Protein fractionation by ammonium sulphate and dialysis

Materials:

Chemical

Prepared crude extract, ammonium sulphate, 0.1 M Tris-HCl, pH 7.4, distal water.

Equipment and Glassware

Beakers, measuring cylinder, centrifuge tubes, dialysis bags, electronic balance, centrifuge, magnetic stirrer.

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 on 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 the dialysis buffer for at least 30 min.
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 or phosphate buffer 0.1 M, pH 7.0.
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.

%	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 liter of solution.