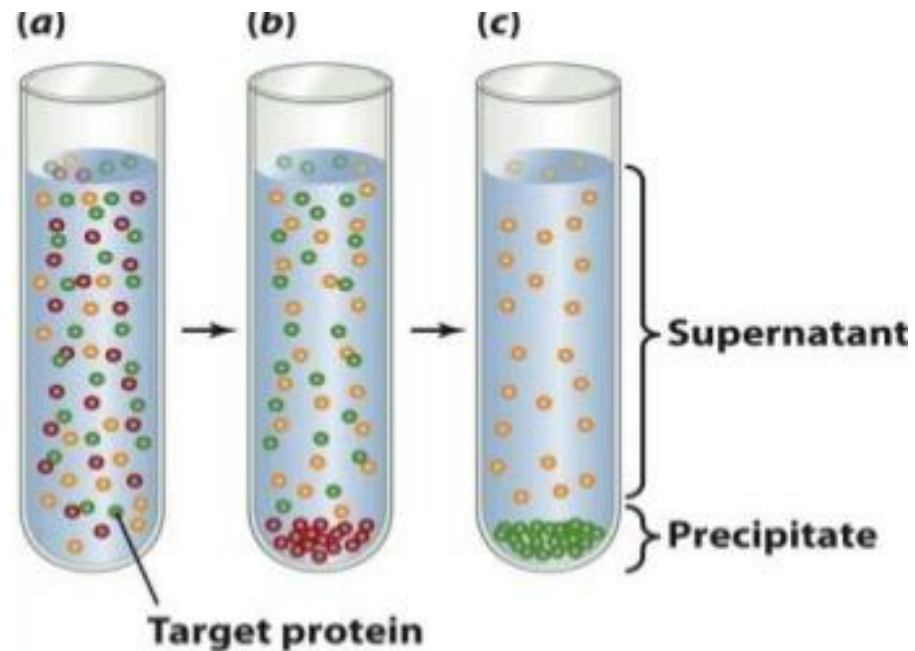
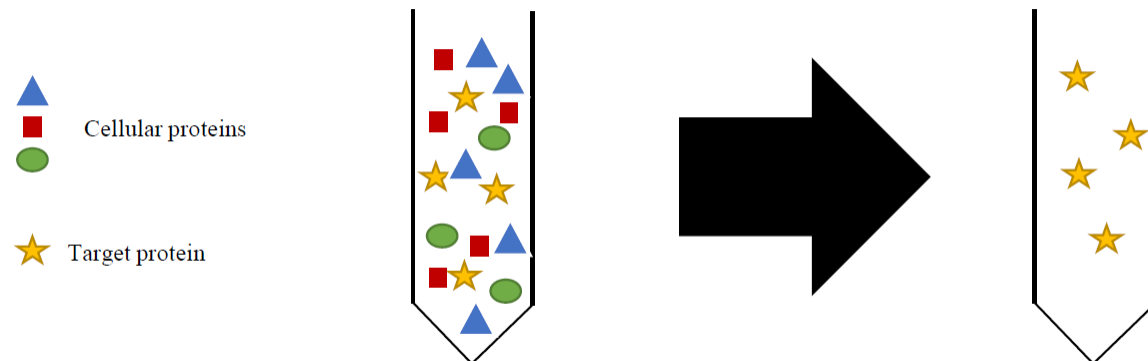


Protein fractionation by ammonium sulphate and dialysis



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 only be 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.



Purification based on solubility:

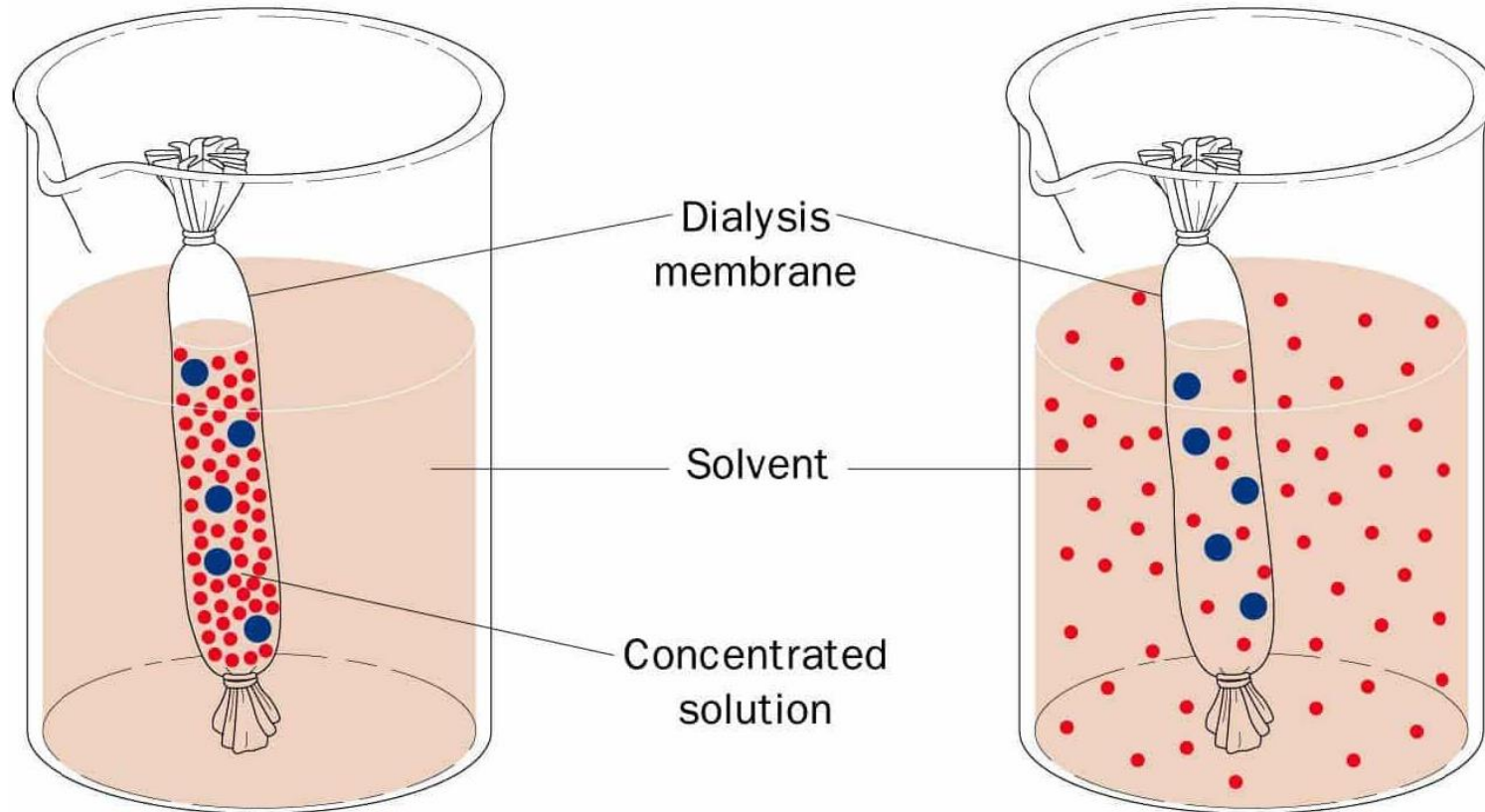
- Several thousand proteins have been purified in active form on the basis of such characteristics as solubility, size, charge, and specific binding affinity.
- The purification of proteins by altering the **solubility** achieved by what called **salting out**.
- The salt concentration at which a protein precipitates differs from one protein to another.
- salting out can be used to **fractionate proteins** (as proteins will precipitate at different points with increases in salt concentration).

Dialysis

- Ammonium sulphate is common substance used to precipitate proteins selectively. Why?
- **How to remove the salt?**
- Proteins can be separated from small molecules (salts) by **dialysis** through a **semipermeable membrane**, such as a cellulose membrane with pores.
- Molecules greater than the pore are retained inside the dialysis bag, whereas smaller molecules and ions traverse the pores of such a membrane and emerge in the dialysate outside the bag (**Osmosis**).
- 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.

(a) At start of dialysis

(b) At equilibrium



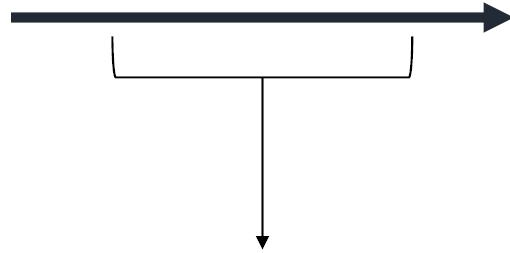
Practical part

Objective:

- Fractionation of animal crude extract by ammonium sulphate.
- 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 not be isolated by only one step)

Principle:

- 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.
- Different proteins will precipitate at different salt concentration, where protein size is inversely correlated with salt concentration.
- A typical protocol consists of adding ammonium sulphate to give specific percentage saturation,
- followed by a period of time for proteins to precipitate and a centrifugation step to collect the precipitate.
- Precipitation of proteins is conventionally carried out at 0°C to avoid possible denaturation of proteins.
- Following fractionation by ammonium sulphate, dialysis is applied to remove salts.

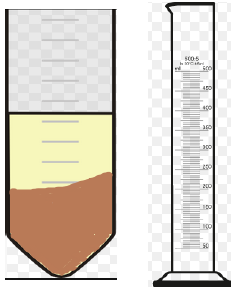
Protocol:

A. Salting out of protein A by 40% ammonium sulphate saturation:

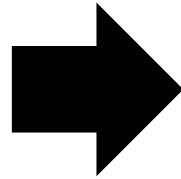
1. Measure the volume of your crude extraction and calculate the weight in g of ammonium sulphate needed to **saturate the solution 40%** using Table 1.
2. Add the required salt to the solution slowly and gradually with small quantities and mix well continuously using magnetic stirrer while the sample is placed in ice.
3. After the addition is completed and the salt is completely dissolved, centrifuge at 3500 rpm for 10 min.
4. Discard the supernatant and dissolve the pellet in 10 ml of extraction buffer (0.1 M Tris-HCl, pH 7.4).

B. Removing of salts molecules by dialysis:

1. Pre-wet the membrane by soaking the dialysis bag in dialysis buffer.
2. Close the dialysis bag from one side and load the sample.
3. Close the other side and place the bag in a beaker filled with 0.1 M Tris-HCl, pH 7.4 buffer.
4. Dialyze for 1 to 2 h at room temperature.
5. Change the dialysis buffer and dialyze for another 1 to 2 h.
6. Change the dialysis buffer and dialyze overnight at 4°C.



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



1	16	20	25	31	37	43	49	55	60	65	71	76	81	86	91	96
2	18	22	27	33	39	45	51	57	62	67	72	77	82	87	92	97
3	20	24	29	35	41	47	53	59	64	69	74	79	84	89	94	99
4	22	26	31	37	43	49	55	61	66	71	76	81	86	91	96	101
5	24	28	33	39	45	51	57	63	68	73	78	83	88	93	98	103
6	26	30	35	41	47	53	59	65	70	75	80	85	90	95	100	105
7	28	32	37	43	49	55	61	67	72	77	82	87	92	97	102	107
8	30	34	39	45	51	57	63	69	74	79	84	89	94	99	104	109
9	32	36	41	47	53	59	65	71	76	81	86	91	96	101	106	111
10	34	38	43	49	55	61	67	73	78	83	88	93	98	103	108	113
11	36	40	45	51	57	63	69	75	80	85	90	95	100	105	110	115
12	38	42	47	53	59	65	71	77	82	87	92	97	102	107	112	117
13	40	44	49	55	61	67	73	79	84	89	94	99	104	109	114	119
14	42	46	51	57	63	69	75	81	86	91	96	101	106	111	116	121
15	44	48	53	59	65	71	77	83	88	93	98	103	108	113	118	123
16	46	50	55	61	67	73	79	85	90	95	100	105	110	115	120	125
17	48	52	57	63	69	75	81	87	92	97	102	107	112	117	122	127
18	50	54	59	65	71	77	83	89	94	99	104	109	114	119	124	129
19	52	56	61	67	73	79	85	91	96	101	106	111	116	121	126	131
20	54	58	63	69	75	81	87	93	98	103	108	113	118	123	128	133
21	56	60	65	71	77	83	89	95	100	105	110	115	120	125	130	135
22	58	62	67	73	79	85	91	97	102	107	112	117	122	127	132	137
23	60	64	69	75	81	87	93	99	104	109	114	119	124	129	134	139
24	62	66	71	77	83	89	95	101	106	111	116	121	126	131	136	141
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26	66	70	75	81	87	93	99	105	110	115	120	125	130	135	140	145
27	68	72	77	83	89	95	101	107	112	117	122	127	132	137	142	147
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