Lab# 3

Salting in and Salting out of proteins and Dialysis

BCH 333 [PRACTICAL]

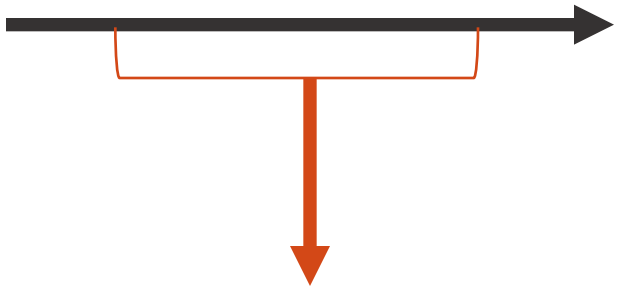
Objectives

1. Salting in and salting out of proteins.
2. To learn the technique of isolation of proteins on the basis of their solubility (salting out).
3. Dialysis of proteins.
4. Determination of protein content by biuret assay.

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.

Whole Tissue



Protein of interest.

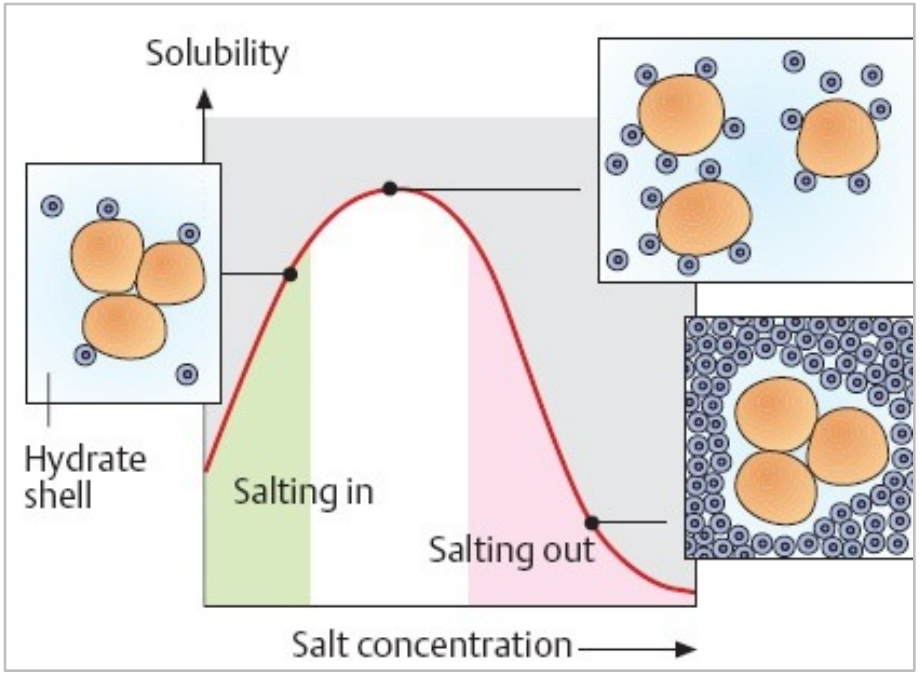
a series of processes to remove other unwanted proteins and components (**Protein can not be isolated by only one step**)

Protein Purification

1. **First Step is tissue homogenization.**

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

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



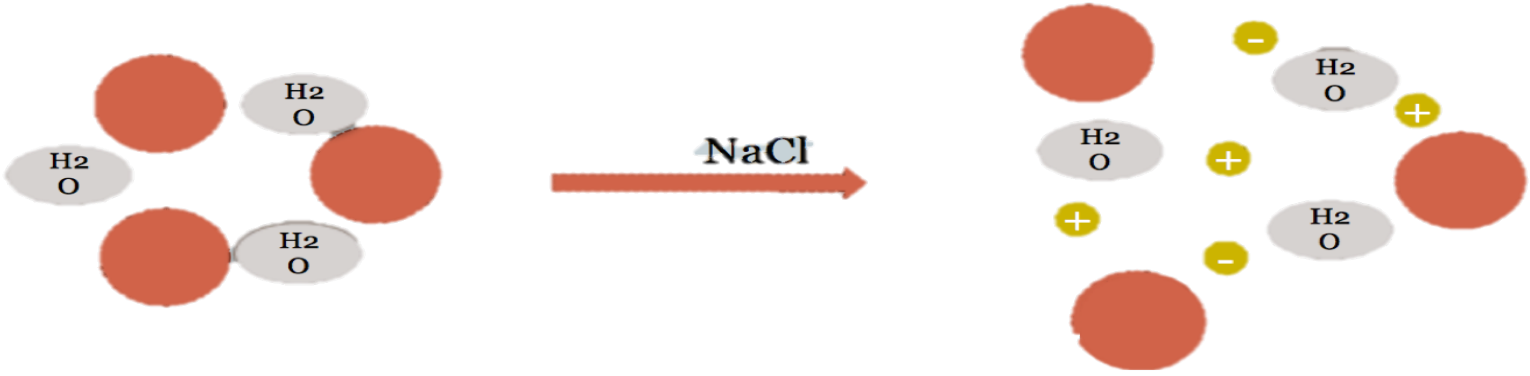
Salting in

Salting in: Refers to the increase of proteins solubility in a solution with low salts concentration.

Low salts concentrations → the solubility of the protein 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.



e.g: the effects of salts such as sodium chloride on increasing the solubility of proteins is often referred to as **salting in**.

Salting out

Salting out; Refers to the precipitation of the proteins at high salts concentration. It is a purification method that relies on the basis of protein solubility [reducing the solubility].

High salts concentrations → increase the ionic strength of a protein solution → decreases the protein solubility thus precipitation.

This could be explained by the following:

The 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 favorable than protein-solvent interaction.

Salting out

- Proteins have characteristic salting out points, and these are used in protein separations in crude extracts.
- Salting out, is a purification method at initial molecule purification its lacks the ability for precise isolation of a specific protein.
- Powerful tool to separate classes of proteins that vary in size, charge, and surface area among other characteristics.
- The proteins are separated after salt addition by centrifugation.

Notes

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

- Increase solute – solvent interaction → increase the solubility.
- Increase solute – solute interaction → decrease the solubility.
- Increase ionic strength → increased salt concentration.

Notes

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 is determined from the salt's fractionation table.

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

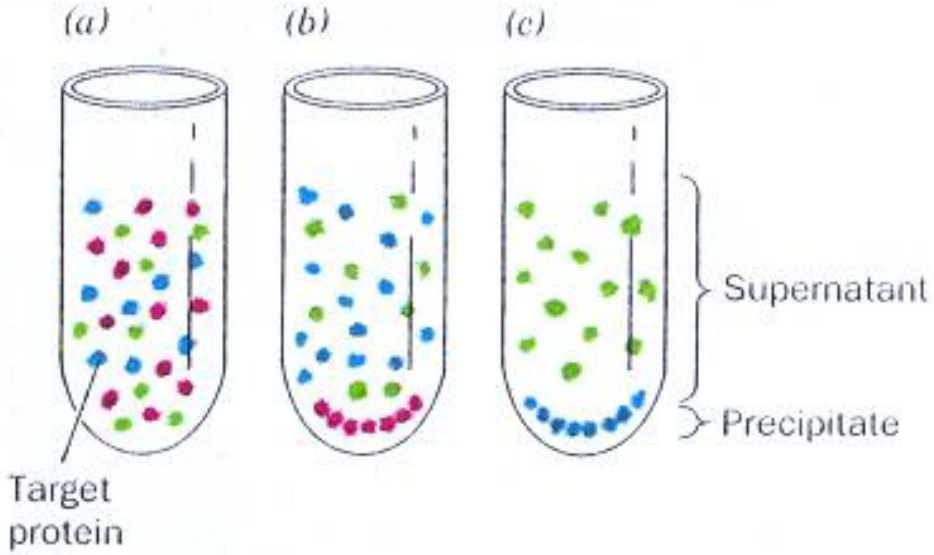


Figure 5-5 Fractionation by salting out. (*a*) The salt of choice, usually ammonium sulfate, is added to a solution of macromolecules to a concentration just below the precipitation point of the protein of interest. (*b*) After centrifugation, the unwanted precipitated proteins (*red spheres*) are discarded 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.

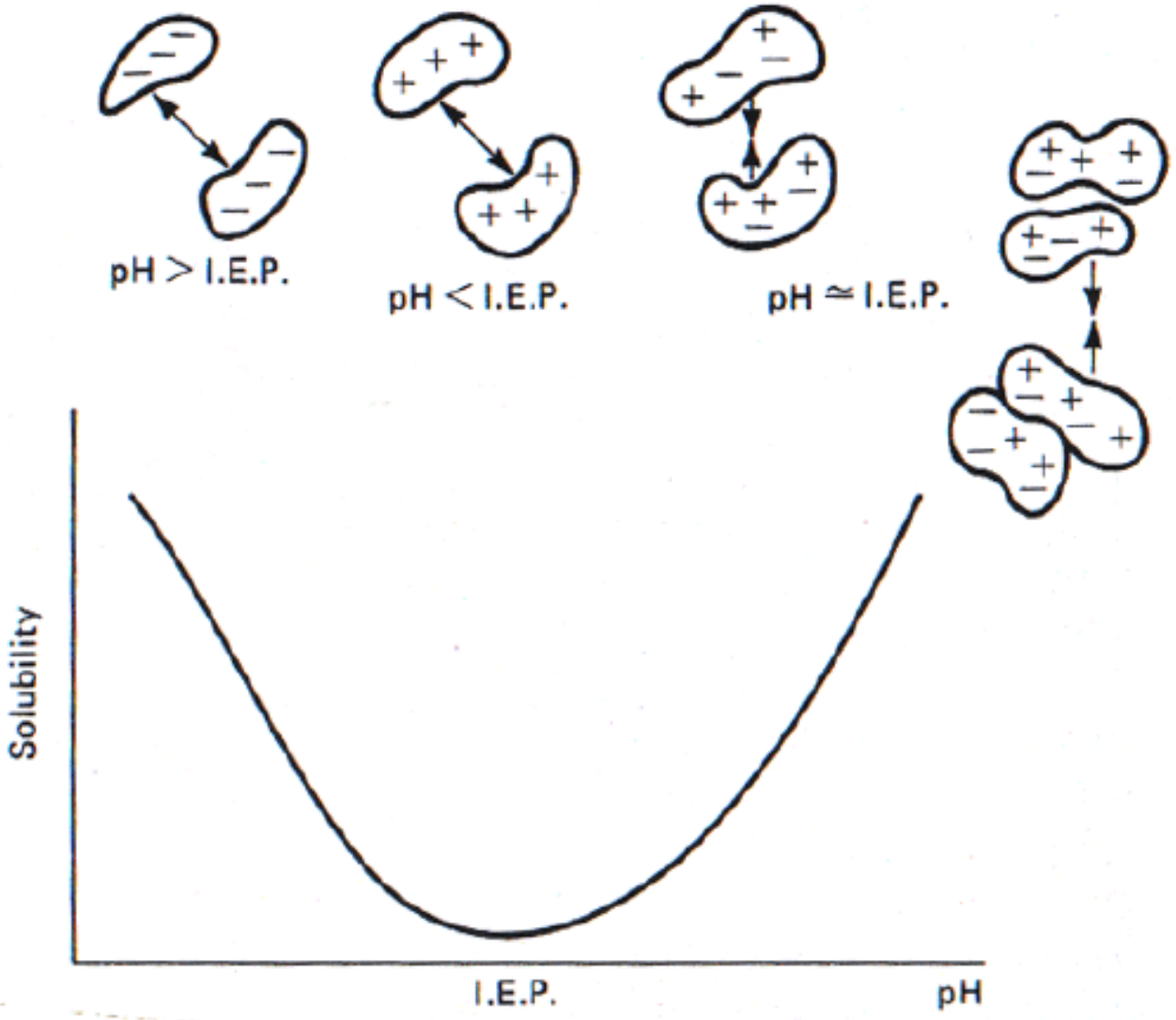
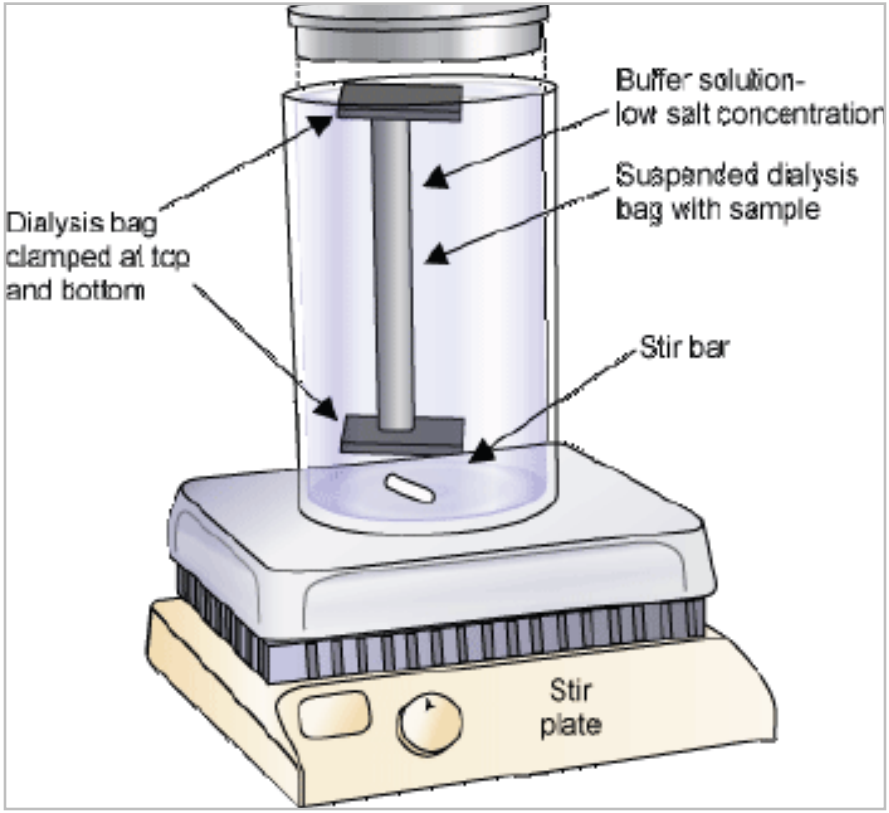


Figure 4.3. Solubility of a globulin-type protein close to its isoelectric point (IEP).

Dialysis

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

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



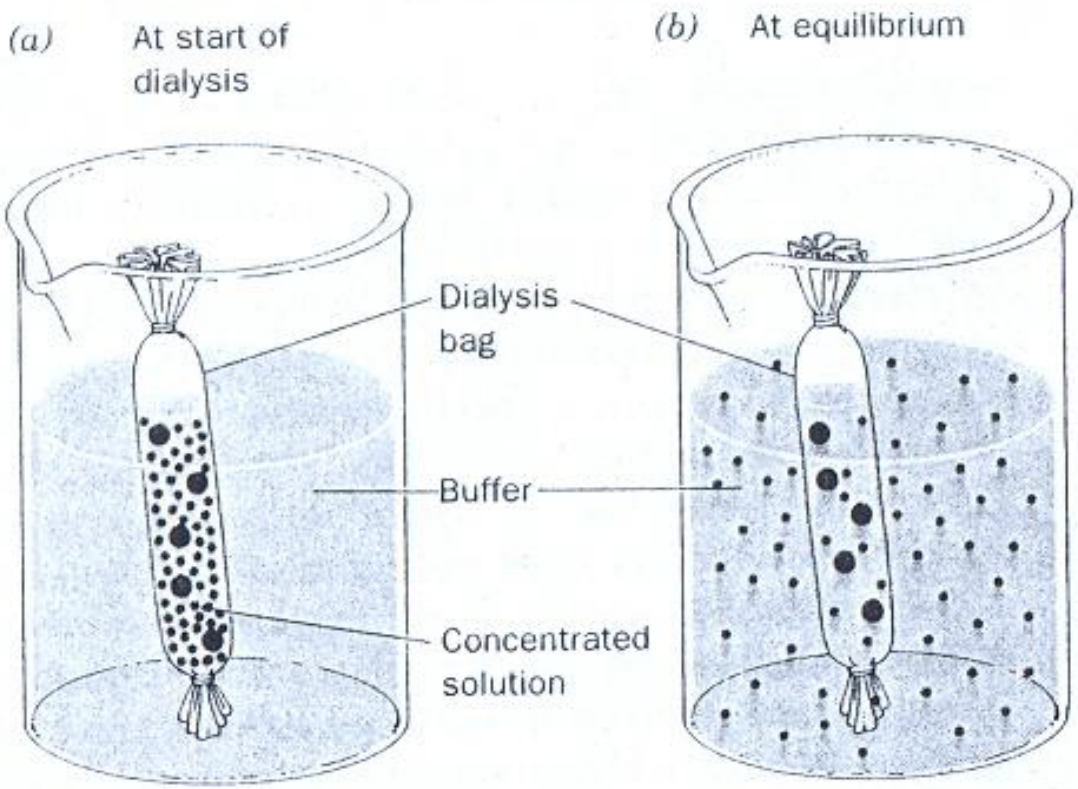
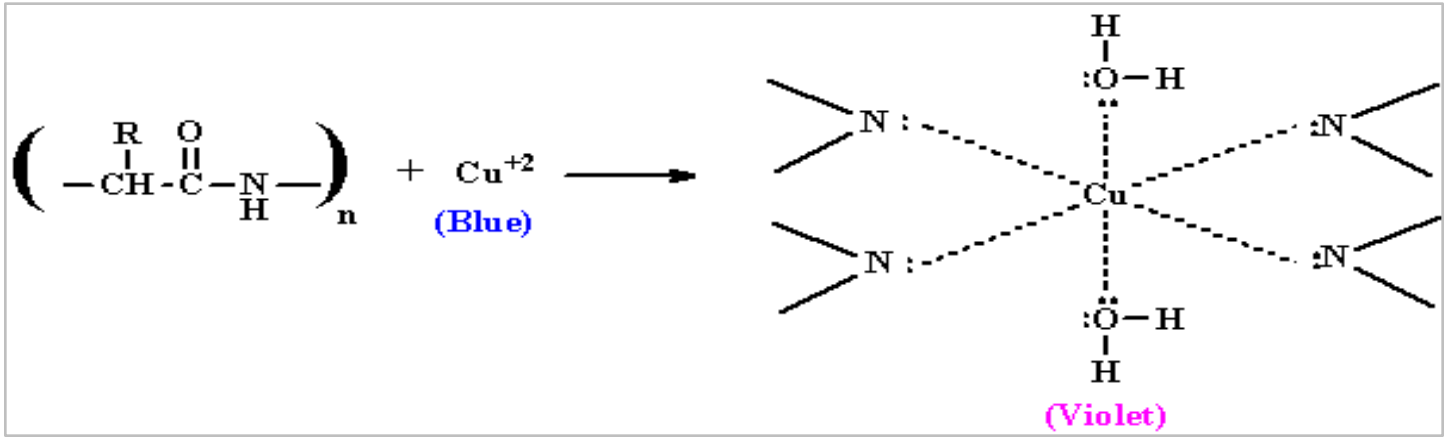


Figure 5-14
The separation of small and large molecules by dialysis. (*a*) Only small molecules can diffuse through the pores in the bag. (*b*) At equilibrium the concentrations of small molecules are nearly the same inside and outside the bag, whereas the macromolecules remain in the bag.

Biuret assay of protein

- The biuret reagent is: alkaline copper sulphate.
- The biuret reagent reacts with peptides and proteins to give a purple colored Cu2+ - peptide complex.
- 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.
- 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

A) Isolation of LDH.
B) Dialysis.
C) Protein assay.

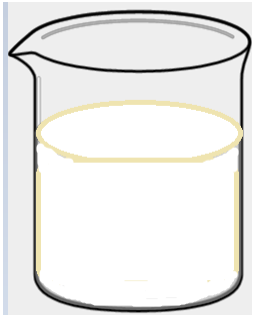
Note:
Lactic Acid Dehydrogenase [LDH], in an important enzyme in the anaerobic metabolism of glucose for the generation of ATP.

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

A- Isolation of LDH:

Skeletal muscle

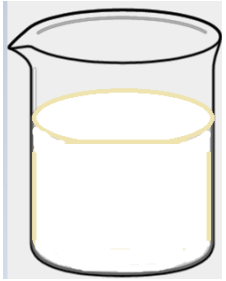
Buffer with suitable pH



Crude extract

????

Homogenate

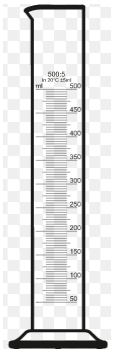


Crude extract

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

Supernatant
(LDH + Other proteins)

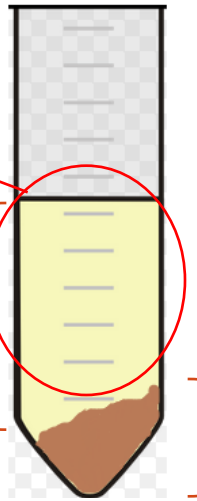
Pellet
(Extraneous proteins)



The volume of the supernatant

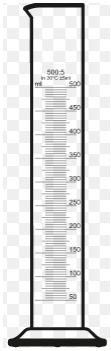
saturate the solution 40%
using ammonium sulfate in grams.

Centrifugation



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

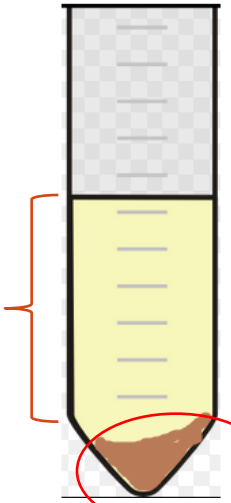
Pellet
(unwanted proteins
That precipitate at 40%)



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%)

B-Dialysis

C-Protein assay:

Determination of protein by Biuret Method