FUNDAMENTALS OF CONTEMPORARY MASS SPECTROMETRY

CHHABIL DASS
University of Memphis
# CONTENTS

## PREFACE

### INSTRUMENTATION

1 **BASICS OF MASS SPECTROMETRY**

1.1 Brief History of Mass Spectrometry 3
1.2 Desirable Features of Mass Spectrometry 5
1.3 Basic Principles of Mass Spectrometry 5
1.4 Anatomy of a Mass Spectrum 7
1.5 Atomic and Molecular Masses 9
   1.5.1 Mass-to-Charge Ratio 10
1.6 General Applications
   - Overview 11
   - Exercises 12
   - References 13

2 **MODES OF IONIZATION**

2.1 Why Ionization Is Required 15
2.2 General Construction of an Ion Source 16
   - Gas-Phase Ionization Techniques 17
2.3 Electron Ionization 17
2.4 Chemical Ionization
  2.4.1 Charge-Exchange Chemical Ionization
  2.4.2 Negative-Ion Chemical Ionization
2.5 Photoionization
2.6 Field Ionization
2.7 Metastable Atom Bombardment Ionization
  Condensed-Phase Ionization Techniques: Ionization of Solid-State Samples
2.8 Field Desorption
2.9 Plasma Desorption Ionization
2.10 Secondary-Ion Mass Spectrometry
2.11 Fast Atom Bombardment
2.12 Laser Desorption/Ionization
2.13 Matrix-Assisted Laser Desorption/Ionization
  2.13.1 MALDI Analysis of Low-Molecular-Mass Compounds
  2.13.2 Atmospheric-Pressure MALDI
  2.13.3 Surface-Enhanced Laser Desorption/Ionization
  2.13.4 Material-Enhanced Laser Desorption/Ionization
  Condensed-Phase Ionization Techniques: Ionization of Liquid-State Samples
2.14 Thermospray Ionization
2.15 Atmospheric-Pressure Chemical Ionization
2.16 Atmospheric-Pressure Photoionization
2.17 Electrospray Ionization
  2.17.1 Mechanism of Electrospray Ionization
  2.17.2 Sample Consideration
  2.17.3 Nanoelectrospray Ionization
2.18 Desorption Electrospray Ionization
  2.18.1 DART Ion Source
Overview
Exercises
Additional Reading
References

3 MASS ANALYSIS AND ION DETECTION

3.1 Mass Resolving Power
3.2 Kinetic Energy of Ions
Mass Analyzers
3.3 Magnetic-Sector Mass Spectrometers
  3.3.1 Working Principle of a Magnetic Analyzer
  3.3.2 Working Principle of an Electrostatic Analyzer
  3.3.3 Working Principle of Double-Focusing Magnetic-Sector Mass Spectrometers
  3.3.4 Performance Characteristics
3.4 Quadrupole Mass Spectrometers
  3.4.1 Working Principle
  3.4.2 Performance Characteristics
  3.4.3 RF-Only Quadrupole
3.5 Time-of-Flight Mass Spectrometers
  3.5.1 Working Principle
  3.5.2 Delayed Extraction of Ions
  3.5.3 Reflectron TOF Instrument
  3.5.4 Orthogonal Acceleration TOF Mass Spectrometer
  3.5.5 Performance Characteristics
3.6 Quadrupole Ion-Trap Mass Spectrometers
  3.6.1 Working Principle
  3.6.2 Operational Modes
  3.6.3 Performance Characteristics
3.7 Linear Ion-Trap Mass Spectrometers
  3.7.1 Rectilinear Ion Trap
3.8 Fourier-Transform Ion Cyclotron Resonance Mass Spectrometers
  3.8.1 Working Principle
  3.8.2 Performance Characteristics
3.9 Orbitrap Mass Analyzers
3.10 Ion Mobility Mass Spectrometers
3.11 Detectors
  3.11.1 Faraday Cup Detector
  3.11.2 Electron Multipliers
  3.11.3 Photomultiplier Detectors
  3.11.4 Postacceleration Detectors
  3.11.5 Low-Temperature Calorimetric Detectors for High-Mass Ions
  3.11.6 Focal-Plane Detectors
Overview
Exercises
4 TANDEM MASS SPECTROMETRY

4.1 Basic Principles of Tandem Mass Spectrometry 119
4.2 Types of Scan Functions 121
4.3 Ion Activation and Dissociation 123
  4.3.1 Collision-Induced Dissociation 124
  4.3.2 Surface-Induced Dissociation 125
  4.3.3 Absorption of Electromagnetic Radiations 126
  4.3.4 Electron-Capture Dissociation 127
4.4 Reactions in Tandem Mass Spectrometry 128
4.5 Tandem Mass Spectrometry Instrumentation 129
  4.5.1 Magnetic-Sector Tandem Mass Spectrometers 129
  4.5.2 Tandem Mass Spectrometry with Multiple-Quadrupole Devices 132
  4.5.3 Tandem Mass Spectrometry with Time-of-Flight Instruments 133
  4.5.4 Tandem Mass Spectrometry with a Quadrupole Ion-Trap Mass Spectrometer 136
  4.5.5 Tandem Mass Spectrometry with an FT–ICR Mass Spectrometer 138
  4.5.6 Tandem Mass Spectrometry with Hybrid Instruments 138

Overview 143
Exercises 145
Additional Reading 146
References 146

5 HYPHENATED SEPARATION TECHNIQUES

5.1 Benefits of Coupling Separation Devices with Mass Spectrometry 152
5.2 General Considerations 153
  5.2.1 Characteristics of an Interface 153
  5.2.2 Mass Spectral Data Acquisition 153
  5.2.3 Characteristics of Mass Spectrometers 155
5.3 Chromatographic Properties 155
5.4 Gas Chromatography/Mass Spectrometry 158
  5.4.1 Basic Principles of Gas Chromatography 158
5.4.2 Interfaces for Coupling Gas Chromatography with Mass Spectrometry 159

5.5 Liquid Chromatography/Mass Spectrometry 161
  5.5.1 Basic Principles of HPLC Separation 161
  5.5.2 Fast-Flow Liquid Chromatography 162

5.6 Interfaces for Coupling Liquid Chromatography with Mass Spectrometry 163
  5.6.1 Moving-Belt Interface 164
  5.6.2 Direct-Liquid Introduction Probe 165
  5.6.3 Continuous-Flow Fast Atom Bombardment Interface 165
  5.6.4 Thermospray Interface 166
  5.6.5 Particle–Beam Interface 167
  5.6.6 Electrospray Ionization Interface 168
  5.6.7 Atmospheric-Pressure Chemical Ionization Interface 171
  5.6.8 Atmospheric-Pressure Photoionization Interface 171
  5.6.9 Coupling LC with TOF–MS 171
  5.6.10 Coupling LC with MALDI–MS 172

5.7 Multidimensional LC/MS 173

5.8 Capillary Electrophoresis/Mass Spectrometry 174
  5.8.1 Basic Principles of Capillary Electrophoresis 175
  5.8.2 Interfaces for Coupling Capillary Electrophoresis with Mass Spectrometry 177

5.9 Affinity Chromatography/Mass Spectrometry 181

5.10 Supercritical-Fluid Chromatography/Mass Spectrometry 183

5.11 Coupling Planar Chromatography with Mass Spectrometry 183
  Overview 185
  Exercises 186
  Additional Reading 187
  References 187

II ORGANIC AND INORGANIC MASS SPECTROMETRY 195

6 ORGANIC MASS SPECTROMETRY 197

6.1 Determination of Molecular Mass 198
  6.1.1 Molecular Mass Measurements at Low-Mass Resolving Power 198
  6.1.2 Molecular Mass Measurements at High-Mass Resolving Power 198
  6.1.3 Molecular Mass Measurements by ESI and MALDI 200
6.1.4 Mass Calibration Standards 201
6.2 Molecular Formula from Accurate Mass Values 201
6.3 Molecular Formula from Isotopic Peaks 203
6.4 General Guidelines for Interpretation of a Mass Spectrum 210
   6.4.1 Odd- and Even-Electron Ions 210
   6.4.2 Recognizing the Molecular Ion 211
   6.4.3 Nitrogen Rule 211
   6.4.4 Value of the Rings Plus Double Bonds 214
   6.4.5 Systematic Steps in Interpreting a Mass Spectrum 215
   6.4.6 Mass Spectral Compilations 216
6.5 Fragmentation Processes 216
   6.5.1 Simple Bond-Cleavage Reactions 219
   6.5.2 Rearrangement Reactions 223
   6.5.3 Fragmentation of Cyclic Structures 227
   6.5.4 Differentiation of Isomeric Structures 232
   6.5.5 Structurally Diagnostic Fragment Ions 235
6.6 Fragmentation Reactions of Specific Classes of Compounds 238
   6.6.1 Hydrocarbons 238
   6.6.2 Alcohols 240
   6.6.3 Ethers 241
   6.6.4 Aldehydes and Ketones 242
   6.6.5 Carboxylic Acids 242
   6.6.6 Esters 243
   6.6.7 Nitrogen-Containing Compounds 244
   6.6.8 Sulfur-Containing Compounds 246
   6.6.9 Halogen-Containing Compounds 246
6.7 Theory of Ion Dissociation 247
6.8 Structure Determination of Gas-Phase Organic Ions 250
   Overview 254
   Exercises 255
   Additional Reading 259
   References 259

7 INORGANIC MASS SPECTROMETRY 263
   7.1 Ionization of Inorganic Compounds 263
   7.2 Thermal Ionization Mass Spectrometry 264
   7.3 Spark-Source Mass Spectrometry 265
   7.4 Glow Discharge Ionization Mass Spectrometry 267
7.5 Inductively Coupled Plasma Mass Spectrometry 268
  7.5.1 Inductively Coupled Plasma Ion Source 268
  7.5.2 Coupling an ICP Source with Mass Spectrometry 269
  7.5.3 Sample Introduction Systems for an ICP Source 270
  7.5.4 Spectral Interferences 271
  7.5.5 Laser Ablation–ICP–MS 273
7.6 Resonance Ionization Mass Spectrometry 273
7.7 Isotope Ratio Mass Spectrometry 275
  7.7.1 Isotope Ratio MS Systems 277
  7.7.2 Applications of Isotope Ratio MS 277
7.8 Accelerator Mass Spectrometry 278
7.9 Isotope Dilution Mass Spectrometry 280
  Overview 281
  Exercises 282
  Additional Reading 283
  References 283

III  BIOLOGICAL MASS SPECTROMETRY 287

8  PROTEINS AND PEPTIDES: STRUCTURE DETERMINATION 289

  8.1 Structure of Proteins 290
  8.2 Determination of the Sequence of a Protein 292
  8.3 General Protocol for Amino Acid Sequence Determination of Proteins 294
    8.3.1 Homogenization and Subcellular Fractionation 295
    8.3.2 Enrichment and Purification of Proteins 295
  8.4 Molecular Mass Measurement of Proteins 297
  8.5 Peptide Mass Mapping 298
    8.5.1 Reduction and Carboxymethylation 299
    8.5.2 Cleavage of Proteins 299
    8.5.3 Mass Spectrometric Analysis of Peptide Maps 302
  8.6 Proteomics 303
    8.6.1 Strategies for Proteomics 304
  8.7 Quantitative Proteomics 310
  8.8 Biomarker Discovery 314
  8.9 De Novo Protein Sequencing 316
  8.10 Determination of the Amino Acid Sequence of Peptides 316
9 PROTEINS AND PEPTIDES: POSTTRANSLATIONAL MODIFICATIONS

Disulfide Bonds in Proteins

9.1 Traditional Approaches to Identify Disulfide Bonds

9.2 Mass Spectrometry-Based Methods to Identify Disulfide Bonds

9.2.1 Determination of the Number of Disulfide Bonds

9.2.2 Generation of Disulfide-Containing Peptides

9.2.3 Identification of Disulfide-Containing Peptides by FAB-MS

9.2.4 Identification of Disulfide-Containing Peptides by MALDI-MS

9.2.5 Identification of Disulfide-Containing Peptides by Electron-Capture Dissociation

9.2.6 Identification of Disulfide-Containing Peptides by Tandem MS

Analysis of Phosphoproteins and Phosphoproteomics

9.3 $^{32}$P Labeling for the Analysis of Phosphoproteins

9.4 Mass Spectrometry Protocol for the Analysis of Phosphoproteins

9.4.1 Cleavage of Purified Phosphoproteins

9.4.2 Fractionation of Peptide Fragments in the Digest

9.4.3 Determination of the Average Number of Phosphate Groups

9.4.4 Identification of Phosphopeptides

9.4.5 Identification of Phosphorylation Sites

Analysis of Glycoproteins

9.5 Structural Diversity of Glycoproteins

9.6 Analysis of Glycoproteins

9.7 Structural Diversity of Glycoproteins

9.8 Analysis of Glycoproteins

9.9 Structural Diversity of Glycoproteins

9.10 Analysis of Glycoproteins

9.11 Structural Diversity of Glycoproteins

9.12 Analysis of Glycoproteins

9.13 Structural Diversity of Glycoproteins

9.14 Analysis of Glycoproteins
9.6.1 Molecular Mass Determination of Glycoproteins 366
9.6.2 Identification of Glycosylation 368
9.6.3 Site of Glycosylation 369
Overview 370
Exercises 370
References 371

10 PROTEINS AND PEPTIDES: HIGHER-ORDER STRUCTURES 379

10.1 Charge-State Distribution 380
10.2 Hydrogen–Deuterium Exchange to Study Conformational States of Proteins 383
10.2.1 Folding and Unfolding Dynamics of Proteins 385
10.2.2 Experimental Measurements of Amide Hydrogen Isotopic Exchange 386
10.3 Chemical Cross-Linking as a Probe for the Three-Dimensional Structure of Proteins 391
10.4 Ion Mobility Measurements to Study Protein Conformational Changes 391
Overview 392
Exercises 392
Additional Reading 393
References 393

11 CHARACTERIZATION OF OLIGOSACCHARIDES 397

11.1 Structural Diversity in Oligosaccharides 398
11.2 Classes of Glycans 400
11.3 Mass Spectrometric Methods for Complete Structure Elucidation of Oligosaccharides 401
11.3.1 Release of Glycans 402
11.3.2 Derivatization of Carbohydrate Chains 402
11.3.3 Composition Analysis by GC/MS 403
11.3.4 Linkage Analysis by GC/MS 403
11.3.5 Rapid Identification by a Precursor-Ion Scan 403
11.3.6 Composition Analysis by Direct Mass Measurement 403
11.3.7 Structure Determination of Oligosaccharides by Sequential Digestion 406
11.3.8 Tandem Mass Spectrometry for Structural Analysis of Carbohydrates 408
12 CHARACTERIZATION OF LIPIDS

12.1 Classification and Structures of Lipids

12.2 Mass Spectrometry of Fatty Acids and Acylglycerols
  12.2.1 Analysis of Fatty Acids
  12.2.2 Analysis of Acylglycerols

12.3 Mass Spectrometry of Phospholipids

12.4 Mass Spectrometry of Glycolipids

12.5 Analysis of Bile Acids and Steroids

12.6 Analysis of Eicosanoids

12.7 Lipidomics

Overview
Exercises
References

13 STRUCTURE DETERMINATION OF OLIGONUCLEOTIDES

13.1 Structures of Nucleotides and Oligonucleotides

13.2 Mass Spectrometry Analysis of Nucleosides and Nucleotides

13.3 Cleavage of Oligonucleotides

13.4 Molecular Mass Determination of Oligonucleotides
  13.4.1 Electrospray Ionization for Molecular Mass Determination
  13.4.2 Matrix-Assisted Laser Desorption/Ionization for Molecular Mass Determination
  13.4.3 Base Composition from an Accurate Mass Measurement

13.5 Mass Spectrometry Sequencing of Oligonucleotides
  13.5.1 Gas-Phase Fragmentation for Oligonucleotide Sequencing
  13.5.2 Solution-Phase Techniques for Oligonucleotide Sequencing

Overview
Exercises
References
14 QUANTITATIVE ANALYSIS

14.1 Advantages of Mass Spectrometry 486
14.2 Data Acquisition 486
   14.2.1 Selected-Ion Monitoring 487
   14.2.2 Selected-Reaction Monitoring 487
14.3 Calibration 488
   14.3.1 External Standard Method 488
   14.3.2 Standard Addition Method 489
   14.3.3 Internal Standard Method 489
14.4 Validation of a Quantitative Method 491
14.5 Selected Examples 492
   14.5.1 Applications of Gas Chromatography/Mass Spectrometry 493
   14.5.2 Applications of Liquid Chromatography/Mass Spectrometry 493
   14.5.3 Applications of MALDI–MS 494

Overview 495
Exercises 497
Additional Reading 498
References 498

15 MISCELLANEOUS TOPICS

15.1 Enzyme Kinetics 501
   15.1.1 Theory 501
   15.1.2 Reaction Monitoring 504
15.2 Imaging Mass Spectrometry 507
   15.2.1 Imaging with SIMS 508
   15.2.2 Imaging with MALDI–MS 509
15.3 Analysis of Microorganisms 511
   15.3.1 Bacterial Identification 511
   15.3.2 Analysis of Viruses 513
15.4 Clinical Mass Spectrometry 513
   15.4.1 Low-Molecular-Mass Compounds as Biomarkers of Disease 514
   15.4.2 Analysis of DNA to Diagnose Genetic Disorders 514
   15.4.3 Proteins as Biomarkers of Disease 515
15.5 Metabolomics 517
15.6 Forensic Mass Spectrometry 517
   15.6.1 Analysis of Banned Substances of Abuse 518
15.6.2 Analysis of Explosives 519
15.6.3 Analysis of Glass and Paints 519
15.6.4 Authenticity of Questioned Documents 519
15.6.5 Mass Spectrometry in Bioterror Defense 520

15.7 Screening Combinatorial Libraries 520
15.7.1 Combinatorial Synthetic Procedures 521
15.7.2 Screening Methods 522

Additional Reading 526
References 526

Appendix A: ABBREVIATIONS 533
Appendix B: PHYSICAL CONSTANTS, UNITS, AND CONVERSION FACTORS 541
Appendix C: ISOTOPES OF NATURALLY OCCURRING ELEMENTS AND THEIR ABUNDANCES 543
Appendix D: REFERENCE IONS AND THEIR EXACT MASSES 551
Appendix E: INTERNET RESOURCES 555
Appendix F: SOLUTIONS TO EXERCISES 557
INDEX 577