

Contents

	Page
Contents	ix
List of Tables	xvi
List of Figures	xviii
Chapter	
1. Introduction	1
1.1 Background and Rationale	1
1.2 Objectives of the research	2
1.3 Benefits	3
2. Affinity Biosensor	4
2.1 Biosensor	4
2.2 Types of affinity biosensor	5
2.2.1 DNA biosensors	7
2.2.2 Receptor biosensors	8
2.2.3 Immunosensors	8
2.2.3.1 Antibody	9
2.2.3.2 Binding forces	10
2.2.3.3 Kinetics	11
2.2.3.4 Labeled immunosensors	12
2.2.3.5 Label-free immunosensors	15
2.3 Detection principles of label-free immunosensors	16
2.3.1 Optical transducer	16
2.3.2 Piezoelectric transducer	17
2.3.3 Electrochemical transducer	19

Contents (Continued)

	Page
3. Electrochemical Detection of Label-free Immunosensors	20
3.1 Potentiometric immunosensor	20
3.2 Amperometric immunosensor	24
3.3 Conductimetric immunosensor	27
3.4 Impedimetric immunosensor	29
3.5 Capacitive immunosensor	32
3.5.1 Current step	41
3.5.2 Potential sweep (Voltage ramp)	43
3.5.3 Potential step	45
4. Performance Criteria	48
4.1 Selectivity	48
4.2 Linear range, sensitivity and limit of detection	48
4.3 Regeneration, stability and reproducibility	50
5. Capacitive Biosensor for Direct Detection of Protein Affinity Reaction	52
5.1 Introduction	52
5.2 Materials	54
5.3 Methods	55
5.3.1 Preparation of Fc-fragments from anti-HSA (IgG) for protein A	55
5.3.1.1 Papain-digestion of anti-HSA IgG	55
5.3.1.2 Purification of Fc-fragments	56
5.3.1.2.1 Gel filtration column (Sephadex G-50)	56
5.3.1.2.2 Protein A affinity column	56

Contents (Continued)

	Page
5.3.2 Determination of Fc-fragments	56
5.3.3 Immobilization	60
5.3.3.1 Pretreatment of gold surface	60
5.3.3.2 Immobilization of anti-HSA or Fc-fragment	61
5.3.4 Capacitance measurement	61
5.3.5 Capacitive biosensor for HSA	65
5.3.6 Capacitive biosensor for protein A	65
5.4 Results and discussion	65
5.4.1 HSA	65
5.4.1.1 Effect of type of buffer solution	65
5.4.1.2 Effect of concentration of anti-HSA	66
5.4.1.3 Linear dynamic range and detection limit	67
5.4.1.4 Selectivity	68
5.4.2 Protein A	69
5.4.2.1 Effect of regeneration solution	70
5.4.2.2 Flow rate	73
5.4.2.3 Sample volume	74
5.4.2.4 Linear dynamic range and detection limit	75
5.5 Conclusions	75
6. Ultra-sensitive Capacitive Biosensor Developed for the Monitoring of Endotoxins in Fermentation Liquid	77
6.1 Introduction	77
6.2 Materials	78
6.3 Methods	78
6.3.1 Preparation of endotoxins from <i>E.coli</i>	78
6.3.2 Endotoxin extraction with phenol-water system	79

Contents (Continued)

	Page
6.3.3 Immobilization of Lectin	79
6.3.4 Capacitance measurement	79
6.3.5 Optimization of the capacitive biosensor	80
6.3.6 Determination of the amount of endotoxin in real sample	80
6.3.7 Comparison between the results obtained from the capacitive biosensor system and LAL-test	80
6.4 Results and discussion	82
6.4.1 Electrochemical performance of the immobilization process	82
6.4.2 Optimization of the flow injection capacitive biosensor	83
6.4.2.1 Regeneration solution	83
6.4.2.2 Sample volume	86
6.4.2.3 Flow rate	87
6.4.2.4 Buffer solution	88
6.4.3 Linear dynamic range and detection limit	90
6.4.4 Comparison between the capacitive biosensor system and LAL-test	92
6.5 Conclusions	95
7. A Comparative Study of Capacitive Immunosensors Based on Self-Assembled Monolayers Formed from Thiourea, Thiocetic Acid and 3-Mercaptopropionic Acid	96
7.1 Introduction	96
7.2 Materials	99
7.3 Methods	99

Contents (Continued)

	Page
7.3.1 Preparation of gold surface	99
7.3.2 SAMs formation	99
7.3.3 Immobilization of anti-AFP	100
7.3.4 Determination of the immobilization yield	105
7.3.5 Capacitance measurement	106
7.4 Results and discussion	108
7.4.1 Immersion times	108
7.4.2 Concentration of thiol solutions	112
7.4.3 Immobilization of anti-AFP	115
7.4.3.1 SATAM and SAMPAM	115
7.4.3.1.1 Electrostatic binding	115
7.4.3.1.2 Covalent binding	116
7.4.3.2 SATUM	116
7.4.4 Electrochemical performance of the process of Anti-AFP immobilization	119
7.4.5 Linear range and detection limit	122
7.4.6 Selectivity	122
7.4.7 Reproducibility	126
7.5. Conclusions	128
8. A Reusable Capacitive Immunosensor for Carcinoembryonic Antigen (CEA) Detection Using Thiourea Modified Gold Electrode	129
8.1 Introduction	129
8.2 Materials	130
8.3 Methods	130

Contents (Continued)

	Page
8.3.1 Immobilization of anti-CEA	130
8.3.2 Capacitance measurement	132
8.3.3 Optimization of the flow injection capacitive immunosensor	134
8.3.4 Determination of the amount of CEA in serum samples	134
8.3.5 Comparison between the results obtained from the capacitive immunosensor system and ELFA technique (VIDAS [®] CEA)	134
8.3.5.1 Regression line analysis	135
8.3.5.2 Wilcoxon signed rank test	136
8.4 Results and discussion	137
8.4.1 Electrochemical performance of the immobilization process	137
8.4.2 Optimization of the flow injection capacitive immunosensor	139
8.4.2.1 Regeneration solution	139
8.4.2.2 Flow rate	141
8.4.2.3 Sample volume	143
8.4.2.4 Buffer solutions	144
8.4.2.4.1 Type	144
8.4.2.4.2 pH	145
8.4.3 Reproducibility	147
8.4.4 Effect of non-specific binding	150
8.4.5 Linear dynamic range, detection limit	152

Contents (Continued)

	Page
8.4.6 Selectivity	152
8.4.7 Comparison between the results obtained from the capacitive immunosensor system and ELFA technique (VIDAS [®] CEA)	154
8.5 Conclusions	158
9. Conclusions	159
References	165
Appendix	202
Vitae	220

List of Tables

Table	Page
5.1. Assayed values of the type, pH and concentration of regeneration solution. The efficiency of protein A removal from the Fc-fragments immobilized on the electrode was studied by injecting 10^{-12} M of protein A standard solution.	72
6.1 Critical values for the Wilcoxon signed rank test; statistic at $P < 0.05$ for $n = 6$ to 37 where n is the number of data pair (Triola, 1998). The null hypothesis can be rejected when the test statistic is \leq the tabulated value.	81
6.2 Assayed and optimized values of the type, pH and concentration of regeneration solution. The efficiency of endotoxin removal from the lectin immobilized on the electrode was studied by injecting 0.1 nM of endotoxin standard.	85
6.3 Assayed and optimized values used in the study of the capacitive biosensor system as a tool for endotoxin analysis.	88
7.1. Performances of anti-AFP covalently immobilized on self-assembled thioctic acid monolayer (SATAM), self-assembled 3-mercaptopropionic acid monolayer (SAMPAM), and self-assembled thiourea monolayer (SATUM). (NA: not applicable)	118
8.1 The efficiency of CEA removal from the anti-CEA immobilized on the electrode studied by injecting 0.1 ng ml^{-1} CEA. The efficiency is given as capacity (in per cent of initial value) of the sensor to respond to a new pulse of CEA.	141
8.2 Assayed and optimized values used in the study of the flow injection capacitive immunosensor system.	147

List of Tables (Continued)

Table	Page
8.3 The Wilcoxon sign rank test for the comparison of the concentration of CEA in sample from the capacitive immunosensor system and ELFA technique (VIDAS [®] CEA). (The null hypothesis (there is no difference between the two methods) is rejected if the test statistic T (the lower of the sum of positive rank or negative rank-shown as italic) is less than or equal to the critical value. The null hypothesis can not be rejected if the test statistic T is greater than the critical value).	157
9.1 Performance of the flow injection capacitive biosensor systems for different analytes studied in this work.	162
9.2 Comparison of the analytical feature for protein A detection	163
9.3 Comparison of the analytical feature for endotoxin detection	163
9.2 Comparison of the analytical feature for alpha-fetoprotein (AFP) detection	164
9.2 Comparison of the analytical feature for carcinoembryonic antigen (CEA) detection	164

List of Figures

Figure	Page
2.1 Major biosensor types	6
2.2 Structure of IgG antibody	10
2.3 Two main types of labeled immunosensors. a). sandwich-type, b). competitive-type sensors. The signal is proportional to analyte concentration in a sandwich sensors, and inversely proportional to analyte concentration in a competitive sensors	14
2.4 Label-free immunosensors	15
2.5 Surface plasmon resonance (SPR) biosensor principle. Binding of biomolecules to the surface increase the refractive index, which induces shift of the SPR-angle. The shift is directly proportional to the mass increase (Adapted from Johansson, 2004).	17
2.6 Quartz crystal microbalance	19
3.1 Schematic diagram of a potentiometric immunosensor using potassium ion selective membrane electrode	22
3.2 Schematic diagram of polymer modified electrode potentiometric immunosensor; reference electrode (RE), working electrode (WE) (Modified from Fu <i>et al.</i> , 2004; Tang <i>et al.</i> , 2004b; 2004c)	23
3.3 Schematic diagram of the pulsed amperometric technique for amperometric immunosensor. (a) amperometric response of antibody (i_{Ab}), (b) amperometric response of antibody-antigen reaction (i_{Ab-Ag}) (Modified from Ramanaviciene and Ramanavicius, 2004; Sargent and Sadil, 1998)	25

List of Figures (Continued)

Figure	Page
3.4 Schematic diagram of chronoamperometric technique for amperometric immunosensor. (a) amperometric response of antibody (i_{Ab}), (b) amperometric response of antibody-antigen reaction (i_{Ab-Ag})	26
3.5 Schematic diagram of a conductimetric immunosensor measures the change of the conductivity of the layer between the electrodes due to antibody-antigen reaction (Modified from Yagiuda <i>et al.</i> , 1996)	27
3.6 Schematic diagram of conductimetric immunosensor measures the change of the polymer modified electrode due to antibody-antigen reaction; reference electrode (RE), working electrode (WE)	28
3.7 Randles' equivalent circuit; (R_s) the ohmic resistance of the electrolyte resistance resulting from the diffusion of redox-probe, (C_{dl}) the double-layer capacitance, (R_{et}) the electron-transfer resistance, and (Z_w) the Warburg impedance (Grant <i>et al.</i> , 2005; Katz and Willner, 2003; Kharitonov <i>et al.</i> , 2000; Tang <i>et al.</i> , 2004a; Yang <i>et al.</i> , 2003)	29
3.8 Typical Nyquist plot of immobilized Ab on the surface of the electrode (a) and Ab-Ag complex on the surface of the electrode (b), (R_s) the electrolyte resistance, ($R_{et(Ab)}$) the electron- transfer resistance of immobilized Ab, and ($R_{et(Ab-Ag)}$) the electron- transfer resistance of Ab-Ag complex (Modified from Katz and Willner, 2003; Kharitonov <i>et al.</i> , 2000; Tang <i>et al.</i> , 2004a)	31

List of Figures (Continued)

Figure	Page
3.9 Schematic representation of (a) model of the double-layer region (IHP is inner Helmholtz plane; OHP is outer Helmholtz plane) and (b) potential profile across the double layer (Redrawn from Bard and Faulkner, 2001; Mark, 1991; Wang, 2000)	33
3.10 Schematic diagram of capacitive immunosensor measures the capacitance as a change of distance between two plates (a) or as a change in dielectric constant using interdigitated electrode (b) (Modified from Gebbert <i>et al.</i> , 1992; Berggren <i>et al.</i> , 2001)	37
3.11 Schematic diagram of capacitive immunosensor measures the change in the capacitance at the electrode/solution interface; reference electrode (RE), working electrode (WE), auxiliary electrode (AE) (Modified from Hu <i>et al.</i> , 2000; Jiang <i>et al.</i> , 2003; Wu <i>et al.</i> , 2005; Yin <i>et al.</i> , 2005(a)).	38
3.12 Common adder potentiostat (Redrawn from Bard and Faulkner, 2001; Wang, 2000)	39
3.13 Models of an electrochemical cell for capacitance detection. (a) Randle's circuit; (R_s) the resistance of the solution, (C_{dl}) the double layer capacitance, (C_p) a pseudo capacitance, due to movement of ions at the interface, (R_{ct}) the charge transfer resistance at the interface. (b) a series $R_s C_{dl}$ equivalent circuit (Berggren and Johansson, 19897; Jiang <i>et al.</i> , 2003; Yang <i>et al.</i> , 2003)	40
3.14 $E-t$ behavior resulting from a current step applied to an $R_s C_{dl}$ circuit (Redrawn from Bard and Faulkner, 2001)	42

List of Figures (Continued)

Figure	Page
3.15 <i>i-t</i> behavior resulting from a linear potential sweep applied to an $R_s C_{dl}$ circuit (Redrawn from Bard and Faulkner, 2001)	44
3.16 Potentiostatic step method to evaluate capacitance (a) shows a potential step and (b) the corresponding current response (Redrawn from Bard and Faulkner, 2001; Wang, 2000)	45
4.1 Schematic of a calibration curve showing relationships for determining linear range, sensitivity and limit of detection (Buck and Lindner, 1994; Eggins, 1996; Swartz and Krull, 1997; Thevenot <i>et al.</i> , 1999; Wang, 2000)	50
5.1 Two affinity binding pairs, human serum albumin (HSA) and anti human serum albumin antibody (anti-HSA), and crystallizable fragment (Fc-fragments) from IgG (anti-HSA) and protein A.	54
5.2 Schematic diagram of digestion of IgG molecule by papain resulting in two Fab-fragments and one Fc-fragment.	55
5.3 a), Separation of Fc-fragment and Fab-fragment from the digested sample solution on gel filtration column. b), Purification of a Fc-fragment from Fab-fragments by protein A affinity column.	58
5.4 Standard Curve of absorbance (562 nm) versus protein sample concentration	59
5.5 Gold electrode surface (\varnothing 3 mm) under optical microscopy before (a) and after polishing with alumina (b).	60

List of Figures (Continued)

Figure	Page
5.6 Schematic view of the flow injection capacitive biosensor system and the capacitive properties of the transducer surface where C_{SAM} ; the capacitance of self-assembled thiocetic acid monolayer, C_P ; the capacitance in protein layer, C_a ; the capacitance of analyte interaction and C_{Total} ; the total capacitance measured at the working electrode/solution interface.	63
5.7 Schematic diagram showing the change in capacitance (ΔC) as a function of time caused by binding between analyte and biorecognition element with subsequent signal increase due to dissociation under regeneration conditions.	64
5.8 Responses of the flow injection capacitive biosensor system for HSA using different buffer solutions.	66
5.9 Sensitivity of the flow injection capacitive biosensor system for HSA using different concentration of anti-HSA in the immobilization.	67
5.10 Capacitance change vs. the logarithm of HSA concentration for a transducer surface with immobilized anti-HSA under optimized conditions ($250 \mu\text{l min}^{-1}$ flow rate, $250 \mu\text{l}$ sample volume, 10 mM borate buffer, $\text{pH } 8.65$).	68
5.11 Capacitance measurement showing the specificity of the HSA-anti-HSA affinity binding system.	69
5.12 Responses of the flow injection capacitive biosensor system for protein A at different flow rates.	73
5.13 Responses of the flow injection capacitive biosensor system for protein A at different sample volume.	74

List of Figures (Continued)

Figure	Page
5.14 Capacitance change vs. the logarithm of protein A concentration for a transducer surface with immobilized Fc-fragments under optimized conditions (100 $\mu\text{l min}^{-1}$ flow rate, 250 μl sample volume, 10 mM borate buffer, pH 8.50).	76
6.1 Cyclic voltammograms of a gold electrode obtained in a 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ containing 0.1 M KCl solution at scan rate of 50 mV s^{-1} . All potentials are given vs SCE. (a) clean gold, (b) thioctic acid covered gold, (c) lectin modified thioctic acid couple gold, and (d) as in (c) but after 1-dodecanethiol treatment.	83
6.2 Responses of the capacitive biosensor system at different sample volume.	86
6.3 Responses of the capacitive biosensor system at different flow rates.	87
6.4 Responses of the flow injection capacitive biosensor system from different buffer solution.	89
6.5 Effect of pH of phosphate buffer solution.	89
6.6 Capacitance change vs. the logarithm of endotoxin concentration for a surface with immobilized lectin. (a) the first optimum conditions; 50 $\mu\text{l min}^{-1}$ flow rate of current buffer (10 mM phosphate buffer pH 7.20) and sample volume 100 μl , (b) the second optimum conditions; 50 $\mu\text{l min}^{-1}$ flow rate of current buffer (10 mM phosphate buffer pH 7.20) and sample volume 250 μl . *Note; the second optimum conditions was performed in duplicates due to the life time of the electrode.	91

List of Figures (Continued)

Figure	Page
6.7 The logarithm of the endotoxin concentration for difference samples analyte . Samples; (Std) endotoxin standard with a concentration of 4.0×10^{-8} M; (A) endotoxin in supernatant; (B) endotoxin in supernatant treated with lysozyme; (C, D, E, and F) endotoxin in extractants from different preparations.	94
7.1 Structure of thiol compounds.	98
7.2 Reaction mechanism for the anti-AFP immobilized on a self-assemble thioctic acid monolayer.	102
7.3 Reaction mechanism for the anti-AFP immobilized on a self-assemble 3-mercaptopropionic acid monolayer.	103
7.4 Reaction mechanism for the anti-AFP immobilized on a self-assemble thiourea monolayer.	104
7.5 Schematic diagram showing the flow injection capacitive immunosensor system.	107
7.6 Cyclic voltammograms for bare gold electrode (a), thioctic acid modified electrode after 0 h (b). All scans were performed in 0.1 M H_2SO_4 , with a scan rate of 100 mV s^{-1} .	109
7.7 Cyclic voltammograms for bare gold electrode (a), thioctic acid modified electrode after 6 h (b). All scans were performed in 0.1 M H_2SO_4 , with a scan rate of 100 mV s^{-1} . Q_{MGE} is the amount of electric charge exchanged during the electroadsorption of oxygen of the modified gold electrode, Q_{BGE} is the amount of electric charge exchanged during the electroadsorption of oxygen of the bare gold electrode.	110

List of Figures (Continued)

Figure	Page
7.8 Cyclic voltammograms for bare gold electrode (a), thioctic acid modified electrode after 24 h (b). All scans were performed in 0.1 M H ₂ SO ₄ , with a scan rate of 100 mV s ⁻¹ . Q _{MGE} is the amount of electric charge exchanged during the electroadsorption of oxygen of the modified gold electrode, Q _{BGE} is the amount of electric charge exchanged during the electroadsorption of oxygen of the bare gold electrode.	111
7.9 The effect of incubation times of thiol solutions for the formation of SAMs on gold electrode surfaces.	112
7.10 Cyclic voltammograms for the reduction of the Au-S bond, (a) bare gold electrode, (b) 10 mM thioctic acid modified electrode, (c) 50 mM thioctic acid modified electrode, (d) 100 mM thioctic acid modified electrode, (e) 250 mM thioctic acid modified electrode. All scans were performed in 0.1 M KOH with a scan rate of 100 mV s ⁻¹ .	114
7.11 The effect of concentration of thiol solutions for the formation of SAMs on gold electrode surfaces.	115
7.12 The effect of the concentration (a) and incubation times (b) of glutaraldehyde to activated self-assemble thiourea monolayer for anti-AFP immobilization.	117
7.13 Cyclic voltammograms of a gold electrode obtained in a 5 mM K ₃ [Fe(CN) ₆] containing 0.1 M KCl solution at scan rate of 0.1 V s ⁻¹ . All potentials are given vs. Ag/AgCl reference electrode. The voltage range was -0.3 to 0.8 V. (a) clean gold electrode, (b) self-assembled thiol monolayer electrode, (c) anti-AFP self-assembled thiol monolayer electrode, and (d) as in (c) but after 1 dodecanethiol treatment.	120

List of Figures (Continued)

Figure	Page
7.14 Cyclic voltammograms of a gold electrode obtained in a 5 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl solution at scan rate of 0.1 V s^{-1} . All potentials are given vs. Ag/AgCl reference electrode. The voltage range was -0.3 to 0.8 V. (a) Clean gold electrode, (b) self-assembled thiourea monolayer (SATUM) electrode, (c) glutaraldehyde-amine SATUM, (d) anti-AFP glutaraldehyde-amine SATUM, and (e) as in (d) but after 1-dodecanethiol treatment.	121
7.15 Responses of the anti-AFP to Alpha-fetoprotein (AFP), Carcinoembryonic antigen (CEA) and Human serum albumin (HSA) using self-assemble thioctic acid monolayer (SATAM).	123
7.16 Responses of the anti-AFP to Alpha-fetoprotein (AFP), Carcinoembryonic antigen (CEA) and Human serum albumin (HSA) using self-assemble 3-mercaptopropionic acid monolayer (SAMPAM).	124
7.17 Responses of the anti-AFP to Alpha-fetoprotein (AFP), Carcinoembryonic antigen (CEA) and Human serum albumin (HSA) using self-assemble thiourea monolayer (SATUM).	125
7.18 The reproducibility of the anti-AFP on self-assemble thioctic acid monolayer (SATAM).	127
7.19 The reproducibility of the anti-AFP on self-assemble 3-mercaptopropionic acid monolayer (SAMPAM).	127
7.20 The reproducibility of the anti-AFP on self-assemble thiourea monolayer (SATUM).	128

List of Figures (Continued)

Figure	Page
8.1 Reaction mechanism for anti-CEA immobilized covalently on a self-assemble thiourea monolayer (SATUM) modified on gold electrode. (a) Gold surface was modified with thiourea, (b) Amino groups of SATUM are activated by glutaraldehyde, (c) Covalent binding between carbonyl groups of the activated SATUM and free amino groups of anti-CEA, (d) Block any pinholes or bare spots on electrode surface with 10-dodecanethiol.	131
8.2 Schematic representation of the different layers on the electrode surface showing a series of capacitances determining the total capacitance; (C_{SAM}) the capacitance related to the self-assembled thiourea monolayer (SATUM), (C_p) the capacitance of protein layer, (C_a) the capacitance as a result of analyte interaction, (C_{Total}) the total capacitance measured at the working electrode/solution interface.	132
8.3 The decrease in capacitance (ΔC_1) resulting from the binding between CEA and anti-CEA with subsequent signal increase due to dissociation under regeneration conditions.	133
8.4 The use of a regression line to compare two analytical methods; (a) shows perfect agreement between the two methods for all the samples; (b)–(f) illustrate the result of various type of systematic errors of the slope and/or the interception. (Redrawn from Miller and Miller, 1993)	136

List of Figures (Continued)

Figure	Page
8.5 Cyclic voltammograms of a gold electrode obtained in a 5 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl solution at a scan rate of 0.1 V s^{-1} . All potentials are given vs. Ag/AgCl reference electrode. The voltage range was -0.3 to 0.8 V. (a) Clean gold electrode, (b) self-assembled thiourea monolayer (SATUM) electrode, (c) Glutaraldehyde-amine SATUM, (d) Anti-CEA-glutaraldehyde-amine SATUM, and (e) as in (d) but after 1-dodecanethiol treatment.	138
8.6 Responses of the flow injection capacitive immunosensor system at different flow rates.	142
8.7 Responses of the flow injection capacitive immunosensor system at different sample volume.	143
8.8 Responses of the flow injection capacitive immunosensor system at different of buffer solution.	144
8.9 Effect of the pH of Tris-HCl buffer solution.	146
8.10 Reproducibility of the response from the anti-CEA modified electrode to injections of a fixed volume of a standard solution of CEA (10 ng/ml) with regeneration and reconditioning steps between each individual assay.	148
8.11 Capacitance change vs. the concentraion of CEA for a transducer surface with immobilized anti-CEA under optimized conditions ($100 \mu\text{l min}^{-1}$ flow rate, $200 \mu\text{l}$ sample volume, 10 mM Tris-HCl buffer, pH 7.00); (a) first preparation, (b) second preparation, (c) third preparation.	149

List of Figures (Continued)

Figure	Page
8.12 The decrease in capacitance resulting from the binding between CEA and anti-CEA; (a) standard CEA (0.05 ng ml^{-1}), (b) serum sample.	151
8.13 Capacitance change vs. the logarithm of CEA and AFP concentration for a transducer surface with immobilized anti-CEA under optimized conditions ($100 \text{ } \mu\text{l min}^{-1}$ flow rate, $200 \text{ } \mu\text{l}$ sample volume, 10 mM Tris-HCl buffer, pH 7.00).	153
8.14 Capacitance change vs. concentration of CEA. The insert shows the relationship between the capacitance change and the concentration of CEA in the concentration range from 0.01 to 0.07 ng ml^{-1} .	154
8.15 Comparison between the results obtained from the capacitive immunosensor system and ELFA technique (VIDAS [®] CEA) in serum samples.	156