



Advanced laboratory technique

SEPARATION TECHNIQUES, SEPARATION OF PROTEINS ,SEPARATION OF AMINO ACIDS

Sawa University

College of health and medical techniques

Department of Medical Laboratories

. Stage third

جامعة ساوة الاهلية

كلية التقنيات الصحية والطبية

قسم تقنيات المختبرات الطبية

المرحلة / الثالثة

محاضرة رقم: 6

¹ Lecture No : 6

Practical

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Objective

Protein Separation

- Common protein separation techniques
- Types of Chromatography

2- Amino Acid Separation

- Common amino acid separation techniques

Separation techniques:

Essential in biochemistry to **isolate and purify proteins, amino acids**, and other biomolecules from complex mixtures.

These techniques exploit differences in physical and chemical properties such as size, charge, solubility, and binding affinity.

1. Protein Separation:



Proteins are separated to study their structure , function, and interactions.
Common protein separation techniques include:

- a) Chromatography
- b) Electrophoresis
- c) Ultracentrifugation

Types of Chromatography:

1. **Affinity Chromatography:** Utilizes specific binding interactions between a protein and a ligand.
 2. **Ion-Exchange Chromatography:** Separates proteins based on charge; proteins are eluted by changing the pH or ionic strength.
 3. **Size-Exclusion Chromatography (Gel Filtration):** Separates based on molecular size; smaller molecules enter pores and elute slower.
- Applications:** Protein purification for structural and functional analysis

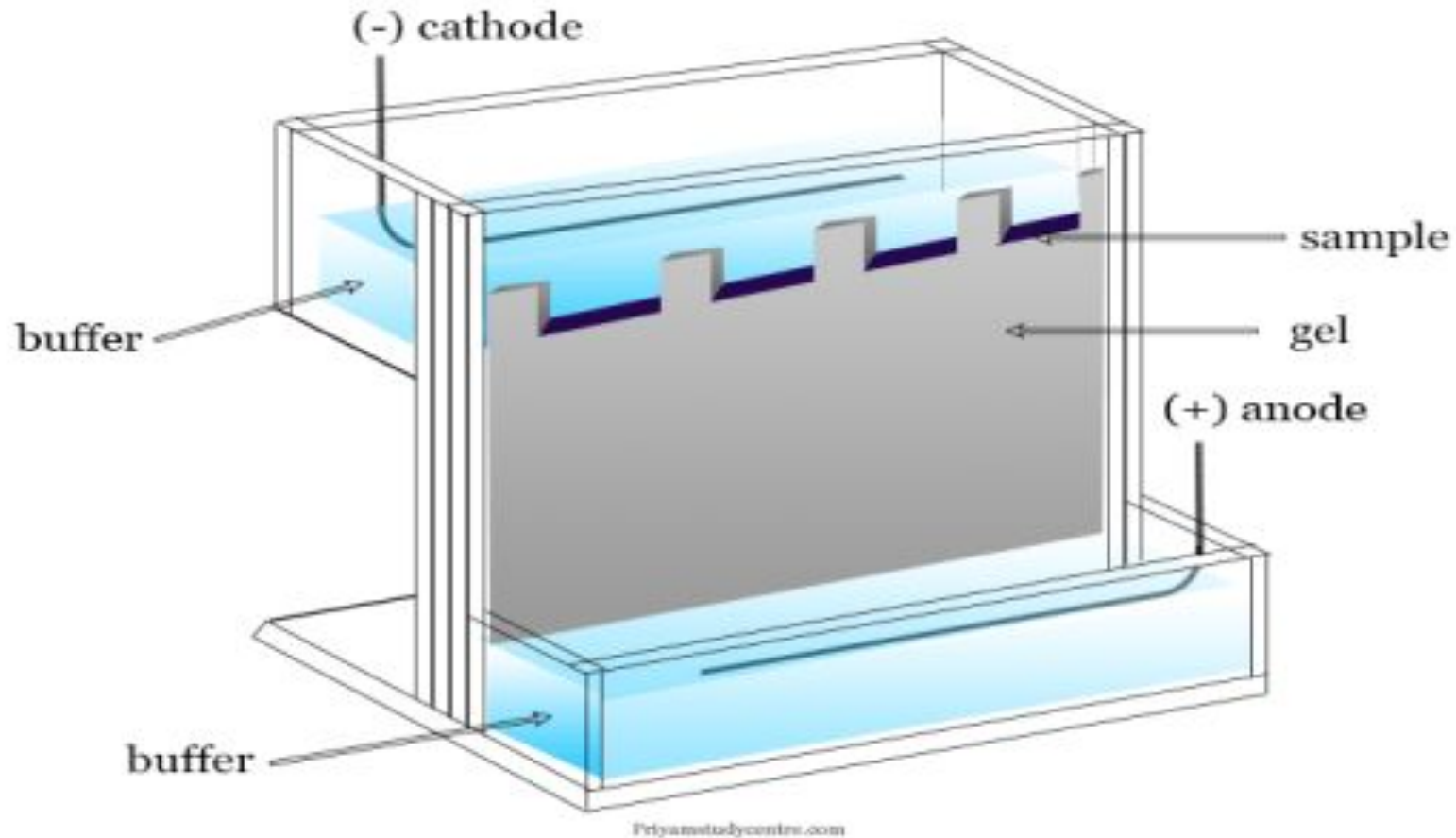
b) Electrophoresis

SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis): Separates proteins by size after denaturation.

Isoelectric Focusing (IEF): Separates proteins based on their isoelectric points (pI) in a pH gradient.

Applications: Determining molecular weight, identifying protein purity, and assessing protein mixtures

b) Electrophoresis



c) Ultracentrifugation



Uses high-speed centrifugation to separate proteins based on their sedimentation rates, which relate to size and density.

Applications: Analysis of protein complexes and large macromolecules

2. Amino Acid Separation: amino acids can be separated from protein hydrolysates for quantitative and qualitative analysis.

Common amino acid separation techniques include:

a) Ion-Exchange Chromatography

Separates amino acids based on charge differences, usually after derivatization for better detection.

Applications: Quantification and analysis of amino acid content in food and biological samples.

b) High-Performance Liquid Chromatography (HPLC)

Often used with fluorescent or UV detectors, especially after amino acids are derivatized for enhanced separation.

Applications: Precise separation and quantification of amino acids in mixtures.

c) Thin-Layer Chromatography (TLC)



A simpler and cost-effective technique to separate amino acids on a thin layer of adsorbent material.

Applications: Qualitative analysis of amino acids in biological samples

Any questions?

*Thank
You*