CONTENTS

PREFACE xvii
ACKNOWLEDGMENTS xix
CONTRIBUTORS xxi

1 Critical Evaluation of the Use of Surfactants in Capillary Electrophoresis 1
Jessica L. Felhofer, Karin Y. Chumbimuni-Torres, Maria F. Mora, Gabrielle G. Haby, and Carlos D. García

1.1 Introduction 1
1.2 Surfactants for Wall Coatings 4
  1.2.1 Controlling the Electroosmotic Flow 4
  1.2.2 Preventing Adsorption to the Capillary 5
1.3 Surfactants as Buffer Additives 6
  1.3.1 Micellar Electrokinetic Chromatography 6
  1.3.2 Microemulsion Electrokinetic Chromatography 8
  1.3.3 Nonaqueous Capillary Electrophoresis with Added Surfactants 9
1.4 Surfactants for Analyte Preconcentration 9
  1.4.1 Sweeping 10
  1.4.2 Transient Trapping 11
  1.4.3 Analyte Focusing by Micelle Collapse 12
  1.4.4 Micelle to Solvent Stacking 12
  1.4.5 Combinations of Preconcentration Methods 12
  1.4.6 Cloud Point Extraction 12
1.5 Surfactants and Detection in CE 14
  1.5.1 Mass Spectrometry 14
  1.5.2 Electrochemical Detection 15
1.6 Conclusions 16
References 17
2 Sample Stacking: A Versatile Approach for Analyte Enrichment in CE and Microchip-CE
Bruno Perlatti, Emanuel Carrilho, and Fernando Armani Aguiar

2.1 Introduction 23
2.2 Isotachophoresis 24
2.3 Chromatography-Based Sample Stacking 25
2.4 Methods Based on Electrophoretic Mobility and Velocity Manipulation (Electrophoretic Methods)
2.4.1 Field-Enhanced Sample Stacking (FESS) 27
2.4.2 Field-Enhanced Sample Injection (FESI) 27
2.4.3 Large-Volume Sample Stacking (LVSS) 28
2.4.4 Dynamic pH Junction 28
2.5 Sample Stacking in Pseudo-Stationary Phases 29
2.5.1 Field-Enhanced Sample Stacking 29
2.5.2 Hydrodynamic Injection Techniques
2.5.2.1 Normal Stacking Mode (NSM) 30
2.5.2.2 Reverse Electrode Polarity Stacking Mode (REPSM) 30
2.5.2.3 Stacking with Reverse Migrating Micelles (SRMM) 30
2.5.2.4 Stacking Using Reverse Migrating Micelles and a Water Plug (SRW) 31
2.5.2.5 High-Conductivity Sample Stacking (HCSS) 31
2.5.3 Electrokinetic Injection Techniques
2.5.3.1 Field-Enhanced Sample Injection (FESI–MEKC) 32
2.5.3.2 Field-Enhanced Sample Injection with Reverse Migrating Micelles (FESI–RMM) 32
2.5.4 Sweeping 32
2.5.5 Combined Techniques
2.5.5.1 Dynamic pH Junction: Sweeping 33
2.5.5.2 Selective Exhaustive Injection (SEI) 33
2.5.6 New Techniques 33
2.6 Stacking Techniques in Microchips 33
2.7 Concluding Remarks 36
References 37

3 Sampling and Quantitative Analysis in Capillary Electrophoresis
Petr Kuba, Andrus Seisman, and Mihkel Kaljurand

3.1 Introduction 41
3.2 Injection Techniques in CE
3.2.1 Hydrodynamic Sample Injection
3.2.1.1 Principle 43
3.2.1.2 Advantages and Performance 44
3.2.1.3 Disadvantages 44
3.2.2 Electrokinetic Sample Injection 44
3.2.2.1 Principle 44
3.2.2.2 Advantages and Performance 45
3.2.2.3 Disadvantages 45
3.2.3 Bias-Free Electrokinetic Injection 45
3.2.4 Extraneous Sample Introduction Accompanying Injections in CE 46
3.2.5 Sample Stacking 48
3.2.5.1 Principle 48
3.2.5.2 Advantages and Performance 49
3.2.5.3 Disadvantages 50
3.2.6 Alternative Batch Sample Injection Techniques 50
3.2.6.1 Rotary-Type Injectors for CE 50
3.2.6.2 Hydrodynamic Sample Splitting as Injection Method for CE 51
3.2.6.3 Electrokinetic Sample Splitting as Injection Method for CE 52
3.2.6.4 Dual-Opposite End Injection in CE 52

3.3 Micromachined/Microchip Injection Devices 53
3.3.1 Droplet Sampler Based on Digital Microfluidics 53
3.3.2 Wire Loop Injection 54

3.4 Automated Flow Sample Injection and Hyphenated Systems 55
3.4.1 Introduction 55
3.4.2 Advantages and Performance 56
3.4.3 Disadvantages 57

3.5 Computerized Sampling and Data Analysis 57

3.6 Sampling in Portable CE Instrumentation 58

3.7 Quantitative Analysis in CE 59
3.7.1 Introduction 59
3.7.2 Quantitative Analysis with HD Injection 59
3.7.3 Quantitative Analysis with EK Injection 60
3.7.4 Validation of the Developed CE Methods 61
3.7.5 Computer Data Treatment in Quantitative Analysis 61

3.8 Conclusions 62

References 62

4 Practical Considerations for the Design and Implementation of High-Voltage Power Supplies for Capillary and Microchip Capillary Electrophoresis 67
Lucas Blanes, Wendell Karlos Tomazelli Coltro, Renata Mayumi Saito, Claudimir Lucio do Lago, Claude Roux, and Philip Doble

4.1 Introduction 67
4.1.1 High-Voltage Fundamentals 67
4.1.2 Electroosmotic Flow Control 68
4.1.3 Technical Aspects 70
4.1.4 Construction of Bipolar HVPS from Unipolar HVPS 70
4.1.5 Safety Considerations 71
4.1.6 HVPS Commercially Available 71
4.1.7 Practical Considerations 72
4.1.8 Alternative Sources of HV 72
4.1.9 HVPS Controllers for MCE 72

4.2 High-Voltage Measurement 73
4.3 Concluding Remarks 74

References 74

5 Artificial Neural Networks in Capillary Electrophoresis 77
Josef Havel, Eladia Maria Peña-Méndez, and Alberto Rojas-Hernández

5.1 Introduction 77
5.2 Optimization in CE: From Single Variable Approach Toward Artificial Neural Networks 77
5.2.1 Limitations of “Traditional” Single Variable Approach 79
5.2.2 Multivariate Approach with Experimental Design and Response Surface Modeling 79
5.2.2.1 Experimental Design 79
5.2.2.2 Response Surface Modeling 80
## 5.3 Artificial Neural Networks in Electromigration Methods

5.3.1 Introduction—Basic Principles of ANN

5.3.2 Optimization Using a Combination of ED and ANN
   5.3.2.1 Testing of ED–ANN Algorithm
   5.3.2.2 Practical Applications of ED–ANN

5.3.3 Quantitative CE Analysis and Determination from Overlapped Peaks
   5.3.3.1 Evaluation of Calibration Plots in CE Using ANN to Increase Precision of Analysis
   5.3.3.2 ANN in Quantitative CE Analysis from Overlapped Peaks

5.3.4 ANN in CEC and MEKC

5.3.5 ANN for Peptides Modeling

5.3.6 Classification and Fingerprinting

5.3.7 Other Applications

5.4 Conclusions

Acknowledgments

References

## 6 Improving the Separation in Microchip Electrophoresis by Surface Modification


6.1 Introduction

6.2 Strategies for Improving Separation
   6.2.1 Selection of an Adequate Technique: ME
   6.2.2 Microchannel Design
   6.2.3 Selection of an Appropriate ME Material
   6.2.4 Optimization of the Working Conditions
   6.2.5 Surface Modification
     6.2.5.1 Surface Micro- and Nanostructuring
     6.2.5.2 Employment of Energy Sources
     6.2.5.3 Chemical Surface Modification

6.3 Chemical Modifiers
   6.3.1 Surfactants
   6.3.2 Ionic Liquids
   6.3.3 Nanoparticles
   6.3.4 Polymers

6.4 Conclusions

Acknowledgments

References

## 7 Capillary Electrophoretic Reactor and Microchip Capillary Electrophoretic Reactor: Dissociation Kinetic Analysis Method for “Complexes” Using Capillary Electrophoretic Separation Process

Toru Takahashi and Nobuhiko Iki

7.1 Introduction

7.2 Basic Concept of CER

7.3 Dissociation Kinetic Analysis of Metal Complexes Using a CER
   7.3.1 Determination of the Rate Constants of Dissociation of 1:2 Complexes of Al$^{3+}$ and Ga$^{3+}$ with an Azo Dye Ligand 2,2'-Dihydroxyazobenzene-5,5'-Disulfonate in a CER
7.4 Expanding the Scope of the CER to Measurements of Fast Dissociation Kinetics with a Half-Life from Seconds to Dozens of Seconds: Dissociation Kinetic Analysis of Metal Complexes Using a Microchip Capillary Electrophoretic Reactor (μCER) 133

7.5 Expanding the Scope of the CER to the Measurement of Slow Dissociation Kinetics with a Half-Life of Hours 135

7.5.1 Principle of LS-CER 135

7.5.2 Application of LS-CER to the Ti(IV)–Catechin Complex 136

7.5.3 Application of LS-CER to the Ti(IV)–Tiron Complex 138

7.6 Expanding the Scope of CER to Measurement of the Dissociation Kinetics of Biomolecular Complexes 139

7.6.1 Dissociation Kinetic Analysis of [SSB–ssDNA] Using CER 139

7.7 Conclusions 142

References 142

8 Capacitively Coupled Contactless Conductivity Detection (C\textsuperscript{4}D) Applied to Capillary Electrophoresis (CE) and Microchip Electrophoresis (MCE) 145

José Alberto Fracassi da Silva, Claudimir Lucio do Lago, Dosil Pereira de Jesus, and Wendell Karlos Tomazelli Coltro

8.1 Introduction 145

8.2 Theory of C\textsuperscript{4}D 145

8.2.1 Basic Principles of C\textsuperscript{4}D 145

8.2.2 Simulation 146

8.2.3 Basic Equation for Sensitivity 147

8.2.4 Equivalent Circuit of a CE-C\textsuperscript{4}D System 147

8.2.5 Practical Guidelines 148

8.3 C\textsuperscript{4}D Applied to Capillary Electrophoresis 148

8.3.1 Instrumental Aspects in CE 149

8.3.2 Coupling C\textsuperscript{4}D with UV–Vis Photometric Detectors in CE 149

8.3.3 Fundamental Studies in Capillary Electrophoresis Using C\textsuperscript{4}D 149

8.3.4 Fundamental Studies on C\textsuperscript{4}D 149

8.3.5 Applications 150

8.4 C\textsuperscript{4}D Applied to Microchip Capillary Electrophoresis 151

8.4.1 Geometry of the Detection Electrodes 151

8.4.1.1 Embedded Electrodes 151

8.4.1.2 Attached Electrodes 153

8.4.1.3 External Electrodes 153

8.4.2 Applications 154

8.4.2.1 Bioanalytical Applications 154

8.4.2.2 On-Chip Enzymatic Reactions 155

8.4.2.3 Food Analysis 155

8.4.2.4 Explosives and Chemical Warfare Agents 155

8.4.2.5 Other Applications 156

8.5 Concluding Remarks 156

Acknowledgments 157

References 157

9 Capillary Electrophoresis with Electrochemical Detection 161

Blanaid White

9.1 Principles of Electrochemical Detection 161

9.1.1 Amperometric Detection 161

9.1.2 Potentiometric Detection 162
11.8.2 Electrophoretic Mobility of Proteins 210
11.8.3 Peak Profiles and Derivable Thermodynamic Aspects of Protein Re-/Unfolding 211
11.8.4 Dipeptides as a Case Study for Isomerization 213
11.8.5 Denaturation Factors and Strategies Applied in CE 214
   11.8.5.1 Separation Electrolyte, Injection Solution, and Sample Storage 215
   11.8.5.2 Denaturation by Urea, Dithiothreitol, and GdmCl 215
   11.8.5.3 Effects of pH and Organic Solvents 216
   11.8.5.4 Temperature 216
   11.8.5.5 Electrical Field 218
   11.8.5.6 Detergents 218
   11.8.5.7 Ligands and Ions—Case Studies on Potential Amyloidogenic β2m 221
11.8.6 β-Amyloid Peptides 222
   11.8.6.1 Prions 223
11.9 Comparison Between CE and HPLC 223
11.10 Conclusive Discussion and Method Evaluation 223
   11.10.1 General Aspects 223
   11.10.2 HPLC 224
   11.10.3 CE 224
References 225

12 Capillary Electromigration Techniques for the Analysis of Drugs and Metabolites in Biological Matrices: A Critical Appraisal 229
Cristiane Masetto de Gaitani, Anderson Rodrigo Moraes de Oliveira, and Pierina Sueli Bonato

12.1 Introduction 229
12.2 Strategies to Obtain Reliable Capillary Electromigration Methods for the Bioanalysis of Drugs and Metabolites 230
   12.2.1 Selectivity and Detectability 230
   12.2.1.1 Efficiency 232
   12.2.1.2 Sample Preparation 233
   12.2.1.3 Detectors 235
   12.2.2 Repeatability 236
12.3 Selected Applications of Capillary Electromigration Techniques in Bioanalysis 238
   12.3.1 Pharmacokinetics and Metabolism Studies 238
   12.3.2 Enantioselective Analysis of Drugs and Metabolites 240
   12.3.3 Biopharmaceuticals or Biotechnology-Derived Pharmaceuticals 240
   12.3.4 Therapeutic Drug Monitoring 241
   12.3.5 Clinical and Forensic Toxicology 242
12.4 Concluding Remarks 243
References 243

13 Capillary Electrophoresis and Multicolor Fluorescent DNA Analysis in an Optofluidic Chip 247
Chaitanya Dongre, Hugo J.W.M. Hoekstra, and Markus Pollnau

13.1 Introduction 247
13.2 Optofluidic Integration in an Electrophoretic Microchip 248
16 Rapid Analysis of Charge Heterogeneity of Monoclonal Antibodies by Capillary Zone Electrophoresis and Imaged Capillary Isoelectric Focusing

Yan He, Jim Mo, Xiaoping He, and Margaret Ruesch

16.1 Introduction
16.2 Capillary Zone Electrophoresis
   16.2.1 Separation and Detection Strategy
      16.2.1.1 Capillary Construction
      16.2.1.2 Buffer Composition
      16.2.1.3 Separation Voltage and Field Strength
      16.2.1.4 Detection
   16.2.2 Applications
16.3 Imaged Capillary Isoelectric Focusing
   16.3.1 Method Development and Optimization
      16.3.1.1 Carrier Ampholyte
      16.3.1.2 Additives
      16.3.1.3 Focusing Time and Voltage
      16.3.1.4 Salt Concentration
      16.3.1.5 Protein Concentration
   16.3.2 iCE Method Validation
   16.3.3 Applications
      16.3.3.1 Cell Line Development Support
      16.3.3.2 Formulation Screening
      16.3.3.3 Characterization of Acidic Species
   16.4 Summary
References

17 Application of Capillary Electrophoresis for High-Throughput Screening of Drug Metabolism

Román Řemínek, Jochen Pauwels, Xu Wang, Jos Hoogmartens, Zdeněk Glatz, and Ann Van Schepdael

17.1 Introduction
17.2 Sample Deproteinization
17.3 On-line Preconcentration
17.4 Method Development
   17.4.1 Dynamic Coating of Inner Capillary Wall
   17.4.2 Short-End Injection
   17.4.3 Strong Rinsing Procedure
   17.4.4 Optimized Method
17.5 Method Validation
17.6 Method Applications
   17.6.1 Drug Stability Screening
   17.6.2 Kinetic Study
17.7 Conclusions
Acknowledgments
References

18 Electrokinetic Transport of Microparticles in the Microfluidic Enclosure Domain

Qian Liang, Chun Yang, and Jianmin Miao

18.1 Introduction
18.2 Numerical Model
18.2.1 Problem Description 320
18.2.2 Mathematical Model 320
18.3 Numerical Simulation 322
18.4 Results and Discussion 322
18.4.1 Particle Transport in the Bulk Flow 322
  18.4.1.1 The Particle Velocity in the Confined Domain 322
  18.4.1.2 The Trajectory of Particle Transport within the Confined Domain 323
  18.4.1.3 The Effect of Sidewall Zeta Potential on the Particle Motion 324
18.4.2 Particle Transport Near the Bottom Surface 325
  18.4.2.1 The Effect of the EDL Thickness on the Near Wall Motion of the Particle 325
  18.4.2.2 The Effect of Surface Charge on the Near Wall Transport of the Particle 325
18.5 Model Application 325
18.6 Conclusions 326
References 326

19 Integration of Nanomaterials in Capillary and Microchip Electrophoresis as a Flexible Tool 327
Germain A. Messina, Roberto A. Olsina, and Patricia W. Stege
19.1 Introduction 327
  19.1.1 Historical Overview of Nanotechnology 327
  19.1.2 Nanomaterials 329
    19.1.2.1 Carbon-Based Nanomaterials 329
    19.1.2.2 Metal-Based Nanomaterials 329
    19.1.2.3 Dendrimers 331
    19.1.2.4 Composites 331
19.2 Nanomaterials in Analytical Chemistry 332
19.3 Nanoparticles in Capillary Electrophoresis 333
  19.3.1 Nanoparticles in Capillary Electrochromatography 334
    19.3.1.1 Organic Nanoparticles 334
    19.3.1.2 Inorganic Particles 338
  19.3.2 Nanoparticles in Electrokinetic Chromatography 342
    19.3.2.1 Organic Nanoparticles 343
    19.3.2.2 Inorganic Particles 347
  19.3.3 Nanoparticles in Microchip Electrochromatography 349
19.4 Conclusions 352
References 353

20 Microchip Capillary Electrophoresis to Study the Binding of Ligands to Teicoplanin Derivatized on Magnetic Beads 359
Toni Ann Riveros, Roger Lo, Xiaojun Liu, Marisol Salgado, Hector Carmona, and Frank A. Gomez
20.1 Introduction 359
20.2 Experimental Section 359
  20.2.1 Materials and Methods 359
    20.2.1.1 Equipment and Fabrication of the Microchips 360
    20.2.1.2 Surface Coating 360
    20.2.1.3 Teic Immobilization on Magnetic Microbeads 360
  20.2.2 Procedures 360
    20.2.2.1 FAMCE Studies 360
20.2.2.2 MFAC Studies 361
20.3 Results and Discussion 361
  20.3.1 FAMCE Studies 361
    20.3.1.1 Nonspecific Adsorption Resistance 361
    20.3.1.2 The Binding of DA3 to Teic-Beads 362
  20.3.2 MFAC Studies 363
20.4 Conclusions 364
Acknowledgments 365
References 365

21 Glycomic Profiling Through Capillary Electrophoresis and Microchip Capillary Electrophoresis

Yehia Mechref

21.1 Introduction 367
  21.1.1 Release of N-Glycans from Glycoproteins 368
    21.1.1.1 Chemical Release 368
    21.1.1.2 Enzymatic Release 368
  21.1.2 Release of O-Glycans from Glycoproteins 368
    21.1.2.1 Chemical Release 368
    21.1.2.2 Enzymatic Release 369
21.2 General Considerations of Capillary Electrophoresis and Microchip Capillary Electrophoresis of Glycans 369
  21.2.1 Capillary Electrophoresis–Laser-Induced Fluorescence (CE–LIF) Analysis of Glycans 369
  21.2.2 Interfacing Capillary Electrophoresis and Capillary Electrophoresis to Mass Spectrometry 372
    21.2.2.1 ESI Interfaces for Capillary Electrophoresis 372
    21.2.2.2 Sheathless-Flow Interface 372
    21.2.2.3 Sheath-Flow Interface 373
    21.2.2.4 Liquid Junction Interface 373
    21.2.2.5 MALDI Interfaces for Capillary Electrophoresis 373
    21.2.2.6 CE–MS Analysis of Glycans 374
    21.2.2.7 Glycomic Analysis by CEC–MS 376
21.3 Microchip Capillary Electrophoresis 377
21.4 Conclusions 380
References 381

INDEX 385