

Contents

Preface IX

The Structure of the Book XI

Notes on Contributors XIII

List of Contributors XVII

Part 1 Principles of Gradient Elution 1

- 1 **Aspects of Gradient Optimization 3**
Stavros Kromidas (translated from German by Steve Ross)
 - 1.1 Introduction 3
 - 1.2 Special Features of the Gradient 3
 - 1.3 Some Chromatographic Definitions and Formulas 5
 - 1.4 Detection Limit, Peak Capacity, Resolution – Possibilities for Gradient Optimization 8
 - 1.4.1 Detection Limit 8
 - 1.4.2 Peak Capacity and Resolution 9
 - 1.5 Gradient “Myths” 14
 - 1.6 Examples for the Optimization of Gradient Runs: Sufficient Resolution in an Adequate Time 15
 - 1.7 Gradient Aphorisms 38
- 2 **Instrumental Influences on the Quality and Performance of Gradient Methods and Their Transfer Between Different HPLC Devices 41**
Frank Steiner
 - 2.1 Technical Implementation of the Gradient Elution and the Respective Characteristics 41
 - 2.1.1 Low-Pressure and High-Pressure Gradient Proportioning – Two Fundamentally Different Technical Principles 41
 - 2.1.2 The Role of the Mixing Device in HPG and LPG Systems 42
 - 2.1.3 The Operational Principle of Mixing Devices and the Systematic Characterization of Their Effectiveness 47

- 2.1.4 Effects of Volume Contraction when Mixing Water and Organic Solvents in Gradient Instruments 58
- 2.1.5 Effects of Minimum Leak Rates of Pump Heads in Sensitive Applications and HPG Synchronization Techniques to Correct Them 63
- 2.2 The Determination and Significance of the Gradient Delay Volume of the System 65
 - 2.2.1 The Determination of the GDV and its Dependence on the Specific Operation Conditions of the System 66
 - 2.2.2 The Influence of GDV on the Chromatographic Results 76
 - 2.2.3 Possibilities of the User to Influence System GDV and its Impact on Chromatography 77
- 2.3 The Transfer of Gradient Methods Between Different HPLC Systems 80
 - 2.3.1 Practical Tips for Dealing with Deviating GDVs and Possible Countermeasures 80
 - 2.3.2 The Relevance of the Pressure Dependence of the GDV in Method Transfer 82
 - 2.3.3 Effect of a too High Elution Strength of the Sample Solvent in the Presence of Weakly Eluting Solvent at the Gradient Start 85
- 2.4 Influence of Fluctuations of the Eluent Composition on the Quality of the Detection 87
 - 2.4.1 Influence of a Reference Channel on the Baseline in Diode Array Detectors 88
 - 2.4.2 The Special Challenge in Methods with UV-Absorbing Retained Additives in the Mobile Phase such as TFA 90
- 2.5 Other Kinds of Practical Application of Gradient Systems in HPLC 95
 - 2.5.1 Alternative and Combined Gradient Modes in HPLC 96
 - 2.5.2 Advantages in the Implementation of Isocratic Methods with Gradient Instruments 97
 - 2.5.3 Use of Gradient Systems in Method Development and Method Optimization 98

3 Optimization of a Reversed-Phase Gradient Separation Using EXCEL 103

Hans-Joachim Kuss

Part 2 Specifics of the Gradient in Different Elution Modes 111

4 Gradient Elution of Ionic Compounds 113

Joachim Weiss

- 4.1 Introduction 113
- 4.2 Theoretical Aspects 114
- 4.3 Gradient Types in Ion Chromatography 116
- 4.4 Choice of Eluent 119

- 4.4.1 Possibilities for Optimizing Concentration Gradients 125
- 4.5 Gradient Elution of Anions on Anion Exchangers 126
- 4.6 Gradient Elution of Cations on Cation Exchangers 136
- 4.6.1 pH Gradients for the Separation of Monoclonal Antibodies 144
- 4.7 Gradient Elution of Anions and Cations on Mixed-Mode Stationary Phases 148

- 5 The Gradient in Biochromatography 161**
Oliver Genz
- 5.1 Biomolecules 161
- 5.2 Biochromatography 161
- 5.3 The Gradient in Biochromatography 162
- 5.3.1 A Gradient you Should Definitely Avoid ... 163
- 5.4 Gradients for Different Biochromatographic Techniques 164
- 5.4.1 Gel-Filtration, Size-Exclusion Chromatography (SEC) 164
- 5.4.2 Ion Exchange Chromatography (IEX) 164
- 5.4.3 Hydrophobic Interaction Chromatography (HIC) 168
- 5.4.4 Reversed-Phase Chromatography of Biomolecules 170
- 5.4.5 Affinity Chromatography (AC) 171
- 5.5 Summary 173

- 6 Specifications of Gradients in Hydrophilic Interaction Liquid Chromatography (HILIC) 175**
Thomas Letzel

- 7 Specifications of Gradients in Supercritical Fluid Chromatography 183**
Stefan Bieber and Thomas Letzel
- 7.1 Types of Gradients in SFC 183
- 7.1.1 Mobile Phase Gradients 183
- 7.1.2 Pressure Gradients 184
- 7.1.3 Temperature Gradients 185
- 7.2 Effects of gradients 185

- 8 Aspects of Gradient Elution in LC-MS Analysis 189**
Markus M. Martin
- 8.1 Role and Importance of Gradient Elution for LC-MS 189
- 8.2 Technical Aspects of Gradient Elution in LC-MS Analysis 192
- 8.2.1 Technical Impact of the LC System: System Dispersion, Gradient Proportioning Precision, and How they Affect MS Results 192
- 8.2.2 Technical Impacts of a Mass Spectrometer: LC Gradients and Signal Generation in the MS 198
- 8.2.3 Quantitation in Mass Spectrometry Within a Gradient Separation: Matrix Effects and How to Address Them 204
- 8.2.4 MS Workload Balancing in Gradient Elution – Column Equilibration as a Throughput Bottleneck 206
- 8.2.5 Gradient Delay, Flow Rate, and Column Dimension – How Far Can we Get With Downsizing of Gradient Separations in LC-MS? 208

VIII | Contents

- 8.3 Summary 211
- 8.4 Abbreviations 211

9 Additional Tools for Method Development: Flow and Temperature Gradients 215

Egidijus Machtejevas

- 9.1 Introduction 215
- 9.2 Temperature Gradients 215
- 9.3 Flow Gradients 216
- 9.4 Combination of Flow and Temperature Gradients 217
- 9.5 Case Example 217
- 9.6 Conclusions 219

Index 223