



UNIVERSITI PUTRA MALAYSIA

**DEVELOPMENT OF A CAPACITANCE-BASED BIOSENSOR FOR THE
DETERMINATION OF HISTAMINE CONCENTRATION**

HELMI WASOH @ MOHAMAD ISA

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By

HELMI WASOH @ MOHAMAD ISA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Doctor of Philosophy**

March 2009



*Dedicated to,
teachers and family, for their endless support*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the degree of Doctor of Philosophy

**DEVELOPMENT OF A CAPACITANCE-BASED BIOSENSOR FOR THE
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Chair : Abu Bakar Salleh, PhD

Faculty : Institute of Biocience

Histamine level has been suggested as a rapid fish spoilage indicator. Besides that, a biosensor device with low cost, simple and portable is an advantage for fish freshness monitoring. Therefore, a histamine biosensor device was developed using the enzyme diamine oxidase (DAO) and a capacitance (C) concept. A capacitor with two plates system gives linear relationship between frequency (f) and histamine concentration. It has been proven that the relationship between f , dielectric constant (k) and histamine could be used to develop a histamine biosensor. The behavior of histamine reaction can be monitored in the presence of free enzyme in solution. Based on this relationship, immobilized enzyme electrode was developed by immobilizing 1.0 mg DAO/L in the ratio of DAO:polymer= 1:1. Good sensor response could be observed when the shape of the electrode was in a row and the electrode material was made from copper, with a distance between electrode, $d=2.5$ mm. By using this device, a linear range of histamine from 20 to 100 ppm was obtained ($R^2=0.9897$) with good correlation between biosensor method and HPLC ($R^2 = 0.9998$). For the determination of



histamine in prawn tissues, the recovery obtained was 98.13% after spiking with 150 ppm histamine and 98.79% after spiking with 200 ppm histamine (average RSD, 1.16-5.67%).

To complete the device, electronic reader was developed comprises PIC (peripheral integrated circuit) microcontroller, LCD (light crystal display), capacitor-resistor circuit and *computer-programming* in C code (installed in PIC). By using astable operation of capacitor-resistor circuit, a difference sensor response was generated from difference histamine concentration. During histamine reaction, the physicochemical changes was converted into electrical signal and translated in histamine concentration as part per million (ppm). PIC is very useful in modifying the writing process of *computer-programming* due to its capability and easily reprogrammed.

For the basic test (without enzyme), the device (with electronic reader) gave different (f) values for histamine (50-300 ppm, $RSD \leq 2.01\%$). After the reader was developed successfully, enzyme electrode was designed with the dimension of copper material at $d=2.0$ mm and area, $A=2.5 \times 10$ mm. By immobilizing enzyme onto this electrode, the device showed linear response to histamine concentrations (25 to 100 ppm, $R^2=0.998$, $RSD \leq 2.74\%$). The biosensor response was still 80% of the initial value after 10 days of storage (4°C). The histamine biosensor exhibited reproducibility characteristic with RSD value equals to 8.88% ($n=4$). This device can be used up to 30 times without a major change in sensor response (11.76 ± 3.41 Hz/s). Experiment with prawn tissues shows that the performance of histamine biosensor device is comparable to HPLC with $R^2=0.9895$. The histamine biosensor is a promising device for on site screening of fish freshness.



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

**PEMBANGUNAN BIOPENDERIA BERASASKAN-KAPASITAN UNTUK
PENENTUAN KEPEKATAN HISTAMIN**

Oleh

HELMI WASOH @ MOHAMAD ISA

Mac 2009

Pengerusi : Abu Bakar Salleh, PhD

Fakulti : Institut Biosains

Tahap histamin telah dicadangkan sebagai petunjuk pantas kerosakan ikan. Dengan itu, biopenderia kos murah, ringkas dan mudah alih merupakan kelebihan bagi mengesan ikan segar. Jadi, biopenderia histamin telah dibangunkan sebagai alat petunjuk kesegaran dibantu oleh enzim diamine oksidase (DAO) dan konsep fizik, *kapasitan* (C). Kapasitor dengan sistem dua plat memberikan respon linear antara frekuensi (f) dan kepekatan histamin. Ianya menjadi petunjuk awal bahawa hubungan antara f , pemalar dielektrik (k) dan histamin boleh digunakan untuk membina biopenderia histamin ini. Kelakuan tindak balas histamin didapati boleh dikaji dengan menggunakan DAO bebas dalam larutan. Berdasarkan kemampuan ini, elektrod berenzim pegun dibina menggunakan 1.0 mg DAO/L dan nisbah DAO:polimer=1:1. Respon yang baik diperolehi bila elektrod kuprum dibina memanjang dan jarak plat, $d=2.5$ mm. Alat ini memberikan julat linear dari 20-100 ppm histamin dengan $R^2=0.9897$. Korelasi yang baik diperolehi bagi biopenderia dibandingkan dengan kaedah konvensional HPLC ($R^2=0.9998$). Analisis dengan tisu udang menunjukkan perolehan-semula mampu dicapai pada 98.13%



selepas ditambah 150 ppm histamin dan 98.79% selepas ditambah 200 ppm histamin (purata RSD, 1.16-5.67%).

Bagi melengkapkan pembinaan alat ini, pencatat elektronik dibina, mengandungi PIC (peripheral integrated circuit) kawalan-mikro, LCD (light crystal display), dan *program-komputer* (kod C yang di muat dalam PIC). Dengan menggunakan operasi astable litar kapasitor-perintang, respon biopenderia yang berlainan dijana daripada kepekatan histamin berbeza. Semasa tindakbalas, perubahan fizikokimia larutan ditukar ke isyarat elektrik dan diterjemah kepada kepekatan histamin, bahagian-per-juta (ppm). PIC berguna untuk mengubahsuai penulisan *program-komputer* berdasarkan kemampuannya yang mudah deprogram semula.

Bagi ujikaji awal (tanpa enzim), biopenderia histamin (dengan pencatat elektronik) memberikan bacaan (f) berbeza untuk histamin (50-300pm, $RSD \leq 2.01\%$). Selepas pencatat berjaya dibina, elektrod direkabentuk menggunakan plat kuprum, $d=2.0$ mm dan keluasan, $A=2.5 \times 10$ mm. Penggunaan elektrod berenzim pegun memberikan respon linear bagi histamin antara 25 ke 100 ppm ($R^2=0.998$, $RSD \leq 2.74\%$). Nilai respon biopenderia didapati masih melebihi 80% berbanding nilai asal selepas 10 hari penyimpanan dalam keadaan sejuk (4°C). Biopenderia histamin mempamerkan ciri kebolehasilan dengan nilai $RSD=8.88\%$ ($n=4$). Elektrod yang sama ini juga boleh diulang penggunaannya sebanyak 30 kali tanpa perubahan besar pada respon penderia (11.76 ± 3.41 Hz/s). Analisis dengan tisu udang menunjukkan prestasi analisis alat ini adalah setanding dengan kaedah HPLC ($R^2=0.9895$). Biopenderia histamin ini berpotensi untuk menjadi alat yang sesuai di lapangan untuk mengesan kesegaran ikan.



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I certify that a Thesis Examination Committee has met on 19th March 2009 to conduct the final examination of Helmi Wasoh @ Mohamad Isa on his thesis entitled “Development of a Capacitance-based Biosensor for Determination of Histamine” 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 degree of Doctor of Philosophy.

Members of the Examination Committee were as follows:

Nor Aripin Shamaan, Ph.D.

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Kaida Khalid, Ph.D.

Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mariana Nor Shamsudin, Ph.D.

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Musa Ahmad, Ph.D.

Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 21 Mei 2009



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

Abu Bakar Salleh, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Main supervisor)

Fatimah Abu Bakar, PhD

Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Lee Yook Heng, PhD

Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 8 June 2009



DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

(HELMI WASOH @ MOHAMAD ISA)

Date: 23 June 2009



TABLE OF CONTENTS

	Pg
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xv
LIST OF FIGURES	xvii
LIST OF EQUATION	xxii
LIST OF ABBREVIATION	xxiv
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Biosensor	5
2.2 Transducer	6
2.3 Enzyme electrode	11
2.4 Electrode for Different Fish Freshness Indicator	12
2.4.1 Biogenic Amines	12
2.4.2 Ammonia	13
2.4.3 Hypoxanthine (Hx)	14
2.4.4 Trimethylamine (TMA)	15
2.4.5 Octopine	15
2.4.6 Lipid	16
2.5 One-Substrate Mechanism: The Enzyme-Substrate Complex	16
2.6 Diamine Oxidase	17
2.7 Immobilization of Enzyme	18
2.7.1 Enzyme Immobilization by Electropolymerisation	19
2.7.2 Enzyme Immobilization by Adsorption	20
2.7.3 Enzyme Immobilization by Cross-linking in a Glutaraldehyde Layer	20
2.7.4 Enzyme Immobilisation by Entrapment	21
2.8 Enzyme Immobilization in Freshness Measurement	22
2.8.1 Biogenic Amines Sensor	22
2.8.2 Hypoxanthine	23
2.8.3 Ammonia Sensor	24



2.8.4 Trimethylamine (TMA)	24
2.8.5 Octopine Sensor	24
2.8.6 Lipid Sensor	25
2.9 Fish Spoilage	25
2.9.1 Histamine	28
2.9.2 Conventional and Enzymatic Methods in Fish Freshness Measurement	29
2.9.3 Methods Related to Fish Freshness Measurement	32
Immunosensor	32
Quality Index Method (QIM)	34
Quality Evaluation Sensor (base at a glance)	34
Electronic Nose	35
2.10 Capacitive Sensor in Fish Freshness Measurement	36
2.10.1 The Concept of Capacitive Sensor	38
2.10.2 Instrument Set-up for Capacitive Sensor	39
2.10.3 Commercial Biosensors	43
2.10.4 Capacitor-resistor Circuit	45
2.10.5 Astable Operation	45
2.10.6 PIC (Peripheral Interface Controller) microcontroller	48
2.10.7 Introduction of 555 timer	48
2.10.8 LabVIEW	50
2.11 Trade and Economic Implications	52
2.11.1 Comparison of Border Cases among Major Importers	53
2.11.2 Fish Needed in 2010	55
3 MATERIALS AND METHODS	56
3.1 Chemical and Biochemical Reagents	60
3.2 Instrumentation	62
3.2.1 UV Exposure Unit	62
3.2.2 LabVIEW Software	63
3.2.3 Bench Driller	64
3.2.4 High Performance Liquid Chromatography (HPLC)	64
3.2.5 Soldering Station	65
3.2.6 Programmer	65
3.2.7 IC Timer (NE555)	66
3.2.8 PIC Microcontroller	67
3.3 Preparation of Chemicals	68
3.3.1 Preparation of Phosphate Buffer Solution	68
3.3.2 Preparation of Photocurable Poly(2-Hydroxyethyl Methacrylate) (PhotoHEMA)	68
3.3.3 Preparation of Standard Histamine Solution	69
3.3.4 Preparation of Diamine Oxidase (DAO) Solution	69
3.4 Histamine Biosensor Fabrication and Histamine Detection	69
3.4.1 Development of Capacitance Plate	69
3.4.2 Enzyme Immobilization	71



3.4.3 Procedure for Histamine Determination Using Capacitance Concept	72
3.5 Introduction to Biosensor Fabrication	73
3.5.1 Capacitance Behavior of Histamine in Free Solution	73
3.5.2 Capacitance Behavior of Reaction of Histamine with Free Enzyme	74
3.6 Development of Immobilized Enzyme Electrode	75
3.6.1 Calculation of Sensor Response	75
3.6.2 Effect of Enzyme Immobilization on Capacitance Response to Histamine	76
3.6.3 Effect of Electrode Design on Capacitance Response to Histamine	77
3.7 Determination of Histamine Using Optimized Biosensor	78
3.7.1 Reaction Time of Histamine Biosensor	79
3.7.2 Response Range of Histamine Biosensor	79
3.7.3 Biosensor Calibration Curve	79
3.7.4 Validation of Histamine Biosensor	79
3.8 Electronic Reader Designed for the Histamine Biosensor Device	82
3.8.1 Hardware Design	83
3.8.2 Software Development Tools (<i>Computer-Programming</i>)	98
3.9 Application of Histamine Biosensor Device	111
3.9.1 Response of Reader to Histamine in Free Solution	111
3.9.2 Response of Device to Histamine (With & Without Immobilized Enzyme)	111
3.9.3 Effect of Electrode Design	111
3.9.4 Calibration Curve of the Histamine Biosensor Device	112
3.9.5 Storage Stability of Histamine Biosensor	113
3.9.6 Reproducibility of Histamine Biosensor Device	113
3.9.7 Repeatability of Histamine Biosensor Device	113
3.9.8 Real Sample Analysis using Prawn Tissues	113
3.10 Histamine Biosensor Device	114
4 RESULTS AND DISCUSSION	116
4.1 Introduction to Biosensor Fabrication	116
4.1.1 Development of Capacitance Plate	116
4.1.2 Enzyme Immobilization	116
4.1.3 Procedure for Histamine Determination Using Capacitance Concept	117
4.2 Introduction Test to Histamine Detection	118
4.2.1 General Test for Capacitor-Resistor Circuit	118
4.2.2 Capacitance Behavior of Histamine in Free Solution	119
4.2.3 Capacitance Behavior of Reaction of Histamine with Free Enzyme	123
4.3 Development of Immobilized Enzyme Electrode	127
4.3.1 Calculation of Sensor Response	127

4.3.2	Effect of Enzyme Immobilization on Capacitance Response to Histamine	129
4.3.3	Effect of Electrode Design on Sensor Response to Histamine	139
4.4	Determination of Histamine Using Optimized Biosensor	143
4.4.1	Reaction Time of Histamine Biosensor	143
4.4.2	Response Range of Histamine Biosensor	145
4.4.3	Biosensor Calibration Curve	149
4.4.4	Validation of Histamine Biosensor	150
4.5	Electronic Reader Designed for the Histamine Biosensor Device	157
4.5.1	Calibration of Circuitry	157
4.5.2	Software Development Tools (<i>Computer-Programming</i>)	158
4.6	Application of Histamine Biosensor Device	167
4.6.1	Response of Reader to Histamine in Free Solution	167
4.6.2	Response of Biosensor to Histamine (With & Without Immobilized Enzyme)	170
4.6.3	Effect of Electrode Design	173
4.6.4	Response Range of Histamine Biosensor	181
4.6.5	Calibration Curve of the Histamine Biosensor Device	182
4.6.6	Storage Stability of Histamine Biosensor	183
4.6.7	Reproducibility of Histamine Biosensor	186
4.6.8	Repeatability of Histamine Biosensor	188
4.6.9	Real Sample Analysis Using Prawn Tissues	189
4.7	Trade and Economic Implications	190
4.7.1	Histamine Biosensor Device	191
5	CONCLUSION	193
6	RECOMMENDATION	196
	REFERENCES	200
	APPENDICES	215
	BIODATA OF STUDENT	243



LIST OF TABLES

Table		Pg
2.1	Transducers in sensor technology	7
2.2	Enzyme-based biosensors for fish freshness evaluation	8
2.3	Typical biopolymer/ transducer combinations and the respective transfer signals.	9
2.4	Methods for quality evaluation of fishery products	30
2.5	Seafood import refusals by US FDA from July 2001 to June 2003 (FDA 2002)	53
2.6	Breakdown of Reasons for Border Cases in Japan - 2000/2001 and 2001/2002	54
2.7	Additional Demand for Fish as Food in Asia and The World by 2010 Resulting from Population Growth	55
3.1	Chemicals and reagents used in this project	60
3.2	Electronic components and instruments used in this project	61
3.3	Pin description and function of the LCD display	89
3.4	Histamine biosensor device and its components	115
4.1	Gradient value for slope of standard curve in histamine assay with different distance between electrode plates	142
4.2	The histamine content of prawn sample after dilution determined by histamine biosensor	154
4.3	Precision data for histamine in prawn homogenate samples in HPLC and histamine biosensor analysis	155
4.4	Recoveries for histamine in prawn homogenates in histamine biosensor	156
4.5	Effect of difference capacitor and resistor value to frequency (f)	158
4.6	The different between <i>computer-programming A, B and C</i>	161



4.7	Result of mean difference at the 0.05 level between each capacitance value for <i>computer-programming A, B</i> and <i>C</i> analysed by one-way ANOVA	165
4.8	A comparison of frequency (Hz) for histamine concentration calculated by PIC microcontroller and LabVIEW software	169
4.9	Concentration different between actual and calculated (by <i>computer-programming</i>)	170
4.10	Average and standard deviation for repeatability performance	188
4.11	The Cost involved in Fish as Food needed in 2010	190
4.12	The difference cost for histamine measurement using capacitive biosensor and HPLC (including price that may be charged for necessary service)	191



LIST OF FIGURES

Figure		Pg
2.1	Biosensor with schematically represented internal signal processing	6
2.2	Layer of positive and negative charges on dielectric system	38
2.3	Diagram for determination of capacitance in solution	41
2.4	Astable multivibrator using 555 IC	46
2.5	Astable multivibrator circuit and waveforms	48
2.6	555 Timer	50
2.7	Building block in LabVIEW software	51
2.8	Block diagram in LabVIEW software	51
3.1	A capacitance sensor in histamine solution	56
3.2	Instrumental set up for histamine determination	57
3.3	A capacitance sensor with immobilized enzyme in histamine solution	58
3.4	Biosensor device and electrode in histamine solution	59
3.5	UV exposure unit	62
3.6	Components in LabVIEW software	63
3.7	Bench driller	64
3.8	Soldering station	65
3.9	Universal programmer	65
3.10	NE555-Timer	66
3.11	PIC16f877 microcontroller	67
3.12	Capacitance plate without photoHEMA and DAO	70



3.13	Capacitance plate with photoHEMA and without DAO	70
3.14	Capacitance plate with entrapped DAO in photoHEMA	71
3.15	Immobilization of DAO	71
3.16	LabView software (the command in <i>computer-programming</i>) that was used in this experiment	72
3.17	Overall diagram for histamine determination procedure	73
3.18	The design of electrodes in column (a) and rod (b) shape	77
3.19	PIC Microcontroller, LCD and capacitor-resistor circuit in the process of electronic reader development	84
3.20	PIC16f877 microcontroller pin configuration	86
3.21	NE555 timer pin configuration	87
3.22	Clock circuit with crystal oscillator 4000MHz	88
3.23	LCD schematic diagram with 16 character x 12 line	89
3.24	Power supply circuit	90
3.25	Pinout of the 7805 regulator IC	90
3.26	The 555-timer circuit design	91
3.27	A modification in capacitor-resistor circuit	92
3.28	Overall schematic for the electronic circuitry diagram	94
3.29	Copper board, photoresist film, negative film and PCB	95
3.30	Printed circuit board (PCB) with electronic components	96
3.31	PCB layout for the whole circuit	97
3.32	PCWH compiler IDE	98
3.33	MPLAB IDE	99
3.34a	Flowchart of software development for PIC16f877 microcontroller	100



3.34b	Flowchart of software development for PIC16f877 microcontroller	101
3.35	A brief flow chart for histamine detection by <i>computer-programming</i> in PIC Microcontroller	103
3.36	Command on LCD made by <i>computer-programming</i> and PIC microcontroller for histamine detection	104
3.37	Diagram of <i>computer-programming</i> for complete period of square wave	107
3.38	Diagram for Delay Function	108
3.39	Histamine biosensor device	115
4.1	Frequency of histamine concentration when $d=1.0\pm 0.1\text{cm}$ and $0.5\pm 0.1\text{cm}$	120
4.2	Relation of histamine solution to frequency (f) and dielectric constant (k)	122
4.3	Sensor response (f) for histamine reaction with free enzyme	124
4.4	Dielectric constant (k) for histamine reaction with free enzyme	125
4.5	Condition of reaction in the presence of enzyme	128
4.6	Graph of histamine reaction obtained using a LabVIEW software	129
4.7	Rate of sensor response (Hz/s) vs. weight (mg) of DAO in immobilization system	130
4.8	Rate of sensor response (Hz/s) for different ratio of enzyme solution (aqueous) to polyHEMA in immobilization system	133
4.9	Rate of sensor response (Hz/s) for two electrodes with and without immobilized enzyme	135
4.10	Rate of sensor response (Hz/s) for histamine assay in phosphate buffer solution (0.001 and 0.01M) pH 7.0 and 7.5	138
4.11	Rate of sensor response (Hz/s) Vs. histamine concentration for histamine measurement in buffer solution and deionized water	138
4.12	Rate of sensor response (Hz/s) for different shape of electrodes (column and rod) in different concentration of histamine	139
4.13	Rate of sensor response (Hz/s) for different electrode materials (copper, aluminum and lead)	140



4.14	Optimum reaction time for histamine biosensor (0 to 100ppm histamine)	144
4.15	Rate of sensor response (Hz/s) for histamine (0 to 100ppm histamine) at different time range	144
4.16	Response range for histamine determination (20-300ppm) in the presence of immobilized enzyme using copper plate electrode with ' d '=2.5mm in deionized water	145
4.17	Illustration of the process of histamine determination	147
4.18	Linear range of histamine biosensor in histamine detection using capacitance electrode from copper material, ' d ' = 2.5mm in deionized water	149
4.19	Calibration for histamine measured by HPLC	151
4.20	Correlation study for concentration of histamine determine by biosensor and HPLC	152
4.21	Rate of sensor response (Hz/s) of different dilution of prawn homogenate determined by histamine biosensor	153
4.22	Comparison of histamine concentration in spiked samples determined by biosensor and HPLC	155
4.23	Proteus ISIS professional simulation software	159
4.24	Graph of mean value and each individual value for different capacitance obtained from <i>computer-programming A</i>	162
4.25	Graph of mean value and each individual value for different capacitance obtained from <i>computer-programming B</i>	163
4.26	Graph of mean value and each individual value for different capacitance obtained from <i>computer-programming C</i>	164
4.27	RSD value for different capacitance obtained from <i>computer-programming A</i> and <i>B</i>	166
4.28	Frequency (Hz) for different concentration of histamine measured by PIC microcontroller in histamine biosensor device	168
4.29	Rate of sensor response (Hz/s) in low histamine concentration measured by histamine biosensor device	171



4.30	Rate of sensor response (Hz/s) in high histamine concentration measured by histamine biosensor device	172
4.31	Rate of sensor response (Hz/s) in low histamine concentration measured by copper and aluminum electrode	174
4.32	Rate of sensor response (Hz/s) for histamine assay measured by histamine biosensor device (with and without enzyme) in high concentration of histamine	175
4.33	Rate of sensor response (Hz/s) vs. distance (mm) between plate for low concentration of histamine	176
4.34	Rate of sensor response (Hz/s) vs. distance (mm) for high concentration of histamine	176
4.35	Rate of sensor response (Hz/s) vs. area of electrode plate (mm ²) for low concentration of histamine	179
4.36	Rate of sensor response (Hz/s) versus area (mm ²) for high concentration of histamine	179
4.37	Minimum and maximum value of time period set by microcontroller	181
4.38	Response range of histamine biosensor measured by biosensor device	182
4.39	Calibration curve for histamine measurement measured by biosensor device	183
4.40	Trend of sensitivity (as slope of the calibration curve) of the biosensor in deionized water as a function of time, histamine as a substrate	184
4.41	Graph for reproducibility of histamine measurement with different electrode	187
4.42	The repeatability performance of histamine measurement with same electrode	188
4.43	Correlation study for histamine determined by HPLC and capacitance sensor	189
4.44a	Histamine biosensor device and its electronic components	192
4.44b	Histamine biosensor device	192



LIST OF EQUATIONS

Equation		Pg
2.1	$C = \frac{Q}{\Delta\phi}$	38
2.2	$C_0 = \frac{\epsilon_0 A}{d}$	38
2.3	$C = \frac{kA}{d}$	39
2.4	$f = \frac{1.44}{C(R_1 + 2R_2)}$	42
2.5	$C = \frac{1.44}{f(R_1 + 2R_2)}$	42
2.6	$k = \frac{(1.44)(d)}{f(A)(R_1 + 2R_2)}$	43
2.7	$T_{high} = 0.7(R_1 + R_2)C$	47
2.8	$T_{low} = 0.7R_2C$	47
2.9	$T = \text{period} = T_{high} + T_{low}$	47
2.10	$f = \frac{1}{T} \approx \frac{1.44}{(R_1 + 2R_2)}$	47
3.1	$\text{pH} = \text{pKa} + \log [A^-] / [HA]$	68
3.2	Molarity = Moles/ Liter	68
3.3	$k = \frac{3.19 \times 10^9}{f}$	74
3.4	$\text{Sensor response (Hz/sec)} = \frac{\text{Frequency at 120 sec} - \text{frequency at 60 sec}}{120 \text{ sec} - 60 \text{ sec}}$	75
3.5	$\frac{1}{Ct} = \frac{1}{C_{fixed}} + \frac{1}{C_{sensor}}$	92



3.6	One complete period = (no. of counter) x (int. timer resol.)	105
3.7	Actual period = (integer, 1,2,3....255) x (1μsec)	106
3.8	Calculated period= (integer, 1,2,3....255) x (example 1.6μsec)	106
3.9	Frequency= 1/(period) _{average}	109

