

## Contents

### Preface XIII

<b>1</b>	<b>General Concepts 1</b>
1.1	Introduction 1
1.2	Migration and Retention 2
1.2.1	General 2
1.2.2	Mobile and Stationary Phases 3
1.2.3	Chromatograms 3
1.2.4	Retention Factor 3
1.3	Band Broadening 5
1.3.1	Eddy Diffusion 6
1.3.2	Longitudinal Diffusion 6
1.3.3	Resistance to Mass Transfer 7
1.3.4	Combined Band Broadening in a Column 8
1.3.5	Band Broadening outside the Column 9
1.4	Measuring Column Efficiency 9
1.4.1	Plate Numbers 9
1.4.2	Coupling Columns 10
1.4.3	Plate Height 10
1.4.3.1	Reduced Plate Height 11
1.4.4	Effective Plate Number 11
1.4.5	Asymmetry 11
1.5	Resolution 11
1.5.1	Increasing the Resolution 13
1.6	Peak Capacity 13
1.7	Two-Dimensional Systems 13
1.8	Increased Performance 14
	References 15
<b>2</b>	<b>Gas Chromatography 17</b>
2.1	Introduction 17
2.2	Mobile Phase/Carrier Gas 17
2.3	Injection Systems 19

2.3.1	Packed Column Injector (Evaporation Injector)	20
2.3.2	Injection Systems for Capillary Columns	21
2.3.2.1	Split Injection	21
2.3.2.2	Splitless Injection	22
2.3.2.3	On-Column Injection	22
2.3.2.4	Large-Volume Injectors	23
2.3.2.5	Headspace Techniques	23
2.4	Columns	24
2.4.1	Packed Columns	25
2.4.2	Open Tubular Columns	25
2.5	Detectors	26
2.5.1	Introduction	26
2.5.2	Thermal Conductivity Detector	28
2.5.3	Flame Ionization Detector	28
2.5.4	Nitrogen–Phosphorus Detector	30
2.5.5	Electron Capture Detector	31
2.5.6	Mass Spectrometry	32
2.5.6.1	Positive Ionization	33
2.5.6.2	Negative Ionization	33
2.5.6.3	Gas Chromatography–Mass Spectrometry (GC–MS) Interfacing	33
2.5.7	Other Detectors	35
2.5.7.1	The Flame Photometric Detector	35
2.5.7.2	The Chemiluminescent Detector	35
2.5.7.3	The Electrolytic Conductivity Detector	35
2.5.7.4	The Photoionization Detector	35
2.5.7.5	The Atomic Emission Detector	36
2.5.7.6	Fourier Transform Infrared Detector	36
2.6	Stationary Phases	36
2.6.1	GSC – Adsorption Chromatography	36
2.6.2	GLC – Partition Chromatography	37
2.6.2.1	Matrix	37
2.6.2.2	Choosing the Stationary Phase	37
2.6.2.3	Types of Stationary Phases in GLC	38
2.6.2.4	Stationary Phase (Film) Thickness	40
2.6.2.5	Temperature	41
2.7	Two-Dimensional Separations	42
2.8	Qualitative and Quantitative Analyses	43
2.9	Derivatization	44
	References	46
<b>3</b>	<b>High-Performance Liquid Chromatography (HPLC)</b>	<b>47</b>
3.1	Introduction	47
3.2	Solvents and Solvent Delivery	47
3.2.1	Maintenance	49
3.2.2	Automation	50

3.3	Injection	50
3.3.1	Techniques	50
3.3.1.1	Constant Volume Injection	50
3.3.1.2	Variable Volume Injection	51
3.3.1.3	Volumes and Precision	51
3.3.2	Dilution and Refocusing	51
3.3.2.1	Injection Volume Related to Solvent Elution Strength	51
3.3.2.2	Timed Injection	52
3.3.2.3	Carryover	52
3.3.2.4	Combination with Solid-Phase Extractors	52
3.3.3	Calculation of Maximum Injection Volumes	53
3.3.4	Calculating the Dilution of the Analyte in the Column	54
3.4	Columns	54
3.4.1	Packed Columns	54
3.4.1.1	Column Dimensions and Materials	54
3.4.1.2	Effect on Detection	55
3.4.1.3	Solvent Saving	55
3.4.1.4	Column Efficiency	56
3.4.1.5	Column Lifetime	57
3.4.1.6	Peak Shapes	57
3.4.1.7	Flow and Backpressure	58
3.4.1.8	Conventional Totally Porous Particles	58
3.4.1.9	Core–Shell Particles	58
3.4.1.10	Ultrahigh-Pressure LC (UHPLC or UPLC)	59
3.4.2	Monolithic Columns	59
3.4.3	Microchip Columns	60
3.4.4	Open Tubular Columns	61
3.4.5	Temperature Control	61
3.4.6	Preparative LC and Flash Chromatography	63
3.5	Stationary Phases and Their Properties in HPLC	64
3.5.1	Normal-Phase Materials for Adsorption Chromatography	64
3.5.1.1	Separation Principles	64
3.5.1.2	Silica	65
3.5.1.3	Alumina, Titania, and Zirconia	65
3.5.1.4	Silica with Bonded Polar Functional Groups	66
3.5.1.5	Hydrophilic Interaction Liquid Chromatography (HILIC)	67
3.5.1.6	Carbon Materials	68
3.5.2	Reversed-phase Materials	68
3.5.2.1	Separation Principles	68
3.5.2.2	Retention	69
3.5.2.3	The Solvation Parameter Model	70
3.5.2.4	Silica-based Reversed-phase Materials	71
3.5.2.5	Hybrid Materials and Hydrosilated Materials	72
3.5.2.6	Organic Polymer-based Materials	72
3.5.2.7	Ion Pair Chromatography on Reversed-Phase Columns	72

3.5.2.8	Hydrophobic Interaction Chromatography	73
3.5.3	Ion Exchange Materials	73
3.5.3.1	Elution	74
3.5.3.2	Retention	74
3.5.4	Chromatofocusing	74
3.5.4.1	Ion Chromatography for Inorganic Ions	75
3.5.5	Size Exclusion Materials	76
3.5.5.1	Separation Principles	76
3.5.5.2	Materials	76
3.5.5.3	Mobile Phases	77
3.5.6	Materials for Chiral Separations	77
3.5.6.1	Separation Principle	77
3.5.6.2	Materials	78
3.5.7	Affinity Materials	78
3.5.7.1	Separation Principle	78
3.5.7.2	Affinity Materials for Chromatography and Microarrays	79
3.6	Detectors	80
3.6.1	UV Detection	81
3.6.1.1	Some Common Chromophores	82
3.6.1.2	Choosing the Right Wavelength	82
3.6.1.3	Flow Cells	82
3.6.1.4	Filter Photometric Detection	83
3.6.1.5	Spectrophotometric Detection	83
3.6.1.6	Diode Array Detectors	83
3.6.2	Mass Spectrometric Detection	85
3.6.2.1	Electrospray Ionization	86
3.6.2.2	Atmospheric Pressure Chemical Ionization	88
3.6.2.3	Atmospheric Pressure Photoionization	89
3.6.2.4	Inductively Coupled Plasma Ionization	90
3.6.2.5	Mass Analysis	91
3.6.2.6	The Quadrupole Mass Analyzers	91
3.6.2.7	The Ion Trap Analyzers	92
3.6.2.8	The Time-of-Flight Analyzers	92
3.6.2.9	The FTMS Analyzers	93
3.6.2.10	Fragmentation in Mass Spectrometry	94
3.6.3	Fluorescence Detection	95
3.6.3.1	Filter Fluorimeters	97
3.6.3.2	Spectrofluorimeters	97
3.6.3.3	Chemiluminescence Detection	97
3.6.4	Electrochemical Detection	98
3.6.4.1	Amperometric Detection	98
3.6.4.2	Coulometric Detector	99
3.6.5	Light Scattering Detection	100
3.6.6	Refractive Index Detection	100
3.6.7	Other Detectors	102

3.6.7.1	The Conductivity Detector	102
3.6.7.2	The Corona Discharge Detector	102
3.6.7.3	Radioactivity Detectors	102
3.6.7.4	Ion Mobility Spectrometry	103
3.6.7.5	Chemiluminescent Nitrogen Detector	103
3.6.7.6	Chirality Detection	103
3.7	Increased Performance	103
3.7.1	Speed	103
3.7.2	Efficiency	103
3.7.3	Resolution	103
3.7.4	Detection	103
3.7.5	Column Lifetime	104
	References	104

#### **4 Thin Layer Chromatography (TLC) 105**

4.1	Introduction	105
4.2	Sample Application	105
4.3	Stationary Phases	106
4.3.1	TLC versus HPTLC	106
4.3.2	Adsorbents	107
4.3.3	Chemically Bonded Phases	107
4.4	Mobile Phases	107
4.5	Elution and Development	108
4.5.1	Vertical Linear Development	108
4.5.2	Horizontal Development	109
4.5.3	Two-Dimensional Development	110
4.5.4	Gradient Development	111
4.5.5	Overpressured Layer Chromatography (OPLC)	111
4.6	R <sub>f</sub> Value	111
4.7	Detection	112
4.7.1	Instrumental Detection	113
4.7.2	TLC-MS	114

#### **5 Supercritical Fluid Chromatography 115**

5.1	Introduction	115
5.2	Mobile Phases	118
5.2.1	CO <sub>2</sub> as Mobile Phase	118
5.2.2	Mobile Phase Delivery	119
5.3	Gradient Elution	120
5.4	Injection	121
5.5	Columns	122
5.6	Restrictors	124
5.7	Detectors	124
5.8	Current Performance	125
	References	126

<b>6</b>	<b>Electrophoresis and Potential-Driven Chromatography</b>	127
6.1	Introduction	127
6.2	Theory	127
6.2.1	Secondary Effects	128
6.2.2	Electroosmosis	129
6.3	Gel Electrophoresis Techniques	130
6.3.1	Gels	130
6.3.1.1	Polyacrylamide Gels	130
6.3.1.2	Agarose Gels	131
6.3.2	Instrumentation	131
6.3.2.1	Sample Application	131
6.3.2.2	Separation	132
6.3.2.3	Detection	132
6.3.3	Zone Electrophoresis	133
6.3.4	Isoelectric Focusing	134
6.3.5	Two-Dimensional Separations	134
6.3.6	Selected Applications	134
6.3.6.1	Protein Separations	134
6.3.6.2	Separation of DNA/RNA	135
6.4	Capillary Electrophoresis	135
6.4.1	Instrumentation	136
6.4.1.1	High-Voltage Supply	136
6.4.1.2	Capillaries	136
6.4.1.3	Sample Introduction	137
6.4.1.4	Detection	139
6.4.2	CE Zone Electrophoresis	140
6.4.3	Other CE Separation Principles	142
6.4.3.1	Isoelectric Focusing	142
6.4.3.2	Gel Electrophoresis in CE	142
6.4.3.3	Gel-Free Sieving	142
6.4.3.4	Isotachophoresis	143
6.4.4	Micellar Electrokinetic Capillary Chromatography (MEKC)	143
6.5	Potential-Driven Chromatography (Electrochromatography – CEC)	145
6.5.1	Instrumentation	145
6.5.2	Mobile Phases	145
6.5.3	Columns and Stationary Phases	146
6.5.4	CEC in Separation Science	146
	References	147
<b>7</b>	<b>Chromatography on a Chip</b>	149
7.1	Introduction	149
7.2	Sample Introduction	149
7.3	Columns and Stationary Phases	151
7.3.1	Open Channel Columns	152

7.3.2	Packed Columns	152
7.3.3	Monolithic Columns	152
7.3.4	COMOSS	152
7.4	Flow Management	152
7.5	Detection	153
	Reference	154
<b>8</b>	<b>Field-Flow Fractionation</b>	155
8.1	Introduction	155
8.2	Types of FFF	156
8.2.1	Flow FFF	156
8.2.2	Thermal FFF	157
8.2.3	Sedimentation FFF	158
8.3	Applications	158
	Reference	159
<b>9</b>	<b>Sample Preparation</b>	161
9.1	Introduction	161
9.1.1	Recovery	162
9.1.2	Enrichment	162
9.2	Liquid–Liquid Extraction	164
9.2.1	Back Extraction	167
9.3	Solid-Phase Extraction (SPE)	168
9.3.1	Normal Phase	170
9.3.2	Reversed Phase	172
9.3.3	Ion Exchange	172
9.3.4	Mixed-Mode Ion Exchange	175
9.3.5	MIP	175
9.3.6	RAM	176
9.3.7	SPE Hardware	176
9.3.7.1	Disks	177
9.4	SPME	178
9.4.1	Adsorption/Extraction	178
9.4.2	Desorption/Injection	179
9.4.2.1	SPME–GC	180
9.4.2.2	SPME–HPLC	180
9.4.3	SPME Fiber Materials and Extraction Parameters	180
9.4.3.1	pH	181
9.4.3.2	Ionic Strength	181
9.4.3.3	Water and Organic Solvents	181
9.4.3.4	Temperature	181
9.4.3.5	Agitation	181
9.4.3.6	Extraction Time	182
9.5	Protein Precipitation	182
9.6	Membrane-Based Sample Preparation Techniques	183

9.6.1	Microdialysis	183
9.6.1.1	Perfusion Flow Rate	184
9.6.1.2	Diameter and Length	184
9.6.1.3	Cutoff	184
9.6.1.4	Membrane Chemistry	184
9.6.1.5	Application of Microdialysis	185
9.6.1.6	How to Analyze the Dialysate?	185
9.6.2	LPME	185
9.6.2.1	Two-Phase LPME	186
9.6.2.2	Three-Phase LPME	186
9.6.2.3	Enrichment in LPME	186
9.6.2.4	Donor Phase pH	187
9.6.2.5	Acceptor Phase pH	187
9.6.2.6	Composition of the SLM	187
9.6.2.7	Extraction Time	188
	References	188
<b>10</b>	<b>Quantitation</b>	189
10.1	Introduction	189
10.2	Calibration Methods	192
10.2.1	External Standard	192
10.2.2	Internal Standard	193
10.2.3	Standard Addition	194
10.3	Method Validation	196
10.3.1	Validation Parameters	196
10.3.1.1	Linearity and Range	197
10.3.1.2	Repeatability	197
10.3.1.3	Accuracy	197
10.3.1.4	Selectivity	197
10.3.1.5	Robustness	197
10.3.1.6	Stability	198
10.3.2	Validation Procedure: A Simple Example	198
	Reference	199