

4.2 INSTRUMENTAL AND BIOMEDICAL ANALYSIS (THEORY)

75 hours ; 3 hours/week

The subject to be discussed with special reference to quality control and assurance of pharmaceuticals, its scope and its importance in the pharmaceutical industry. The following analytical techniques should be discussed with suitable examples.

1. Spectroscopy

Electromagnetic radiation, Electromagnetic spectra, Quantization of energy, criteria for absorption of EMR, wavelength, frequency, wave number, Chromophores, Auxochromes, Bathochromic shift, hypsochromic shift, Hyperchromic and hypochromic effects. Theory of electronic, atomic and molecular spectra, Beer and Lambert's law, Derivation and Deviations, Effect of solvent and pH on absorption spectra, Application of Beer's law to single component analysis and multicomponent systems. **6 hours; 8-9 marks**

Absorption Spectroscopy: UV/Visible spectroscopy, Instrumentation and working; Sources of radiation, Wavelength selectors; Filters-Prisms and Gratings, Sample cells, Detectors-Phototube, Photomultiplier tube, Barrier layer cell and Silicon photo diode. Measurement of equilibrium constant and rate constant by spectroscopy, spectrophotometric titrations **7 hours;9-10 marks**

IR Spectroscopy: Theory, Hook's law, vibration modes of in molecule, Instrumentation, Working and Sample handling methods, Sources of radiation, Monochromators, Sample cells and detectors- Bolometers, Thermocouples, Golay cells. IR Frequency - structure correlation, study of characteristic fundamental stretching vibrations of functional groups like Alcohol, Carboxyl, Amide, Amine, Aldehyde, and Ketone **6 hours;8-9 marks**

2. Fluorimetric analysis: Theory, concept of singlet and triplet electronic states, Internal and external conversions, intersystem crossing, factors affecting fluorescence, Quenching. Instrumentation- Fluorimeter, Spectrofluorimeter and Applications Estimation of vitamin B₁₂ by fluorimetry **4 hours;5-6 marks**

3. Flame emission and atomic absorption spectrometry: Theory, Nebulization, flames and flame temperatures, interferences, Hallow cathode lamp, flame spectrometric techniques, applications. **4 hours;5-6 marks**

4. Nephelometry and Turbidimetric Analysis: Theory, General principles, Instrumentation and applications. **2 hours;3-4 marks**

5. Chromatography: Introduction and classification **2 hours;3-4 marks**

A) Paper Chromatography: Introduction, Principle, Technique, Development, methods and applications. **2 hours;3-4 marks**

B) Thin layer chromatography: Introduction, principle, technique, R_f, R_X and R_m values and applications. **2 hours;3-4 marks**

C) Column chromatography: Adsorption column chromatography, development Techniques – Frontal analysis, Displacement analysis and elution analysis. Factors affecting column efficiency, Applications, Partition Chromatography **3 hours;4-5 marks**

D) HPTLC: Basic concept, difference between TLC and HPTLC **2 hours;3-4 marks**

E) Ion exchange chromatography: Ion exchange materials, synthetic ion exchange resins, Properties of ion exchangers, mechanism of ion exchange process, factors affecting ion exchange, applications. **3 hours;4-5 marks**

F) HPLC: Introduction, theory, instrumentation –Solvent treatment systems, Pumps- Reciprocating and displacement pumps, Columns, Guard column, Detectors- UV detectors, Fluorimetric detectors, Refractive index detectors and applications. **4 hours;5-6 marks**

G) Size exclusion chromatography: Theory, gels and applications **2 hours;3-4 marks**

H) Gas chromatography: Introduction, theory, instrumentation-carrier gas, types of columns, Stationary phases in gas liquid chromatography and gas solid chromatography, Detectors- Flame ionization detector, electron capture detector, thermal conductivity detector. Gas chromatogram, Derivatisation techniques like silylation, and esterification. Programmed temperature gas chromatography, Applications. **5 hours;6-7 marks**

I) Electrophoresis: Principles of separation, Factors affecting separation, equipment for paper and gel electrophoresis, Moving boundary electrophoresis, isoelectric focusing electrophoresis, Applications . **3 hours;4-5 marks**

6. Electrometric methods:

A) Potentiometry: Electrochemical cell, construction and working of reference electrode, Normal hydrogen electrode, calomel electrode, silver-silver chloride electrode, Indicator electrodes- Glass electrode, Antimony electrode, Quinhydrone electrode, Potentiometric titrations, methods of detecting end point, applications. **6 hours;8-9 marks**

B) Conductometry: Introduction, conductivity cell, cell constant, conductometric titrations, Applications **2 hours;3-4 marks**

7. NMR: Theory and instrumentation of ^1H NMR, applications. 2 hours;3-4 marks

8. Mass Spectroscopy: Basic concept mass spectroscopy, applications. 2hours;3-4marks

9. X-ray Diffraction: Theory of X-ray diffraction, applications. 2 hours;3-4 marks

10. Quality assurance: Introduction, Sources of quality variation, control of quality variation, Validation methods. **4 hours;5-6 marks**

INSTRUMENTAL AND BIOMEDICAL ANALYSIS (PRACTICALS)
75 hours ; 3 hours/week

Note: At least 18-19 experiments to be covered from the following list

1. Calibration of UV-Visible spectrophotometer
2. Determination of absorption maxima for a given solution of the drug*
3. Determination of isobestic point*
4. Separation and identification of amino acids by paper chromatography*
5. Separation and identification of alkaloids by thin layer chromatography*
6. Separation and Identification of Dyes by radial paper chromatography*
7. U.V. Spectrometric determination of Ibuprofen tablets*
8. U.V. Spectrometric determination of Paracetamol tablets*
9. Colorimetric estimation of Ferrous ions using 1, 10 Phenanthroline**
10. Colorimetric estimation of Sulphanilamide using N-1-Naphthyl Ethylene Diaminedihydrochloride**
11. Colorimetric estimation of salicylic acid**
12. Assay of Dextrose injection by colorimetry**
13. Estimation of quinine sulphate by fluorimetry**
14. Estimation of Riboflavine by fluorimetry**
15. Quenching of Quinine fluorescence by iodide ions*
16. Conductometric titration of Benzoic acid with NaOH**
17. Potentiometric titration of HCl with NaOH**
18. Determination of chloride and sulphate in calcium gluconate by Nepheloturbidimetric analysis**
19. Infrared spectral peaks identification of samples with different functional groups such as -COOH, -COOR, CONHR, -NH₂, -NHR, -OH, -CHO, -C=O*
20. Determination of Sodium / Potassium by flame photometry**

Note: ** Denotes major experiments * Denotes minor experiments

SCHEME OF EXAMINATION

1. Synopsis	10 Marks
2. Major experiment (Experiment indicated by**)	30 Marks
3. Minor experiment (Experiment indicated by*)	20 Marks
4. Viva voce	10 Marks

Total	70 Marks
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INSTRUMENTAL AND BIOMEDICAL ANALYSIS REFERENCE BOOKS (THEORY)

Latest editions and all volumes of

1. Hobarth Willard, Lynne L. Merritt, John A. Dean & Frank A. Settle Jr, Instrumental methods of analysis, 6 edition, CBS Publishers & Distributors, New Delhi
2. Skoog and West, Pharmaceutical Analysis
3. Kenneth A. Connors, A Text book of Pharmaceutical analysis, 3 Edition, John Wiley & Sons, New York (1982)
4. Instrumental approach to chemical analysis – AK Srivatsava, PC Jain, S Chand publishers
5. A.I. Vogel, Text book of Quantitative chemical analysis, ELBS/ Longman, London
6. Stahl.E , Thin layer chromatography-A Hand book, Springer verlag, Berlin
7. Leon Lachman, Lieberman, and J.L.Kanig, Theory and practice of industrial pharmacy
8. Y.R Sharma, Organic spectroscopy

INSTRUMENTAL AND BIOMEDICAL ANALYSIS REFERENCE BOOKS (PRACTICALS)

Latest editions and all volumes of

1. Garratt. D.C. Quantitative analysis of drugs, 3 edition, CBS Publishers & Distributors, New Delhi.(2001).
2. Melean. C.E., Kiser.R.W., Problems and experiments in instrumental analysis.
3. Beckett A.H., Stenlake J.B, Practical Pharmaceutical Chemistry, 4 edition, The Athlone Press, London.
4. KennethA.Connors, A Text book of Pharmaceutical analysis, 3 edition , John Wiley & Sons, New York (1982),.
5. Hobarth Willard, Lynne L. Merritt, John A. Dean , Frank A. Settle Jr,, Instrumental methods of analysis, 6 edition, CBS Publishers & Distributors, New Delhi.
6. Indian Pharmacopoeia, Vol I & II , , Ministry of Health & Family welfare, Govt. of India, Published by Controller of the Publications, New Delhi, 1996.
7. United States Pharmacopoeia.
8. British Pharmacopoeia.
9. Alfonso R. Gennaro, Remington's: The Science & Practice of Pharmacy, Vol I & II – Lippincotts Wiliams & Wilkins.
10. Higuchi T & Hanssen E.B., Text Book of Pharmaceutical Analysis, A Wiley Inter science Publications.
11. William Kemp, Spectroscopy methods, ELBS.
12. John R. Dyer, Applications of Absorption spectroscopy of organic compounds, Prentice Hall of India Pvt. Ltd., New Delhi.
13. R. M. Silverstein, G. Clayton, Terence C. Morill, Spectrometric identification of organic compounds, John Wiley & Sons, New York.

LIST OF MINIMUM EQUIPMENTS REQUIRED

Equipments

1. Colorimeter	01
2. UV Spectrophotometer	01
3. Fourier Transform Infra Red spectrometer	01
4. Fluorimeter	01
5. Nephelo turbidity meter	01
6. Flame photometer	01
7. Potentiometer	02
8. Conductivity meter	02
9. Analytical weighing Balance (1 mg sensitivity)	01
10. Oven	01

Apparatus

1. Volumetric flask 10 ml, 25 ml, 50 ml and 100ml
2. Weighing bottle
3. Pipette – Graduated 10 ml
4. Pipette – Bulb 1ml, 2ml, 5ml and 10 ml
5. Beaker – 100 ml, 250 ml and 500 ml
6. Separating funnel
7. TLC plate
8. Chromatographic paper
9. TLC and Paper chromatography – Development chamber
10. Water bath