

BSC II SEMESTER III
BIOTECHNOLOGY – W.E.F.2016-17

BT 301: BIOPHYSICAL TECHNIQUES

UNIT – I:

Spectrophotometry: Concept of electromagnetic radiation, spectrum of light, absorption of electromagnetic radiations, Concept of chromophores and auxochromes, involvement of orbitals in absorption of electromagnetic radiations, Absorption spectrum and its uses, Beer's law - derivation and deviations, extinction coefficient. Difference between spectrophotometer and colorimeter. Instrumentation of UV and visible spectrophotometry Double beam spectrometer; dual-wavelength spectrometer

- a) Applications of UV and visible spectrophotometry.
- b) Spectrofluorometry: principle, instrumentation and applications. Absorption & emission flame photometry: principle, instrumentation and application.
- c) Principles of IR and Mass spectrometry

UNIT II:

Chromatography: Partition principle, partition coefficient, nature of partition forces, brief account of paper chromatography.

Thin layer chromatography and column chromatography.

Gel filtration: Concept of distribution coefficient, types of gels and glass beads, applications.

Ion-exchange chromatography: Principle, types of resins, choice of buffers, applications including amino acid analyzer.

Affinity chromatography: Principle, selection of ligand, brief idea of ligand attachment, specific and non-specific elution, applications.

Elements of high pressure liquid chromatography.

UNIT III

- a) Migration of ions in electric field, Factors affecting electrophoretic mobility.
- b) Paper electrophoresis: - Electrophoretic run, Detection techniques, Cellulose acetate electrophoresis, High voltage electrophoresis.
- c) Gel electrophoresis: - Types of gels, Solubilizers, Procedure, Column & slab gels, Detection, Recovery & Estimation of macromolecules.

- d) SDS-PAGE Electrophoresis: - applications (determination of molecular weight of proteins, determination of subunit stoichiometry, molecular biology applications).
- e) Isoelectric focussing, Principle, Establishing pH and density gradients, Procedures & applications.
- f) Pulsed-field gel electrophoresis.

UNIT – IV:

Isotopic tracer technique: -

- a) Radioactive & stable isotopes, rate of radioactive decay. Units of radioactivity.
- b) Measurement of radioactivity: - Ionization chambers, proportional counters, Geiger- Muller counter, Solid and liquid scintillation counters (basic principle, instrumentation and technique), Cerenkov radiation.
- c) Principles of tracer technique, advantages and limitations, applications of isotopes in biotechnology distribution studies, metabolic studies, isotope dilution technique, metabolic studies, clinical applications, autoradiography.

UNIT V

Centrifugation:

on:

- a) Basic principles, concept of RCF, types of centrifuges (clinical, high speed and ultracentrifuges).
- b) Preparative centrifugation: Differential and density gradient centrifugation, applications (Isolation of cell components).
- c) Analytical centrifugation: Sedimentation coefficient, determination of molecular weight by sedimentation velocity and sedimentation equilibrium methods.

Biostatistics

Basic concepts of mean, median, mode, Standard deviation and Standard error

B.Sc. II SEMESTER III PRACTICALS

BIOTECHNOLOGY

BT302: BIOCHEMISTRY & BIOPHYSICAL TECHNIQUES

1. Spectrophotometric analysis of DNA denaturation.
2. Determination of absorption spectrum of oxy- and deoxyhemoglobin and methemoglobin.
3. Protein estimation by E280/E260 method.
4. Paper chromatography of amino acids/sugars.
5. TLC of sugars/amino acids.
6. Cellular fractionation and separation of cell organelles using centrifuge.
7. Isolation of mitochondria and assay of marker enzyme.
8. Estimation of Urea by diacetyl monoxime method.
9. Estimation of Sugars by Folin Wu method.
10. Validity of Beer's law for colorimetric estimation of creatinine.
11. Absorption spectrum of NAD & NADH.
12. Preparation of standard buffers and determination of pH of a solution.
13. Titration of a mixture of strong & weak acid.
14. Paper electrophoresis of proteins.
15. Gel electrophoresis of proteins.
16. SDS-PAGE of an oligomeric protein.
17. Calculation of mean, median, and mode (manual/computer aided).
18. Calculation of standard deviation and standard error (manual/computer aided).
19. Biostatistical problem based on standard deviation.

Note: - Mandatory to perform atleast 10 practicals

**BSc II BIOTECHNOLOGY
SEMESTER III
MODEL QUESTION PAPER
COURSE CODE: BT 301
COURSE NAME: Biophysical techniques**

Time 3 Hrs

Marks 75

**Attempt any *five* questions from Part A and *all* questions from Part B
PART A (5x3=15 Marks)**

Note: At least one question must be set from each UNIT

1. Define Beer's law
2. Partition coefficient
3. Nature of resin
4. Principle of pressure pump
5. Concept of RCF
6. Half life
7. Tracer technique advantages
8. Svedberg unit

**PART B (5x12=60 Marks)
Answer the following**

- 9 (a) Describe the layout, principle and applications of Mass spectrophotometer.
Or
(b). Describe the layout, principle and applications of UV/VIS spectrophotometer
- 10 (a) Discuss the principle of gel permeation and add note on its advantages.
Or
(b) Explain the principle of thin layer chromatography and add note on Rf values.
- 11 (a) Define electrophoresis. Add note on paper electrophoresis and its applications.
Or
(b) What are the methods used to determine the pI values. Explain the techniques related to it.
- 12 (a) What are the precautions one has to take in while he is in Radioactive laboratory.
Or
(b) Discuss in detail on the principles and functioning of LSC.
- 13 (a) What is meant by centrifugation? Explain the mechanics involved in preparative centrifugation.
Or
(b) What are methods used to determine the molecular weight of proteins? Discuss any one method.