Bioanalytical techniques form an integral part of applied biology and biomedical sciences. The book provides understanding of the concept and working principles of various bioanalytical techniques used in biomedical sciences, environmental studies, life sciences, pharmaceutical analysis, molecular biology, and biotechnological research, as well as the various instruments used in these processes. Divided into 12 chapters, the book provides a comprehensive account of microscopy, centrifugation, chromatography, electrophoresis, spectroscopy. It also focuses on two main topics: radioisotope and immunodiagnostic techniques. Techniques in molecular biology and recombinant DNA technology have also been described in detail.

Key Features
- Explains analytical instrumentation in a concise manner
- Provides state-of-the-art sophisticated techniques that would be beneficial to researchers in various fields for experimentation
- Encourages reader to analytical thinking and practical application of the technique
Bioanalytical Techniques
Bioanalytical Techniques

Abhilasha Shourie
Shilpa S. Chapadgaonkar

The Energy and Resources Institute
Science and technology by far depend upon experimental procedures involving one or more analytical methods. Experiments are conducted not only to predict phenomena, but also to validate the results. Analysis of chemical and biochemical entities is metaphorically the path to achieve the objectives of research in life sciences. In the past few decades, the field of life science has witnessed rapid advancements through development of highly sophisticated, automated, sensitive, and accurate analytical techniques. A vast range of analytical techniques and respective instruments are available, and therefore it is imperative to understand the principles, limitations, and alternatives of a given technique in order to apply it effectively to obtain useful results.

‘Bioanalytical techniques’ is included as a fundamental paper in most courses in chemistry, biochemistry, biology, pharmaceutical and clinical sciences, environmental, forensic and materials sciences. In the past 10 years of our career, we have felt a profound need for a comprehensive yet intensive textbook on analytical techniques that would serve the purpose of both students and researchers. This book has been written to fulfil the need for a text-cum-reference in undergraduate and postgraduate level curriculum, providing necessary information required to demonstrate the concepts of an analytical technique in all its guises.

The book emphasizes on imparting profound knowledge that is able to meet the current throughput screening demands of scientists and researchers. It consists of 12 chapters, encompassing techniques used for biological and biochemical separation, purification, identification and quantification, all put together to construct a compact package. The chapters have been prepared meticulously using simple yet lucid language. We have included a fairly good
number of state-of-the-art sophisticated techniques that would be beneficial to researchers in various fields for experimentation.

Many typical analytical procedures appear in the book as boxed features which give a snapshot of the techniques being widely used contemporarily in research. Throughout the book, wherever felt required, extended illustrative examples have been incorporated, which are to be read as part of the respective chapter. The format also encourages the reader to analytical thinking and practical application of the technique. This book will certainly prove to be an invaluable reference tool for students, teachers and researchers in the mentioned fields.

We express our gratitude to Almighty and all those who have contributed to the book in any manner. We thank our families for their encouragement and unconditional support. We also thank the editorial team of TERI Press for their continuous effort and faith in us.

We solicit constructive suggestions from all the readers for further improvement of the content of the book.

Dr. Abhilasha Shourie

Dr. Shilpa S. Chapadgaonkar
# Contents

*Preface*  

1. **GENERAL PRINCIPLES OF ANALYTICAL INSTRUMENTATION**  
   1.1 Introduction  
   1.2 Experimental Studies  
   1.3 Experimental Errors  
   1.4 Statistical Parameters for Validation of an Experiment  

2. **SOLUTIONS AND BUFFERS**  
   2.1 Introduction  
   2.2 Units of Concentration  
   2.3 The Concept of pH  
   2.4 Acids and Bases  
   2.5 Henderson–Hasselbalch Equation  
   2.6 Determination of pKₐ  
   2.7 Buffers  

3. **MICROSCOPY**  
   3.1 Historical Background  
   3.2 Nature of Light  
   3.3 Compound Microscope  
   3.4 Image Formation in a Light Microscope  
   3.5 Phase Contrast Microscopy  
   3.6 Fluorescence Microscopy  
   3.7 Electron Microscopy
4. CELL DISRUPTION 69
   4.1 Introduction 69
   4.2 Barriers for Cell Disruption 70
   4.3 Methods of Cell Disruption—an Overview 70
   4.4 Mechanical Methods of Cell Disruption 71
   4.5 Non-Mechanical Methods of Cell Disruption 82
   4.6 Combinations of Cell Disruption Methods 86
   4.7 Selection of Cell Disruption Methods 88
   4.8 Analysis of Cell Disruption 90

5. CENTRIFUGATION 91
   5.1 Introduction 91
   5.2 Principles of Centrifugation 93
   5.3 Centrifuge Machines 102
   5.4 Centrifugal Separations 109
   5.5 Analytical Ultracentrifugation 117
   5.6 Care and Safety of Centrifuges 122

6. CHROMATOGRAPHIC TECHNIQUES 125
   6.1 Introduction 125
   6.2 Types of Chromatographic Techniques 126
   6.3 Planar Chromatography 127
   6.4 Column Chromatography 137
   6.5 Protein Purification Strategies 178

7. ELECTROPHORESIS 181
   7.1 Introduction 181
   7.2 Principles of Electrophoresis 182
   7.3 Free Solution Electrophoresis 187
   7.4 Paper Electrophoresis 188
   7.5 Gel Electrophoresis 190
   7.6 Gel Electrophoresis of Proteins 199
   7.7 Gel Electrophoresis of Nucleic Acids 212
   7.8 Capillary Electrophoresis 218

8. SPECTROSCOPY I 225
   8.1 Electromagnetic Radiations 225
   8.2 Ultraviolet and Visible Light Spectroscopy 228
   8.3 Fluorescence Spectroscopy 238
8.4 Atomic Absorption Spectrometry 243
8.5 X-ray Spectroscopy 246
8.6 Circular Dichroism and Optical Rotatory Dispersion 253

9. SPECTROSCOPY II 259
9.1 Introduction 259
9.2 Infrared Spectroscopy 259
9.3 Nuclear Magnetic Resonance Spectroscopy 267
9.4 Electron Spin Resonance Spectroscopy 276
9.5 Mass Spectroscopy 282

10. RADIOISOTOPE TECHNIQUES 295
10.1 Introduction and Applications 295
10.2 Structure of Atom and Radioactivity 297
10.3 Types of Radioactive Decay 298
10.4 Interaction of Radioactivity with Matter 301
10.5 Kinetics of Radioactive Decay 303
10.6 Units of Radioactivity 306
10.7 Detection and Measurement of Radioactivity 307
10.8 Safety Issues and Radio-waste Management 320

11. IMMUNOCHEMICAL TECHNIQUES 325
11.1 Introduction 325
11.2 Production and Purification of Antibodies 329
11.3 Immunoassay Techniques 336
11.4 Advances in Immunochemical Techniques 353

12. MOLECULAR BIOLOGY TECHNIQUES 355
12.1 Introduction 355
12.2 Isolation of Nucleic Acids 355
12.3 Qualitative and Quantitative Evaluation of Nucleic Acids 364
12.4 Nucleic Acid Hybridization 367
12.5 Restriction Digestion 376
12.6 Nucleic Acid Sequencing 380
12.7 DNA Amplification by Polymerase Chain Reaction 387

Further Readings 393
Index 397
About the Authors 411