

CONTENTS

CONTRIBUTORS	xix
PREFACE	xxiii
CUMULATIVE LISTING OF VOLUMES IN SERIES	xxvii

PART I THEORY AND MODES OF HPCE

CHAPTER 1	CAPILLARY ELECTROPHORESIS: OVERVIEW AND PERSPECTIVE	3
	<i>Barry L. Karger</i>	
	1.1. Introduction	3
	1.2. Modes of Operation	5
	1.3. Capillary Electrophoresis–Mass Spectrometry	16
	1.4. Electric Field Manipulation of Bulk Liquid Flow	18
	1.5. Conclusions	22
	List of Acronyms and Abbreviations	22
	References	23
CHAPTER 2	THEORY OF CAPILLARY ZONE ELECTROPHORESIS	25
	<i>Ernst Kenndler</i>	
	2.1. Capillary Zone Electrophoresis in the Absence of Electroosmotic Flow	27
	2.2. Capillary Zone Electrophoresis in the Presence of Electroosmotic Flow	58
	List of Symbols	70
	List of Acronyms	73
	References	73

CHAPTER 3	MICELLAR ELECTROKINETIC CHROMATOGRAPHY	77
	<i>Morteza G. Khaledi</i>	
3.1.	Introduction	77
3.2.	Pseudostationary Phases	79
3.3.	Migration in Micellar Electrokinetic Chromatography	83
3.4.	Migration Parameters	84
3.5.	Resolution	85
3.6.	Structure–Retention Relationships in Micellar Electrokinetic Chromatography	87
3.7.	Characterization of the Chemical Selectivity of Pseudostationary Phases	89
3.8.	Effects of Chemical Composition of Micellar Solutions	95
3.9.	Multiparameter Optimization	117
3.10.	Conclusions and Future Trends	127
	List of Acronyms	130
	References	131
CHAPTER 4	BAND BROADENING IN MICELLAR ELECTROKINETIC CHROMATOGRAPHY	141
	<i>Joe M. Davis</i>	
4.1.	Introduction	142
4.2.	Measurements of Efficiency	143
4.3.	Plug Size	143
4.4.	The Detection Window and Time Constants	144
4.5.	Micellar Overload	145
4.6.	Longitudinal Diffusion	146
4.7.	Nonequilibrium Dispersion in Micellar Systems	148
4.8.	Electromigrative Dispersion	158
4.9.	Dependence of N on Concentration of Organized Media	158
4.10.	Dependence of N on Electrolyte Concentration and Composition	164
4.11.	Dependence of N on Organic Solvents	165

4.12. Efficiency of Pseudostationary Phases Other Than Simple Micelles	171
4.13. General Observations	174
4.14. Conclusions	175
Addendum	176
List of Acronyms and Abbreviations	178
References	179
CHAPTER 5 CAPILLARY GEL ELECTROPHORESIS	185
<i>Paul Shieh, Nelson Cooke, and Andras Guttman</i>	
5.1. Introduction	185
5.2. Theory and Operation Parameters	187
5.3. Applications	196
5.4. Conclusion	217
List of Acronyms and Abbreviations	218
References	218
CHAPTER 6 CAPILLARY ISOELECTRIC FOCUSING	223
<i>John E. Wiktorowicz</i>	
6.1. Introduction	223
6.2. Capillary Isoelectric Focusing	226
6.3. Modes of Isoelectric Point Determination	241
6.4. Future Considerations and Direction	245
Addendum	247
List of Acronyms	247
References	248
CHAPTER 7 CAPILLARY ISOTACHOPHORESIS	251
<i>Ludmila Křivánková and Petr Boček</i>	
7.1. Introduction	251
7.2. Principles of Isotachopheresis	252
7.3. Electrolyte Systems	256
7.4. Sample Stacking	258
7.5. The Isotachopheresis–Capillary Zone Electrophoresis Combination	260
7.6. Dynamics of Destacking	265

7.7. Sample-Induced Isotachopheresis (Stacking)	267
7.8. Conclusion	273
List of Acronyms and Abbreviations	274
References	275
CHAPTER 8 CAPILLARY ELECTROCHROMATOGRAPHY	277
<i>Kathleen A. Kelly and Morteza G. Khaledi</i>	
8.1. Introduction	277
8.2. Column Preparation	279
8.3. Detection	283
8.4. Theory	285
8.5. Stationary Phase Considerations	287
8.6. Mobile Phase Considerations	289
8.7. Applications	290
8.8. Conclusions and Future Trends	295
Addendum	296
List of Acronyms	297
References	297
PART II DETECTION SYSTEMS IN HPCE	
CHAPTER 9 CAPILLARY ELECTROPHORETIC DETECTORS BASED ON LIGHT	303
<i>Louann Cruz, Scott A. Shippy, and Jonathan V. Sweedler</i>	
9.1. Introduction	304
9.2. Requirements of Capillary Electrophoretic Detectors	305
9.3. Absorbance	308
9.4. Fluorescence	321
9.5. Other Capillary Electrophoretic Optical Detectors	331
9.6. Recent Developments	341
9.7. Conclusions	347
List of Acronyms and Abbreviations	347
References	348

CHAPTER 10	ELECTROCHEMICAL DETECTION IN HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS	355
	<i>Barbara Rhoden Bryant, Franklin D. Swanek, and Andrew G. Ewing</i>	
	10.1. Introduction	355
	10.2. Methods Used to Electrically Isolate the Electrochemical Detector	356
	10.3. Modes of Electrochemical Detection	359
	10.4. Applications of High-Performance Capillary Electrophoresis with Electrochemical Detection	363
	10.5. Future Directions	369
	List of Acronyms	371
	References	371
CHAPTER 11	INDIRECT DETECTION IN CAPILLARY ELECTROPHORESIS	375
	<i>Hans Poppe and Xiaoma Xu</i>	
	11.1. Introduction	375
	11.2. Measurement Considerations	377
	11.3. The Transfer Ratio and System Zones	383
	11.4. Overload: Electromigration Dispersion	390
	11.5. Noise Induced by the Electrophoretic Process and System Peaks	393
	11.6. Choice of the Background Electrolyte	395
	11.7. Instrumental Improvements	397
	11.8. Applications and Some Special Aspects	398
	11.9. Summary	400
	List of Acronyms	401
	References	401
CHAPTER 12	HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY	405
	<i>Kenneth B. Tomer, Leesa J. Deterding, and Carol E. Parker</i>	
	12.1. Introduction	406

12.2. Advantages of Mass Spectrometric Detection	407
12.3. Instrumentation	407
12.4. Disadvantages of Mass Spectrometric Detection	414
12.5. Potential Solutions to Disadvantages of Mass Spectrometric Detection	415
12.6. Applications	429
12.7. Summary	435
Addendum No. 1	436
Addendum No. 2	438
List of Acronyms	440
References	441

PART III OPERATIONAL ASPECTS AND SPECIAL TECHNIQUES IN HPCE

CHAPTER 13 SAMPLE INTRODUCTION AND STACKING	449
<i>Ring-Ling Chien</i>	
13.1. Introduction	449
13.2. Sample Introduction in Capillary Zone Electrophoresis	451
13.3. On-Column Sample Stacking	456
13.4. Stacking in Sample Introduction	464
13.5. Applications of Sample Stacking	471
13.6. Conclusions	477
Addendum	477
List of Acronyms and Abbreviations	478
References	478
CHAPTER 14 COATED CAPILLARIES IN HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS	481
<i>Gerhard Schomburg</i>	
14.1. Introduction	481
14.2. The Status of Capillary Surfaces in Capillary Electrophoresis: Influence on Electroosmotic Flow	483

14.3. Influence of Electroosmotic Flow on Efficiency and Resolution of Capillary Electrophoretic Separations	484
14.4. Analyte–Wall Interaction and the Performance of Analytical Capillary Electrophoretic Separations	486
14.5. Surface-Modification Procedures by Coating in Fused-Silica Capillaries	488
14.6. Methods and Applications of Dynamic Surface Modifications	492
14.7. Methods and Applications of Permanent Surface Modifications	507
14.8. Recent Developments in the Dynamic and Permanent Modification of Capillary Surfaces	516
List of Acronyms and Abbreviations	519
References	520
CHAPTER 15 NONAQUEOUS CAPILLARY ELECTROPHORESIS	525
<i>Joseph L. Miller and Morteza G. Khaledi</i>	
15.1. Introduction	525
15.2. Influence of Nonaqueous Solvents in Capillary Electrophoresis	527
15.3. Applications	540
15.4. Conclusions and Future Trends	553
List of Acronyms and Abbreviations	553
References	554
CHAPTER 16 METHOD VALIDATION IN CAPILLARY ELECTROPHORESIS	557
<i>K. D. Altria</i>	
16.1. Introduction	557
16.2. Specific Validation Aspects	559
16.3. Conclusions	577
List of Acronyms	577
References	578

CHAPTER 17	TWO-DIMENSIONAL SEPARATIONS IN HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS	581
	<i>Thomas F. Hooker, Dorothea J. Jeffrey, and James W. Jorgenson</i>	
17.1.	Introduction	582
17.2.	Two-Dimensional Separation Theory	583
17.3.	Comprehensive Two-Dimensional Liquid Chromatography–Capillary Electrophoresis	588
17.4.	Three-Dimensional Size Exclusion Chromatography–Reversed-Phase Liquid Chromatography–High-Speed Capillary Zone Electrophoresis	605
17.5.	Future Directions	607
	List of Acronyms	610
	References	610
CHAPTER 18	MICROFABRICATED CHEMICAL SEPARATION DEVICES	613
	<i>Stephen C. Jacobson and J. Michael Ramsey</i>	
18.1.	Introduction	613
18.2.	Modular System Design	614
18.3.	Fabrication Techniques	615
18.4.	Fluid Manipulation and Injection	617
18.5.	Microchip Electrophoresis	620
18.6.	DNA Separations	623
18.7.	Chromatographic Separations	626
18.8.	Integrated Structures for Chemical and Biochemical Reactions and Analysis	627
18.9.	The Future	629
	Addendum	631
	List of Acronyms	631
	References	632

PART IV APPLICATIONS OF HPCE

CHAPTER 19	PEPTIDE ANALYSIS BY CAPILLARY ELECTROPHORESIS: METHODS DEVELOPMENT AND OPTIMIZATION, SENSITIVITY ENHANCEMENT STRATEGIES, AND APPLICATIONS	637
	<i>Gregory M. McLaughlin, Kenneth W. Anderson, and Dietrich K. Hauffe</i>	
19.1.	Introduction	638
19.2.	Effects of Capillary Dimensions, Applied Voltage, and Temperature	639
19.3.	Effects of pH and Ionic Strength	643
19.4.	Buffer Additives	647
19.5.	Use of Ion-Pairing Reagents	650
19.6.	Use of Micellar Electrokinetic Chromatography for Hydrophobic and Neutral Species	651
19.7.	Chiral Selectors	653
19.8.	Sensitivity-Enhancement Strategies	654
19.9.	Use of Coated Capillaries and Wall Coatings	656
19.10.	Methods Development Strategy	657
19.11.	Use of Capillary Electrophoresis and High-Performance Liquid Chromatography as Complementary Techniques	659
19.12.	Enhanced Detection Methods	662
19.13.	Physicochemical Measurements	663
19.14.	Concluding Remarks and Future Trends	666
	Addenda	666
	List of Acronyms, Abbreviations, and Symbols	670
	References	671
CHAPTER 20	CAPILLARY ELECTROPHORESIS OF PROTEINS	683
	<i>Fred E. Regnier and Shen Lin</i>	
20.1.	Conventional Electrophoresis of Proteins	684

20.2.	Electrophoresis of Proteins in Fused-Silica Capillaries	685
20.3.	Surface Modification of Capillaries	686
20.4.	Zone Electrophoresis in Open-Tubular Capillaries	703
20.5.	Capillary Isoelectric Focusing	712
20.6.	Capillary Gel Electrophoresis	713
20.7.	Micellar Electrokinetic Chromatography	715
20.8.	Selectivity	715
20.9.	Mass Spectrometry	716
20.10.	The Future	719
	List of Acronyms and Abbreviations	720
	References	722
CHAPTER 21	CAPILLARY ELECTROPHORESIS OF CARBOHYDRATES	729
	<i>Milos V. Novotny</i>	
21.1.	Introduction	729
21.2.	The Goals of Analytical Glycobiology	730
21.3.	Instrumental Aspects	732
21.4.	Sample Derivatization	736
21.5.	Electromigration Mechanisms	744
21.6.	Selected Applications	753
	List of Acronyms and Abbreviations	761
	References	761
CHAPTER 22	DNA SEQUENCING BY MULTIPLEXED CAPILLARY ELECTROPHORESIS	767
	<i>Edward S. Yeung and Qingbo Li</i>	
22.1.	Highly Multiplexed DNA Sequencing	768
22.2.	Acceleration of Electrophoretic Runs	774
22.3.	Base Calling and Data Handling	780
22.4.	System Integration	783
22.5.	Future Prospects	785
	List of Acronyms and Symbols	786
	References	787

CHAPTER 23	CHIRAL SEPARATIONS BY CAPILLARY ELECTROPHORESIS	791
	<i>Fang Wang and Morteza G. Khaledi</i>	
23.1.	Introduction	791
23.2.	Chiral Resolution	793
23.3.	Types of Chiral Selector	795
23.4.	Effect of the Chiral Selector Concentration	806
23.5.	pH and Ionic Strength	807
23.6.	Organic Solvents	808
23.7.	The Counter–Electroosmotic Flow Scheme	811
23.8.	Capillary Electrochromatography	812
23.9.	Other Methods	813
23.10.	Chiral Separation Efficiency	814
23.11.	Conclusions and Future Trends	816
	Addendum	817
	List of Acronyms	818
	References	819
CHAPTER 24	CAPILLARY ELECTROPHORESIS OF INORGANIC IONS	825
	<i>Jeffrey R. Mazzeo</i>	
24.1.	Introduction	825
24.2.	Indirect-Ultraviolet Detection: General Principles	826
24.3.	Direct-Ultraviolet Detection: Anion Determinations	841
24.4.	Direct-Ultraviolet Detection: Cation Determinations	843
24.5.	Other Detection Modes	844
24.6.	Concluding Remarks	847
	List of Acronyms and Symbols	849
	References	849
CHAPTER 25	THE ANALYSIS OF PHARMACEUTICALS BY CAPILLARY ELECTROPHORESIS	853
	<i>K. D. Altria</i>	
25.1.	Introduction	853

25.2. Application Areas of Capillary Electrophoresis in Pharmaceutical Analysis	854
25.3. Future Directions	873
25.4. Conclusions	875
List of Acronyms	875
References	875
CHAPTER 26 ON-LINE IMMUNOAFFINITY CAPILLARY ELECTROPHORESIS FOR THE DETERMINATION OF ANALYTES DERIVED FROM BIOLOGICAL FLUIDS	879
<i>Norberto A. Guzman, Andy J. Tomlinson, and Stephen Naylor</i>	
26.1. Introduction	879
26.2. Nonspecific On-Line Preconcentration Capillary Electrophoresis	881
26.3. Specific On-Line Preconcentration Capillary Electrophoresis	883
26.4. Conclusions	894
List of Acronyms	894
References	895
CHAPTER 27 MICROBIOANALYSIS USING ON-LINE MICROREACTORS-CAPILLARY ELECTROPHORESIS SYSTEMS	899
<i>Larry Licklider and Werner G. Kuhr</i>	
27.1. Introduction	899
27.2. Sampling Single Biological Cells	900
27.3. On-Capillary Assays	904
27.4. On-Line Capillary Microreactors-Capillary Electrophoresis Systems	909
27.5. Conclusions	919
List of Acronyms and Symbols	920
References	920
CHAPTER 28 ELECTROPHORETICALLY MEDIATED MICROANALYSIS	925
<i>Bryan J. Harmon and Fred E. Regnier</i>	
28.1. Coupling On-Line Chemical Reactions to Capillary Electrophoresis	926
28.2. Determinations of Enzymes by Electrophoretically Mediated Microanalysis	928

28.3. Enzymatic Determinations of Substrates by Electrophoretically Mediated Microanalysis	938
28.4. Complexometric Determinations of Inorganic Ions by Electrophoretically Mediated Microanalysis	941
28.5. Determinations of Single Cells by Electrophoretically Mediated Microanalysis	941
List of Acronyms	942
References	942

PART V PHYSICOCHEMICAL STUDIES

CHAPTER 29 AFFINITY CAPILLARY ELECTROPHORESIS: USING CAPILLARY ELECTROPHORESIS TO STUDY THE INTERACTIONS OF PROTEINS WITH LIGANDS	947
<i>Jinming Gao, Milan Mrksich, Mathai Mammen, and George M. Whitesides</i>	
29.1. Background	948
29.2. Principles of Affinity Capillary Electrophoresis	950
29.3. Technical Issues	952
29.4. Carbonic Anhydrase as a Model Protein	953
29.5. Determination of Binding Affinity	953
29.6. Determination of Kinetic Parameters for Binding	963
29.7. Using Affinity Capillary Electrophoresis to Measure the Effective Charge of a Protein	963
29.8. Determination of Stoichiometry of Binding	964
29.9. Prospects and Limitations of Affinity Capillary Electrophoresis	966
Addendum	968
List of Acronyms or Abbreviations	969
References	970
CHAPTER 30 DETERMINATION OF PHYSICOCHEMICAL PARAMETERS BY CAPILLARY ELECTROPHORESIS	973
<i>Pier Giorgio Righetti</i>	
30.1. Introduction	973
30.2. Determination of pK Values of Weak Electrolytes	975

30.3.	Determination of pK Values of Amphoteric Compounds	980
30.4.	Assessment of pK Values of Silanols	981
30.5.	Determination of pI Values of Proteins	982
30.6.	Determination of Absolute Mobility and Its Relation to the Charge/Mass Ratio in Peptides (and Proteins)	984
30.7.	Determination of Binding Constants	988
30.8.	Determination of Diffusion Constants	991
30.9.	Viscosity Measurements	992
30.10.	Determination of T_m Values of Nucleic Acids	992
30.11.	Conclusions	995
	List of Acronyms and Symbols	995
	References	996

CHAPTER 31	APPLICATIONS OF MICELLAR ELECTROKINETIC CHROMATOGRAPHY IN QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP STUDIES: ESTIMATION OF LOG P_{ow} AND BIOACTIVITY	999
	<i>Morteza G. Khaledi</i>	
31.1.	Introduction	999
31.2.	Solute–Micelle Interactions and Hydrophobicity	1001
31.3.	Relationships Between Micellar Electrokinetic Chromatography Retention and log P_{ow}	1002
31.4.	Role of the Pseudostationary Phase	1005
31.5.	Prediction of Retention in Micellar Electrokinetic Chromatography from Solute Hydrophobicity	1011
31.6.	Quantitative Retention–Activity Relationships in Micellar Electrokinetic Chromatography	1011
31.7.	Conclusions	1012
	List of Acronyms	1013
	References	1014
INDEX		1015