAb immobilization and <i>V. harveyi</i> binding of the mixed SAMs; x = NH ₂ for CE and x = OH for ME.....	43
4.16 Frequency shifts due to the MAb immobilization (Δf_i) and the <i>V. harveyi</i> binding (Δf_b) of the MPA-CE mixed SAM as a function of dilution ratio (%MPA).....	44
4.17 Frequency shifts due to the MAb immobilization (Δf_i) and the <i>V. harveyi</i> binding (Δf_b) of the MPA-ME mixed SAM as a function of dilution ratio (%MPA).....	45
4.18 RAIRS spectra of the gold-coated substrates before and after MAb immobilization and <i>V. harveyi</i> binding	46
4.19 Frequency shift due to <i>V. harveyi</i> binding (Δf_b) of the MAb-immobilized substrate prepared from MPA monolayer as a function of <i>V. harveyi</i> concentration.....	47
4.20 Frequency shift due to bacteria binding (Δf_b) on the MAb-immobilized substrate prepared from MPA monolayer.....	48
4.21 Frequency shift due to <i>V. harveyi</i> binding (Δf_b) of the MAb-immobilized substrate prepared from MPA monolayer after 1 cycle of regeneration in 0.1 M glycine/HCl buffer solution (pH = 2.3) as a function of regeneration time.....	49

LIST OF TABLES

Table		Page
1.1	Transduction modes in biosensors.....	8
A-1	Water contact angle and frequency shift due to SAM formation (Δf_s) of 10 mM MPA as a function of time.....	57
A-2	Frequency shift due to SAM formation of alkanethiol (Δf_s) as a function of concentration.....	57
A-3	Water contact angle of SAM of alkanethiol as a function of thiol concentration.....	57
A-4	Water contact angle and frequency shift due to the activation (Δf_a) of the MPA-modified substrate as a function of immersion time using 15:45 mM of NHS/EDCI.....	58
A-5	Water contact angle due to the activation (Δf_a) of the MPA-modified substrate as a function of NHS/EDCI concentration using 30 min immersion time.....	58
A-6	Frequency shift due to the activation (Δf_a) of the MPA-modified substrate as a function of NHS/EDCI concentration using 30 min immersion time.....	59
A-7	Frequency shift due to the MAb immobilization (Δf_i) on the NHS-modified substrate as a function of immobilization time using 0.1 mg/mL MAb.....	59
A-8	Frequency shift due to the MAb immobilization (Δf_i) as a function of MAb concentration using the immobilization time of 15h.....	59
A-9	Frequency shift due to <i>V. harveyi</i> binding (Δf_b) with the MAb-immobilized substrate after the treatment with blocking reagents. The concentration of <i>V. harveyi</i> is 10^5 CFU/mL.....	60

Table	Page	
A-10	Frequency shift due to <i>V. harveyi</i> binding (Δf_b) with MAb-immobilized substrate as a function of MAb concentration used in the immobilization step after the treatment with 1%BSA.....	60
A-11	Frequency shifts due to MAb immobilization (Δf_i) and <i>V. harveyi</i> binding (Δf_b) of the MPA-CE mixed SAM as a function of dilution ratio (%MPA).....	60
A-12	Frequency shifts due to MAb immobilization (Δf_i) and <i>V. harveyi</i> binding (Δf_b) of the MPA-ME mixed SAM as a function of dilution ratio (%MPA).....	61
A-13	Frequency shift due to <i>V. harveyi</i> binding (Δf_b) of the MAb-modified substrate prepared from MPA monolayer as a function of <i>V. harveyi</i> concentration.....	61
A-14	Frequency shift due to bacteria binding (Δf_b) of the MAb-immobilized substrate prepared from MPA monolayer.....	62
A-15	Frequency shift due to <i>V. harveyi</i> binding (Δf_b) of the MAb-immobilized substrate prepared from MPA monolayer after 1 cycle of regeneration in 0.1 M glycine/HCl buffer solution (pH 2.3) as a function of regeneration time.....	62

LIST OF ABBREVIATION

BSA	: Bovine serum albumin
DTDPA	: 3,3-Dithiodipropionic acid
EDCI	: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
CE	: Cysteamine
MAB	: Monoclonal antibody
ME	: 2-mercaptoethanol
mg	: miligram
MHz	: Megahertz
min	: minute
mL	: milliliter
mM	: millimolar
MPA	: 3-mercaptopropionic acid
MUA	: 11-mercaptoundecanoic acid
MALDI-TOF	: Matrix-assisted laser desorption ionization time-of flight
NHS	: <i>N</i> -hydroxysuccinimide
PBS	: Phosphate buffer saline
QCM	: Quartz crystal microbalance
SAM	: Self-assembled monolayer
<i>V. hareyi</i>	: <i>Vibrio harveyi</i>
<i>V. paraheamolyticus</i>	: <i>Vibrio paraheamolyticus</i>
<i>V. vulnificus</i>	: <i>Vibrio vulnificus</i>
θ	: Contact angle
μL	: microliter
Δf_s	: A frequency shift due to SAM formation
Δf_a	: A frequency shift due to the activation
Δf_i	: A frequency shift due to the immobilization
Δf_b	: A frequency shift due to the bacteria binding