ADVANCED LABORATORY TECHNIQUES

SEPARATION TECHNIQUES (PROTEINS, AMINO ACIDS)

جامعـــــــة ســـــــــاوة الاهلية — Sawa University

كليـــــة التقنيـــات الصحيـة والطبيـة — College of health and medical techniques

قسم تقنيات المختبرات الطبية — Department of Medical Laboratories

المرحلـــــــــــــة الثالثة — third Stage .

محاضرة رقم 7

Lecture No. 7

الجانب النظري

Theoretical

تدريسي المادة : م.م طيبة شاكر

(INTRODUCTION)

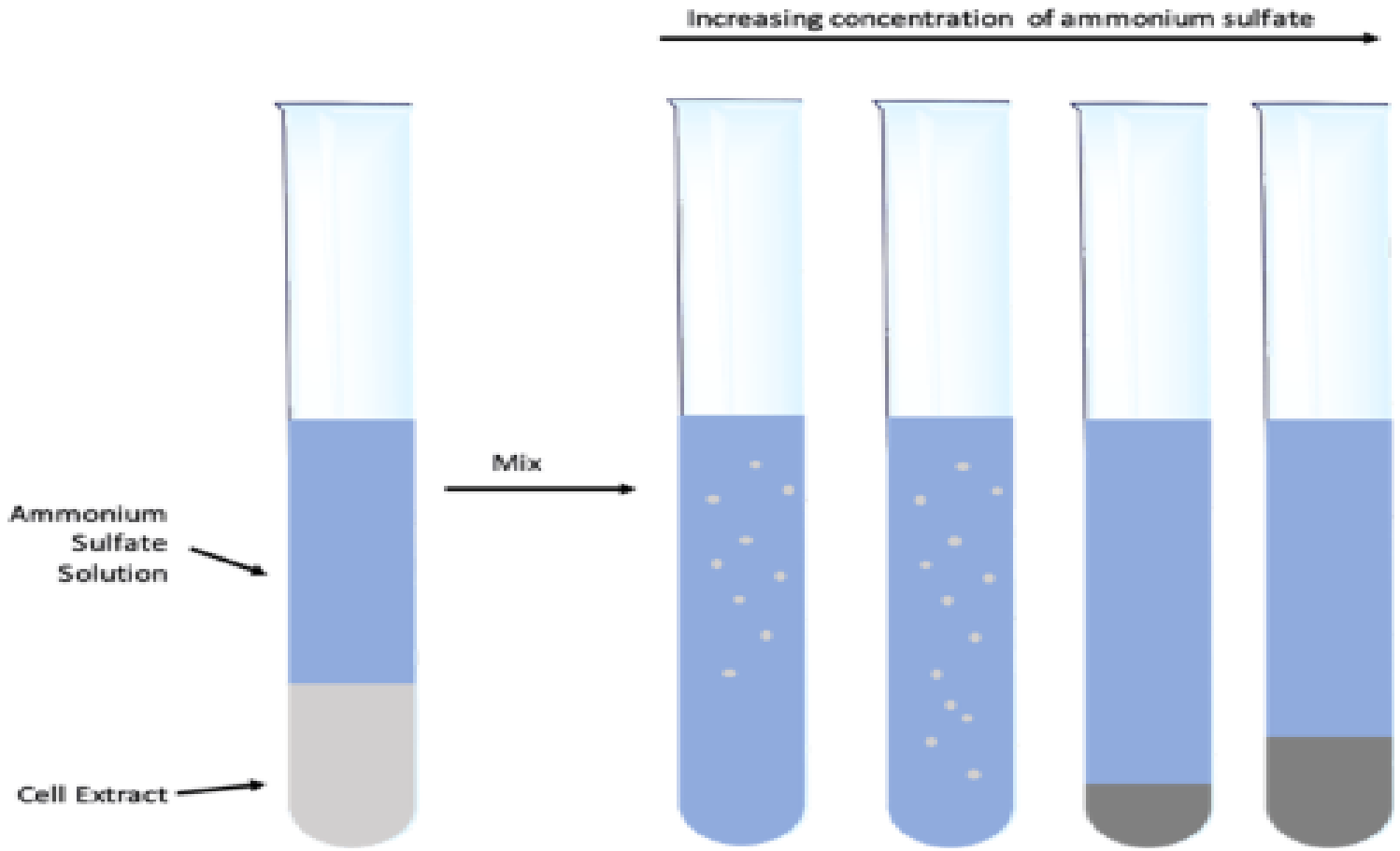
- **Biological samples** often contain **complex mixtures of proteins and amino acids**. To **study**, **identify**, or **purify them**, scientists use **separation techniques** that **exploit the physical or chemical differences among biomolecules—such as charge, size, solubility, and binding affinity.**
- **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.**
- **There are several techniques used to separate proteins based on their unique properties**

The **most common methods for protein separation** :

A. **Ammonium Sulphate Precipitation**:One of the **earliest steps in protein purification**. Based on **reducing protein solubility by increasing salt concentration ("salting out").**Different proteins precipitate at different saturation levels of ammonium sulphate.

Advantages: simple, inexpensive, and scalable. Used for concentrating immunoglobulins or enzyme preparation.

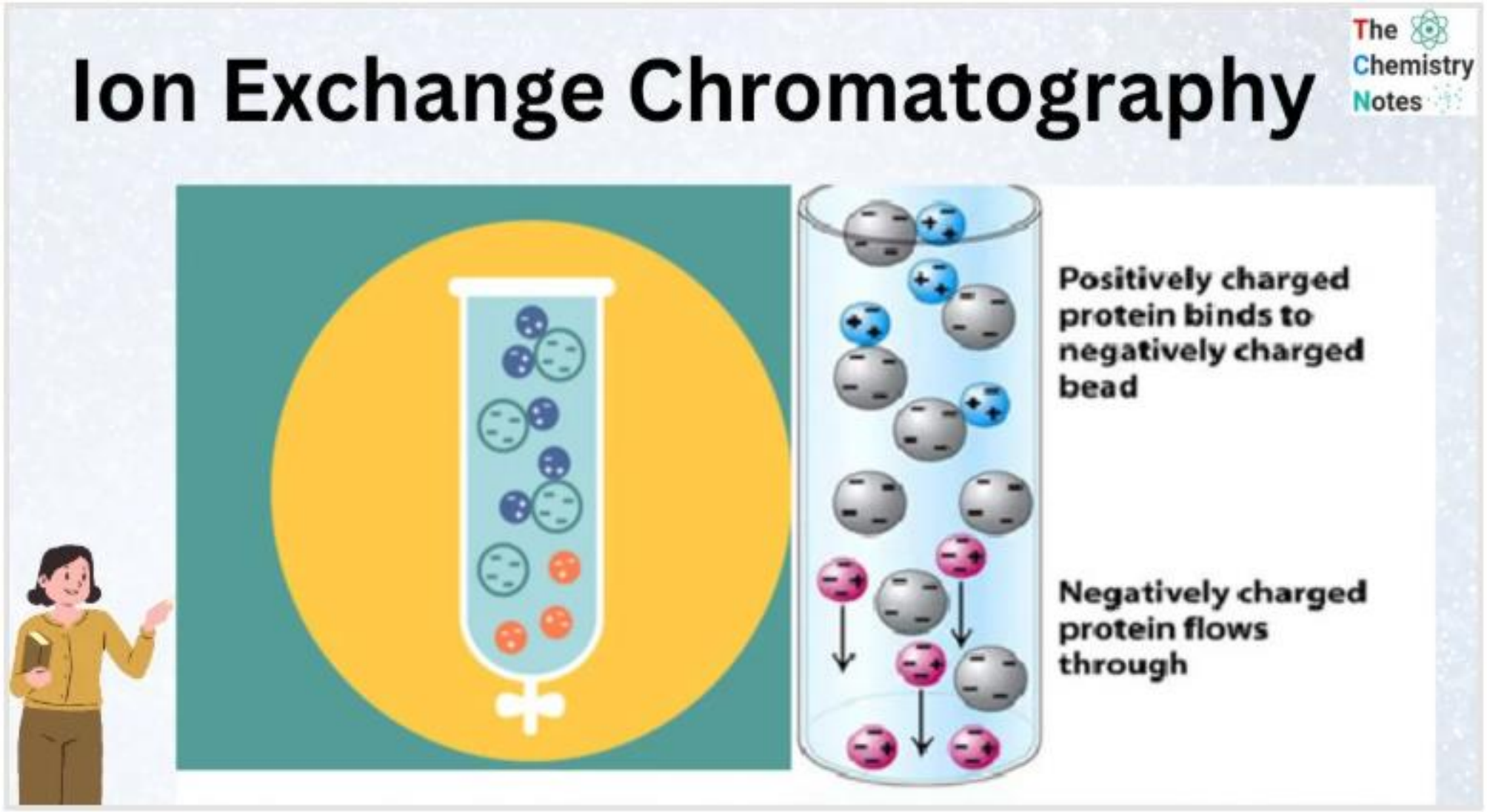
Cold Precipitation: Uses cold temperatures to precipitate proteins .



Protein Purification: Salting Out

B. Ion-exchange chromatography

- Separates proteins **based on their *net surface charge*** at **a given ph**.
- Uses charged matrices:
  - **Anion exchangers** (e.G., Deae-cellulose) bind negatively charged proteins.
  - **Cation exchangers** (e.G., Cm-cellulose) bind positively charged proteins.
- Commonly applied for the purification of serum or monoclonal antibodies.



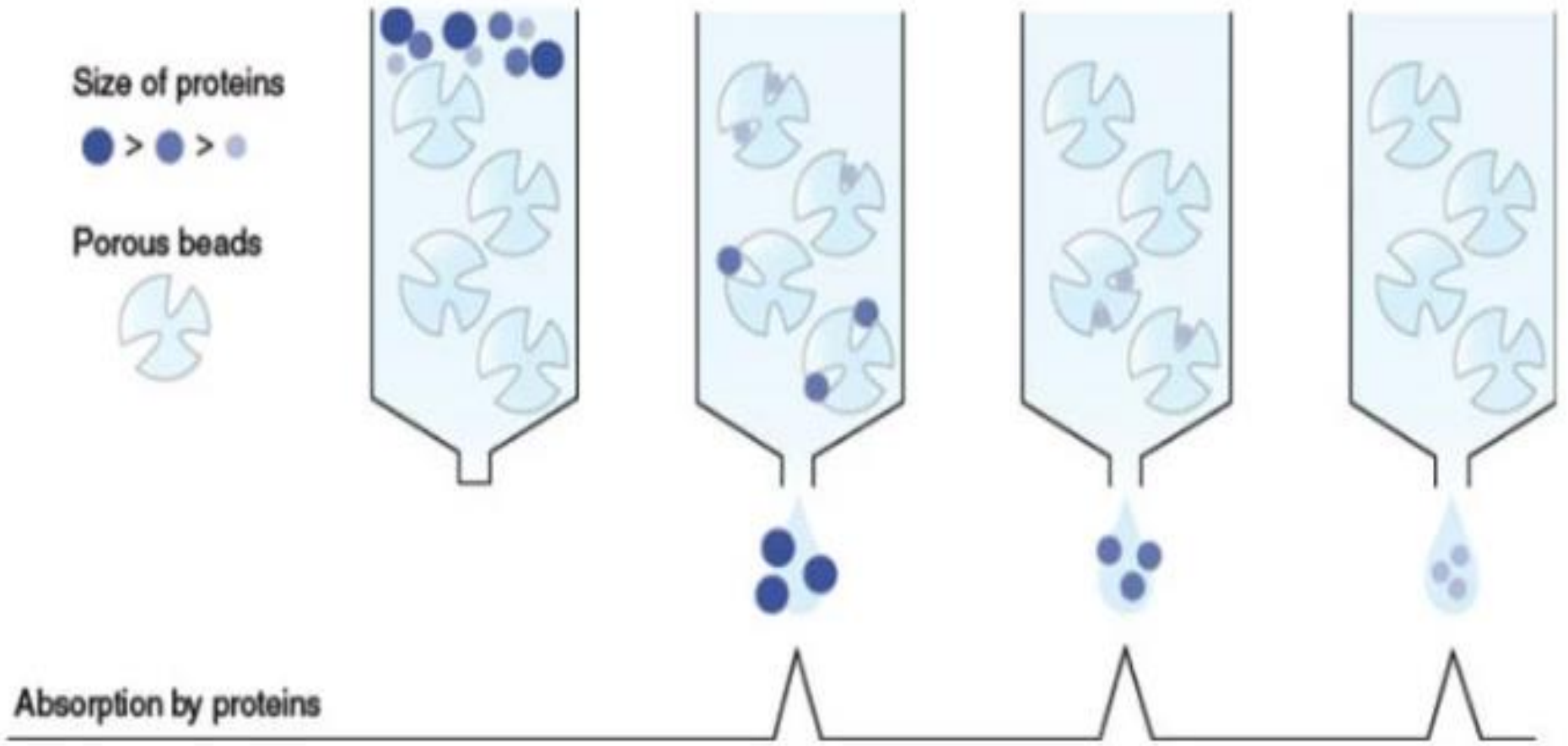
Ion Exchange Chromatography

Gel filtration (size-exclusion chromatography):

•Separates proteins according to **molecular size and shape.**
•A porous matrix allows smaller molecules to enter the pores, while larger molecules elute first.

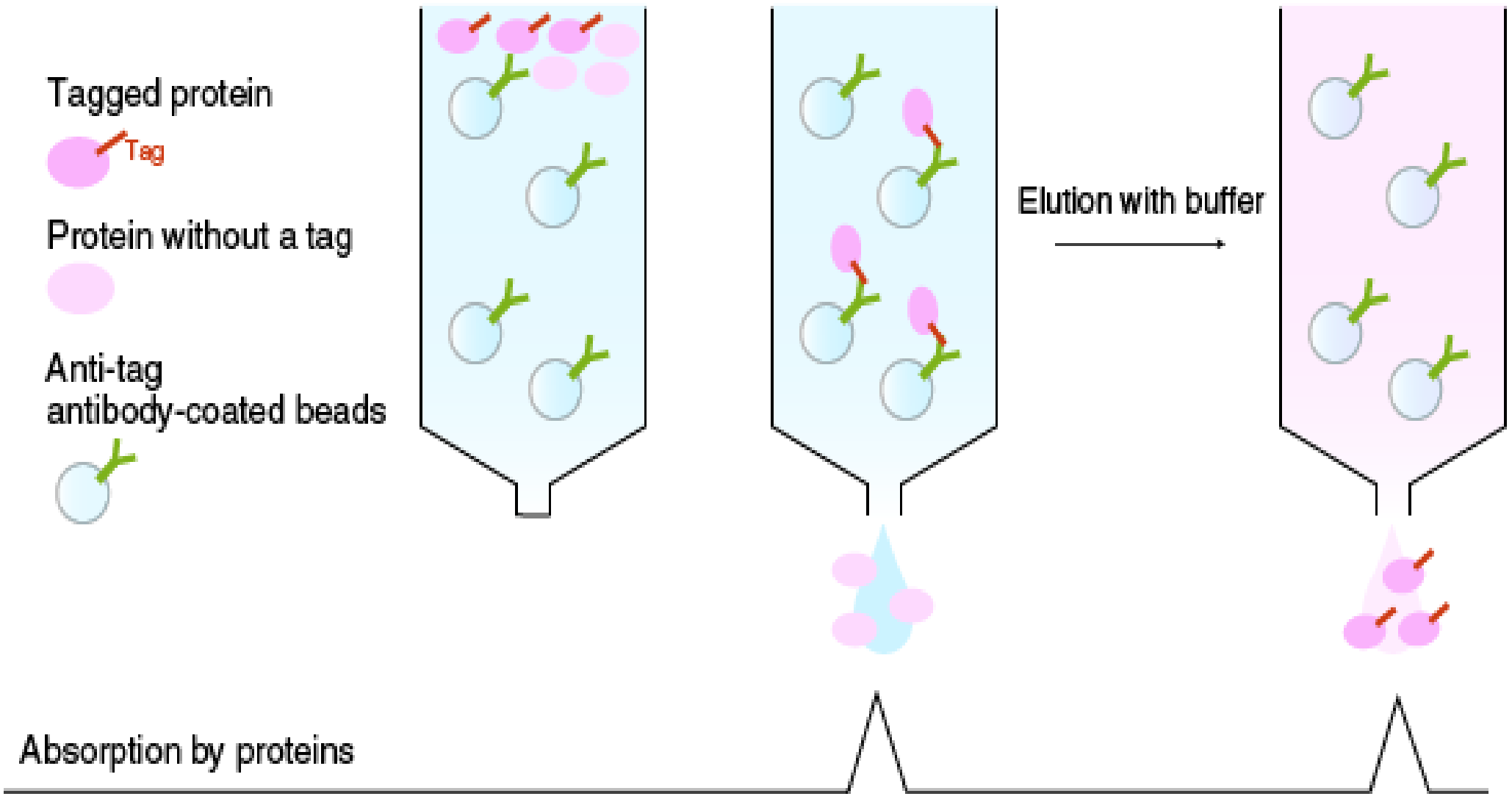
•**Advantages:**
- **Gentle on protein structure.**
- **Allows buffer exchange and desalting**.

Gel Filtration Chromatography



Affinity chromatography

- Relies on **specific biological interactions** (e.G., Antigen–antibody, enzyme–substrate).
- **Ligands** are covalently attached to an inert matrix such as **agarose beads**.
- **Highly selective**—provides large purification gains in a single step.
- Example: protein a or g columns for antibody purification.


Tagged protein
Tag
Protein without a tag
Anti-tag
antibody-coated beads
Elution with buffer
Absorption by proteins

Electrophoresis:

-SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis):

separates

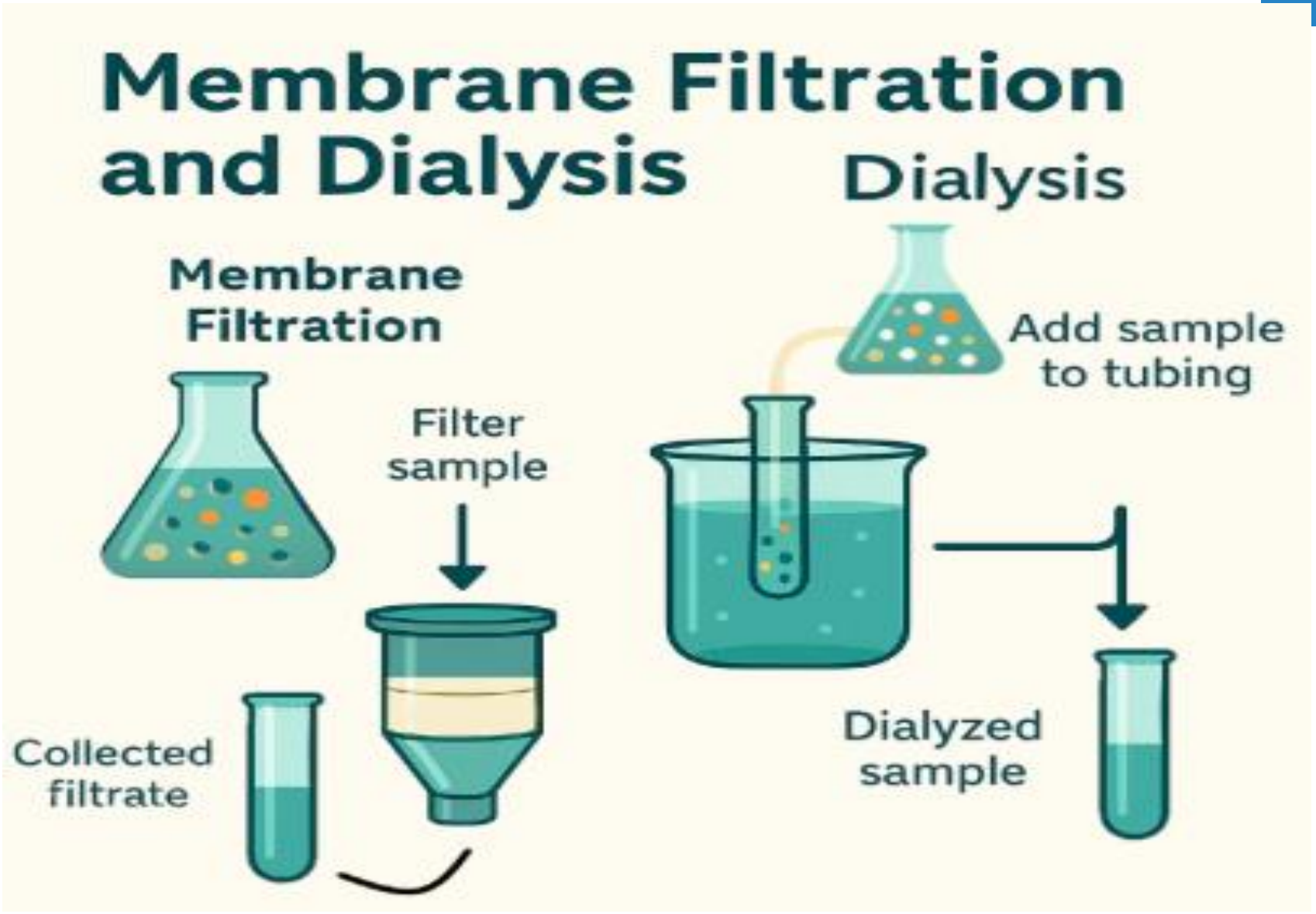
Proteins based on **their molecular weight**

- Capillary electrophoresis: separates proteins based on their **charge-to-mass ratio**

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.


Membrane Filtration and Dialysis
Membrane Filtration
Filter sample
Collected filtrate
Dialysis
Add sample to tubing
Dialyzed sample

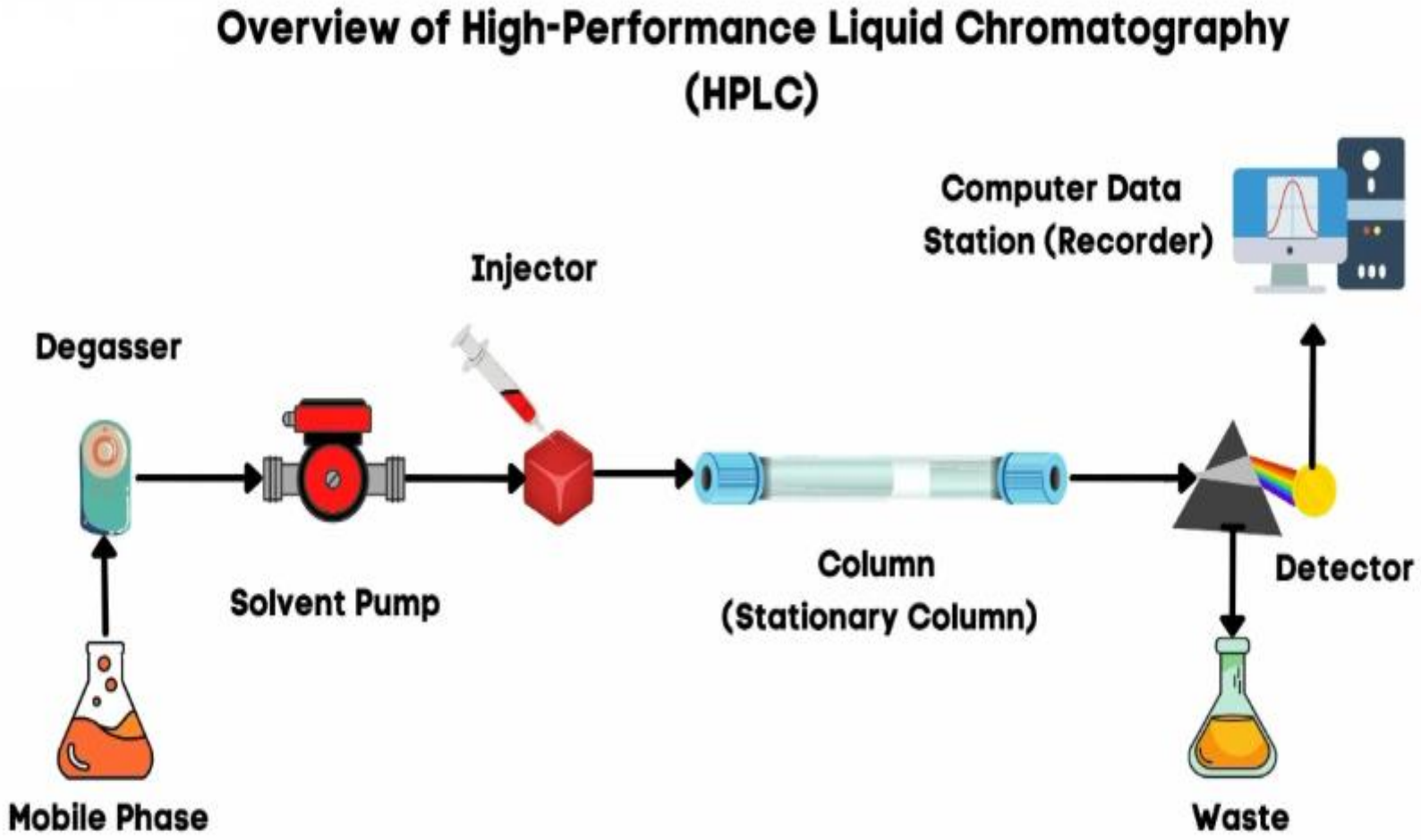
Separation of Amino Acids:

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.

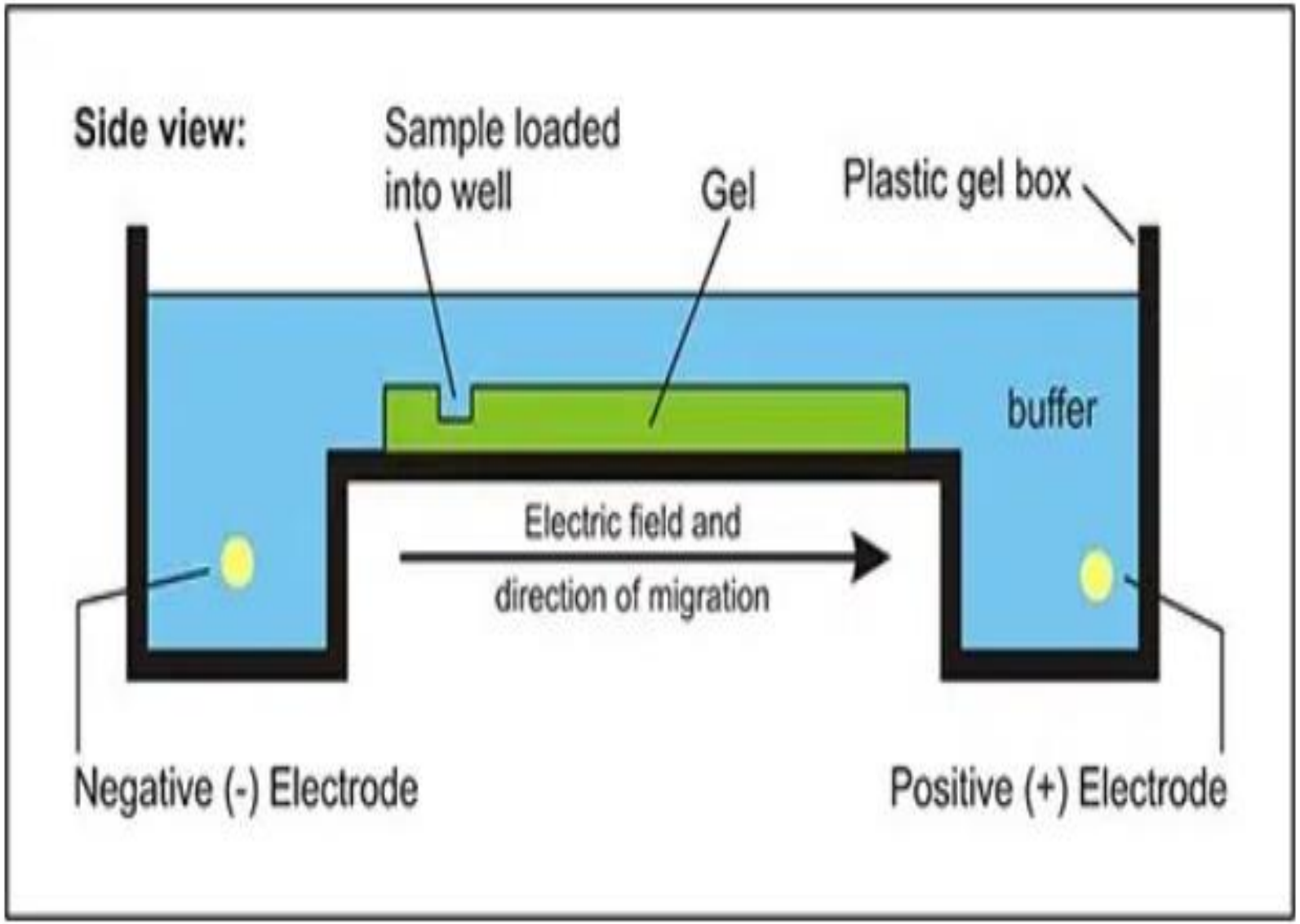


High-Performance Liquid Chromatography (HPLC)Modern, sensitive technique for quantitative separation. Can separate amino acids and peptides within minutes. Uses either ion-exchange or reversed-phase columns with UV detection.

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.


Side view:
Sample loaded into well
Gel
Plastic gel box
buffer
Electric field and direction of migration
Negative (-) Electrode
Positive (+) Electrode

Factors affecting separation efficiency:

- ✓ ph and buffer composition .
- ✓ Temperature .
- ✓ flow rate and particle size of the matrix.
- ✓ sample concentration and volume.

QUIZ:

Why is SDS-PAGE often used after chromatography in protein purification workflows?

ANY QUESTION?

Thank You