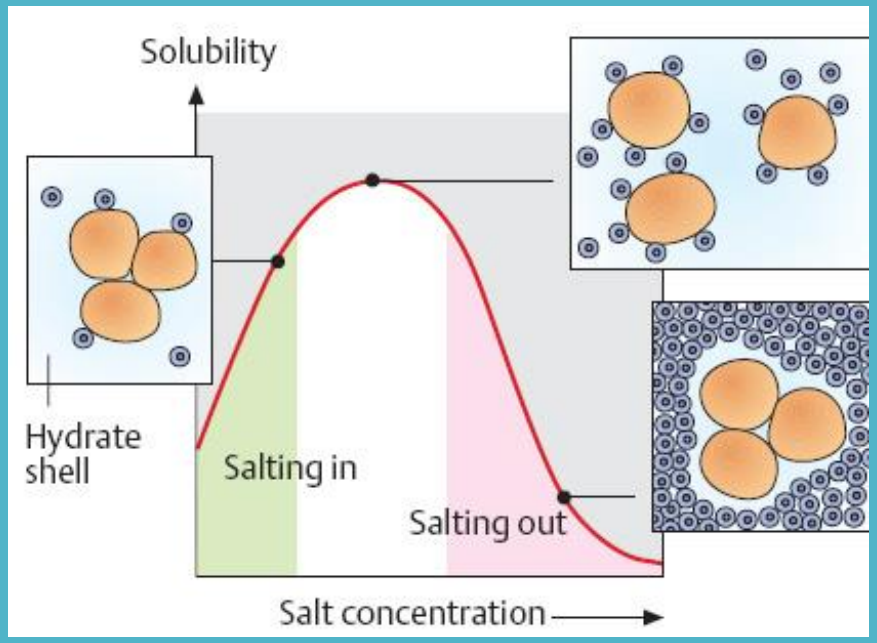
Salting in and salting out of proteins and dialysis

Experiment 3



objectives

To learn one of the technique for protein isolation on the basis of their solubility. This experiment consists of two parts

Part I: salting in, salting out of proteins and dialysis of proteins.

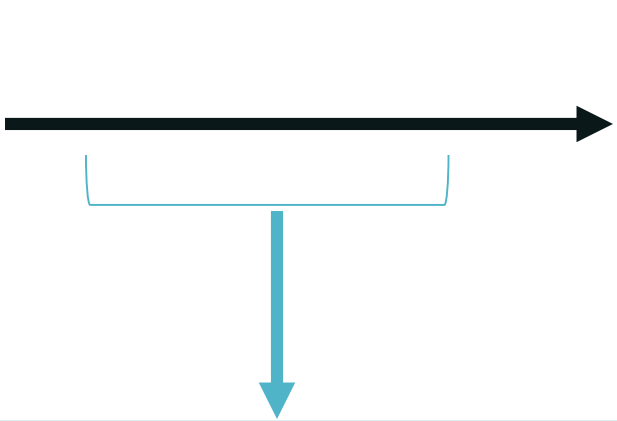
Part II: Determination of protein content by biuret assay

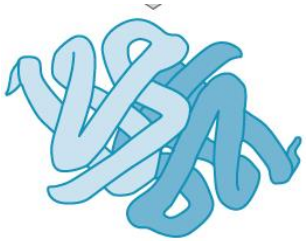
In this lab you will try to isolate Lactate Dehydrogenase from a skeletal muscle of chicken.

Protein Isolation and 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.

Whole Tissue





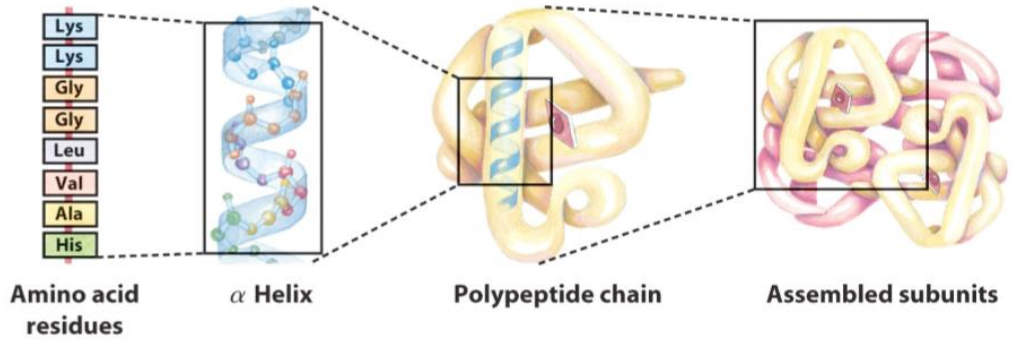
Protein to be studied

a series of processes to remove other unwanted proteins and components (**Protein can not be isolated by only one step only**)

Isolation of proteins

1. First Step is tissue homogenization
2. Isolation techniques utilize different properties of proteins:

- Solubility (salt, pH, temperature)
- Charge
- Size
- Binding properties (Ligands)



Protein precipitation by salt

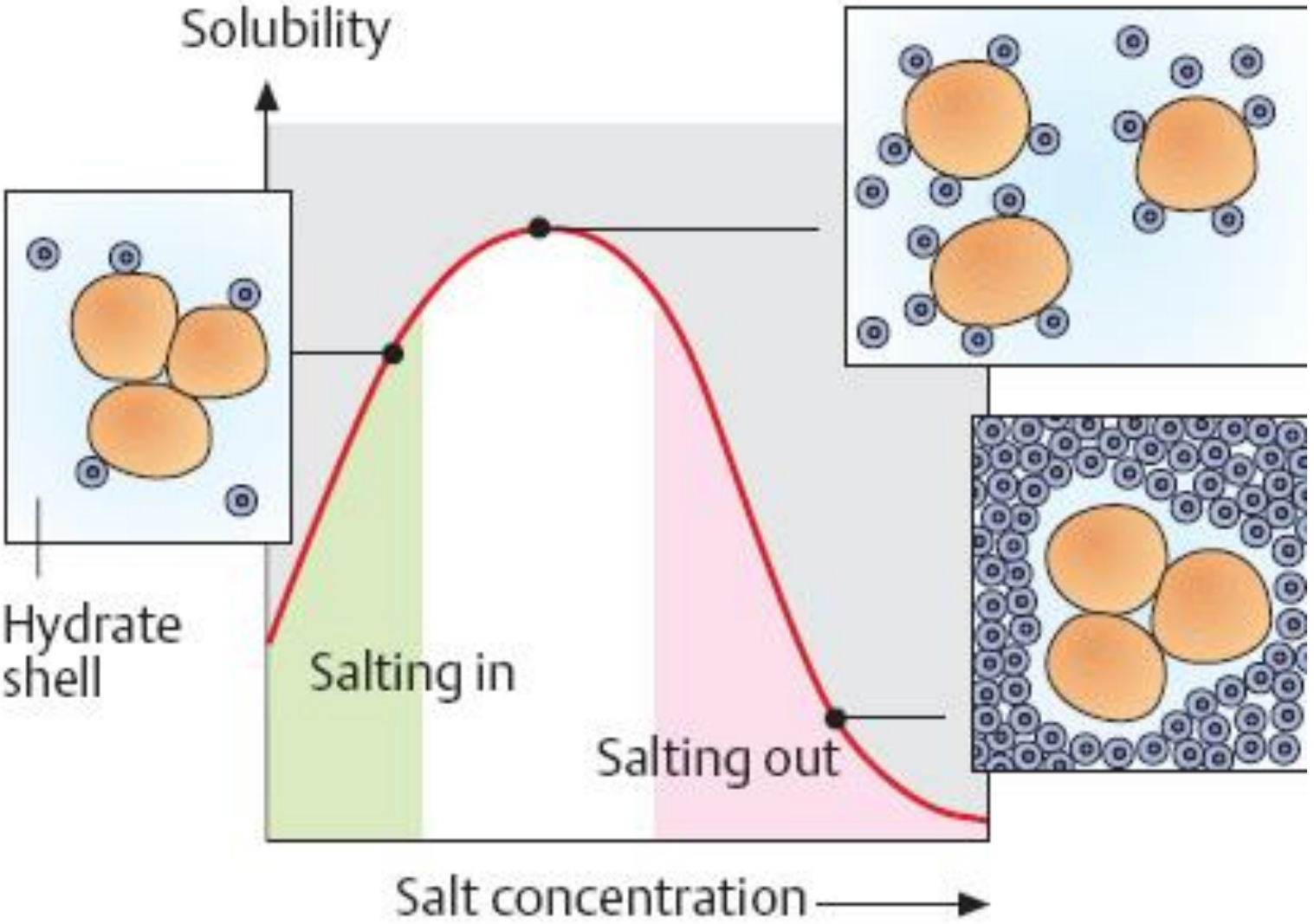
- Proteins show a variation in solubility depending on the ionic environment of their solution . (salts)

increased salt concentration → Increase ionic strength

- When low concentrations of salt is added to a protein solution the solubility increases .

- Beyond a certain point after continuing addition of salt, the protein solubility start to decrease leading to exclusion of protein out of the solution in the form of precipitate.

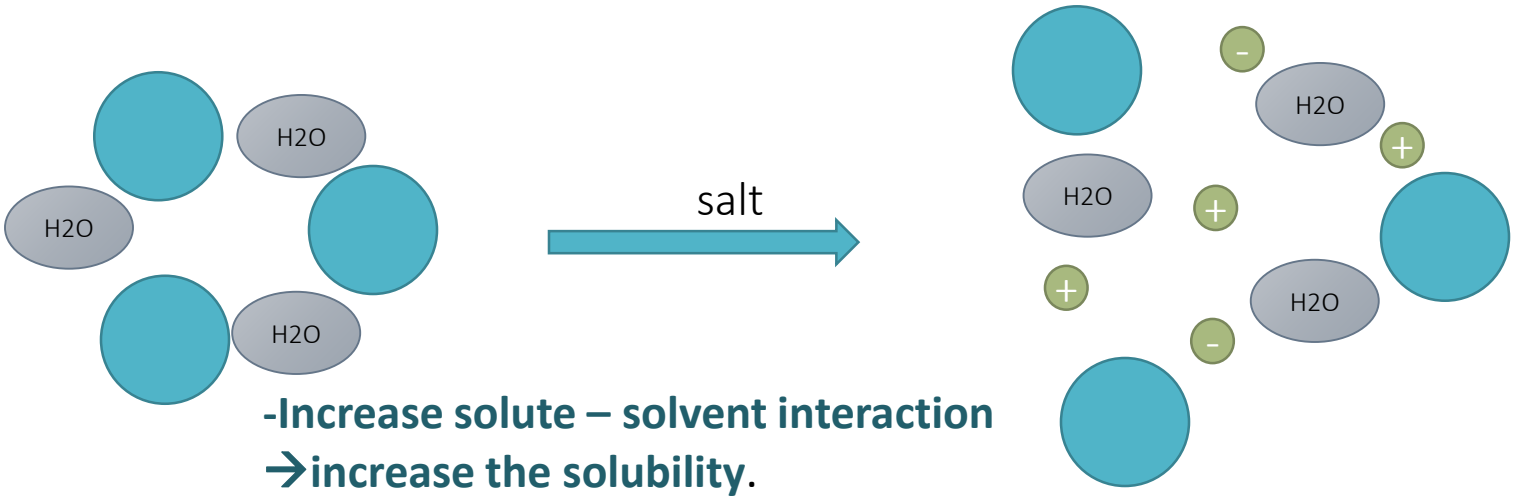
- The point where the precipitation start is different between different protein


Solubility
Hydrate shell
Salting in
Salting out
Salt concentration

Salting In

- Low concentrations of salt⟶ the solubility increases. 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.



-Increase solute – solvent interaction
→increase the solubility.

Salting out

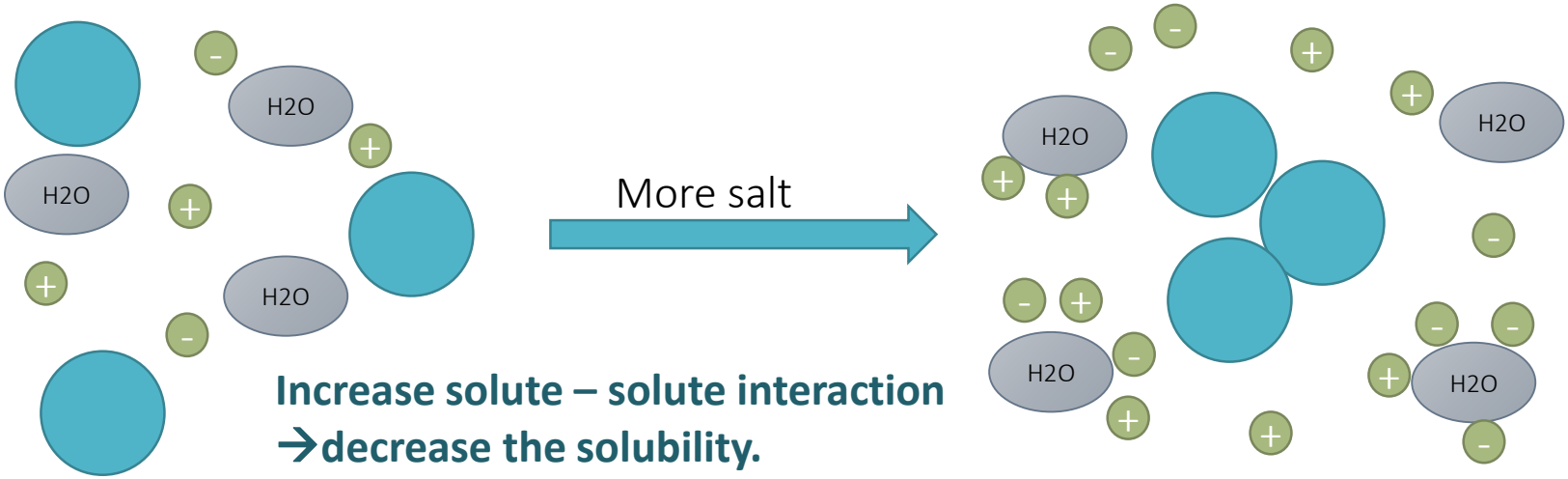
High concentration of salts ⟶ the solubility decreases, and protein precipitates.

This could be explained by the following:

1. because the excess ions (not bound to the protein) compete with proteins for the solvent.

2. The decrease in solvation allows the proteins to aggregate and precipitate . **The protein molecules tend to associate with each other because protein-protein interactions become energetically more favorable than protein-solvent interaction.**

Salting out



Increase solute – solute interaction
→decrease the solubility.

Salting out and protein isolation and purification

- Salting out with ammonium sulfate is a technique that is used as an **early step** in purification scheme.

- **Significant purification is not achieved**, but broad ammonium sulfate cut at least a volume of unwanted proteins.

- Different protein molecules precipitate at different concentrations of salt solution because different proteins have different compositions of amino acids
- **Example**: Some proteins will precipitate at 50% saturation with ammonium sulphate

- The amount of salt needed to saturate the solution with ammonium sulphate is determined from the salt's fractionation table.

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Note: that the table indicate the grams of the salts to be added to one liter of solution.

%	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

- Unwanted proteins can be removed from a protein solution mixture by salting out

- After removing the precipitate by filtration or centrifugation, the desired protein can be precipitated by altering the salt concentration to the level at which the desired protein becomes insoluble.

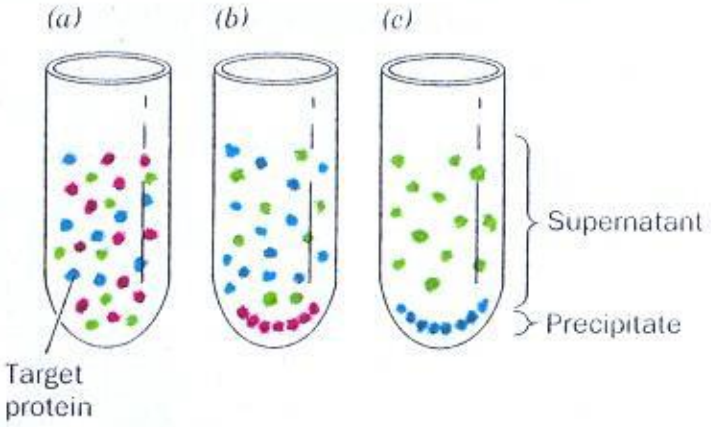


Figure 5-5 Fractionation by salting out. (*a*) The salt of choice, usually ammonium sulfate, is added to a solution of macromolecules to a concentration just below the precipitation point of the protein of interest. (*b*) After centrifugation, the unwanted precipitated proteins (*red spheres*) are discarded and more salt is added to the supernatant to a concentration sufficient to salt out the desired protein (*green spheres*). (*c*) After a second centrifugation, the protein is recovered as a precipitate, and the supernatant is discarded.

Ammonium sulfate

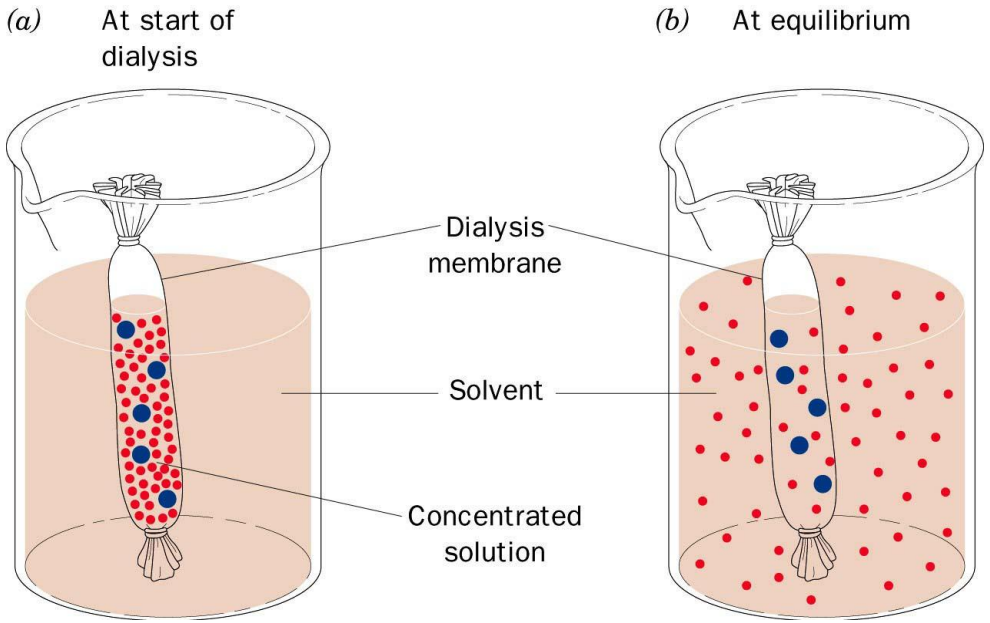
The salt commonly used is ammonium sulfate because:

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

Dialysis

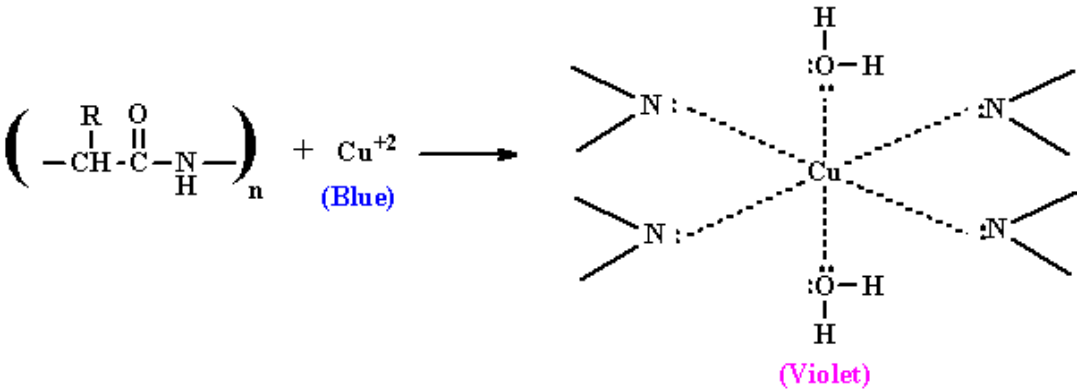
- Removal of salt molecules from the isolated protein solution through a semi permeable dialysis bag

- The salt molecules move from the more concentrated solution (from inside the dialysis bag) to the less concentrated solution (e.g. distilled water).



Biuret assay of protein

The biuret reagent is: alkaline copper sulphate.



This colored complex can be measured quantitatively by a spectrophotometer in the visible region.

The color obtained is directly proportional to the number of peptide bonds present in the protein.

The assay is called Biuret because the reaction is positive with the biuret reagent. In this experiment the amount of isolated protein from the skeletal muscle is determined by the biuret assay and from the standard curve of bovine serum albumin (BSA).

Practical part

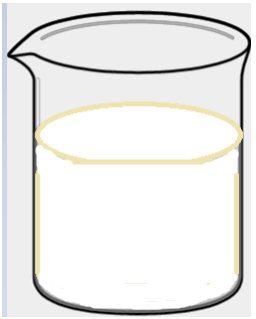
Note:
Lactic Acid Dehydrogenase [LDH], in an important enzyme in the anaerobic metabolism of glucose for the generation of ATP.

$$\text{Pyruvate} + \text{NADH} \xrightarrow[\text{LDH}]{} \text{Lactic acid} + \text{NAD}^{+}$$

A- Isolation of LDH:

Skeletal muscle

Buffer with suitable pH



Crude extract

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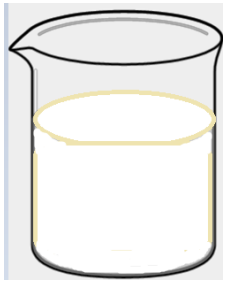
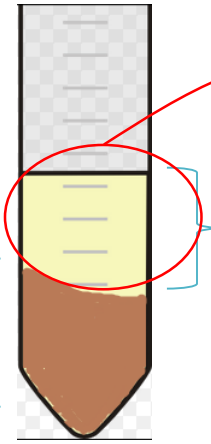
Homogenate



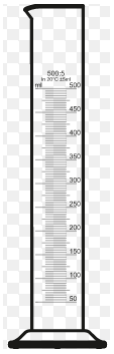
Crude extract

Centrifuge at 2000 rpm
for 10 min. at 4˚C.



Supernatant
(LDH + Other proteins)

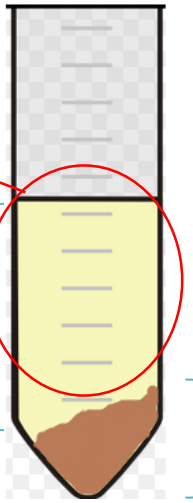
Pellet
(Extraneous proteins)



The volume of the supernatant

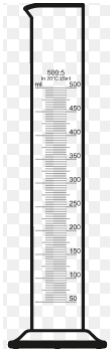
saturate the solution 40%
using ammonium sulfate in grams.

Centrifugation



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

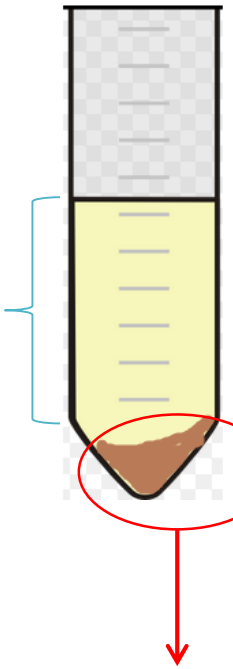
Pellet
(unwanted proteins)



The volume of the supernatant

saturate the solution 60%
using ammonium sulfate in grams.

Centrifugation



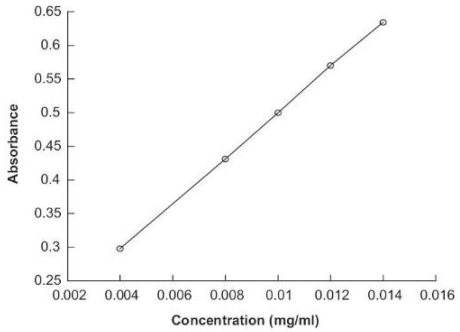
Supernatant (60% sat.)
(unwanted proteins)

Pellet
(LDH + other proteins)

B-Dialysis

C-Protein assay:

Determination of protein by Biuret Method



References

- Biochemical Methods