

## TABLE OF CONTENTS

	<b>Page</b>
ACKNOWLEDGEMENT	iii
ABSTRACT (ENGLISH)	iv
ABSTRACT (THAI)	vi
TABLE OF CONTENTS	viii
LIST OF TABLES	xiii
LIST OF ILLUSTRATIONS	xix
ABBREVIATIONS AND SYMBOLS	xxiii
 <b>CHAPTER 1 : INTRODUCTION</b>	 <b>1</b>
1.1 Biosensors	1
1.1.1 History	1
1.1.2 Definition	3
1.1.3 Principle of biosensors	3
1.2 Plant tissue-based biosensors	6
1.2.1 History and principle	6
1.2.2 Source of biocatalysts	9
1.2.3 Application of plant tissue-based bioelectrodes	11
1.3 Flow injection analysis (FIA)	14
1.3.1 Theory and principle of FIA	14
1.3.2 The basic components of a FIA system	16
1.3.3 Application of plant tissue-base biosensors in FIA system	18
1.4 Batch injection analysis (BIA)	19
1.4.1 Principle of BIA	19
1.4.2 The basic components of a BIA system	19
1.5 Research aims	22

	<b>Page</b>
<b>CHAPTER 2: EXPERIMENTAL</b>	<b>23</b>
2.1 Instrument and apparatus	23
2.2 Chemicals and plant materials	24
2.2.1 Chemicals	24
2.2.2 Plant materials	27
2.3 Preparation of standard solutions and reagents	28
2.4 Batch system	30
2.4.1 Component of the batch system	30
2.4.2 Electrode construction of the batch system	31
2.5 FIA System	32
2.5.1 Component of the FIA system	32
2.5.2 Electrode construction of the FIA system	34
2.6 BIA System	34
2.6.1 Component of the BIA system	34
2.6.2 Electrode construction of the BIA system	35
2.6.3 Construction of the BIA system	37
2.7 Procedure	38
2.7.1 Asparagus based amperometric sensor for fluoride determination	38
2.7.2 Sunflower based amperometric biosensors for glycolic acid determination incorporating with flow injection system	40
2.7.3 Sunflower based amperometric biosensors for glycolic acid determination incorporating with batch injection system	41
2.8 Some of analytical characteristics of bioelectrode	43

	Page
<b>CHAPTER 3 : RESULT AND DISCUSSION</b>	<b>45</b>
<b>3.1 Asparagus based amperometric sensor for fluoride determination</b>	<b>45</b>
3.1.1 Cyclic voltammetry	45
3.1.2 Optimization of experimental variables in batch system	47
3.1.2.1 Effect of pH in batch system	47
3.1.2.2 Effect of applied potential in batch system	49
3.1.2.3 Effect of tissue loading in batch system	50
3.1.2.4 Effect of mediator loading in batch system	52
3.1.2.5 Effect of H <sub>2</sub> O <sub>2</sub> concentration in batch system	53
3.1.2.6 Summary of the optimum conditions in batch system	55
3.1.3 Analytical characteristics of bioelectrode in batch system	56
3.1.3.1 Calibration, detection limit and reproducibility	56
3.1.3.2 Kinetic parameters in batch system	59
3.1.3.3 Response time of the bioelectrode in batch system	63
3.1.3.4 Stability of the bioelectrode in batch system	64
3.1.3.5 Interference studies in batch system	65
3.1.3.6 Percentage recoveries in batch system	67
3.1.4 Determination of fluoride content in commercial fluoride tablets	68
<b>3.2 Sunflower based amperometric biosensors for glycolic acid determination incorporating with flow injection system</b>	<b>71</b>
3.2.1 Cyclic voltammetry	71
3.2.2 Optimization of experimental variables in FIA system	73
3.2.2.1 Effect of applied potential in FIA system	73
3.2.2.2 Effect of pH in FIA system	74

	Page
3.2.2.3 Effect of buffer types in FIA system	76
3.2.2.4 Effect of tissue loading in FIA system	77
3.2.2.5 Effect of mediator loading in FIA system	79
3.2.2.6 Effect of carrier flow rate in FIA system	80
3.2.2.7 Effect of injection volume in FIA system	82
3.2.2.8 Summary of the optimum conditions in FIA system	83
3.2.3 Analytical characteristics of the FI method based on the sunflower leaf tissue bioelectrode	85
3.2.3.1 Calibration, detection limit and reproducibility	85
3.2.3.2 Michaelis and Menten constant in FIA system	88
3.2.3.3 Sample throughput in FIA system	90
3.2.3.4 Stability of the bioelectrode in FIA system	91
3.2.3.5 Interference studies in FIA system	92
3.2.3.6 Percentage recoveries in FIA system	94
3.2.4 Determination of glycolic acid in urine samples in FIA system	95
<b>3.3 Sunflower based amperometric biosensors for glycolic acid determination incorporating with batch injection system</b>	<b>97</b>
3.3.1 Cyclic voltammetry	97
3.3.2 Optimization of experimental variables in BIA system	97
3.3.2.1 Effect of applied potential in BIA system	97
3.3.2.2 Effect of pH in BIA system	99
3.3.2.3 Effect of buffer system in BIA system	100
3.3.2.4 Effect of tissue loading in BIA system	102
3.3.2.5 Effect of mediator loading in BIA system	103
3.3.2.6 Effect of stirring rate in BIA system	105
3.3.2.7 Effect of tip-electrode distance in BIA system	106

	<b>Page</b>
3.3.2.8 Effect of injection volume in BIA system	108
3.3.2.9 Summary of the optimum conditions in BIA system	109
3.3.3 Analytical characteristics of the proposed BIA method on the sunflower leaf tissue bioelectrode	111
3.3.3.1 Calibration, detection limit and reproducibility	111
3.3.3.2 Michaelis and Menten constant in BIA system	114
3.3.3.3 Response time of the bioelectrode in BIA system	115
3.3.3.4 Stability of the bioelectrode in BIA system	115
3.3.3.5 Interference studies in BIA system	117
3.3.3.6 Percentage recoveries in BIA system	119
3.3.4 Determination of glycolic acid in urine samples in BIA system	120
<b>CHAPTER 4 : CONCLUSION AND SUGGESTION FOR FURTHER WORK</b>	<b>122</b>
<b>REFERENCES</b>	<b>126</b>
<b>APPENDICES</b>	
APPENDIX A	131
APPENDIX B	137
<b>VITA</b>	<b>143</b>

## LIST OF TABLES

Table	Page
1.1 Bioreceptors for biosensors	5
1.2 Examples of transducer types	6
1.3 Highlights of the history of plant tissue-based biosensors	7
1.4 Sources of biocatalysts	9
1.5 A brief review of plant tissue-based bioelectrodes	12
1.6 A brief review of plant tissue-base biosensors in a FIA system	18
1.7 Comparison of the performance characteristics of BIA and FIA	21
2.1 Experimental variables and their ranges studied of fluoride sensor in batch system	39
2.2 Experimental variables and their ranges studied for glycolic acid biosensor in FIA system	41
2.3 Experimental variables and their ranges studied for glycolic acid biosensor in BIA system	42
3.1 Effect of pH on the sensitivity of the bioelectrode in batch system	48
3.2 Effect of applied potential on the sensitivity of the bioelectrode in batch system	49
3.3 Effect of amount of asparagus tissue on the sensitivity of the bioelectrode in batch system	51
3.4 Effect of mediator loading on the sensitivity of the bioelectrode in batch system	52

Table	Page
3.5 Effect of $\text{H}_2\text{O}_2$ concentration effect on the bioelectrode in batch system	54
3.6 Experimental variables, their ranges studied and optimum values of fluoride sensor	55
3.7 Current response of standard fluoride	57
3.8 Replicate measurements of fluoride using the sample bioelectrode in batch system	58
3.9 Replicate measurements of fluoride using five-independently made bioelectrodes in batch system	59
3.10 Current response of standard $\text{H}_2\text{O}_2$ and $\frac{\text{current}}{[\text{H}_2\text{O}_2]}$	60
3.11 Current response of $\text{H}_2\text{O}_2$ at different concentration of fluoride	62
3.12 $\frac{1}{[\text{H}_2\text{O}_2]}$ and $\frac{1}{\text{current}}$ at different concentration of fluoride for Lineweaver-Burk plot	62
3.13 Possible interferences tested with the fluoride bioelectrode	66
3.14 Recoveries of fluoride added to fluoride tablet samples	67
3.15 Determination of fluoride in fluoride tablet samples by the proposed method	69
3.16 Determination of fluoride in fluoride tablet samples by the proposed method and fluoride ion selective electrode (ISE) using a standard addition method	69
3.17 Calculation for t-test (in batch system)	70
3.18 Effect of applied potential on glycolic acid bioelectrode in FIA system	73
3.19 Influence of pH value on glycolic acid bioelectrode in FIA system	75
3.20 Effect of buffer type on glycolic acid bioelectrode in FIA system	76

Table	Page
3.21 Effect of sunflower leaf tissue loading on glycolic acid bioelectrode in FIA system	78
3.22 Effect of mediator loading on glycolic acid bioelectrode in FIA system	79
3.23 The influence of carrier flow rate on glycolic acid bioelectrode in FIA system	81
3.24 Effect of injection volume on glycolic acid bioelectrode in FIA system	82
3.25 Experimental variables, their ranges studied and optimum values for glycolic acid bioelectrode in FIA system	84
3.26 Peak height of standard glycolic acid in FIA system	86
3.27 Replicate measurement of glycolic acid using the same bioelectrode in FIA system	87
3.28 Replicate measurement of glycolic acid using five-independently made bioelectrode in FIA system	88
3.29 Current response of standard glycolic acid and $\frac{\text{current}}{[\text{glycolic acid}]}$ in FIA system	89
3.30 Possible interferences tested with the glycolic acid bioelectrode in FIA system	93
3.31 Recoveries of glycolic acid added to a 10 fold-dilution of pretreated urine samples in FIA system	94
3.32 Results of glycolic acid determination in 10 fold-dilution of pretreated urine samples by the proposed method (FIA system)	95
3.33 Results of glycolic acid determination in 10 fold-dilution of pretreated urine samples by the proposed method (FIA system) and HPLC method	96
3.34 Calculation for t-test (in FIA system)	96



<b>Table</b>	<b>Page</b>
3.35 Effect of applied potential on glycolic acid bioelectrode in BIA system	98
3.36 Effect of pH value on glycolic acid bioelectrode in BIA system	99
3.37 Effect of buffer systems on glycolic acid bioelectrode in BIA system	101
3.38 Effect of tissue loading on glycolic acid bioelectrode in BIA system	102
3.39 Effect of mediator loading on glycolic acid bioelectrode in BIA system	104
3.40 Effect of stirring rate on glycolic acid bioelectrode in BIA system	105
3.41 Effect of tip-electrode distance on glycolic acid bioelectrode in BIA system	107
3.42 Effect of injection volume on glycolic acid bioelectrode in BIA system	108
3.43 Experimental variables, their ranges studied and optimum condition for glycolic acid determination in BIA system	110
3.44 Peak height of standard glycolic acid in BIA system	112
3.45 Replicate measurement of glycolic acid using the same bioelectrode in BIA system	113
3.46 Replicate measurement of glycolic acid using five-independently made bioelectrode in BIA system	113
3.47 Current response of standard glycolic acid and $\frac{\text{current}}{[\text{glycolic acid}]}$ in BIA system	114
3.48 Possible interferences tested with the glycolic acid bioelectrode in BIA system	118

Table	Page
3.49 Recovery of glycolic acid added to a 10 fold-dilution of pretreated urine samples in BIA system	119
3.50 Determination of glycolic acid in 10 fold-dilution of pretreated urine samples by the proposed method (BIA system)	120
3.51 Determination of glycolic acid in 10 fold-dilution of pretreated urine samples by the proposed method (BIA system) and HPLC method	121
3.52 Calculation for t-test (in BIA system)	121
A.1 Potentials of fluoride standard solutions (2.0-10.0 mg l <sup>-1</sup> )	133
A.2 Triplicate measurement of potential for fluoride determination in fluoride tablet samples by the fluoride ion selective electrode (ISE) using a standard addition method	134
A.3 Determination of fluoride in fluoride tablet samples by fluoride ion selective electrode (ISE) using a standard addition method	134
A.4 High performance liquid chromatographic determination of glycolic acid in standard solution containing 0.5, 1.0, 2.0 and 3.0 mM glycolic acid for comparing with those obtained by FIA system (section 3.2.4)	139
A.5 Results of glycolic acid determination in 10 fold-dilution of of pretreated urine sample by HPLC for comparing with those obtained by FIA system (section 3.2.4)	140
A.6 Peak heights of 0.1, 0.5, 1.0, 1.5 and 2.0 mM glycolic acid for construction of calibration graph for glycolic acid determination comparing with those obtained by BIA system (section 3.3.4)	141

Table	Page
A.7 Result of glycolic acid determination in 10 fold-dilution of of pretreated urine sample HPLC for comparing with those obtained by BIA system (section 3.3.4)	142

## LIST OF ILLUSTRATIONS

Figure	Page
1.1 The various stages of determination with a biosensors	4
1.2 A single-line FIA manifold	17
1.3 Comparison of FIA and BIA operation	21
2.1 Asparagus (a) and sunflower leave (b)	27
2.2 Experimental set-up of batch system for fluoride determination	30
2.3 Electrochemical cell of batch system	31
2.4 Experimental set-up of flow injection system for the glycolic acid determination	33
2.5 Flow-through thin-layer electrochemical cell with a home-made working electrode	33
2.6 Experimental set-up of BIA system for glycolic acid determination	36
2.7 A home-made electrochemical cell of the BIA system	36
3.1 Cyclic voltammogram for $\text{H}_2\text{O}_2$ bioelectrode	46
3.2 Reaction sequence within the asparagus tissue-based and ferrocene-mediated bioelectrodes	47
3.3 Effect of pH on the sensitivity of the bioelectrode in batch system	48
3.4 Effect of applied potential on the sensitivity of the bioelectrode in batch system	50
3.5 Effect of amount of asparagus tissue on the sensitivity of the bioelectrode in batch system	51

Figure	Page
3.6 Effect of mediator loading on the sensitivity of bioelectrode in batch system	53
3.7 Effect of $\text{H}_2\text{O}_2$ concentration on the bioelectrode in batch system	54
3.8 Typical current-time recording of fluoride in batch system	56
3.9 Calibration graph of fluoride	58
3.10 Eadie-Hofstee plot for the $\text{H}_2\text{O}_2$ bioelectrode	60
3.11 Lineweaver-Burk plot for fluoride inhibitor on bioelectrode	63
3.12 The response time ( $t_{90}$ ) of $4 \text{ mg l}^{-1}$ fluoride	64
3.13 Stability of fluoride bioelectrode	65
3.14 Cyclic voltammograms for glycolic acid	71
3.15 Reaction sequence of glycolic acid within the sunflower leaf tissue-based ferrocene mediated bioelectrodes	72
3.16 Effect of applied potential on glycolic acid bioelectrode in FIA system	74
3.17 Influence of pH value on glycolic acid bioelectrode in FIA system	75
3.18 Effect of buffer type on glycolic acid bioelectrode in FIA system	77
3.19 Effect of sunflower leaf tissue loading on glycolic acid bioelectrode in FIA system	78
3.20 Effect of mediator loading on glycolic acid bioelectrode in FIA system	80
3.21 The influence of carrier flow rate on glycolic acid bioelectrode in FIA system	81
3.22 Effect of injection volume on glycolic acid bioelectrode in FIA system	83

<b>Figure</b>	<b>Page</b>
3.23 Typical response of glycolic acid in FIA system	85
3.24 Calibration graph of glycolic acid in FIA system	87
3.25 Eadie-Hofstee plot for the glycolic acid bioelectrode in FIA system	90
3.26 Stability of glycolic acid bioelectrode in FIA system	91
3.27 Effect of applied potential on glycolic acid bioelectrode in BIA system	98
3.28 The influence of pH value on glycolic acid bioelectrode in BIA system	100
3.29 The dependent of buffer system on glycolic acid bioelectrode in BIA system	101
3.30 Effect of sunflower leaf tissue loading on glycolic acid bioelectrode in BIA system	103
3.31 Effect of mediator loading on glycolic acid bioelectrode in BIA system	104
3.32 Effect of stirring rate on glycolic acid bioelectrode in BIA system	106
3.33 Effect of tip-electrode distance on glycolic acid bioelectrode in BIA system	107
3.34 Effect of injection volume on glycolic acid bioelectrode in BIA system	109
3.35 Typical response of glycolic acid in BIA system	111
3.36 Calibration graph glycolic acid in BIA system	112
3.37 Eadie-Hofstee plot for the glycolic acid bioelectrode in BIA system	115
3.38 Stability of glycolic acid bioelectrode in BIA system	116

Figure	Page
A.1 Calibration graph of standard fluoride solutions (2.0-10.0 mg l <sup>-1</sup> )	133
A.2 Chromatogram of 1.0 mM standard glycolic acid	138
A.3 Calibration graph for glycolic acid (0.5-3.0 mM)	139
A.4 Chromatogram of 10 fold-dilution of pretreated urine sample	140
A.5 Calibration graph for glycolic acid (0.1-2.0 mM)	141

## ABBREVIATIONS AND SYMBOLS

Abs	Absorbance
BIA	batch injection analysis
C	carrier
D	detector
DL	detection limit
FIA	flow injection analysis
HPLC	high performance liquid chromatography
I	injection valve
i.d.	inner diameter
ISE	ion selective electrode
P	pump
R	reagent
RSD	relative standard deviation
S	sample
S/N	signal to noise ratio
SD	standard deviation
Std.	standard
t	time
TISAB	Total ionic strength adjustment buffer
W	waste
w/w	weight by weight