

การพัฒนาแบบรับรู้และวิธีการวิเคราะห์แบบโวลแทมเมตริกสำหรับประยุกต์กับระบบ

โพลีอินเจคชันลิควิด โครมาโทกราฟีและไมโครชิพอะพอลาร์ไอส์อิเล็กโทรโฟรีซิส

นางสาววิณา เสียงเพราะ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

สาขาวิชาเคมี ภาควิชาเคมี

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2549

ISBN 974-14-2497-3

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

DEVELOPMENT OF NEW VOLTAMMETRIC SENSING SYSTEMS
AND ANALYTICAL METHODS AS APPLIED TO FLOW
INJECTION LIQUID CHROMATOGRAPHY AND
MICROCHIP CAPILLARY ELECTROPHORESIS

Miss Weena Siangproh

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic Year 2006

ISBN 974-14-2497-3

490109

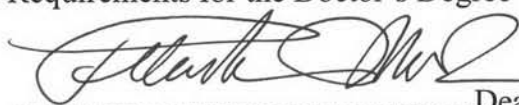
Thesis Title DEVELOPMENT OF NEW VOLTAMMETRIC SENSING
 SYSTEMS AND ANALYTICAL METHODS AS APPLIED
 TO FLOW INJECTION LIQUID CHROMATOGRAPHY
 AND MICROCHIP CAPILLARY ELECTROPHORESIS

By Miss Weena Siangproh

Field of Study Chemistry


Thesis Advisor Associate Professor Orawon Chailapakul, Ph. D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial
Fulfillment of the Requirements for the Doctor's Degree



.....Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE



.....Chairman
(Associate Professor Siri Varothai, Ph. D., D.I.C.)



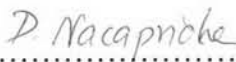
.....Thesis Advisor
(Associate Professor Orawon Chailapakul, Ph. D.)



..... Member
(Associate Professor Thawatchai Tuntulani, Ph.D.)



..... Member
(Assistant Professor Narong Praphairaksit, Ph.D.)



.....Member
(Assistant Professor Duangjai Nacapricha, Ph. D.)

วิชา เคมี เพราะ: การพัฒนาระบบรับรู้และวิธีการวิเคราะห์แบบ โวลแทมเมตริกสำหรับประยุกต์กับระบบ โฟลว์อินเจกชันลิกวิด โครมาโทกราฟีและไมโครชิพอะพอลาร์ไรโอเล็กโทโรไฟเรซิส (DEVELOPMENT OF NEW VOLTAMMETRIC SENSING SYSTEMS AND ANALYTICAL METHODS AS APPLIED TO FLOW INJECTION LIQUID CHROMATOGRAPHY AND MICROCHIP CAPILLARY ELECTROPHORESIS) อาจารย์ที่ปรึกษา: รศ.ดร. อรรพรรณ ชัยลภากุล ; 163 หน้า, ISBN 974-14-2497-3

งานวิจัยนี้มุ่งความสำคัญไปที่การพัฒนาโวลแทมเมตริกเทคนิคชนิดใหม่สำหรับระบบของไหล โดยแบ่งงานวิจัยออกเป็นสองส่วน ได้แก่ ส่วนที่หนึ่งการพัฒนาโวลแทมเมตริกเทคนิคชนิดใหม่โดยใช้ ขั้วไฟฟ้าฟิล์มบางโบรอนโคปัลไดมอนด์สำหรับสารประกอบไฮออลกับการประยุกต์ทางโฟลว์อินเจกชันและไฮเพอร์ฟอร์แมนส์ลิกวิด โครมาโทกราฟี ในการศึกษาเบื้องต้นได้ทำการศึกษาการเกิดปฏิกิริยาทางเคมีไฟฟ้าของยา ที่มีหมู่ไฮออลเป็นองค์ประกอบที่ขั้วไฟฟ้าฟิล์มบาง โบรอนโคปัลไดมอนด์ด้วยไซคลิกโวลแทมเมตริก เปรียบเทียบ ผลการทดลองที่ได้กับขั้วไฟฟ้ากลาสคาร์บอน พบว่าขั้วไฟฟ้าฟิล์มบางโบรอนโคปัลไดมอนด์ให้ผลของ สัญญาณไซคลิกโวลแทมโมแกรมสำหรับปฏิกิริยาออกซิเดชันของยาที่มีหมู่ไฮออลเป็นองค์ประกอบแบบไม่ ผันกลับที่ชัดเจนและให้ค่ากระแสไฟฟ้าที่สูง เมื่อเปรียบเทียบกับขั้วไฟฟ้ากลาสคาร์บอน จากนั้นได้นำระบบ โฟลว์อินเจกชันซึ่งต่อกับขั้วไฟฟ้าฟิล์มบางโบรอนโคปัลไดมอนด์ไปประยุกต์สำหรับการวิเคราะห์ปริมาณยาที่มี หมู่ไฮออลเป็นองค์ประกอบในสารมาตรฐานและสารตัวอย่างยาเตรียม จากการศึกษาเปอร์เซ็นต์การกลับคืน ของสารมาตรฐานที่เติมลงในสารตัวอย่างพบว่าได้ผลการทดลองในช่วง 95 ถึง 110 เปอร์เซ็นต์ นอกจากนี้ยัง ได้ ทำการศึกษาการเกิดปฏิกิริยาออกซิเดชันของไฮโมซิสเทอีนที่ขั้วไฟฟ้าฟิล์มบางโบรอนโคปัลไดมอนด์แบบที่ขัง ไม่ผ่านกระบวนการออกซิไดซ์และแบบที่ผ่านกระบวนการออกซิไดซ์ โดยใช้เทคนิคไซคลิกโวลแทมเมตริก จากการทดลองพบว่าในภาวะที่สารละลายมีฤทธิ์เป็นกรดไฮโมซิสเทอีนสามารถเกิดปฏิกิริยาออกซิเดชันได้ เฉพาะกรณีที่ใช้ขั้วไฟฟ้าฟิล์มบางโบรอนโคปัลไดมอนด์แบบที่ผ่านกระบวนการออกซิไดซ์เท่านั้น ที่ภาวะ สารละลายมีฤทธิ์เป็นเบสไฮโมซิสเทอีนสามารถเกิดปฏิกิริยาออกซิเดชันได้ที่ขั้วไฟฟ้าทั้งสองแบบ และจาก การนำระบบโฟลว์อินเจกชันและไฮเพอร์ฟอร์แมนส์ลิกวิด โครมาโทกราฟีต่อกับขั้วไฟฟ้าฟิล์มบางโบรอนโคปัล ไดมอนด์แบบที่ผ่านกระบวนการออกซิไดซ์ พบว่าขั้วไฟฟ้าฟิล์มบางโบรอนโคปัลไดมอนด์ให้สัญญาณที่มีความ แตกต่างกันน้อยกว่า 2 เปอร์เซ็นต์ ที่ภาวะเป็นกรด และจากการวิเคราะห์ไฮโมซิสเทอีนด้วยเทคนิคไฮเพอร์ ฟอร์แมนส์ลิกวิด โครมาโทกราฟีให้ค่าขีดจำกัดต่ำสุดในการตรวจวัดที่ 1 พิโคโมล สำหรับงานวิจัยในส่วนที่สอง คือ การนำระบบไมโครชิพอะพอลาร์ไรโอเล็กโทโรไฟเรซิสต่อกับระบบตรวจวัดทางเคมีไฟฟ้า เพื่อประยุกต์สำหรับ การแยกและตรวจวัดสารมลพิษในสิ่งแวดล้อม ได้แก่ สารประกอบไฮดรอกซีน สารประกอบฟีนอลและสารวัตถุ ระเบิด นอกจากนี้ได้ออกแบบระบบไมโครชิพอะพอลาร์ไรโอเล็กโทโรไฟเรซิสสำหรับการตรวจวัดสารทั้ง แบบต่อเนื่องและแบบไม่ต่อเนื่อง พบว่าความแม่นยำและความเที่ยงของวิธีวิเคราะห์มีประสิทธิภาพดีให้ผลการ วิเคราะห์อยู่ในช่วงที่ยอมรับได้ คือน้อยกว่า 5 เปอร์เซ็นต์

ภาควิชาเคมี..... ลายมือชื่อนิสิต..... จักรก ได้เขียนเพราะ.....
 สาขาวิชา.....เคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา..... อรรพรรณ ชัยลภากุล.....
 ปีการศึกษา.....2549.....

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my dear advisor, Assoc. Prof. Dr. Orawon Chailapakul, for the interesting subject of my Ph.D thesis, for her fruitful discussions and helpful criticism throughout the course of my work, as well as for giving me an opportunity to realize own ideas and to know the value of working. I truly appreciate her support, guidance and standing by me throughout my studies. I would like to thank her for the encouragement and always wishing for my successful future. I wish to thank her also for the very good laboratory equipment and her assistance on numerous organizational and administrative obstacles during this work. Completion of this dissertation would not have been possible without her supports.

I am very grateful to Prof. Joseph Wang (Arizona State University, USA) for providing me the opportunity to collaborate with his research group and for the interesting discussions in the area of microsystems.

Furthermore, I wish to thank Assoc. Prof. Dr. Siri Varothai for giving me an honor of being the thesis chairman. I really appreciate my committee members, Assoc. Prof. Dr. Thawatchai Tuntulani, Assist. Prof. Dr. Narong Praphairaksit, and Assist. Prof. Dr. Duangjai Nacapricha, for their comments and valuable time during my doctoral program.

I would like to acknowledge the Thailand Research Fund through the Royal Golden Jubilee Ph.D Program (Grant Number PHD/0289/2545), and Thailand-U.S. Educational Foundation (Fulbright) for financial support during my Ph.D course.

I would like to thank also all staffs in the Department of Chemistry, Faculty of Science, Srinakharinwirot University and Chulalongkorn University for the very good knowledge.

My very special thank goes to my all friends, especially those members in the electrochemical research group for their very good corporation in work, sharing of colorful emotion and friendly working atmosphere.

Last, but not least, my heartfelt regards and gratitude are due to my beloved parents for their endless love, eternal understanding and immeasurable care throughout my life.

CONTENTS

	PAGE
ABSTRACT (IN THAI)	iv
ABSTRACT (IN ENGLISH)	v
ACKNOWLEDGEMENT	vi
CONTENTS	vii
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS AND SYMBOLS	xxiii
CHAPTER I INTRODUCTION	1
1.1 Introduction.....	1
1.2 Research Objective.....	2
1.3 Scope of Research.....	2
CHAPTER II THEORY AND LITURATURE SURVEY	4
2.1 Fundamental of Electrochemistry.....	4
2.1.1 Potentiometry.....	5
2.1.2 Voltammetry.....	6
2.1.2.1 Cyclic Voltammetry.....	7
2.1.3 Amperometry.....	9
2.1.4 Hydrodynamic Voltammetry.....	10
2.1.5 Working Electrode: Boron-doped diamond thin film electrode.....	11
2.1.5.1 Preparation and Availability of Conductive Diamond Films by CVD processes.....	12
2.1.5.2 Characterization of Conductive Diamond Films.....	12
2.1.5.2.1 Microscopy.....	12
2.1.5.2.2 Raman Spectroscopy.....	13
2.1.5.2.3 X-ray Photoelectron Spectroscopy.....	13
2.2 Flow Injection Analysis (FIA).....	13
2.3 High Performance Liquid Chromatography.....	15

	PAGE
2.3.1 Ion-Pair Chromatography.....	16
2.4 Microchip Capillary Electrophoresis.....	17
2.4.1 Capillary Electrophoresis.....	17
2.4.1.1 Background Theory.....	17
2.4.1.2 Electroosmotic Flow.....	18
2.4.1.3 Injection Scheme.....	20
2.4.1.3.1 Hydrodynamic Injection.....	20
2.4.1.3.2 Electrokinetic Injection.....	20
2.4.1.4 Separation Modes.....	21
2.4.1.4.1 Capillary Zone Electrophoresis.....	22
2.4.1.4.2 Micellar Electrokinetic Chromatography.....	22
2.4.2 Introduction to Micro Total Analysis Systems.....	23
2.4.3 Personal Laboratories for the 21 st Century.....	23
2.4.4 Capillary Electrophoresis on Microchip.....	24
2.5 Electrochemical Detection under Flow -Through Condition.....	27
2.6 Thiol-Containing Drugs.....	28
2.6.1 Tiopronin.....	30
2.6.2 Captopril.....	32
2.7 Homocysteine.....	34
CHAPTER III EXPERIMENTAL.....	36
3.1 Instruments and Equipments.....	36
3.2 Apparatus for Microchip Capillary Electrophoresis System.....	38
3.3 Chemical and Reagents	38
3.4 Preparation of Solution.....	40
3.4.1 Preparation of 0.1 M Phosphate Buffer.....	41
3.4.2 Preparation of 10 mM Phosphate Buffer.....	41
3.4.3 Preparation of 100 mM MES Buffer pH 4.....	42
3.4.4 Preparation of 100 mM Borate Buffer pH 9.....	42
3.4.5 Preparation of 30 mM SDS.....	42
3.4.6 Stock Standard Solution of Thiol-Containing Drugs.....	43
3.4.7 Thiol-Containing Drugs for Calibration.....	43

	PAGE
3.4.8 Stock Standard Solution of Aminothiols.....	44
3.4.9 Stock Standard Solution of Phenolic Compounds.....	44
3.4.10 Stock Standard Solution of Explosive Compounds.....	45
3.4.12 Mobile Phase for HPLC-ECD.....	45
3.4.13 Aminothiol Mixture Standard Solutions for Calibration.....	45
3.5 Real Sample Analysis.....	46
3.6 Preparation of CoPc-Modified Carbon Paste	47
3.7 Procedures of Electrochemical Detection.....	48
3.7.1 Batch Analysis.....	48
3.7.1.1 Cyclic Voltammetry.....	48
3.7.1.2 Background Current.....	49
3.7.1.3 pH Dependence.....	49
3.7.1.4 The Electrochemical Oxidation of Thiol-Containing Drugs.....	49
3.7.1.5 Effect of Scan Rate.....	49
3.7.1.6 The Analytical Performance.....	49
3.7.2 Flow Injection with Amperometric Detection.....	50
3.7.2.1 Hydrodynamic Voltammetry.....	50
3.7.2.2 Calibration and Linear Range.....	51
3.7.2.3 Limit of Detection.....	51
3.7.2.4 Repeatability.....	51
3.7.3 High performance liquid chromatography.....	52
3.7.3.1 Optimum Potential for HPLC/ECD.....	53
3.7.3.2 Calibration Curve and Linear Range for HPLC.....	53
3.7.3.3 Limit of Detection of HPLC.....	54
3.7.3.4 Precision.....	54
3.7.3.5 Accuracy.....	54
3.7.4 Microchip CE Layout.....	54
3.7.5 End Column Amperometric Detection.....	55
CHAPTER IV RESULTS AND DISCUSSION.....	56
4.1 Boron-Doped Diamond Thin Film Electrodes for Thiol-Containing	

	PAGE
Drugs Analysis Coupled with Flow Injection Analysis.....	56
4.1.1 Cyclic Voltammetry.....	56
4.1.2 Effect of pH on the Electrochemical Behavior of Thiol- Containing Drugs.....	58
4.1.3 Scan Rate and Concentration Dependence.....	60
4.1.4 Flow injection with Amperometric Detection.....	64
4.1.4.1 Hydrodynamic Voltammetry.....	64
4.1.4.2 Flow Injection Analysis.....	66
4.1.5 Application: Quantitation of Sample.....	69
4.1.6 Comparison to the Other Methods.....	74
4.1.7 Summary.....	76
4.2 The Electrooxidation of Homocysteine at Anodized Thin Film Diamond Electrode with Amperometric HPLC.....	77
4.2.1 Cyclic Voltammetry.....	78
4.2.2 Concentration and Sweep Rate Dependence.....	79
4.2.3 Hydrodynamic Voltammetry.....	80
4.2.4 Flow Injection Analysis.....	82
4.2.5 HPLC Amperometric Results.....	84
4.2.6 Sensitivity, Linear Dynamic Range, and Detection Limit....	88
4.2.7 Summary.....	90
CHAPTER V MICROCHIP CAPILLARY ELECTROPHORESIS COUPLED WITH ELECTROCHEMICAL DETECTION...	91
5.1 Introduction.....	91
5.2 Experimental Section.....	94
5.2.1 Electrode Preparation.....	94
5.2.1.1 Capillary Electrode Construction.....	94
5.2.1.2 Screen Printed Electrode Fabrication.....	94
5.2.2 Microchip Apparatus.....	95
5.2.2.1 Apparatus for Normal Chip Layout.....	95
5.2.2.2 Apparatus for Continuous Monitoring.....	95
5.2.3 Electrophoresis Procedure.....	97

	PAGE
5.2.4 End-Column Amperometric Detection.....	98
5.2.5 Safety Considerations.....	99
5.3 Results and Discussion.....	99
5.3.1 Microchip Capillary Electrophoresis/Electrochemical Detection of Hydrazine.....	99
5.3.1.1 Comparison of Unmodified and CoPc-Modified Carbon Paste Capillary Electrodes.....	100
5.3.1.2 Capillary Electrophoresis Optimization.....	102
5.1.3.3 Influence of CoPc Loading.....	104
5.1.3.4 Hydrodynamic Voltammetry.....	104
5.3.1.5 Analytical Figures.....	107
5.3.1.6 Summary.....	109
5.3.2 Microchip Device for Rapid Screening and Fingerprint Identification of Phenol Pollutants.....	110
5.3.2.1 pH Effect.....	112
5.3.2.2 The Effect of Separation Voltage.....	112
5.3.2.3 The Influence of Washing Time and Switching Voltage	114
5.3.2.4 Analytical Figures.....	116
5.3.2.5 Summary.....	118
5.3.3 Microchip for Separation and Detection of Plastic Explosives.....	118
5.3.3.1 Electrochemical characteristics of RDX and PETN.....	119
5.3.3.2 Condition for Separation and Detection of RDX and PETN.....	121
5.3.3.3 Effect of Detection Potential.....	125
5.3.3.4 Analytical Figures.....	126
5.3.3.5 Summary.....	128
5.3.4 Continuous Monitoring with Microfabricated Capillary Electrophoresis Chip Devices.....	128
5.3.4.1 Carry-Over Effect.....	131
5.3.4.2 Influence of Sampling Time.....	133

	PAGE
5.3.4.3 Influence of Sample Flow Rate.....	134
5.3.4.4 Effect of Separation Voltages.....	135
5.3.4.5 Analytical Performances.....	135
5.3.4.6 Summary.....	138
CHAPTER VI CONCLUSIONS AND FUTURE PERSPECTIVES.....	139
6.1 Boron-Doped Diamond Thin Film Electrode for Thiol-Containing Drugs Analysis Coupled with Flow Injection Analysis System.....	139
6.2 The Electrochemical Oxidation of Homocysteine at Anodized Boron-Doped Diamond Thin Film Electrode with Amperometric HPLC.....	140
6.3 Microchip Capillary Electrophoresis Coupled with Electrochemical Detection.....	141
6.4 Suggestions for Future Work.....	143
REFERENCES.....	144
VITAE.....	163

LIST OF TABLES

TABLE	PAGE
3.1 List of Chemicals and Reagents and Their Suppliers.....	39
3.2 Compositions of 0.1 M Phosphate Buffer Solution.....	41
3.3 The Compositions of 10 mM Phosphate Buffer Solution.....	42
3.4 Compositions of Tiopronin Standard Solution.....	43
3.5 Compositions of Seven Amino Thiol Mixture Standard Solution.....	46
3.6 List of Thiol-Containing Drugs Employed in This Work.....	47
3.7 Compositions of %CoPc Loading in Carbon Paste.....	47
3.8 Parameter of the HPLC Condition.....	53
4.1 Comparison of Electrochemical Data Obtained from the Cyclic Voltammograms of 1 mM Captopril for the Diamond Electrode at Various pH values.....	59
4.2 Comparison of Electrochemical Data Obtained from the Cyclic Voltammograms of 2 mM Tiopronin for the Diamond Electrode at Various pH Values.....	60
4.3 Precision and Accuracy in the Determination of Commercially Available Tablets Doses form.....	70
4.4 Recovery of Captopril Tablet Sample with Amperometric Detection using Diamond Electrode Applied to Flow Injection System.....	71
4.5 Recovery of Tiopronin Tablet sample with Amperometric Detection using Diamond Electrode Applied to Flow Injection System.....	74
4.6 Comparison of Data for Tiopronin Determination.....	75
4.7 Response Characteristics for Thiol and Disulfide by HPLC Amperometric detection	89

LIST OF FIGURES

FIGURE	PAGE	
2.1	Electrode is concerned with the exchange of electrons between electrodes and species in solution (these are frequently ions). Ionics is the discipline concerned with the behavior of ions in solution.....	4
2.2	Schematic diagram of an electrochemical cell for potentiometric measurement	6
2.3	Potential-time waveforms are used in various electroanalytical techniques. Waveforms based on (a) square, (b) linear, and (c) triangular potential-time patterns are used in square wave, linear sweep, and cyclic voltammetry, respectively.....	7
2.4	The current-potential (i-E) plot is called a cyclic voltammogram.....	8
2.5	Amperometry (E-t) waveform.....	10
2.6	Schematic diagram of a generic description of FIA.....	15
2.7	Schematic of HPLC instrument.....	16
2.8	Schematic representation of electrophoresis.....	17
2.9	Representation of osmotic flow and migration of neutral, cationic, and anionic solutes in a capillary due to electroosmotic flow and electrophoretic mobility....	19
2.10	Separation mechanisms in (a) CZE and (b) MEKC.	22
2.11	Typical amperometric readout during automated flow injection assays of ethanol at an enzyme carbon-paste electrode. Peak a through h: 2×10^{-5} M to 1.6×10^{-4} M ethanol.....	28
2.12	Thiol-containing drugs structure.....	30
3.1	The electrochemical cell for cyclic voltammetric study	48
3.2	The thin layer flow cell.....	50
3.3	The thin layer flow cell for HPLC system.....	52
3.4	Microchip capillary electrophoresis coupled with electrochemical detection layout.....	55

FIGURE

PAGE

4.1	Cyclic voltammograms for a) BDD and b) GC versus Ag/AgCl in 1.0 mM captopril in 0.1 M phosphate buffer pH 9 (solid lines) and 0.1 M phosphate buffer pH 9 (dashed lines) Sweep rate, 50 mV/s; area of electrode, 0.07cm ² Scan rate = 20 mV/s.....	57
4.2	Cyclic voltammograms for a) BDD and b) GC versus Ag/AgCl in 2.0 mM tiopronin in 0.1 M phosphate buffer pH 8 (solid lines) and 0.1 M phosphate buffer pH 8 (dashed lines) Sweep rate, 50 mV/s; area of electrode, 0.07cm ² ...	58
4.3	Cyclic voltammogram for captopril in 0.1 M phosphate buffer (pH 9) at boron-doped diamond electrode for a series of captopril concentrations. The potential sweep rate was 50mV/ s; area of electrode, 0.07cm ² . The calibration curve was shown in the inset.....	61
4.4	Cyclic voltammogram for 1 mM tiopronin in 0.1 M phosphate buffer (pH 8) at boron-doped diamond electrode for a series of potential sweep rates; area of electrode, 0.07 cm ² . The calibration curve of relationship between current (μA) and (sweep rate) ^{0.5} was also in the inset of this figure.....	62
4.5	Cyclic voltammogram for 1 mM captopril in 0.1 M phosphate buffer (pH 9) at boron-doped diamond electrode for a series of potential sweep rates; area of electrode, 0.07 cm ² . The calibration curve of relationship between current (μA) and (sweep rate) ^{0.5} was also in the inset of this figure.....	63
4.6	Cyclic voltammogram for tiopronin in 0.1 M phosphate buffer (pH 8) at boron-doped diamond electrode for a series of captopril concentrations. The potential sweep rate was 50mV/ s; area of electrode, 0.07cm ² . The calibration curve was shown in the inset.....	63
4.7	(a) Hydrodynamic voltammogram of (-●-) 0.1 M phosphate buffer (pH 9, background current) and (-▼-) 100 μM of captopril in 0.1 M phosphate buffer (pH 9) with four injections of analysis, using 0.1 M phosphate buffer (pH 9) as a carrier solution. (b) Hydrodynamic of signal- to- background ratio. The flow rate was 1 mL/ min.....	65

FIGURE	PAGE	
4.8	(a) Hydrodynamic voltammogram of (-▲-) 0.1 M phosphate buffer (pH 8, background current) and (-●-) 100 μM of tiopronin in 0.1 M phosphate buffer (pH 8) with four injections of analysis, using 0.1 M phosphate buffer (pH 8) as a carrier solution. (b) Hydrodynamic of signal- to- background ratio. The flow rate was 1mL/ min.....	66
4.9	Flow injection analysis with amperometric detection results for a diamond electrode using 20-ml of captopril at various concentrations. The mobile phase was 0.1 M phosphate buffer (pH 9) at a flow rate of 1 mL/ min. The calibration curve was shown in the inset.....	67
4.10	Flow injection analysis with amperometric detection results for a diamond electrode using 20-μL of tiopronin at various concentrations. The mobile phase was 0.1 M phosphate buffer (pH 8) at a flow rate of 1 mL/min. The calibration curve was shown in the inset.....	68
4.11	Flow injection with amperometric detection results for 10 nM captopril in 0.1 M phosphate buffer (pH 9). The flow rate was 1 mL/min.....	68
4.12	Flow injection with amperometric detection results for 10 nM tiopronin in 0.1 M phosphate buffer (pH 8). The flow rate was 1 mL/min.....	69
4.13	Flow injection analysis results for determination of captopril in commercially available tablets. Using the standard addition for the added captopril: (a) 0.00 mg/ml, (b) 0.43 mg/ml, (c) 0.87 mg/ml, (d) 1.30 mg/ml, (e) 1.74 mg/ml, (f) 2.17 mg/ml.....	70
4.14	Flow injection analysis results for determination of tiopronin in commercially available tablets. Using the standard addition for the added captopril: (a) 0.00 mg/ml, (b) 0.65 mg/ml, (c) 0.98 mg/ml, (d) 1.31 mg/ml, (e) 1.63 mg/ml, (f) 1.96 mg/ml.....	72
4.15	Precision results from flow injection - amperometric detection of tiopronin at concentrations of : a, 0 μg/mL; b, 0.98 μg/mL; and c, 1.63 μg/mL (n = 10).....	73

FIGURE	PAGE	
4.16	(A) Cyclic voltammograms for 1 mM homocysteine in 0.1 M phosphate buffer (pH 9) at (a) an as-deposited boron-doped thin film electrode (dotted line) and (b) an oxidized (anodized) boron-doped thin film electrode (solid line). (B) Cyclic voltammograms for 1 mM homocysteine in 0.05 M phosphate buffer (pH 2.7) at (a) an as-deposited boron-doped thin film electrode (dotted line) and (b) an oxidized boron-doped thin film electrode (solid line).....	78
4.17	Cyclic voltammogram for homocysteine in 0.1 M phosphate buffer (pH 2.7) at anodized boron-doped diamond electrode for a series of homocysteine concentrations. The potential sweep rate was 50mV/ s; area of electrode, 0.07cm ² . The calibration curve was shown in the inset.....	79
4.18	Cyclic voltammogram for 1 mM homocysteine in 0.1 M phosphate buffer (pH 2.7) at anodized boron-doped diamond electrode for a series of potential sweep rates; area of electrode, 0.07 cm ² . The calibration curve of relationship between current (μA) and (sweep rate) ^{0.5} was also in the inset of this figure.....	80
4.19	(a) Hydrodynamic voltammograms at the anodized BDD electrode for 20 μL injections of homocysteine, homocystine, cysteine, cystine, methionine, reduced glutathione and oxidized glutathione (each 20 μM) in 2% acetonitrile–0.05 M phosphate buffer (pH 2.7). The flow rate was 1 mL/ min	81
4.20	Flow injection traces with amperometric detection of homocysteine at various concentrations: a, 100 μM; b, 50 μM; c, 10 μM; d, 5 μM; e, 1 μM; f, 0.5 μM; g, 0.1 μM; h, 0.05 μM; i, 0.01 μM; and j, 0.005 μM. The calibration curve (peak current vs. concentration) was shown in the inset. Other conditions were the same as in Figure 4.10.....	83
4.21	Precision results from flow injection – amperometric detection of homocysteine at concentrations of : a, 5 μM; b, 1 μM; c, 0.5 μM;.....	84

FIGURE	PAGE	
4.22	High-performance liquid chromatographic-amperometric chromatograms of aminothiols, in order of elution: 1, cystine; 2, cysteine; 3, homocysteine; 4, reduced glutathione; 5, methionine; 6, homocystine; 7, oxidized glutathione. The mobile phase was 1 mM OSA in 3% acetonitrile–0.05 M phosphate buffer (pH 2.7).....	85
4.23	High-performance liquid chromatographic-amperometric chromatograms of aminothiols, in order of elution: 1, cystine; 2, cysteine; 3, homocysteine; 4, reduced glutathione; 5, methionine+homocystine; 6, oxidized glutathione. The mobile phase was 0.1 mM OSA in 3% acetonitrile–0.05 M phosphate buffer (pH 2.7).....	86
4.24	High-performance liquid chromatographic-amperometric chromatograms of aminothiols, in order of elution: 1, cystine (1.95 min); 2, cysteine (2.53 min); 3, homocysteine (4.59 min); 4, reduced glutathione (5.16 min); 5, methionine (8.50 min); 6, homocystine (12.0 min); 7, oxidized glutathione (13.80 min). The mobile phase was 0.2 mM OSA in 3% acetonitrile–0.05 M phosphate buffer (pH 2.7). (A) 100 μ M, (B) 20 μ M and (C) 0.5 μ M.....	87
4.25	The detection limit of homocysteine from HPLC /amperometric detection at anodized boron-doped diamond thin film electrode compared to the mobile phase.....	88
5.1	Electroferograms for a mixture containing 50 μ M hydrazine (a), 100 μ M 1,1 dimethylhydrazine (b), and 100 μ M phenylhydrazine (c), obtained at unmodified (A) and 5 % CoPc-modified (B) carbon paste electrodes. Separation and injection voltages, + 1000 V; injection time, 3 s; detection potential, +0.5 V (vs Ag/AgCl). Phosphate buffer (10 mM and pH 6.5) was used as running buffer.....	102
5.2	Influence of pH value of 10 mM phosphate run buffer on the separation and detection for mixture at the 5 % CoPc-modified carbon paste capillary electrodes. pH 6.2 (a), pH 6.5 (b), pH 6.8 (c), pH 7.3 (d), and pH 7.6 (e). Other conditions, as in Figure 5.1.....	103

FIGURE	PAGE	
5.3	Effect of CoPc loading in carbon paste on the current response of 50 μM hydrazine (a) and 100 μM phenylhydrazine (b). Other conditions, as in Figure 5.1.....	105
5.4	Hydrodynamic voltammograms for 50 μM hydrazine (a) and 100 μM phenylhydrazine at 5 % CoPc-modified carbon paste electrodes. Other conditions, as in Figure 5.1.....	106
5.5	Hydrodynamic voltammograms for 50 μM hydrazine at unmodified (A) and 5 % CoPc-modified (B) carbon paste electrodes. Other conditions, as in Figure 5.1.....	107
5.6	(A) Electropherograms detailing the current response to increasing concentrations of hydrazine (a), and phenylhydrazine (b) in steps of 20 μM and 40 μM , respectively. (B) The resulting calibration plots. Other conditions were same as Figure 5.1.....	108
5.7	Schematic diagram of the ‘total’ (A) and ‘individual’ (B) on-chip assays of phenolic compounds. (a) Reservoir with 10 mM phosphate buffer (pH 5), (b) reservoir with 10 mM phosphate buffer (pH 8), (c) sample solution, (d) separation channel, (e) amperometric detector. Initial operation using the acidic buffer leads to rapid ‘total’ measurements (A) followed by switching to the basic buffer for separating and detecting ‘individual’ phenols.....	111
5.8	Influence of the pH of the buffer on the amperometric response for a mixture of (1) phenol (50 μM), (2) 2-chlorophenol (100 μM), (3) 2,3-dichlorophenol (200 μM) and (4) 4-nitrophenol (200 μM). pH buffer: (a) 9.0, (b) 8.0, (c) 7.0, (d) 6.0 and (e) 5.0; buffer concentration 10 mM; separation voltage 1500 V; injection voltage 1500 V for 3 sec; detection potential +1.1 V vs. Ag/AgCl wire.....	113
5.9	Effect of the separation voltage on the flow-injection response for a mixture of phenol (50 μM) and 2-chlorophenol (100 μM). Separation voltages: (a) 4000, (b) 3500, (c) 3000, (d) 2500, (e) 2000 and (f) 1500 V. pH buffer, 5.0. Other conditions, same as Figure 5.7.....	114

FIGURE

PAGE

- 5.10** Influence of the switching time and the switching voltage upon the peak resolution between (1) phenol (50 μM) and (2) 2-chlorophenol (100 μM). (A) Electropherograms recorded using switching voltage of 4000 V and a washing time of (a) 25, (b) 50, (c) 75, (d) 100, (e) 125 and (f) 150 sec. (B) Influence of the switching voltage; electropherograms recorded using a 150 sec washing time and switching voltages of (a) 1000, (b) 1500, (c) 2000, (d) 3000 and (e) 4000 V. Running buffer of pH, 8.0. Other conditions, same as Figure 5.8..... 115
- 5.11** Electropherograms depicting the precision of alternate 'total' (A, B, C) and 'individual' (D, E, F) assays using a mixture containing (a) 50 μM phenol and (b) 100 μM 2-chlorophenol. Separation voltage, 4000 (A, B, C) and 1500 V (D, E, F). Running buffer of pH 5 (A, B, C) and pH 8.0 (D, E, F). Other conditions, same as Figure 5.7..... 117
- 5.12** Cyclic voltammograms of 200 ppm RDX (A) and 300 ppm PETN (B) in 20 mM MES pH 4 (solid line) and 20 mM MES pH 4 (dash line), over the 0.0 and -1.0 V range. Scan rate, 50 mV/s..... 120
- 5.13** Separation and detection of Explosive compounds: (1) 10 ppm RDX, and (2) 200 ppm PETN. Separation buffer, 20 mM MES (pH 4) containing 25 mM SDS; separation and injection voltages, + 1500 V; injection time, 3 s; detection potential, -0.8 V (vs Ag/AgCl wire) at bare carbon screen-printed electrode. 121
- 5.14** Effect of pH of running buffer upon the separation and response of 10 ppm RDX (1) and 200 ppm PETN (2), (a) pH=8.0, (b) pH=7.0, (c) pH=6.0, and (d) pH=4.0. Separation buffer, 20 mM MES containing 25 mM SDS; separation and injection voltage, + 1500 V; injection time, 3 s; detection potential, - 0.8 V (vs Ag/AgCl wire) at bare carbon screen-printed electrode..... 122
- 5.15** Effect of electrolyte concentration based on the separation and response of 10 ppm RDX (1) and 200 ppm PETN (2), (a) 10 mM, (b) 20 mM, and (c) 30 mM. Separation buffer, 20 mM MES (pH 4) containing 25 mM SDS. Other conditions as same as Figure 5.14..... 123

FIGURE	PAGE	
5.16	Effect of SDS concentrations upon the separation and detection of 10 ppm RDX (1) and 200 ppm PETN (2), (a) 10 mM, (b) 15 mM, (c) 20 mM, (d) 25 mM, and (e) 30 mM. Other conditions, as in Figure 5.14.....	124
5.17	Hydrodynamic voltammograms of 10 ppm RDX (●) and 200 ppm PETN (▲). Conditions, as in Figure 5.12.....	125
5.18	Electroprerograms for mixture containing increasing levels of (1) RDX, and (2) PETN in steps of 2 and 25 ppm, respectively (a-e). The calibration was also shown insete. Other conditions, as in Figure 5.14.....	126
5.19	Repetitive simultaneous measurements of a mixture containing (1) 10 ppm RDX, and (2) 200 ppm PETN as recorded with amperometric mode. Other conditions, as in Figure 5.13.....	127
5.20	(A) Structure of the microfluidic chip system with on-line continuous monitoring capability. The separation channel (a) was joined by a sharp sample inlet tip (b), side sharp running-buffer inlet tip (c) and a detector compartment (d). The sample was introduced by pumping from the reservoir with a peristaltic pump (P) through the Tygon tubing (e) onto the separation channel through a sharp inlet tip. The sample solution was drained through a plastic tubing (g) that was placed 1 mm from the sample inlet tip. The running buffer was introduced by capillary action through the plastic tubing (f). The detector compartment (d) was designed to accommodated the platinum wire ground electrode (h), the screen-printed carbon working electrode (i), platinum wire auxiliary electrode (k), and the Ag/AgCl wire reference electrode (j). Two platinum wires (l ₁ , l ₂) were inserted into the plastic tubing (e, f), respectively, serving as contacts to the high voltage power supply. (B) Steps involved in the new microchip assay: Plug patterns during the (I) introduction of the running buffer into the channel; (II) sample injection; (III) sample separation and detection.....	130

FIGURE	PAGE	
5.21	<p>(A) Typical electropherograms showing the separation for an explosive mixture [Left; TNB 10 (a), DNB 15 (b) and TNT 20 (c) ppm] and phenolic compounds [Right; phenol 100 (a), 2-chlorophenol 100 (b) and 2,3-dichlorophenol 200 (c)]. Sampling time, 3s; running buffer (for explosives), 15 mM sodium borate solution (pH 9.2) containing 15 mM SDS, and (for phenolic compounds), borate/phosphate buffer (10 mM each, pH 8.0). Sample flow rate, 0.5 mL/min; detection potential, -0.5 V (Left) and +0.9 V (Right) (vs. Ag/AgCl wire); injection and separation voltages, 2000 V (Left) and 1500 V (Right). (B) Electropherograms for repetitive introduced samples containing 10 ppm (a) and 5 ppm (b) TBN (in connection to 2 sample reservoirs and a T connector). Other conditions, as in Figure 5.21A (Left).....</p>	132
5.22	<p>Effect of the sampling time upon the response for 10 ppm TNB. Sampling time 1, 2, 3, 5, 10, 15, and 20 s (a-g); running buffer, 15 mM sodium borate (pH 9.2) Other conditions, as in Figure 5.20A (Left).....</p>	133
5.23	<p>Influence of sample flow rate on the response for a 10 ppm TNB solution. Flow rate, 0.2 (a), 0.5 (b), 0.9 (c), 1.3 (d), and 1.8 (e) mL/ min. Other conditions, in Figure 5.21A (Left).....</p>	134
5.24	<p>(A) Influence of the separation field strength upon the response of a 10 ppm TNB solution. Running buffer, 15 mM sodium borate (pH 9.2). (B) Effect of the sampling voltage on the response for 10 ppm TNB using a separation voltage of 2000V. Other conditions, as in Figure 5.21A (Left).</p>	135
5.25	<p>Response for 40 repetitive injections of a 15 ppm TNB solution. Sampling time, 2 s; running buffer, 15 mM sodium borate (pH 9.2); Top inset shows the response for two such injections. Other conditions, as in Figure 5.20A.....</p>	136
5.26	<p>Electropherograms detailing the response to sample mixtures containing increasing concentrations of explosives [A; TNB(a) and TNT(b) in 2 ppm steps (I-VI)] and phenolic compounds [B; phenol (a), 2-chlorophenol (b) and 2,3-dichlorophenol (c) in steps of 25 μM (a,b) and 50 μM (c) steps (I-VI)]. Insets show the corresponding calibration plots. Other conditions, as in Figure 5.21.....</p>	137