

Contents

Syllabus

vii

Preface

ix

UNIT I

1. Ultraviolet Visible Spectroscopy 3

- Introduction 3
- Instrumental methods of analysis 3
- Merits of instrumental methods of analysis 3
- Demerits of instrumental methods of analysis 3
- Ultraviolet visible (UV-Vis) spectroscopy 4
- Electromagnetic spectrum (UV electromagnetic radiations) 4
- Principle 4
- Theory 5
- Block diagram 5
- Electronic transitions 6
- Absorbing species containing σ , π and n electrons 6
- Chromophore and auxochrome in the UV spectroscopy 8
- Chromophore 8
- Category 8
- Identification of chromophores 9
- Auxochromes 9
- Interactions of chromophores and auxochromes with UV radiation 10
- Absorption and intensity shifts in the UV spectroscopy (spectral shifts) 10
- Solvent effect 10
- Solvent effects on transitions 12
- Choice of a solvent for ultraviolet spectroscopy 12
- Lambert-Beer law (laws of absorbance or law of photochemistry) 12
- Beer-Lambert law equation 13
- Derivation of Lambert-Beer law 14
- Beer-Lambert law applications 15
- Importance 15
- Limitations 15
- Deviations 16
- Reasons for deviation from Beer-Lambert law 16
- Types of UV-Vis spectrophotometer 17
- Instrumentation 17

Light source	18
Wavelength selectors	20
Sample cells/sample holders	21
Detectors	22
Procedure	23
Application of UV spectroscopy	24
Advantages	25
Disadvantages	26
Spectrophotometric titrations	26
Single component analysis	27
Methods of calculation	28
Multi-component analysis	28
Woodward-Fieser rules	29
Advantages	30
Spectrophotometer vs spectrometer	31

2. Fluorimetry

33

Introduction	33
Luminescence	33
Characteristics of a fluorophore	34
Principle	35
Theory	35
Electronic states: Singlet, doublet and triplet electronic state	35
Jablonski energy level diagram	36
Internal conversion and external conversion	38
Quenching/fluorescence quenching	38
Types of quenching	39
Factors affecting fluorescence	40
Instruments	40
Spectrofluorometer working	42
Instrumentation	42
Sources of radiation	42
Wavelength selector	43
Sample cells	43
Detectors	44
Readout devices	44
Applications	44
Fluorometric determination of thiamine	45
Thiamine determination	45

UNIT II

3. IR Spectroscopy	51
Introduction	51
Fingerprint region in IR spectroscopy	52
Principle	53
Theory	53
Block diagram	54
Molecular vibrations/mode of vibrations	54
Number of vibrational modes	56
Vibrational coupling	56
Vibrational frequency	56
Factors affecting vibrational frequencies in IR	56
Sample handling	59
Sample cell and problems in sample handling	59
Sample preparation techniques/sample handling techniques/ sampling of IR spectroscopy	60
Types of sample preparation techniques in IR	60
Types of IR instrument (spectrophotometer)	62
Instrumentation of IR spectroscopy	62
Sources of radiations	63
Wavelength selector	64
Sample cell/cuvette	64
Detectors	65
Procedure	67
Applications of IR spectroscopy	67
Advantages of interferometer (FTIR) over dispersive instrument (IR)	70
4. Flame Photometry	72
Introduction	72
Block diagram	72
Schematic block diagram of the flame photometer	72
Principle	72
Theory	73
Interferences	74
Instrumentation	75
Sources of radiation	75
Nebulizer (part of a sample delivery system)	77
Wavelength selector (monochromators and filters)	78
Detectors	78
Working procedure	78
The processes occurring during flame photometer analysis (flame photometry)	79
Applications	79
Advantages	80
Disadvantages	80

5. Atomic Absorption Spectroscopy 82

- Introduction 82
- Principle 83
- Theory 83
- Calibration curve 83
- Interferences in atomic absorption spectroscopy 84
- Instrumentation 84
- Source 84
- Nebulizer and atomizer 85
- Wavelength selector 86
- Detector and readout device 86
- Working procedure 87
- Applications 87

6. Nepheloturbidometry 90

- Introduction 90
- Tyndall effect 90
- Principle 91
- Theory 92
- Choice of the method 93
- Instrumentation 93
- Sources of radiation 93
- Wavelength selectors 94
- Sample cells/sample cuvette/sample holder 94
- Detectors 94
- Procedure 94
- Applications 95
- Difference between nephelometry and turbidimetry 96

UNIT III

7. Introduction to Chromatography 99

- Introduction 99
- Principle of chromatography 99
- Preparative chromatography vs analytical chromatography 100
- Difference between preparative and analytical chromatography 100
- Chromatography working procedure 100
- Terminology 100
- Choice of solvent/selection of a solvent 101
- Applications of chromatography 101

8. Adsorption Chromatography 103

Introduction 103
Column adsorption chromatography 104
Principle 104
Methodology (instrumentation and procedure) 104
Types of adsorption chromatography 106
Applications 106
Advantages 106
Disadvantages 106

9. Partition Chromatography 108

Introduction 108
Column partition chromatography 108
Principle 108
Methodology (instrumentation and procedure) 109
Types of partition chromatography 110
Applications 110
Advantages 111
Disadvantages 111

10. Thin Layer Chromatography 113

Introduction 113
Principle 113
Selection of stationary phase/choice of adsorbents for TLC 114
Choose a solvent system for TLC 115
Methodology (instrumentation and procedure) 115
Retention factor (R_f) 116
Factors affecting R_f value in TLC chromatography 117
Applications of TLC 118
Other applications of TLC 118
Advantages 119
Disadvantages 119

11. High-Performance Thin Layer Chromatography 121

Introduction 121
Principle 121
Instrumentation 121
Working procedure 122
Fingerprinting 123
HPTLC fingerprinting 123
Applications 123
Advantages of HPTLC 124
Limitations of HPTLC 124
HPTLC vs TLC 124

12. Paper Chromatography 127

- Introduction 127
- Principle 127
- Methodology (instrumentation and procedure) 128
- Instrumentation 128
- R_f value 128
- Factors affecting R_f value 129
- Applications 130
- Advantages 131
- Disadvantages 131

13. Electrophoresis 133

- Introduction 133
- Principle 133
- Instrumentation of electrophoresis 134
- Working procedure of electrophoresis 134
- Electrophoretic Mobility (μ ; $\text{cm}^2 \text{V}^{-1} \text{S}^{-1}$) 135
- Factors affecting electrophoresis or electrophoretic mobility 135
- Applications of electrophoresis 137
- Advantages of electrophoresis 137
- disadvantage of electrophoresis 137
- Paper electrophoresis 137
- Principle 138
- Instrumentation 138
- Procedure 138
- Observation 139
- Applications 139
- Advantages 139
- Disadvantages 139
- Gel electrophoresis 139
- Principle 140
- Instrumentation 140
- Procedure 141
- Types of gel electrophoresis 141
- Applications 141
- Advantages 141
- Disadvantages 141
- Cellulose acetate electrophoresis 142
- Agarose gel electrophoresis 142
- Polyacrylamide gel electrophoresis 142
- Types of PAGE 142

Capillary electrophoresis	143
Principle	143
Instrumentation	144
Procedure	144
Modes of capillary electrophoresis	145
Applications	145
Advantages	146
Disadvantages	146

UNIT IV

14. Column Chromatography **149**

Introduction	149
Principle	149
Instrumentation	149
Working procedure	150
Applications	150
Advantages	151
Disadvantages	151

15. Gas Chromatography **152**

Introduction	152
Two major types	153
A schematic block diagram of gas chromatography	153
Principle	153
Theory	154
Instrumentation	155
Procedure	158
GC: derivatization	159
Limitations	159
Types of derivatization	160
General reaction of derivatization	160
Types of derivatization techniques	161
Reagents	162
Temperature programming	162
Isothermal analysis	163
Drawbacks of temperature programming	163
Applications of GC	164
Advantages	165
Limitations	165
Gas chromatography–mass spectrometry (GC-MS)	165
Applications	166

16. High-Performance Liquid Chromatography 167

- Introduction 167
- Principle 168
- Theory 168
- Types of HPLC 168
- Instrumentation 169
- Procedure 171
- Applications 172
 - Pharmaceutical applications 172
 - Environmental applications 172
 - Applications in forensics 172
 - Food and flavor 172
 - Applications in clinical tests 172
 - Applications in analytical chemistry 173
 - Applications in chemistry 173
- Benefits of HPLC 173
- Limitations 173
- Reversed-phase high-performance liquid chromatography (RP-HPLC) 173
- Factors affecting reversed-phase chromatography 173
- Difference between HPLC and HPTLC 174
- Advantages of HPLC over gas chromatography 174
- Advantages of HPLC over TLC 175

UNIT V

17. Ion-Exchange Chromatography 179

- Introduction 179
- Principle 180
- Types of ion-exchangers 180
- Properties of all ion-exchangers 180
- Resins 181
- General requirements of resins 181
- Ion-exchange GEL 182
- Regeneration of the ion-exchange resin 182
- Factors affecting ion-exchange 183
- Methods of ion-exchange chromatography 183
- Instrumentation 184
- Procedure 185
- Applications 185
- Advantages 186
- Limitations 186

18. Gel-Filtration Chromatography	188
Introduction	188
Principle	188
Theory	189
Gel-filtration chromatography media	190
Instrumentation	190
Procedure	191
Applications	192
Advantages	192
Disadvantages	192
19. Affinity Chromatography	194
Introduction	194
Principle	194
Theory	194
Instrumentation	195
Components of affinity chromatography/specificity of affinity chromatography	195
Working procedure	196
Types of affinity chromatography	197
Applications	197
Advantages	198
Limitations	198
Model Question Papers (Theory)	201
Practical Instrumental Methods of Analysis	205
Experiments	207
Experiment No. 1: Determination of absorption maxima and effect of solvents on absorption maxima of organic compounds	207
Experiment No. 1.1: To determine λ_{max} of paracetamol solution	209
Experiment No. 2: To perform an estimation of dextrose by colorimetry	211
Experiment No. 2.1: Study the instrumentation and working of colorimetry	213
Experiment No. 2.2: To determine λ_{max} of KMnO_4 solution by colorimetry	216
Experiment No. 3: Estimation of sulfanilamide by colorimetry	217
Experiment No. 4: Simultaneous estimation of ibuprofen and paracetamol by UV spectroscopy	219
Experiment No. 5: To perform an assay of paracetamol by UV spectrophotometry	222
Experiment No. 6: Estimation of quinine sulfate by fluorimetry	224
Experiment No. 7: Study of quenching of fluorescence	226
Experiment No. 8: Determination of sodium by flame photometry	228
Experiment No. 9: Determination of potassium by flame photometry	230
Experiment No. 10: Determination of chlorides and sulphate by nepheloturbidometry	232

Experiment No. 11: To perform the paper chromatography for the separation of amino acids present in the given sample 235

Experiment No. 12: To perform the separation and analysis of some sugars (glucose, fructose) by using thin layer chromatography (TLC) 238

Experiment No. 12.1: To separate and identify alkaloids by thin layer chromatography 239

Experiment No. 13: To separate the plant pigments by using column chromatography 241

Experiment No. 14: To perform an assay of azithromycin by HPLC in the tablet dosage form 243

Experiment No. 15: Introduction of gas chromatography 245

Experiment No. 16: To determine the exact equivalent point and normality of 0.1 N HCl by potentiometrically titrating it with 0.1 N NaOH solution (potentiometric titration of strong acid vs strong base) 246

Experiment No. 17: Demonstration of HPTLC 247

Experiment No. 18: Demonstration of FTIR 252

References	258
Basic Concepts, Important Points and Topics	259
Questions and Answers for the Viva and Practical Exam	265
<i>Index</i>	293