



SHREE H. N. SHUKLACOLLEGE OF SCIENCE

(AFFILIATED TO SAURASHTRA UNIVERSITY)

Shree H.N. Shukla College Campus Nr. Lalpari lake, Behind old Marketing Yard,
Amargadh, Bhichari, Rajkot-360001, Ph. No-9727753360

PAPER NO 401 BIOCHEMISTRY – CBCS BIOPHYSICAL AND BIOCHEMICAL TECHNIQUES

Credit: 4

Theory: 6 lectures/ week Total Lectures: 60

Objectives:

To enable the students to

1. Understand the principles of various biophysical techniques and their working.
2. Apply this knowledge for characterization, separation, identification and purification of different biomolecules.

Unit 1: Spectroscopic techniques

[12 Hrs]

- Electromagnetic radiation and spectra. Quantitative aspects of light absorption; Beer- Lamberts laws of light absorption with their limitations. Extinction coefficients.
- Instrumentation, principles, components and working of single and double beam colorimeter and spectrophotometer.
- Advantages of double beam instruments. Applications of UV-Visible spectroscopy.

Unit 2: Hydrodynamic techniques

[12 Hrs]

- Sedimentation- the concepts of Centrifugal force (F) and Relative centrifugal force (RCF).
- Different types of rotors and centrifuges.
- Preparative and analytical centrifugation- instrumentation, techniques, and their applications.
- Differential centrifugation and its applications in isolation of cell organelles. Principle of density gradient centrifugation, materials used to prepare density gradient and applications of density gradient centrifugation.

Unit 3: Radio isotopic techniques

[12 Hrs]

- Radioactive decay by emission of alpha, beta and gamma radiations with suitable examples. Half life of radio isotopes. Types of radioisotopes commonly used in biochemistry, units of radioactivity.
- Techniques for measurement of radioactivity (gas ionization and liquid scintillation counting). Overview of GM counter, Liquid Scintillation counter and gamma counters.
- Biological applications of radioisotopes. Biological hazards of radiation and safety measures in handling radioisotopes.

Unit 4: Chromatography

[12 Hrs]

- General principles, materials, methods and applications of the following techniques:
 - Paper and thin-layer chromatography techniques.
 - Ion exchange chromatography.
 - Molecular sieve chromatography.
 - Affinity chromatography
 - Gas-Liquid chromatography (GLC)
 - High performance liquid chromatography (HPLC)

Unit 5: Electrophoresis

[12 Hrs]

- Basic principles of electrophoresis and factors affecting electrophoretic mobility.
- Principle, materials, apparatus used and applications of Agarose and Polyacrylamide gel electrophoresis (PAGE).
- Principle and applications of SDS PAGE, Native v/s SDS PAGE, Isoelectric focusing and 2D Gel electrophoresis.



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• Text Books

1. Upadhyay, A., Upadhyay, K., & Nath, N. (2009). Biophysical chemistry (principles and techniques). Mumbai: Himalaya Pub. House.

2. Wilson K. and Walker J. (2010) Principles and Techniques of Biochemistry and Molecular Biology 7th Edition, Cambridge: Cambridge University Press.

Reference Books

1. Conn Erice, E. and Stumpf Paul, K. (2007). Outlines of Biochemistry, [5th Edition]. John Wiley & Sons, New Delhi.

2. Freifelder, D. (1986). Physical biochemistry: Applications to biochemistry and molecular biology. San Francisco: W.H. Freeman.

3. Van Holde, K. E., Johnson, W. C., & Ho, P. S. (2006). Principles of physical biochemistry. Prentice-Hall.

Practicals 401:

1) Introduction to principle and working of Colorimeter and spectrophotometer.

2) Determination of absorption spectrum and absorption maxima of given compound.

3) Verification of Beer's Law of light absorption using colored solutions.

4) Introduction to principle and working of centrifuge.

5) Separation of amino acids using paper chromatography. Determination of R_f values and identification of amino acids from mixtures.

6) Separation of lipids by thin layer chromatography.

7) Separation of compounds using column chromatography.

8) Agarose Gel electrophoresis of DNA.

