

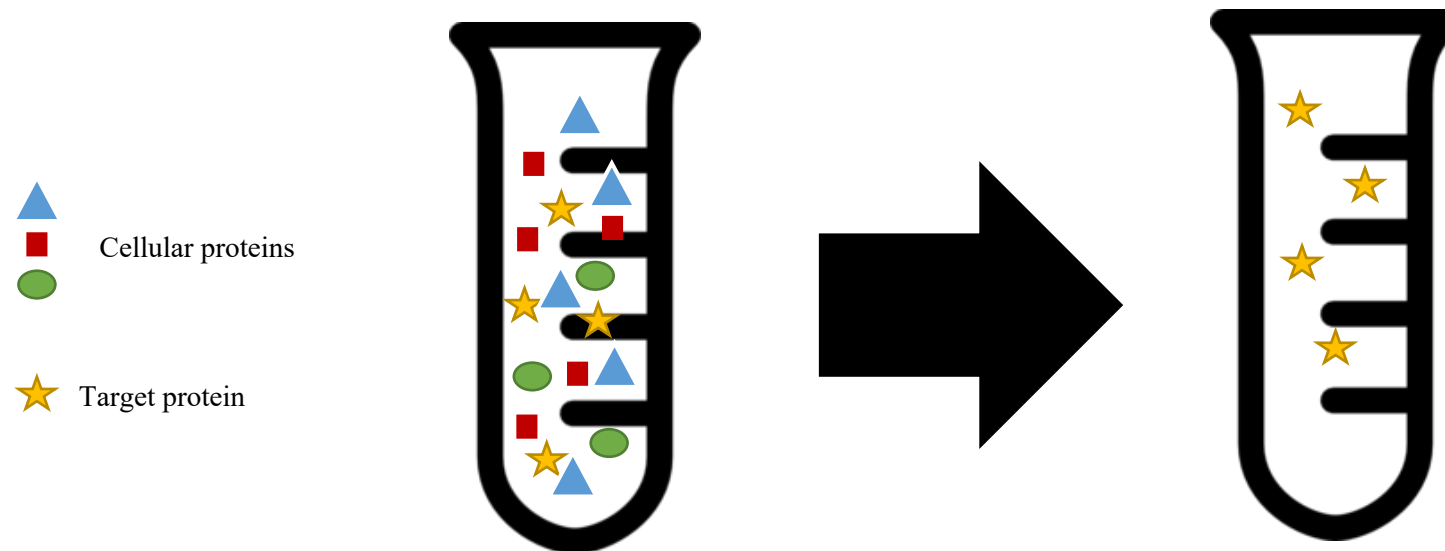
Biochemistry of Proteins BCH 303 [Practical]

**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

---

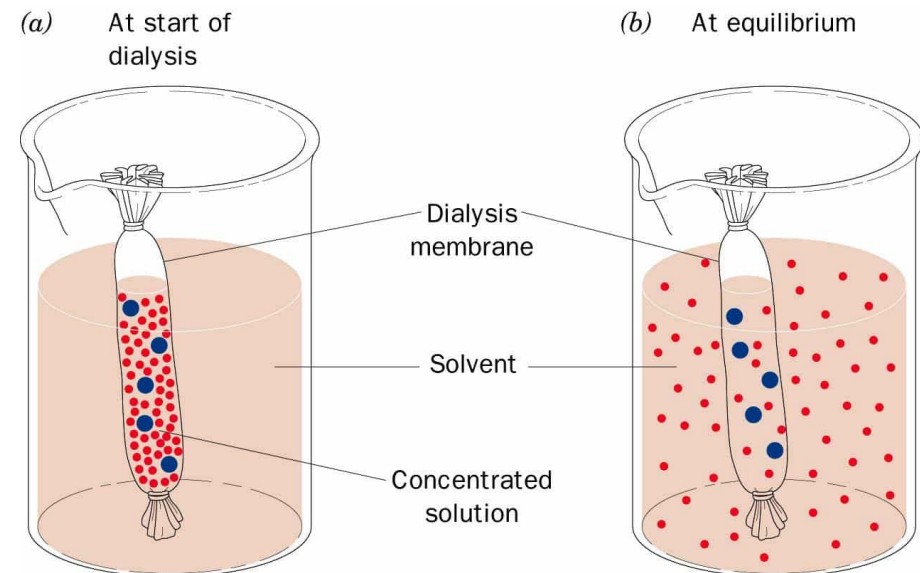
**Ammonium sulphate** is common substance used to precipitate proteins selectively:

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

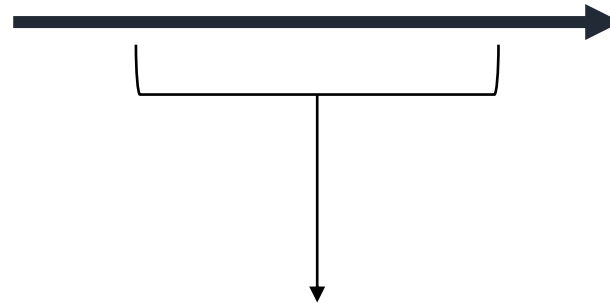
---

## 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*.

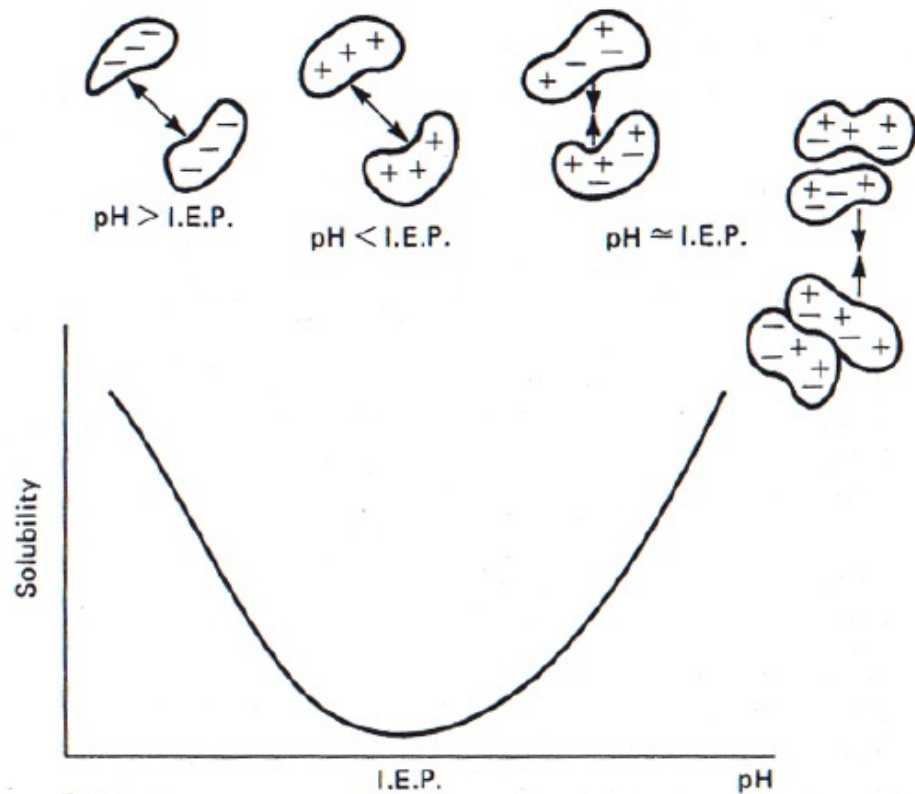


Figure 4.3. Solubility of a globulin-type protein close to its isoelectric point (IEP).

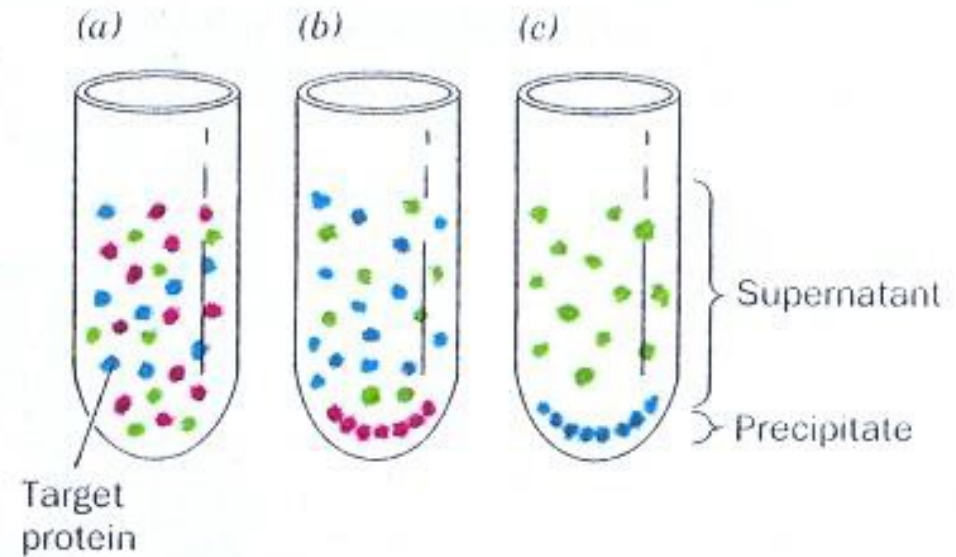


Figure 5-5 Fractionation by salting out. (a) The salt of choice, usually ammonium sulfate, is added to a solution of macromolecules to a concentration just below the precipitation point of the protein of interest. (b) After centrifugation, the unwanted precipitated proteins (*red spheres*) are discarded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

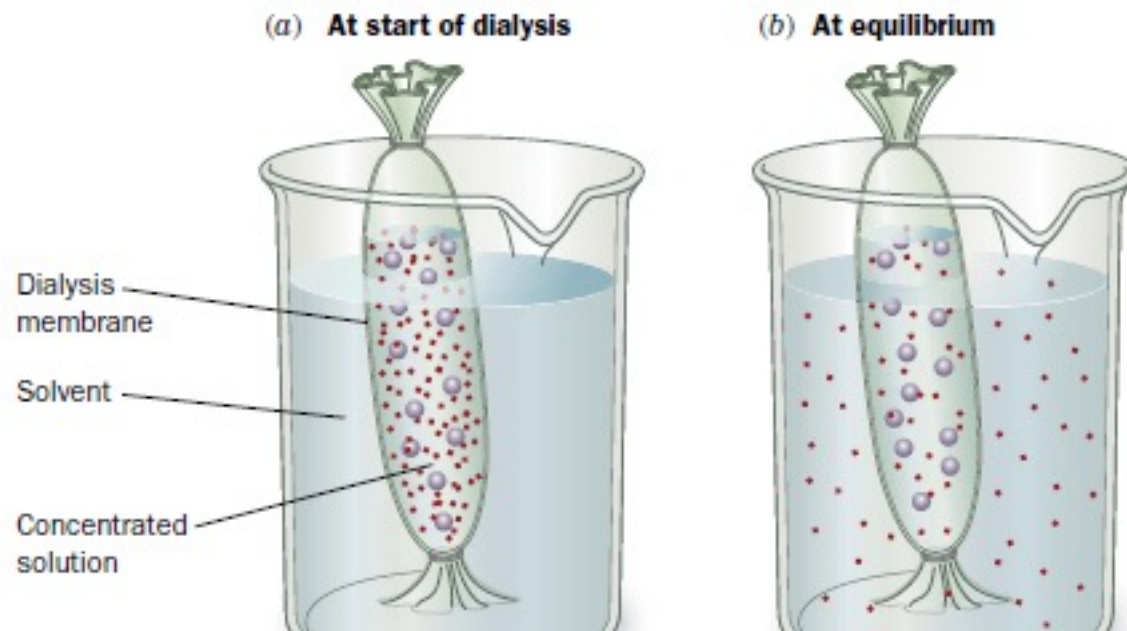
## During dialysis:

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?



**Section 2** Chemical Properties of Water

**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  $(\text{NH}_4)_2\text{SO}_4$  are needed to saturate 1L of solution

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

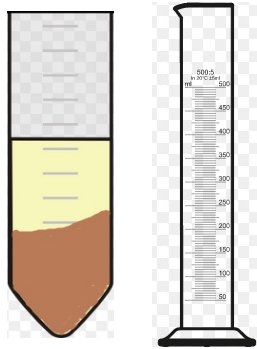
$$\frac{243\text{g} \times \dots \text{ml}}{1000 \text{ ml}} = \dots \dots \text{g}$$

132 g of  $(\text{NH}_4)_2\text{SO}_4$  are needed to saturate 1L of solution

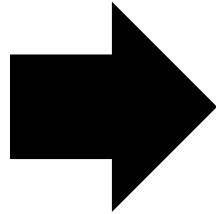
? g are needed to saturate ..... ml of supernatant

$$\frac{132\text{g} \times \dots \text{ml}}{1000 \text{ ml}} = \dots \dots \text{g}$$

# Method

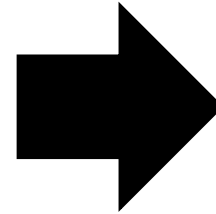


(1)  
**Measure** the volume of the  
"supernatant"  
(Crude extract)



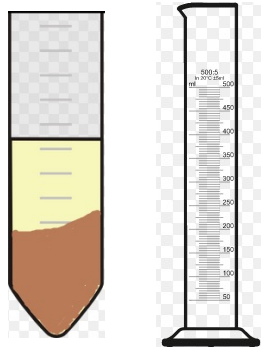
1	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
0	59	84	114	144	176	196	209	243	277	313	351	380	430	472	516	561	610	662	713	767
10	28	57	86	116	137	150	153	216	251	288	326	365	406	448	494	540	582	640	694	754
15	38	57	88	107	120	133	135	200	235	272	310	349	390	432	476	520	562	620	674	734
20	49	59	78	91	103	115	118	183	218	255	292	330	370	412	454	496	538	596	650	710
25	60	48	61	60	60	60	60	125	158	193	230	267	307	348	390	432	474	532	586	646
30	71	59	60	62	64	67	68	137	170	205	242	280	320	360	402	444	486	544	598	658
35	82	70	68	74	77	81	82	147	180	215	252	290	330	370	412	454	496	554	608	668
40	93	80	76	84	87	91	92	157	190	225	262	300	340	380	422	464	506	564	618	678
45	104	90	85	94	97	101	102	167	200	235	272	310	350	390	432	474	516	574	628	688
50	115	100	94	104	107	111	112	177	210	245	282	320	360	400	442	484	526	584	638	698
55	126	110	103	114	117	121	122	187	220	255	292	330	370	410	452	494	536	594	648	708
60	137	120	112	124	127	131	132	197	230	265	302	340	380	420	462	504	546	604	658	718
65	148	130	121	134	137	141	142	207	240	275	312	350	390	430	472	514	556	614	668	728
70	159	140	131	144	147	151	152	217	250	285	322	360	400	440	482	524	566	624	678	738
75	170	150	141	154	157	161	162	227	260	295	332	370	410	450	492	534	576	634	688	748
80	181	160	151	164	167	171	172	237	270	305	342	380	420	460	502	544	586	644	698	758
85	192	170	161	174	177	181	182	247	280	315	352	390	430	470	512	554	596	654	708	768
90	203	180	171	184	187	191	192	257	290	325	362	400	440	480	522	564	606	664	718	778
95	214	190	181	194	197	201	202	267	300	335	372	410	450	490	532	574	616	674	728	788

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

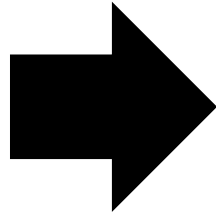


(3)  
**Add** the required salt to the  
solution slowly and gradually  
with continuous mixing

# Method

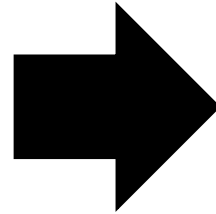


(4)  
**Centrifuge**  
then collect the supernatant  
and measure its volume



1	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
0	59	84	114	144	176	196	209	243	277	313	351	380	430	472	516	561	610	662	713	767
10	28	57	86	116	137	150	153	216	251	288	326	365	406	448	494	540	592	643	694	747
15		38	67	96	107	120	123	185	220	256	294	333	373	415	459	506	556	605	657	709
20			49	78	91	123	125	189	225	262	300	340	382	424	471	520	569	619	671	723
25				48	61	63	125	158	193	230	267	307	348	390	436	485	533	583	633	684
30					39	62	84	107	162	198	235	273	314	356	401	448	496	546	596	647
35						42	43	107	142	177	214	252	292	333	378	424	471	519	567	616
40							62	84	128	164	200	238	278	319	364	411	457	506	554	603
45								61	81	132	168	205	245	288	335	383	431	480	529	578
50									65	86	134	171	210	250	293	339	387	435	484	533
55										66	101	137	176	214	256	302	349	397	446	495
60											67	102	141	179	220	264	311	359	407	456
65												68	105	143	182	227	275	323	371	420
70													70	107	147	190	237	285	333	382
75														69	73	110	154	199	247	295
80															74	115	159	206	254	302
85																77	117	157	207	255
90																	78	118	158	208
95																		79	119	209
																			80	210
																				81
																				82
																				83
																				84
																				85

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

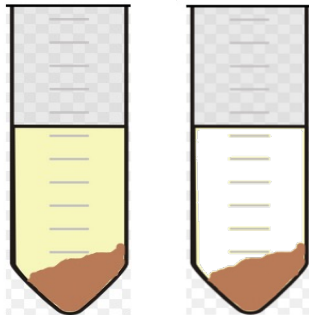


(6)  
Add the required salt to the  
solution slowly and gradually  
with continuous mixing

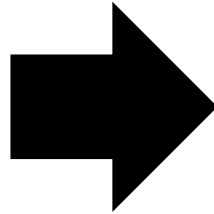


# Method

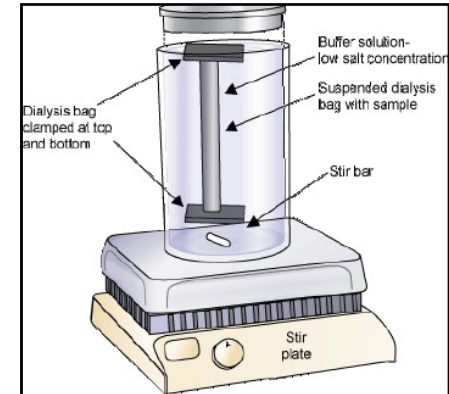
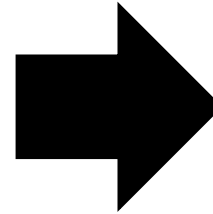
---



(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.