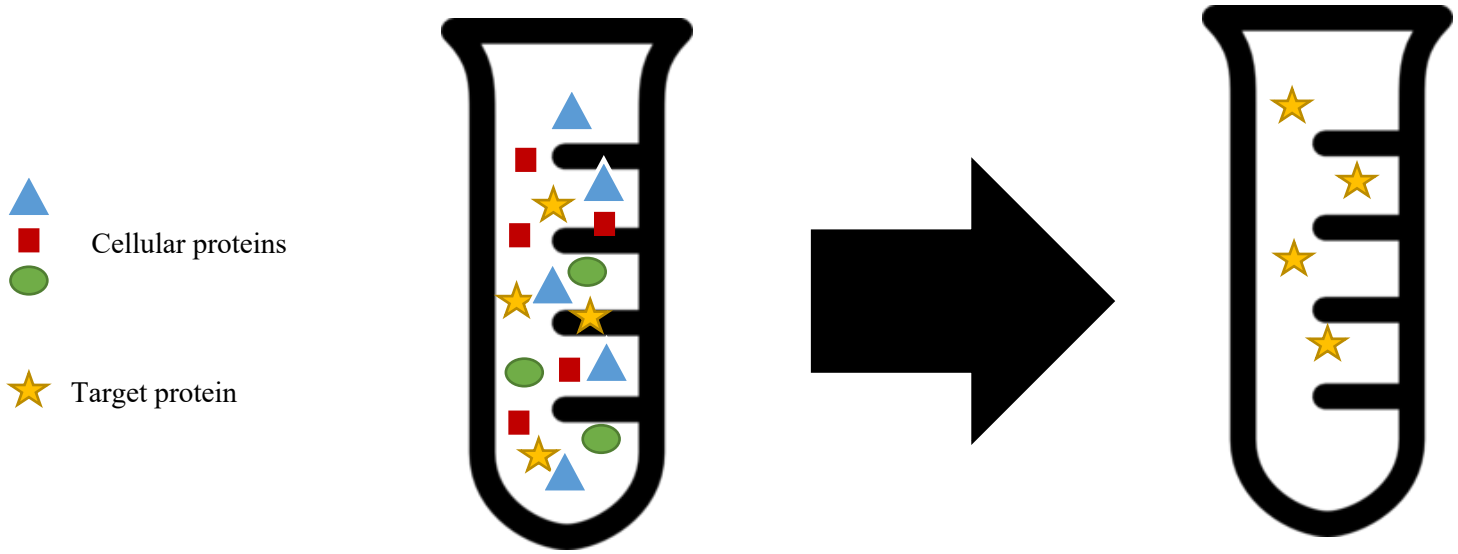
Biochemistry of Proteins BCH 303 [Practical]

Lab (5) Protein fractionation by ammonium sulphate and dialysis

Protein purification

- **Purification** should yield a sample of protein containing only one type of molecule, the protein in which the biochemist is interested ➔**This protein sample may be only a fraction of 1% of the starting material.**
- *How is the biochemist able to isolate a particular protein from a complex mixture of proteins?*
- Isolation techniques utilize different properties of proteins. *Like what?*



Protein purification

- Several thousand proteins have been purified in active form on the basis of such characteristics as:

1. Solubility
2. Size
3. Charge
4. Specific binding affinity

- Protein mixtures are subjected to a series of separations, each based on a different property to yield a pure protein.
- At each step in the purification, the preparation is assayed, and the protein concentration is determined. *(coming lab)*

💡 PAUSE AND THINK ➔ What will happen for the protein concentration during the purification scale?

Purification based on solubility

- *Salting out?*
- The salt concentration at which a protein precipitates **differs from one protein to another.**

Applications:

1. Can be used to fractionate proteins
2. Concentrating dilute solutions of proteins, *How?*

- Salting out is an effective means for initial molecule purification,

→ but lacks the ability for precise isolation of a specific protein.

- The type of salt being used, and the concentration of the salt can be varied to selectively precipitate the molecule.

The type of salt used in precipitation

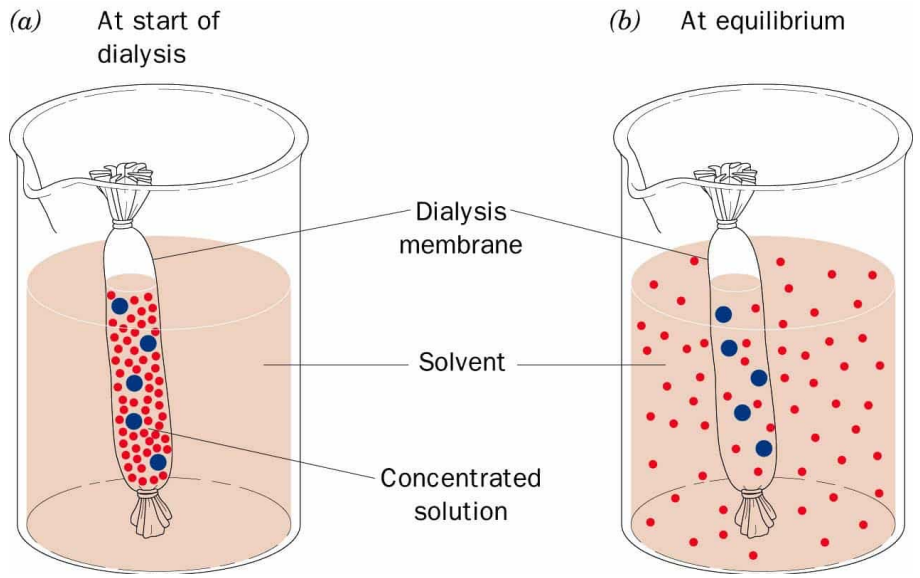
Ammonium sulphate is common substance used to precipitate proteins selectively:

1. It is very soluble in water
2. Its relative freedom from temperature effects
3. It has no harmful effects of proteins like irreversible denaturation

- ***……. How to remove ammonium sulphate salt ?***

Dialysis

- Proteins can be separated from small molecules (salts) by **dialysis**
- Dialysis utilizes a semipermeable membrane, such as a cellulose membrane with pores.
- Molecules having dimensions significantly greater than the pore diameter are retained inside the dialysis bag.
- Smaller molecules and ions traverse the pores of such a membrane and emerge in the dialysate outside the bag.



Practical part 🧪

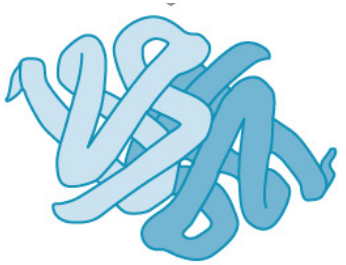
Protein fractionation by ammonium sulphate and dialysis

Objectives:

- Fractionation of animal crude extract by ammonium sulphate.
- Removing of salts ions using dialysis.

Whole tissue

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



Protein of interest
(Target protein)

Principle

- The most effective region of salting out is at the isoelectric point of the protein → minimum protein solubility in solutions of constant ionic strength. *Why?*
- Different proteins will precipitate at different salt concentration → where protein size is inversely correlated with salt concentration. *How?*

A typical fractionation protocol consists of:

1. Adding ammonium sulphate to give specific percentage saturation
2. Waiting a period of time for proteins to precipitate
3. Centrifugation step to collect the precipitate

Precipitation of proteins is conventionally carried out at 0°C to avoid possible *denaturation of proteins*.

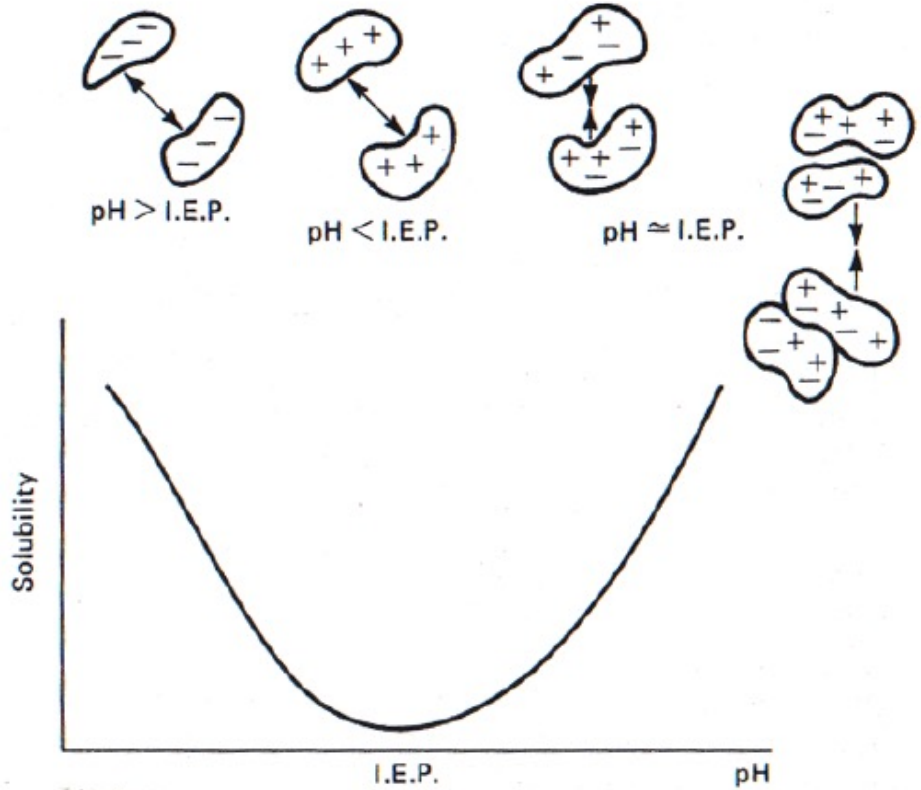


Figure 4.3. Solubility of a globulin-type protein close to its isoelectric point (IEP).

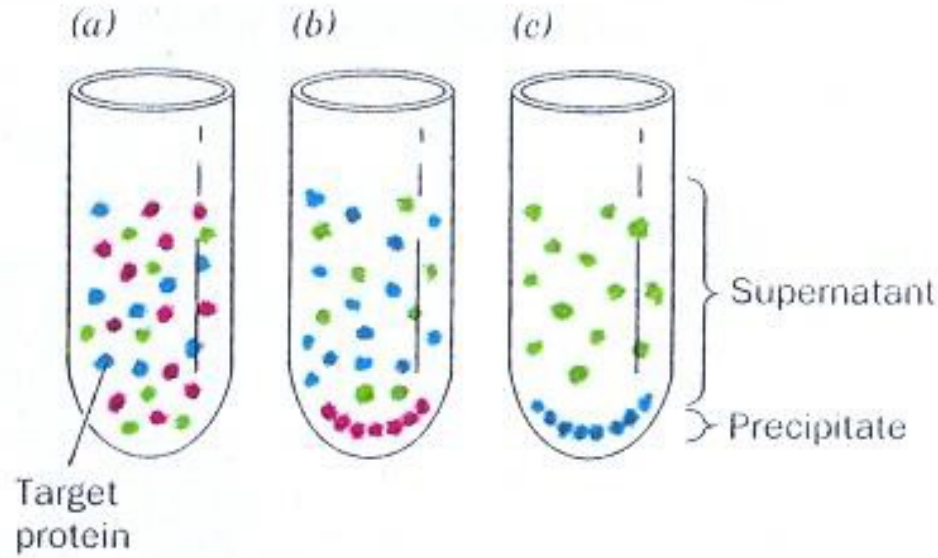


Figure 5-5 Fractionation by salting out. (*a*) The salt of choice, usually ammonium sulfate, is added to a solution of macromolecules to a concentration just below the precipitation point of the protein of interest. (*b*) After centrifugation, the unwanted precipitated proteins (*red spheres*) are discarded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

Principle

- Following fractionation by ammonium sulphate → **dialysis** is applied to remove salts.

During dialysis:

1. Small, unwanted salts ions removed from proteins in a solution by selective and passive diffusion through a semi-permeable membrane.
2. 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

→ Salt molecules move from the more concentrated solution (from inside the dialysis bag) to the less concentrated solution (e.g. buffer).

- The movement of the salt molecules **will stop** → when the solution reaches the equilibrium. At this point, the buffer is changed to drive the diffusion and salts movements.

💡 PAUSE AND THINK → What if you did not change the buffer? Why?

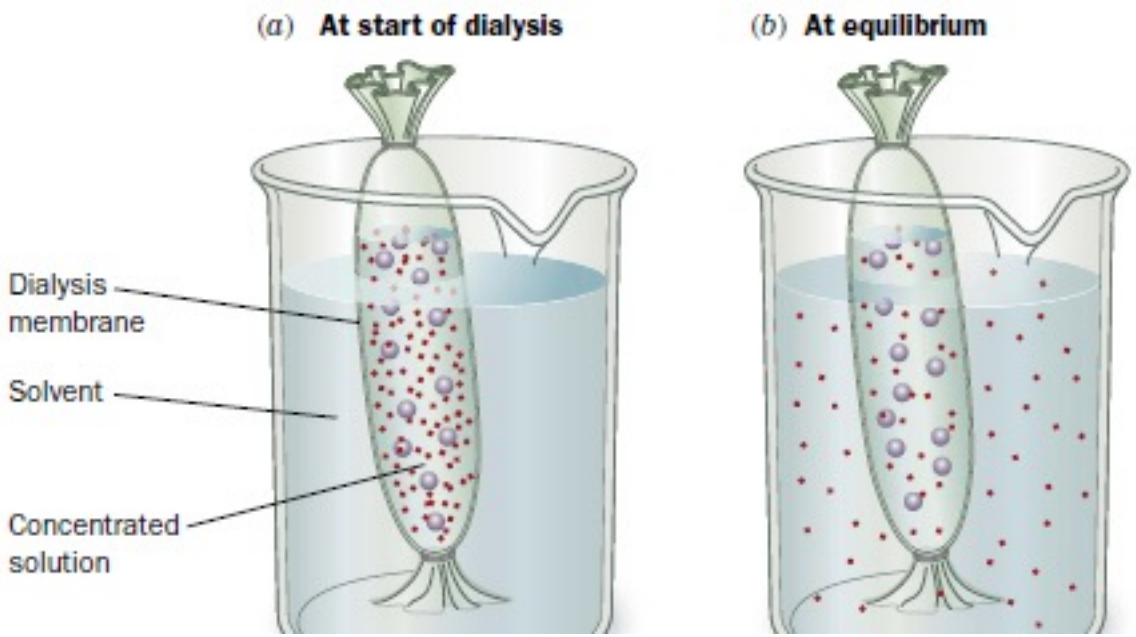


FIG. 2-14 Dialysis. (*a*) A concentrated solution is separated from a large volume of solvent by a dialysis membrane (shown here as a tube knotted at both ends). Only small molecules can diffuse through the pores in the membrane. (*b*) At equilibrium, the concentrations of small molecules are nearly the same on either side of the membrane, whereas the macromolecules remain inside the dialysis bag.

Using salt fractionation table

%	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

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

243 g of $(NH_4)_2SO_4$ are needed to saturate 1L of solution

? g are needed to saturate **….. ml** of crude extract

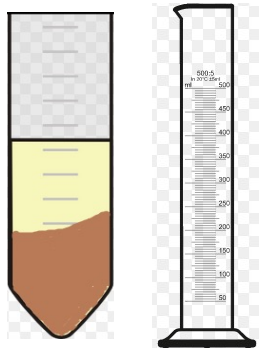
$$\frac{243\text{g x … ml}}{1000\ ml} = \ldots\ldots g$$

132 g of $(NH_4)_2SO_4$ are needed to saturate 1L of solution

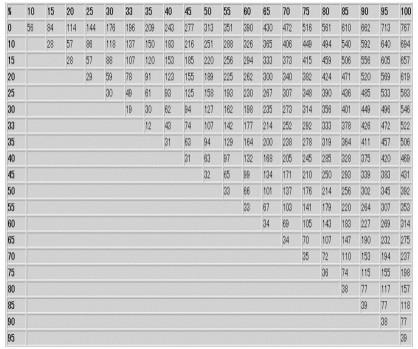
? g are needed to saturate **….. ml** of supernatant

$$\frac{132\text{g x … ml}}{1000\ ml} = \ldots\ldots g$$

Method



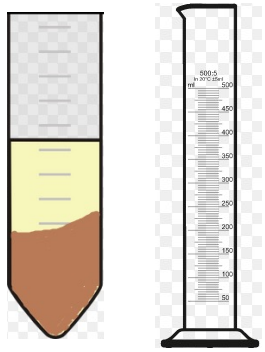
(1)
Measure the volume of the "supernatant“ (Crude extract)



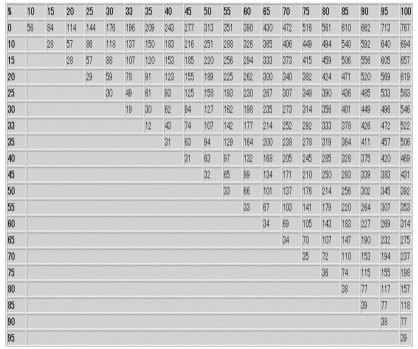
(2)
Calculate the required amount of ammonium sulphate salt needed to saturate the solution **40%.**

(3)
Add the required salt to the solution slowly and gradually with continuous mixing

Method



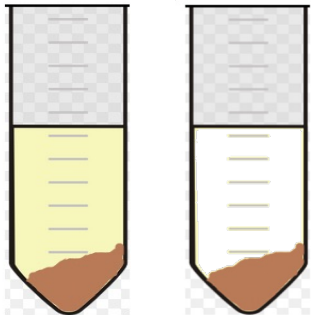
(4)
Centrifuge
then collect the supernatant
and measure its volume



(5)
Calculate the required amount of ammonium sulphate salt needed to saturate the solution **60%.**

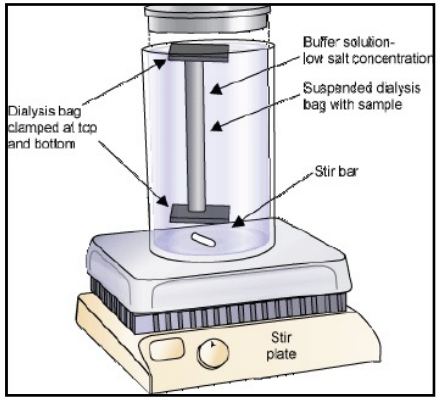
(6)
Add the required salt to the solution slowly and gradually with continuous mixing

Method



(7)
Centrifuge
then collect the pellet and discard the supernatant.

(8)
Dissolve the pellet



(9)
Dialysis

Homework

- Calculate and write the procedure, how would you precipitate by 30% ammonium sulphate saturation followed by 50% saturation, if your crude extract volume was 25 ml and the supernatant volume was 15 ml.