

**Advanced laboratory technique**  
**Lab/8**  
**Protein and Amino acid Separation**  
**Techniques**  
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# INTRODUCTION

**Separation techniques** : are the processes used to separate components of a mixture based on their physical or chemical properties. These techniques can include methods such as filtration, distillation, chromatography, centrifugation, electrolysis, ultrafiltration, sedimentation, and solvent extraction.

# SEPARATION OF PROTEINS

- ❑ There are several techniques used to separate proteins based on their unique properties.
- ❑ The most common methods for protein separation :

## **1-Chromatography:**

- Ion-Exchange Chromatography: Separates proteins based on their charge
- Size-Exclusion Chromatography: Separates proteins based on their size

# SEPARATION OF PROTEINS

## **2-Electrophoresis:**

- SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis): Separates proteins based on their molecular weight
- Capillary Electrophoresis: Separates proteins based on their charge-to-mass ratio

## **3-Ultrafiltration:**

- Dialysis: Separates proteins based on their size using a semi-permeable membrane
- Ultrafiltration: Uses pressure to force proteins through a membrane, separating them based on size.

# SEPARATION OF PROTEINS

## **4-Centrifugation :**

- Differential Centrifugation: Separates proteins based on their size and density by spinning at different speeds
- Density Gradient Centrifugation: Uses a gradient of a dense substance to separate proteins based on their buoyant density.

## **5- Precipitation**

- Ammonium Sulfate Precipitation: Separates proteins by adding ammonium sulfate to precipitate specific proteins out of solution.
- Cold Precipitation: Uses cold temperatures to precipitate proteins .

# SEPARATION OF AMINO ACIDS

- ❑ Separating amino acids is a crucial step in many biochemical and analytical processes.
- ❑ The most common methods for amino acids separation :

## **1. Chromatography:**

-Ion-Exchange Chromatography: Separates amino acids based on their charge. It uses resins that bind amino acids differently depending on their ionic properties.

-High-Performance Liquid Chromatography (HPLC): A high-resolution method that separates amino acids based on their size, polarity, and interaction with the stationary phase.

## **2-Electrophoresis:**

-Paper Electrophoresis: Separates amino acids based on their movement in an electric field through a medium like paper or gel.

-Capillary Electrophoresis: Uses a capillary tube filled with a buffer solution. Amino acids are separated based on their size and charge as they move through the capillary under an electric field.

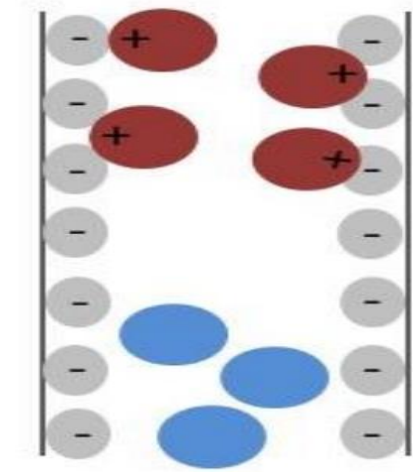
# SEPARATION OF AMINO ACIDS

## **3. Ultrafiltration**

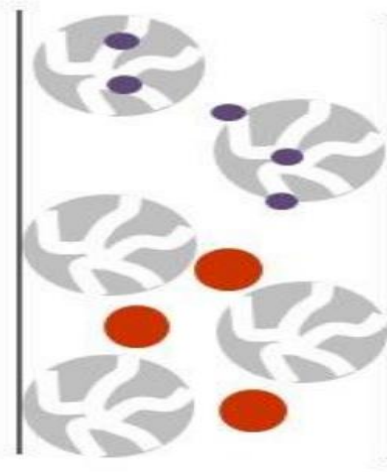
- Dialysis: Separates amino acids based on their size by using a semi-permeable membrane.
- Ultrafiltration: Uses pressure to force amino acids through a membrane, separating them based on size and molecular weight.

## **4. Precipitation:**

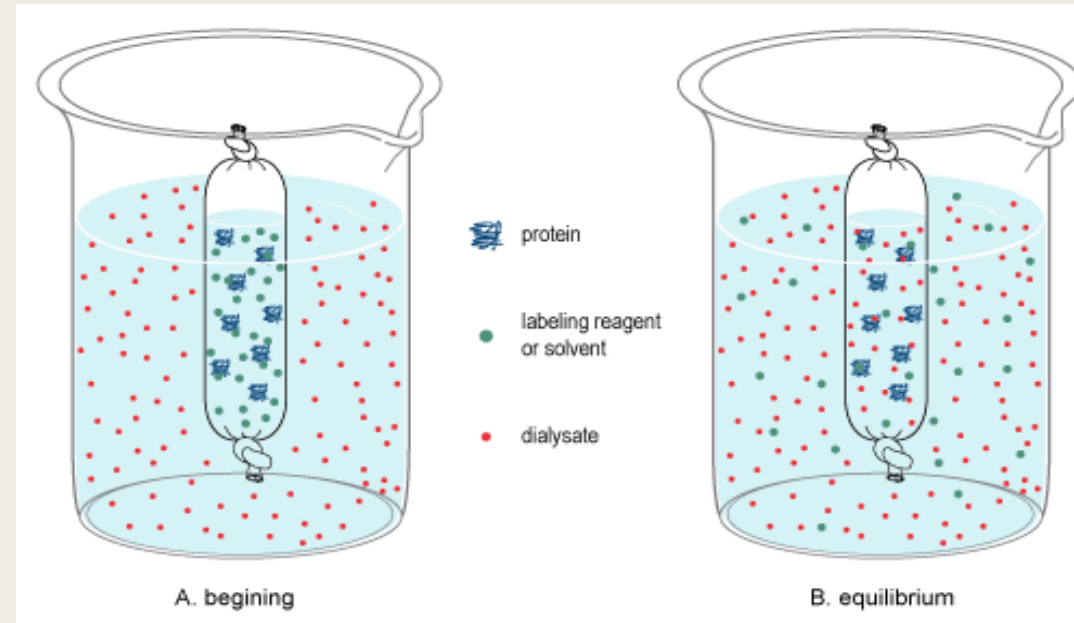
- Chemical Precipitation: Certain reagents can selectively precipitate specific amino acids out of a solution.



**Ion exchange**  
(surface charge)

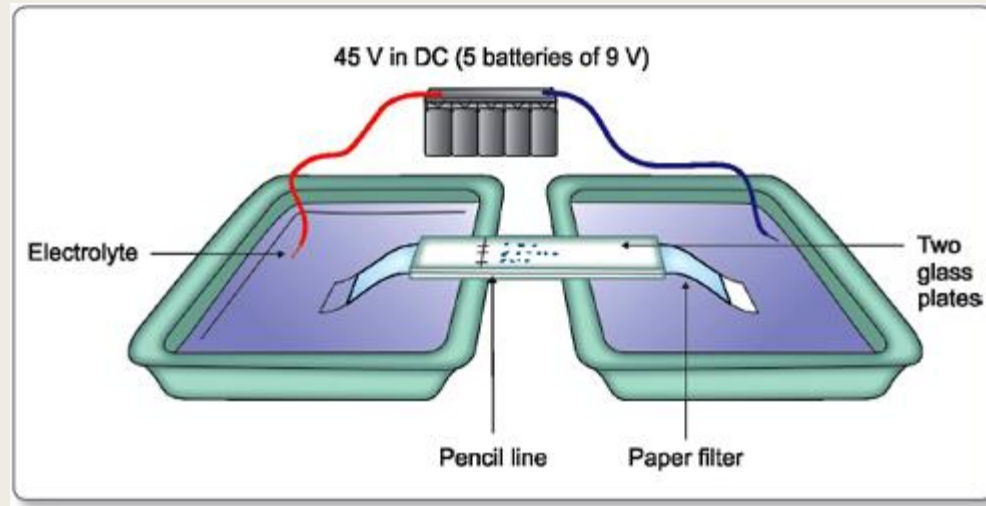


**Size exclusion**  
(hydrodynamic size)



## Chromatography

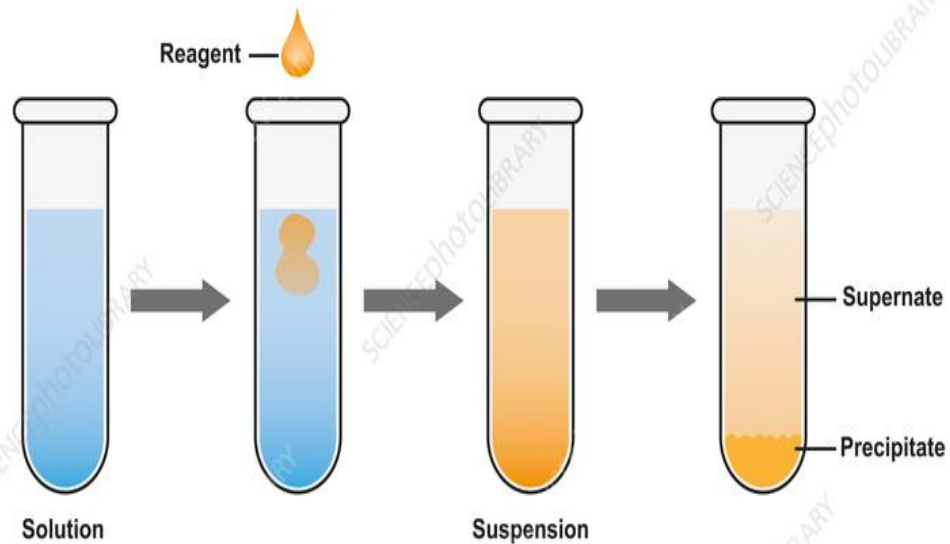
## Dialysis



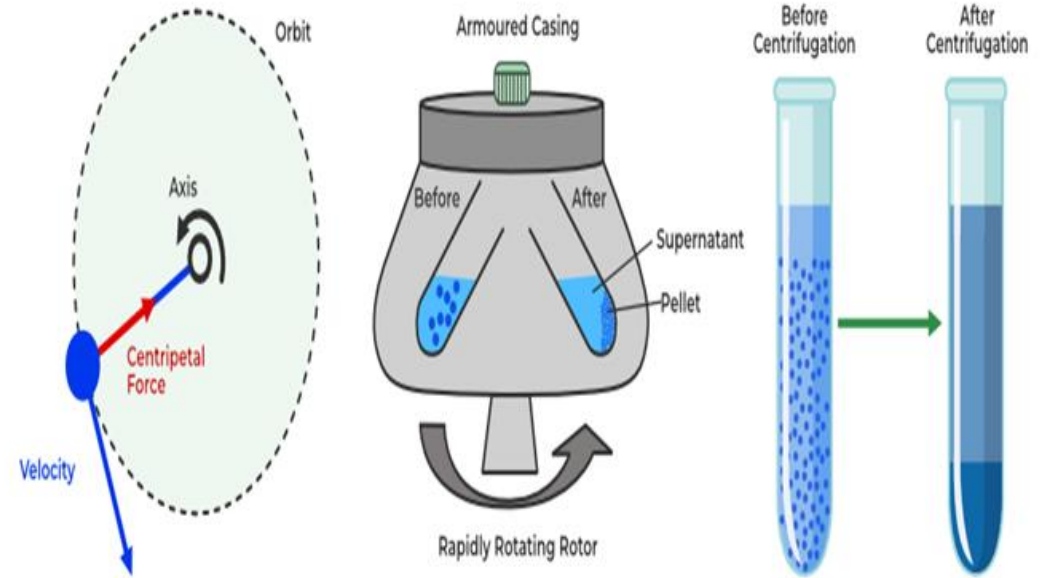
## Paper Electrophoresis



## Chemical Precipitation



Precipitation



Centrifugation

Thank

you

