



محاضرة رقم: 6

Lecture No : 6¹

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

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

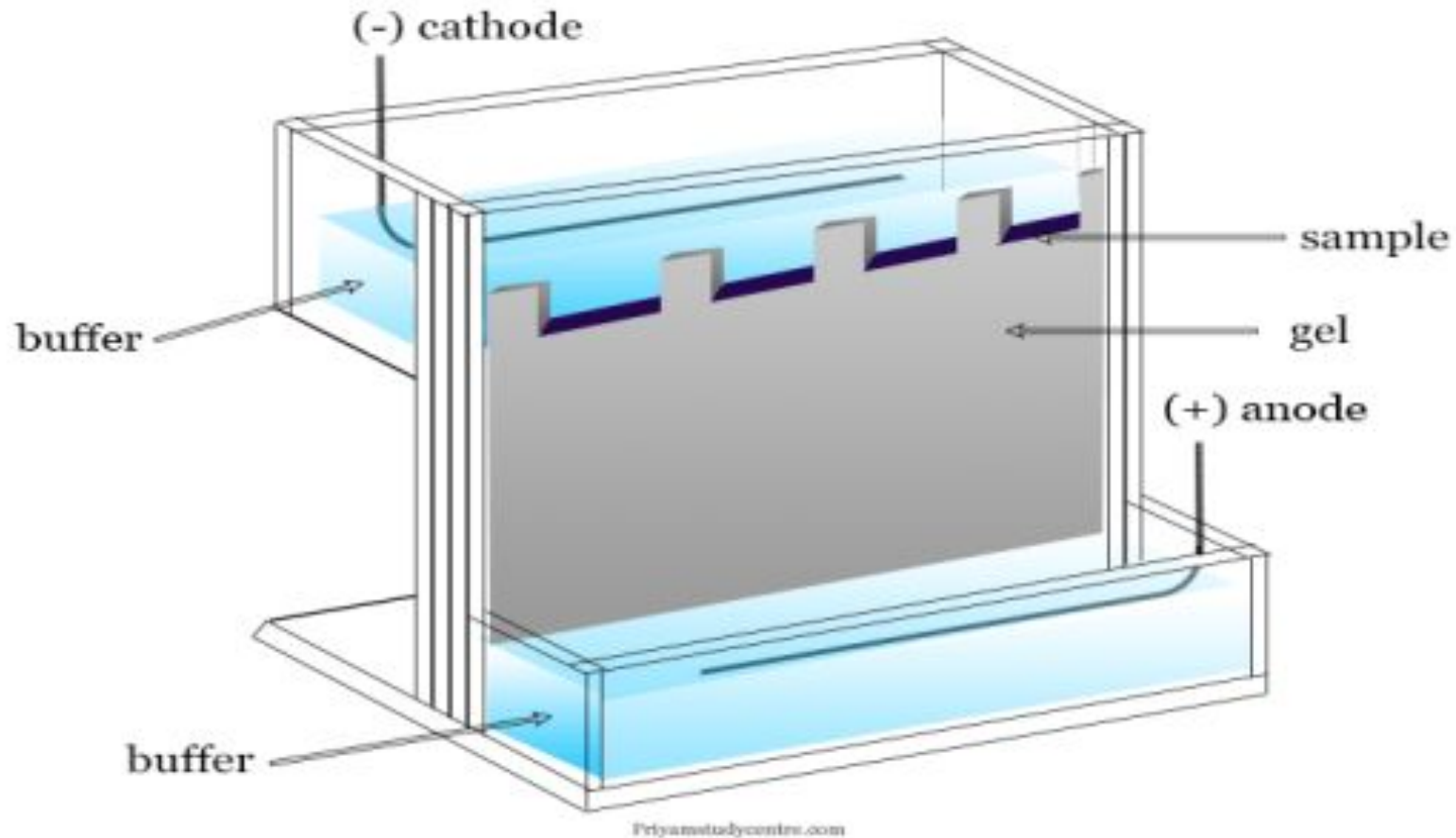
b) Electrophoresis

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*