PROTEIN EXTRACTION, FRACTIONATION BY SALT AND DIALYSIS

Lab#2

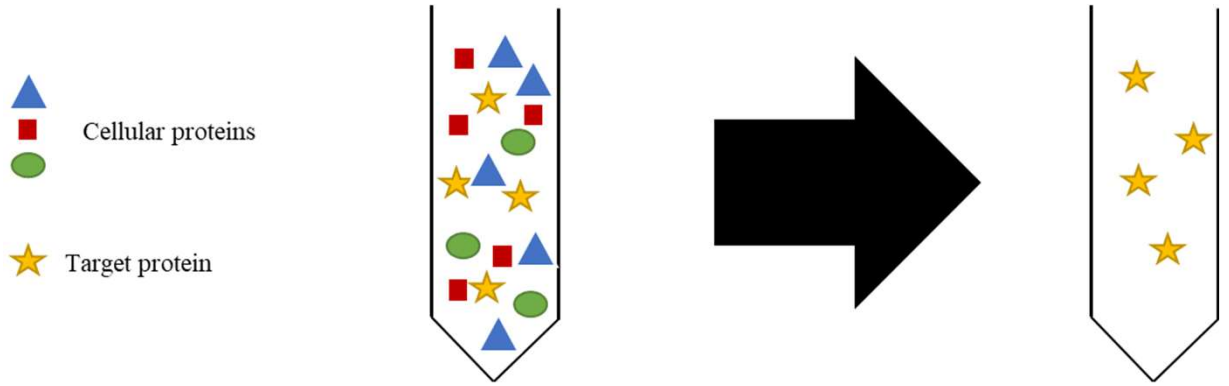
BCH 333

PROTEIN PURIFICATION:

- Protein purification is a series of processes intended to ***isolate*** one or a few proteins from a complex mixture, usually cells, tissues or whole organisms.

- Purification should yield a sample of protein containing only one type of molecule, the protein in which the ***biochemist is interested.***

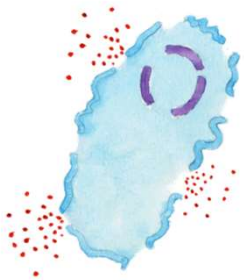
➔How is the biochemist able to isolate a particular protein from a complex mixture of proteins?



ISOLATION OF PROTEINS:

1. *First step is tissue homogenization:*

➔Cell lysis is the first step in cell fractionation, organelle isolation and protein extraction and purification. There are different ***Mechanical*** and ***non-mechanical methods***.



2. *Isolation techniques utilize different properties of proteins:*

- Solubility.
- Charge.
- Size.
- Binding properties (Ligands).

PURIFICATION BASED ON SOLUBILITY:

- Proteins contain various sequences and compositions of amino acids ➔Therefore, their solubility to water differs ***depending on the level of hydrophobic or hydrophilic properties*** of the surface.

- Extrinsic factors that influence protein solubility:

➔ionic strength, pH, temperature.

- Depend on the ionic strength (salt concentration), salts can affect the protein solubility in <u>two ways</u>:

  1. Salting in. "increase the solubility"
  2. Salting out. "decrease the solubility"

SALTING IN:

- When the solubility of proteins ***increased*** in *low* concentration of salts, it called salting in.

- ***Why when low concentrations of salt is added to a protein solution, the solubility increased ?***

➔This could be explained by the following:

Salt molecules stabilize protein molecules by decreasing the electrostatic energy between the protein molecules which increase the solubility of proteins. This occur because the additional ***ions shield the protein's multiple ionic charges***, thereby weakening the attractive forces between individual protein molecules ➔ and thus preventing proteins aggregation and precipitation.

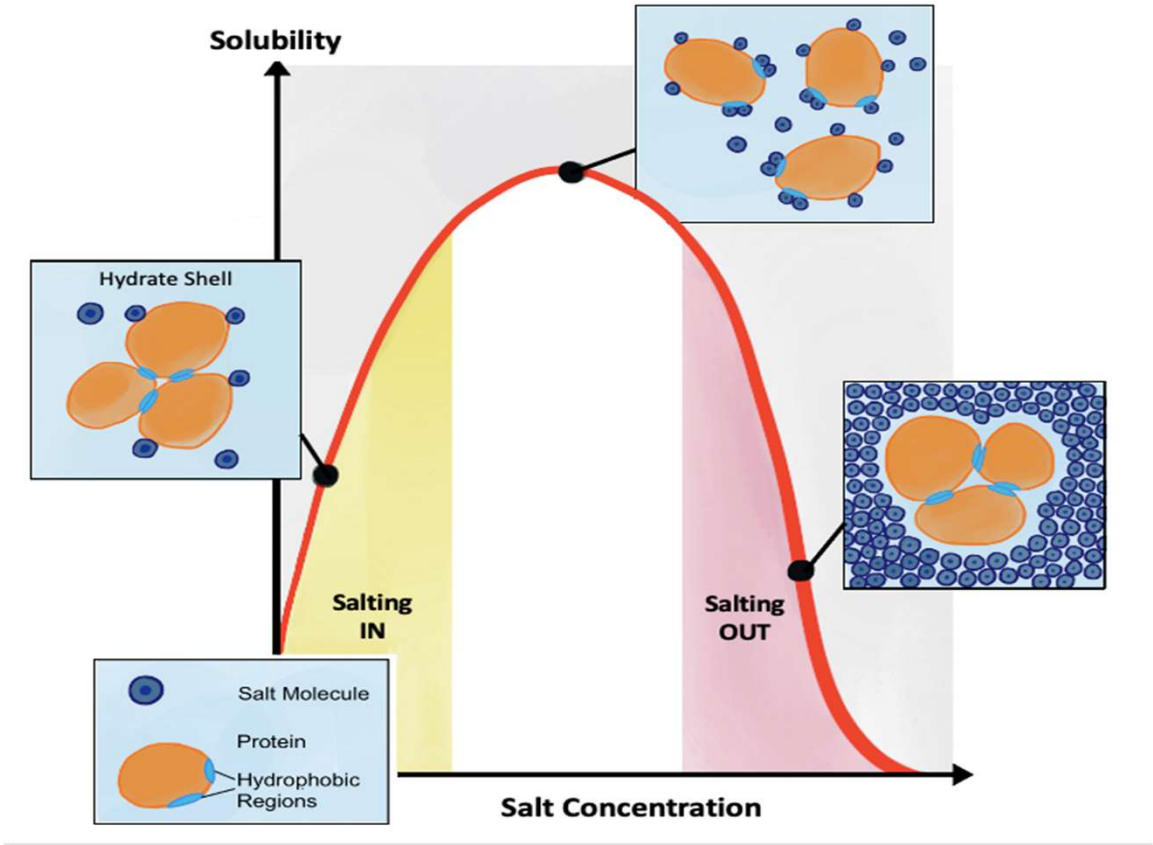
SALTING OUT:

- When the solubility of proteins ***decreased*** in ***high*** concentration of salts, it called salting out.
- It also called Salt-induced precipitation.

- ***Why when high concentrations of salt is added to a protein solution, the solubility decreased and the protein precipitated ?***

➔This could be explained by the following:

The excess salt molecules ***compete*** with the protein molecules in binding with water. In this case, the protein molecules tend to associate with each other because protein-protein interactions become energetically more favourable than protein-solvent interaction.

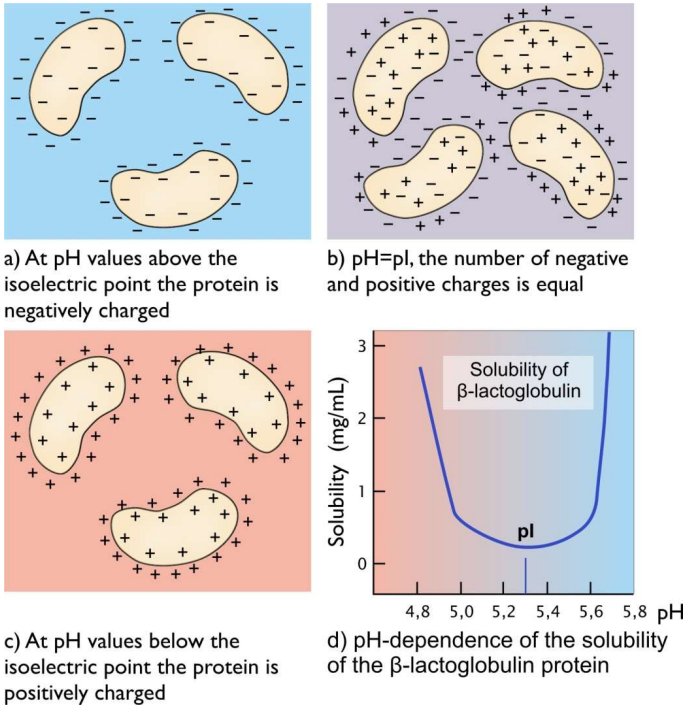


- Increasing solute – solvent interaction →increase the solubility.
  (achieved by low salt concentration)

- Increase solute – solute interaction →decrease the solubility.
  (achieved by high salt concentration)

SALTING OUT CONT':

- The most effective region of salting out is ***at the isoelectric point of the protein***, because all proteins exhibit <u>minimum solubility</u> in solutions of constant ionic strength at their isoelectric points.



a) At pH values above the isoelectric point the protein is negatively charged

b) pH=pI, the number of negative and positive charges is equal

c) At pH values below the isoelectric point the protein is positively charged

d) pH-dependence of the solubility of the β-lactoglobulin protein

PURIFICATION BASED ON SOLUBILITY CONT':

- The purification of proteins by altering the solubility achieved by what called ***salting out.***
- The salt concentration at which a protein precipitates differs from one protein to another
  ➔Hence, salting out can be used to fractionate proteins.
- Salting out is a powerful tool to separate classes of proteins that vary in size, charge, and surface area among other characteristics.
- In fact, salting out is an effective means for ***initial molecule purification***, but lacks the ability for precise isolation of a specific protein.
- In salting-out, proteins are separated after salt addition by ***centrifugation.***

THE TYPE OF SALT USED IN PRECIPITATION :

- The salt commonly used is ***ammonium sulfate*** because:

1. Its large solubility in water.
2. Its relative freedom from temperature effects.
3. It has no harmful effects on most of the proteins.

- The amount of salt needed to isolate a specific protein (at specific saturation percentage) is determined from the ***salt's fractionation table.***

USING SALT'S FRACTIONATION TABLE:

Table 1. Quantities of ammonium sulphate required in (g) to reach given degrees of saturation in one litre of solution.

Initial saturation →

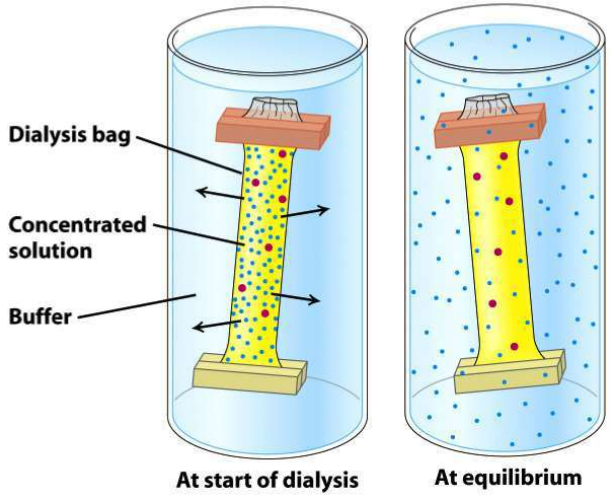
%	10	15	20	25	30	33	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0	56	84	114	144	176	196	209	243	277	313	351	390	430	472	516	561	610	662	713	767
10		28	57	86	118	137	150	183	216	251	288	326	365	406	449	494	540	592	640	694
15			28	57	88	107	120	153	185	220	256	294	333	373	415	459	506	556	605	657
20				29	59	78	91	123	155	189	225	262	300	340	382	424	471	520	569	619
25					30	49	61	93	125	158	193	230	267	307	348	390	436	485	533	583
30						19	30	62	94	127	162	198	235	273	314	356	401	449	496	546
33							12	43	74	107	142	177	214	252	292	333	378	426	472	522
35								31	63	94	129	164	200	238	278	319	364	411	457	506
40									31	63	97	132	168	205	245	285	328	375	420	469
45										32	65	99	134	171	210	250	293	339	383	431
50											33	66	101	137	176	214	256	302	345	392
55												33	67	103	141	179	220	264	307	353
60													34	69	105	143	183	227	269	314
65														34	70	107	147	190	232	275
70															35	72	110	153	194	237
75																36	74	115	155	198
80																	38	77	117	157
85																		39	77	118
90																			38	77
95																				39

← Final saturation

….. How to remove the added salts?

DIALYSIS:

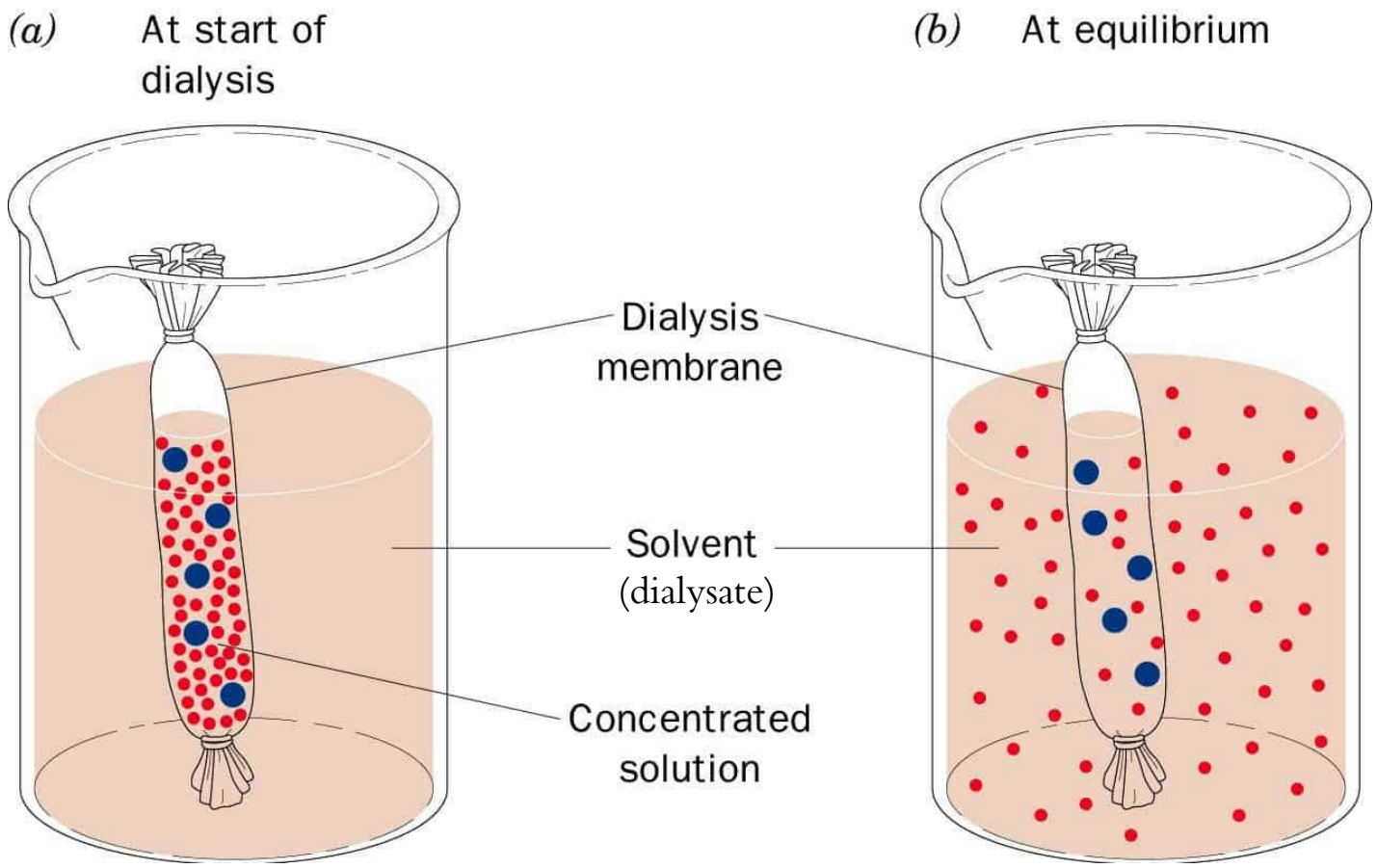
- Following fractionation by ammonium sulphate, dialysis is applied to remove salts.
- Removal of salt molecules from the isolated protein solution through a semi permeable bag is called ***dialysis.***
- During dialysis, the small, unwanted salts ions removed from proteins in a solution by ***selective and passive diffusion through a semi-permeable membrane.***
- The separation based on the molecules size.



DIALYSIS CONT':

- Sample molecules (proteins) that are larger than the membrane-pores are ***retained*** on the sample side of the membrane, but small molecules and buffer salts ***pass freely*** through the membrane.
- The salt molecules move from the more concentrated solution (from inside the dialysis bag) to the less concentrated solution (e.g. buffer or water).
- The movement of the salt molecules will stop when the solution reaches the equilibrium.
  ➔At this point, the buffer (dialysate) is changed to drive the diffusion and salts movements

*PAUSE AND THINK ➔ What if you did not change the buffer? Why?

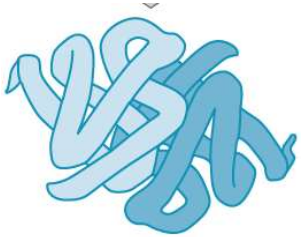

(a) At start of dialysis
(b) At equilibrium
Dialysis membrane
Solvent (dialysate)
Concentrated solution

Practical part

OBJECTIVES:

- Preparation of animal crude extract.
- Fractionation of animal crude extract by ammonium sulphate to isolate LDH.
- Removing of salts ions using dialysis.

Whole tissue



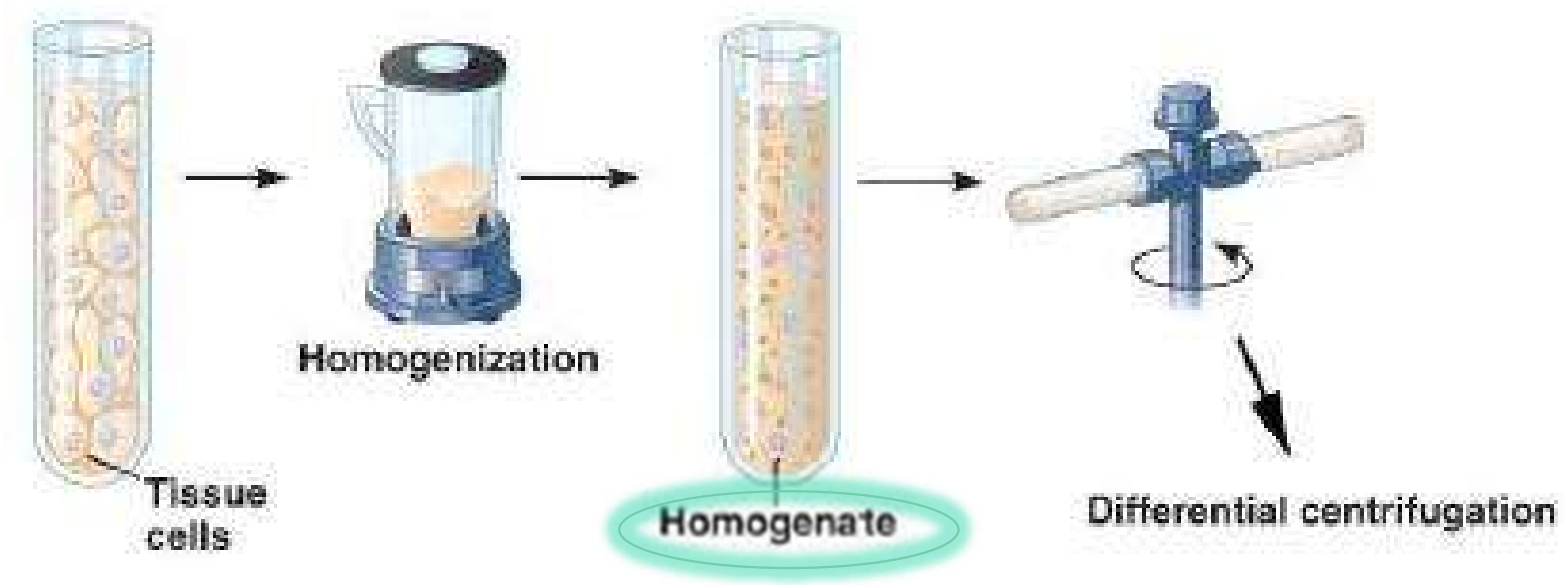
Protein of interest
(Target protein)

A series of processes to remove other unwanted proteins and components

(Protein can't be isolated by only one step)

1
PREPARATION OF CRUDE EXTRACT

Tissue homogenization and preparation of crude extract



- Here, the tissue is cut into small pieces and blended, in the presence of buffer to disrupt the tissue, and then ***centrifuged to remove debris.***

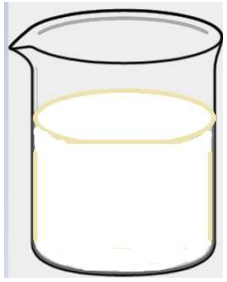
Lactate dehydrogenase (LDH) extraction from animal source

Skeletal muscle

Buffer with suitable pH

(1) Preparation of homogenate

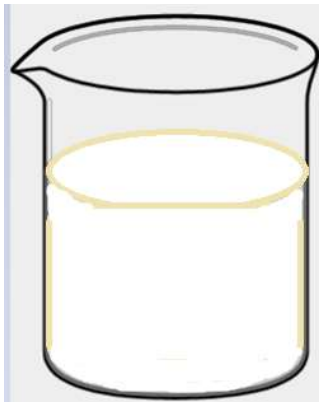
- Cell disrupting will be achieved using mechanical method, the animal cells are susceptible to shear forces using blenders.



Homogenate

(2)
Preparation of crude extract

- Centrifugation to remove cells debris.



Homogenate

Centrifuge at 4000 rpm
for 5 min. at 4°C.

Supernatant

Crude extract

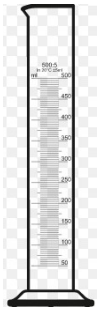
(LDH + Other proteins)

Pellet

(Cell debris)

2
Fractionation by ammonium sulphate to isolate LDH and dialysis

Supernatant
Crude extract
(LDH + Other proteins)



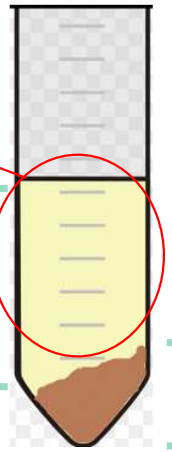
The volume of the supernatant

(1)
Saturation at 40%

- LDH precipitated at 60%, but the solution first will be saturated 40% to remove unwanted proteins.

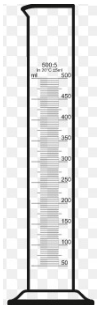
Saturate the solution 40%
using ammonium sulfate in grams.

Centrifugation



Supernatant (40% sat.)
(LDH + Other proteins)

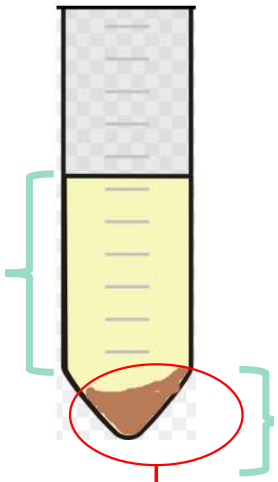
Pellet
(unwanted proteins
that precipitated at 40%)



The volume of the supernatant

Saturate the solution 60%
using ammonium sulfate in grams.

Centrifugation



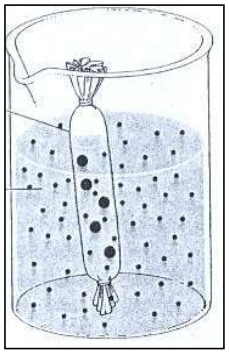
Supernatant (60% sat.)
(unwanted proteins)

Pellet
(LDH + other proteins that precipitate at 60%)

(2)
Saturation at 60%

- To precipitated the targeted protein (LDH).

Dialysis



(3)
Dialysis

- To remove the salt ions.

Next Lab:
Determination of protein concentration.