Contents

1	Outline of the History	1
	Bogusław Buszewski and Ewelina Dziubakiewicz	
	References	3
2	Principles of Electromigration Techniques	5
	Ewelina Dziubakiewicz and Bogusław Buszewski	
2.1	Electrophoretic Mobility	6
2.2	Electroosmotic Flow	7
2.3	Mechanism of Electrophoretic Separation	10
2.4	Parameters of Electrophoretic Separation	12
	2.4.1 Migration Time	12
	2.4.2 Efficiency	13
	2.4.3 Selectivity	14
	2.4.4 Resolution	14
2.5	Optimization of Electrophoretic Separation Conditions	15
	2.5.1 Polarization of Electrodes	15
	2.5.2 Applied Voltage	16
	2.5.3 Temperature	16
	2.5.4 Capillary	17
	2.5.5 Buffer	19
	References	22
3	Equipment	27
	Michał Szumski	
3.1	HV Power Supply	28
3.2	Capillaries	29
3.3	Thermostating of the Capillary	32
3.4	Vials	32
3.5	Sample Injection	33
3.6	Detection in Electromigration Techniques	34

3.6.1	Spectrophotometric Detection	36
3.6.2	Fluorescence Detection	39
3.6.3	RI Detection.	49
3.6.4	Electrochemical Detection	50
3.6.5	MS Detection	57
3.6.6	NMR Detection	60
3.6.7	Summary	62
3.7	Automation and Robotization	62
	References	66
4	Qualitative and Quantitative Analysis: Interpretation of Electropherograms	69
	Michał Szumski and Bogusław Buszewski	
4.1	Qualitative Analysis	69
4.1.1	Sources of Errors	70
4.2	Quantitative Analysis	71
4.2.1	External Standard Method (Calibration Curve).	71
4.2.2	Internal Standard Method.	72
4.2.3	Remarks Regarding Peak Shape	74
	References	75
5	Micellar Electrokinetic Chromatography.	77
	Edward Bald and Paweł Kubalczyk	
5.1	Introduction	77
5.2	Principles of Separation.	78
5.2.1	Efficiency	81
5.2.2	Selectivity	82
5.2.3	Retention Coefficient	82
5.3	Factors Influencing the Separation Process.	83
5.3.1	Surfactants	83
5.3.2	Modifiers	86
5.3.3	pH	87
5.3.4	Elution Order	88
5.4	Equipment and Analytical Procedure.	88
5.4.1	Detection	89
5.4.2	Method Development	89
5.5	Summary	90
	References	91
6	Isotachophoresis	93
	Przemysław Kosobucki and Bogusław Buszewski	
6.1	Theoretical Background.	93
6.1.1	Quantitative and Qualitative Analysis	95
6.1.2	Separation Conditions	97

6.2	Instrumentation	100
6.2.1	Separation Column	100
6.2.2	Detection	102
6.2.3	Discrete Spacers	104
6.3	Applications	106
6.4	Coupling of ITP with Different Analytical Techniques	107
6.4.1	Isotachophoresis-Capillary Zone Electrophoresis (ITP-CZE)	108
6.4.2	Isotachophoresis-High Performance Liquid Chromatography (ITP-HPLC).	109
6.4.3	Isotachophoresis-Mass Spectrometry (ITP-MS).	109
6.4.4	Isotachophoresis-Nuclear Magnetic Resonance (ITP-NMR)	111
6.5	Miniaturization	113
6.6	Summary	115
	References	115
7	Capillary Isoelectric Focusing	119
	Michał J. Markuszewski, Renata Bujak and Emilia Daghir	
7.1	Introduction	119
7.2	CIEF Methodology	120
7.2.1	Sample Preparation and Injection	120
7.2.2	Focusing	121
7.2.3	Two-Step CIEF	122
7.2.4	Single-Step CIEF	124
7.2.5	Capillary Choice.	124
7.2.6	Detection Methods in CIEF	125
7.3	Application of CIEF	126
7.3.1	CIEF in Hemoglobin Analysis	126
7.3.2	CIEF in Protein Analysis.	127
7.3.3	CIEF in Glycoprotein and Immunoglobulin Analysis.	128
	References	130
8	Two-dimensional Gel Electrophoresis (2DE)	133
	Ewa Kłodzińska and Bogusław Buszewski	
8.1	Introduction	134
8.2	Theoretical Basis of 2DE.	135
8.3	Stages of Analytical Procedures Before the 2D Electrophoresis Gel Analysis	137
8.3.1	Sample Preparation	137
8.3.2	Protein Solubilization	142
8.3.3	Chaotropic Substances.	142
8.3.4	Detergents	142

8.3.5	Reducing Agents	143
8.3.6	Protein Enrichment	143
8.3.7	Simplified Fractionation Procedure	144
8.4	First Dimension: Isoelectric Focusing	145
8.4.1	Forming pH Gradient	146
8.4.2	Rehydration and Sample Loading.	150
8.5	Second Dimension: Gel Electrophoresis in Denaturing Conditions	152
8.5.1	Methods of Protein Dyeing (Staining): Detection . . .	153
8.6	Protein Identification by Mass Spectrometry	154
8.7	Computer in the Visualization of Analysis Results	155
8.7.1	2D Database.	156
8.8	Summary	157
	References	157
9	Electrochromatographic Methods: Capillary	
	Electrochromatography	159
	Michał Szumski	
9.1	Theory.	160
9.2	CEC Equipment	168
9.3	Problem of Bubble Formation in CEC.	171
9.4	Performing CEC Separation	172
9.5	CEC Columns	173
9.5.1	Packed Columns.	175
9.5.2	Fritless Columns.	177
9.5.3	Monolithic Columns	178
9.5.4	Polymeric Monoliths	178
9.5.5	Monolithic Silica Beds	181
9.5.6	Organic–Inorganic Mixed Mode Monoliths (PSG).	182
9.5.7	Open-Tubular Columns	184
9.6	CEC Among Contemporary Separation Techniques	186
	References	186
10	Electrochromatography Methods: Planar	
	Electrochromatography	191
	Adam Chomicki, Tadeusz H. Dzido, Paweł Płocharz and Beata Polak	
10.1	Introduction	191
10.2	Development of the Method.	192
10.3	Chambers for Pressurized Planar Electrochromatography. . . .	194
10.4	Advantages and Disadvantages of Pressurized Planar Electrochromatography	196
	References	200

11 Non-Aqueous Capillary Electrophoresis	203
Michał Szumski and Bogusław Buszewski	
11.1 Solvents	204
11.2 Separations in Wide Bore Capillaries	206
11.3 Separation of Large Molecules Insoluble in Water	207
11.4 Separation of Uncharged Compounds	209
11.5 Separation of Optically Active Compounds	210
11.6 Summary	212
References	212
12 Methods of Analyte Concentration in a Capillary	215
Paweł Kubalczyk and Edward Bald	
12.1 Analytes Concentration in Capillary Zone Electrophoresis	217
12.1.1 Field Amplified Sample Stacking	217
12.1.2 Field Amplified Sample Injection	219
12.1.3 Large Volume Sample Stacking	219
12.1.4 pH-Mediated Sample Stacking	221
12.1.5 Stacking by Transient Isotachopheresis Mechanism	221
12.1.6 Stacking by Transient Pseudo Isotachopheresis	222
12.1.7 Stacking by Dynamic pH Junction	222
12.1.8 Concentration by Counter-Flow Gradient Electrofocusing	225
12.2 Sample Concentration in Micellar Electrokinetic Chromatography	225
12.2.1 Field Amplified Sample Stacking	226
12.2.2 Sweeping	226
12.2.3 Stacking with High Salt Concentration	228
12.2.4 Electrokinetic Sample Injection	228
12.2.5 Field Enhanced Sample Injection	230
12.3 Summary	230
References	230
13 Stereoisomers Separation	237
Piotr Wieczorek	
13.1 Mechanism of Chiral Separation	239
13.2 Chiral Selectors	241
13.3 Cyclodextrins	241
13.4 Chiral Crown Ethers	242
13.5 Chiral Surfactants	243
13.6 Macrocyclic Antibiotics	244
13.7 Chiral Metals Complexes	245
13.8 Natural and Synthetic Polymers	245

13.9	Application of Electromigration Techniques on Stereoisomers Analysis	249
13.10	Summary	249
	References	250
14	“Lab-on-a-Chip” Dedicated for Cell Engineering	253
	Elżbieta Jastrzębska, Aleksandra Rakowska and Zbigniew Brzózka	
14.1	Miniaturization	253
14.2	Analytical Techniques in Miniaturization.	254
14.3	Cell Cultures	255
14.4	Microsystems in Tissue Engineering	256
14.5	Design and Development of Microsystems.	256
	14.5.1 Construction Materials.	256
	14.5.2 Fabrication of Microsystem	257
	14.5.3 Casting	258
	14.5.4 Hot Embossing.	258
	14.5.5 Injection Molding	258
	14.5.6 Laser Micromachining.	258
	14.5.7 Micromilling	259
	14.5.8 Microsystems Sealing	259
14.6	Essential Parameters in Microsystem for Cell Culture	259
	14.6.1 Geometry of Microsystem	259
	14.6.2 Sterilization and Culture Condition	260
	14.6.3 Gradient Generation	260
	14.6.4 Other Parameters	262
14.7	Cell Culture in Microscale	262
14.8	Cytotoxicity Tests in Microsystems.	264
	References	267
15	Application of Electromigration Techniques: Metabolomics–Determination of Potential Biomarkers Using Electromigration Techniques	271
	Michał J. Markuszewski, Małgorzata Waszczuk-Jankowska, Wiktoria Struck and Piotr Kośliński	
15.1	Determination of Nucleosides in Biological Samples as Markers of Carcinogenesis.	274
15.2	Micellar Electrokinetic Chromatography	275
	15.2.1 Selected Methods of Detection	277
15.3	Capillary Electrochromatography	278
15.4	Bioinformatic Method of Analysis of the Electrophoretic Data	279
15.5	Determination of Pteridines Compounds in Cancer Diagnosis	282
	References	283

16 Applications of Electromigration Techniques: Electromigration Techniques in Detection of Microorganisms.	287
Ewelina Dziubakiewicz and Bogusław Buszewski	
References	296
17 Applications of Electromigration Techniques: Applications of Electromigration Techniques in Food Analysis	299
Piotr Wieczorek, Magdalena Ligor and Bogusław Buszewski	
17.1 Polyphenols	300
17.2 Pigments	304
17.3 Vitamins	307
17.4 Food Additives	307
17.5 Amino Acids, Peptides, Proteins	308
17.6 Carbohydrates	317
17.7 Nucleic Acids	317
17.8 Biogenic Amines, Natural Toxins and Other Contaminations	318
17.9 Pesticides	319
17.10 Antibiotics	321
17.11 Summary	323
References	323
18 Application of Electromigration Techniques in Environmental Analysis	335
Edward Bald, Paweł Kubalczyk, Sylwia Studzińska, Ewelina Dziubakiewicz and Bogusław Buszewski	
18.1 Introduction	335
18.2 Detectors	336
18.3 Analytes Concentration in a Sample	337
18.4 Microchips	338
18.5 Environmental Contaminants Determination	338
18.6 Pesticides	339
18.7 Polycyclic Aromatic Hydrocarbons	340
18.8 Phenols	340
18.9 Amines	341
18.10 Carboxylic Acids	342
18.11 Explosives	342
18.12 Pharmaceuticals	344
18.13 Ionic Liquids	345
18.14 Summary	347
References	347
Index	355