ST.JOSEPH’S COLLEGE FOR WOMEN (AUTONOMOUS), VISAKHAPATNAM

II SEMESTER **BIOCHEMISTRY** TIME: 4HRS/WK

BCH 2802 (3) **ANALYTICAL TECHNIQUES** MARKS:100

w.e.f. 2021-2022 (20AI) **SYLLABUS**

**OBJECTIVES: To enable the students to-**

* Learn methods of tissue homogenization and types of centrifugation methods available for separation and analysis of biomolecules
* Grasp the principles of Chromatography and appreciate its application in biochemistry.
* Correlate the Beer Lambert’s law usage in various scpetroscometry and colorimetry techniques
* Interpret the electrophoretic mobility of molecules to their behavior in gel separation techniques
* Correlate knowledge on nucleic acids with their physiological role and analyze quality of porphyrins and appreciate their biological significance

**COURSE OUTCOMES- Students will be able to**

**CO1:**Select appropriate centrifugation method after homogenization of tissue sample under analysis

**CO2**:Choose Chromatographic techniques based on the physical properties of the biomolecule

**CO3:**Demonstrate the principles of Spectroscopy and other photometric techniques

**CO4:**Performimmunoelectrophoretic and other electrophoretic techniques for separation of Biomolecules

**CO5:** Isolate and characterize microorganisms

**UNIT-I:CELL HOMOGENIZATION AND CENTRIFUGATION :** Methods of tissue homogenization: (Potter-Elvejham, mechnical blender, sonicator and enzymatic). Centrifugation techniques, principles and applications- differential, density gradient. Ultra-centrifugation- preparative and analytical.

**UNIT-II:CHROMATOGRAPHIC TECHNIQUES :** Types of chromatographic techniques, Principle and applications - Paper chromatography- solvents, Rf value, applications; Thin layer chromatography- principle, choice of adsorbent and solvent, Rf value, applications; Gel filtration, Ion- exchange- principle, resins, action of resins, experimental techniques, applications, separation of metal ions; Affinity chromatography.

**UNIT-III: SPECTROSCOPY AND TRACER TECHNIQUES:** Electromagnetic radiation, Beer-Lambert’s law. Colorimetry and Spectrophotometry, spectro fluorimetry, flame photometry. Tracer techniques: Radio isotopes, units of radio activity, half life, β and γ- emitters, use of radioactive isotopes in biology, ELISA, RIA.

**UNIT-IV:ELECTROPHORESIS :** Electrophoresis- principles and applications of paper, polyacrylamide (native and SDS) and agarose gel electrophoresis, isoelectric focusing, immune-electrophoresis-types and applications.

**UNIT-V: MICROBIAL TECHNIQUES:** Microscopy: Basic principles of light microscopy, phase contrast, electronmicroscope and fluorescent microscope and their applications. Preparation of different growth media, isolation and culturing and preservation of microbes, Gram**’**s staining- Gram positive and Gram negative bacteria, motility and sporulation, Sterilization techniques-Physical methods, chemical methods, radiation methods, ultrasonic and. Antibiotic resistance.

**RECOMMENDED BOOKS:**

1. Principles and Techniques of practical Biochemistry. Eds. Williams and Wilson.
2. Techniques in Molecular biology Ed. Walker &Gastra, Croom Helm, 1983.
3. Principles of instrumental analysis, 2nd Ed, Holt-Sanders, 1980.
4. An introduction to spectroscopy for Biochemistry. Ed. Brown S.N., Academic press
5. Analytical Biochemistry, Holmes and Hazel peck, Longman, 1983.
6. An introduction to practical biochemistry. David T. Plummer, Tata Mac Grew-Hill.
7. Biophysical chemistry, Edshall& Wyman, Academic press Vol. II & I.
8. A textbook of quantitative inorganic analysis including elementary instrumental analysis, Vogel ELBS.
9. Biochemical calculations Seigel, IH, 2nd Edit, John Wiley & sons Inc., 1983.
10. Analytical Biochemistry by Friefelder David

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ST.JOSEPH’S COLLEGE FOR WOMEN ( AUTONOMOUS ) , VISAKHAPATNAM

II SEMESTER **BIOCHEMISTRY** TIME:2HRS/WEEK

BCH 2852 (2) **ANALYTICAL TECHNIQUES** Max.Marks:50

w.e.f. 2021-2022 (20AI) **PRACTICALS**

**OBJECTIVES:** To enable the students to-

* Learn to isolate and quantitate nucleic acids
* Isolate proteins, carbohydrates and cholesterol from sources
* Choose the richest source for biomolecules
* Imbibe and perform simple chromatographic techniques for quality analysis
* Comprehend and characterize the given mixture of biomolecules

**COURSE OUTCOMES- The students will be able to**

**CO1:** Isolate and quantitate RNA and DNA from tissues and culture

**CO2:** Select richest source for Biomolecules and isolate them

**CO3:** Separate biomolecules using simplest of the chromatography techniques

**CO4:** Implement the principle of Ion exchange chromatography

**CO5:** Demonstrate components of a mixture using electrophoretic techniques

**LIST OF EXPERIMENTS:**

1. Isolation of RNA and DNA from tissue/culture.
2. Qualitative Identification of DNA,RNA and Nitrogen Bases
3. Isolation of egg albumin from egg white.
4. Isolation of cholesterol from egg yolk.
5. Isolation of starch from potatoes.
6. Isolation of casein from milk.
7. Separation of amino acids by paper chromatography.
8. Determination of exchange capacity of resin by titrimetry.
9. Separation of serum proteins by paper electrophoresis.

**RECOMMENDED BOOKS:**

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2. Techniques in Molecular biology Ed. Walker &Gastra, Croom Helm, 1983.
3. Principles of instrumental analysis, 2nd Ed, Holt-Sanders, 1980.
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