Protein precipitation

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كيمياء الحيوية – انزيمات

قسم علوم الأغذية – كلية الزراعة

جامعة البصرة

2019

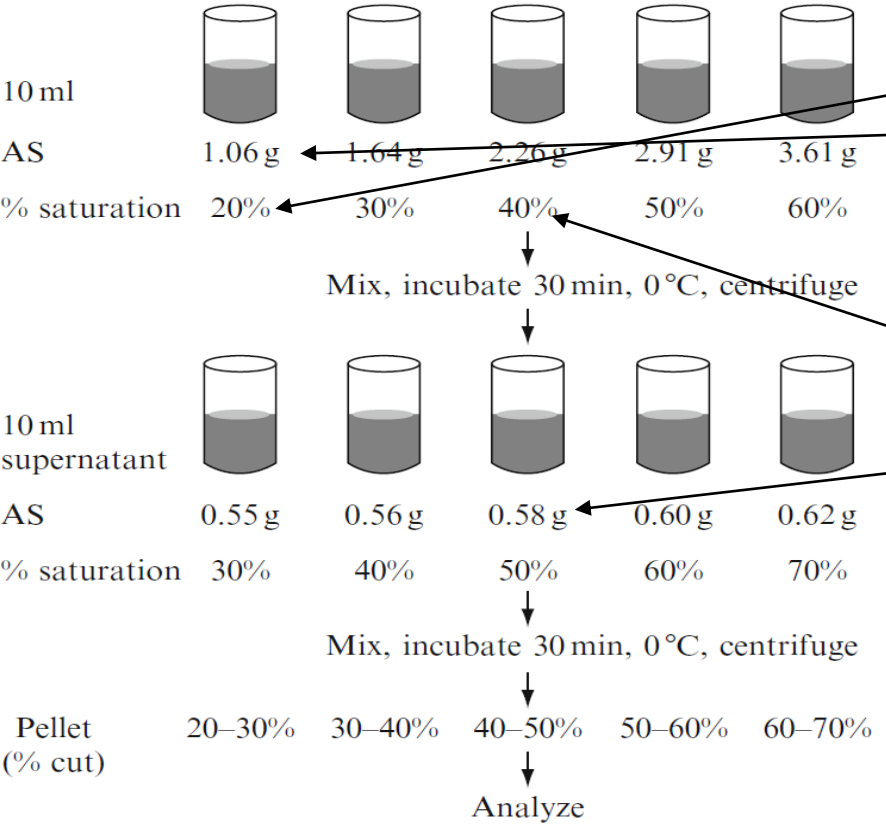
Ammonium sulfate

- Ammonium sulfate is the most common salt used:
  - Because it is unusually soluble in cold buffers (our extractions are kept cold!).
  - In research laboratories as a first step in protein purification because it provides some crude purification of proteins separating non-proteins and some unwanted proteins out.
  - Because it yields a precipitated protein slurry that is usually very stable, so the purification can be stopped for a few hours while the student gets some sleep

Table 20.1 Final concentration of ammonium sulfate: Percentage saturation at 0 °C[a]

Initial concentration of ammonium sulfate (percentage saturation at 0 °C)	Percentage saturation at 0 °C																
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
	Solid ammonium sulfate (g) to be added to 1 l of solution																
0	106	134	164	194	226	258	291	326	361	398	436	476	516	559	603	650	697
5	79	108	137	166	197	229	262	296	331	368	405	444	484	526	570	615	662
10	53	81	109	139	169	200	233	266	301	337	374	412	452	493	536	581	627
15	26	54	82	111	141	172	204	237	271	306	343	381	420	460	503	547	592
20	0	27	55	83	113	143	175	207	241	276	312	349	387	427	469	512	557
25		0	27	56	84	115	146	179	211	245	280	317	355	395	436	478	522
30			0	28	56	86	117	148	181	214	249	285	323	362	402	445	488
35				0	28	57	87	118	151	184	218	254	291	329	369	410	453
40					0	29	58	89	120	153	187	222	258	296	335	376	418
45						0	29	59	90	123	156	190	226	263	302	342	383
50							0	30	60	92	125	159	194	230	268	308	348
55								0	30	61	93	127	161	197	235	273	313
60									0	31	62	95	129	164	201	239	279
65										0	31	63	97	132	168	205	244
70											0	32	65	99	134	171	209
75												0	32	66	101	137	174
80													0	33	67	103	139
85														0	34	68	105
90															0	34	70
95																0	35
100																	0

[a] Reprinted from Englard and Seifter (1990), which was adapted from Dawson *et al.* (1969).



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Acetone Precipitation

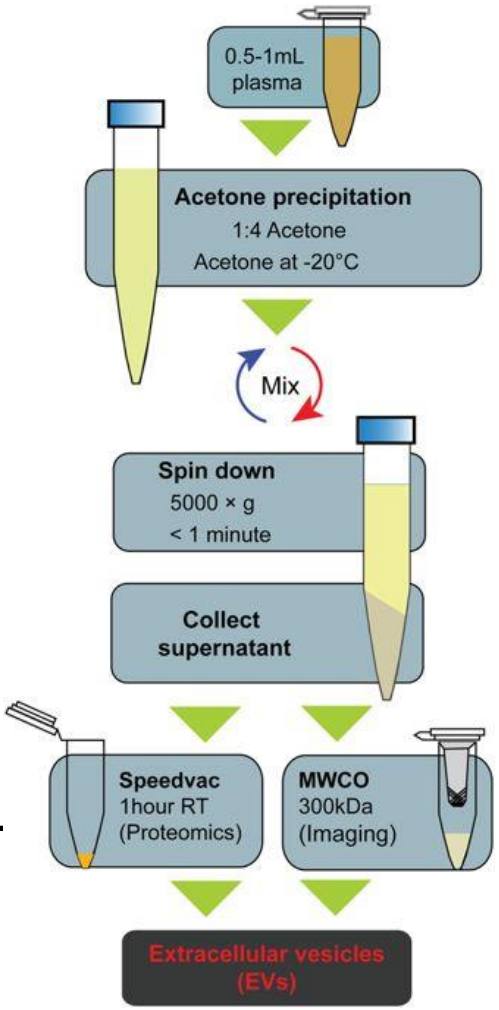
Most universal

Materials:

- Sample of interest
- Cold (-20°C) acetone, a volume four times that of the protein samples to be precipitated
- Centrifuge tube
- Centrifuge

Method:

1. Cool the required volume of acetone to -20°C.
2. Add four times the sample volume of cold (-20°C) acetone to the tube.
3. Vortex tube and incubate for 60 minutes at -20°C.
4. Centrifuge 10 minutes at 13,000-15,000 x g.
5. Decant and properly dispose of the supernatant, being careful to not dislodge the protein pellet.
6. Add 0.5 ml cold acetone, vortex briefly.
7. Centrifuge 10 minutes at 13,000-15,000 x g.
8. Aspirate acetone, avoiding pellet with care.
9. Allow the acetone to evaporate from the uncapped tube at room temperature for 30 minutes. Do not over-dry pellet, or it may not dissolve properly.



Chloroform/Methanol Precipitation

Useful method for removal of salt and detergents

Materials:

- **Samples of interest**
- **Methanol**
- **Chloroform**
- **Centrifuge tube**
- **Centrifuge and rotor for the tubes used, minimum 13,000 x g required**

Method:

1. To 100uL protein sample in a 1.5mL eppendorf tube:

2. Add 400 uL methanol and vortex thoroughly.

3. Add 100 uL chloroform and vortex.

4. Add 300 uL H2O—mixture will become cloudy with precipitate—and vortex.

5. Centrifuge 5 minute at 14,000 x g. Result is three layers: a large aqueous layer on top, a circular flake of protein in the interphase, and a smaller chloroform layer at the bottom.

6. Remove top aqueous layer carefully, trying not to disturb the protein flake.

7. Add 400 uL methanol and vortex.

8. Centrifuge 5 minutes at 14,000 x g, which will slam dandruffy precipitate against the tube wall.

9. Remove as much methanol as possible. Be careful, because the pellet is delicate. You should be able to remove all but a few uL of methanol with care, which will speed drying.

10. Briefly dry the pellets in vacuum centrifuge.

Ethanol Precipitation

Useful method to concentrate proteins and removal of Guanidine Hydrochloride before SDS-PAGE

Materials:

- Samples of interest
- Ethanol (cold)
- Centrifuge tube
- Centrifuge

Method:

1. Add to 1 volume of protein solution 9 volumes of cold ethanol 100%.
2. Mix and keep at least 60 min. at -20°C. (Suggestion: leave ON).
3. Centrifuge 10 minutes at 15,000 x g.
4. Carefully discharge supernatant and retain the pellet: dry tube by inversion on tissue paper (pellet may be difficult to see).
5. Wash pellet with cold ethanol (keep at -20°C).
6. Vortex and repellet samples for 10 min at 15,000 x g.
7. Aspirate ethanol, avoiding pellet with care
8. Dry samples under vacuum (speed vac) or dry air to eliminate any ethanol residue (smell tubes).

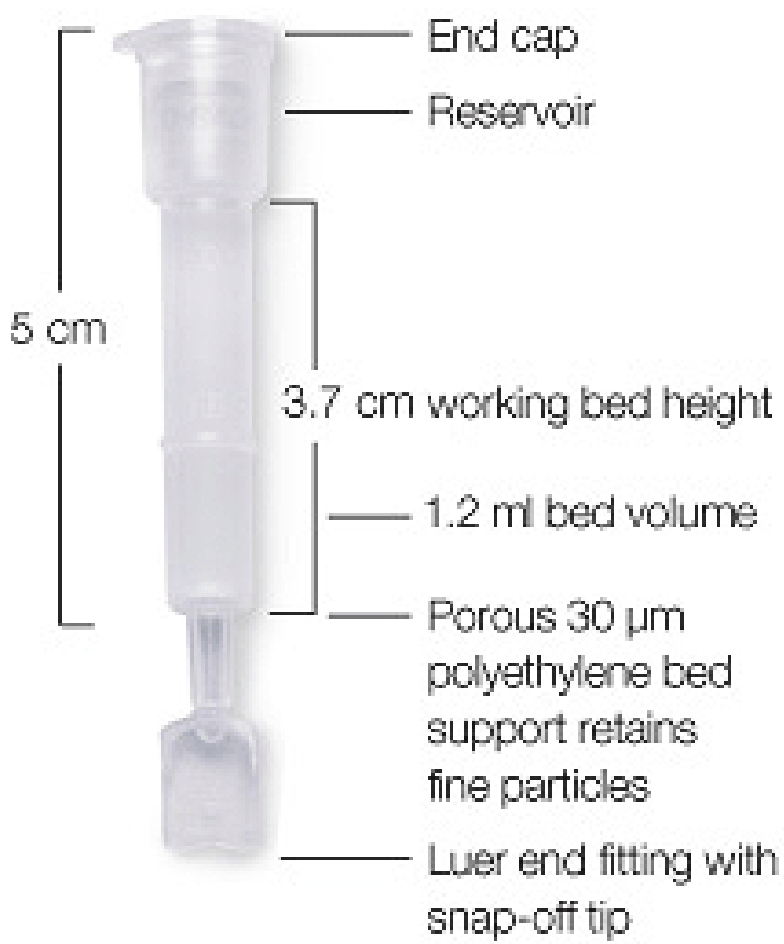
TCA Precipitation

Materials:

- Samples of interest
- TCA = trichloroacetic acid, 100% solution stored at 4°C
- Acetone (cold)
- Centrifuge tube
- Centrifuge

Method:

1. Add 100% TCA from cold stock solution to the sample(s) of interest such that the final mixture is 20% TCA
2. Vortex and incubate for 30 minutes on ice.
3. Centrifuge 10 minutes at 13,000-15,000 x g. at 4 °C.
4. Aspirate the supernatant carefully, to not disturb the pellet (may be seen on the side or bottom of the tube).
5. Add 0.5 ml cold acetone, vortex briefly.
6. Centrifuge 10 minutes at 13,000-15,000 x g. at 4 °C.
7. Aspirate acetone, avoiding pellet with care.
8. Allow the acetone to evaporate from the uncapped tube at room temperature for 30 minutes. Do not over-dry pellet, or it may not dissolve properly


End cap
Reservoir
5 cm
3.7 cm working bed height
1.2 ml bed volume
Porous 30 µm polyethylene bed support retains fine particles
Luer end fitting with snap-off tip


End cap
Reservoir
3 cm
2 cm working bed height
0.8 ml bed volume
Luer end fitting with snap-off tip

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