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Short Communications

Separation of Protein and Polyethylene Glycol in Water Solutions by a Desalting Technique

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Various methods for precipitation of proteins have been used as a means for purifying proteins from a mixture. The types of reagents for these precipitations are inorganic salts like ammonium sulfate or sodium sulfate, organic solvents, polyelectrolytes and nonionic hydrophilic polymers. In the latter group, polyethylene glycol (PEG) is most commonly used. This procedure is very useful because rigid control of ionic strength and temperature is not very critical, the proteins are not denaturated and the yields are generally high.

PEG precipitations often lead to very viscous fractions which are difficult to handle. This problem was seen in our laboratory when we isolated albumin from serum by precipitation of the other proteins with PEG after a method of Gambal.[1] Undiluted serum was stirred at 4 °C and powdered PEG 6000 was added slowly to a concentration of 25 % (w/V). The mixture was stirred for 24 h and then centrifuged. Under these conditions albumin is the only protein that can be detected in the supernatant. PEG can be removed from the albumin solution by prolonged dialysis, but Gambal instead recommended gel filtration on a Sephadex G-75 column. However, in our experiments even an extensive dilution of the supernatant before gel filtration leads to a very viscous solution which would clog the Sephadex column. Instead we tried to separate albumin and PEG by a desalting technique and found that addition of, for instance, ammonium sulfate will cause the supernatant to decompose into two phases. This phenomenon may be due to the reduced solubility of PEG in water by addition of salt. The observation that the protein and the PEG appeared unevenly distributed among the two phases motivated us to examine the relative amounts of protein and PEG in the two phases for different types and concentrations of PEG.

Experimental. PEG 1550 pract. (*M* 1300–1600; m.p. 34–41 °C) and PEG 4000 pract. (*M* 3000–3700; m.p. 53–56 °C) were purchased from Serva, Heidelberg, W. Germany, PEG 6000 was from the British Drug

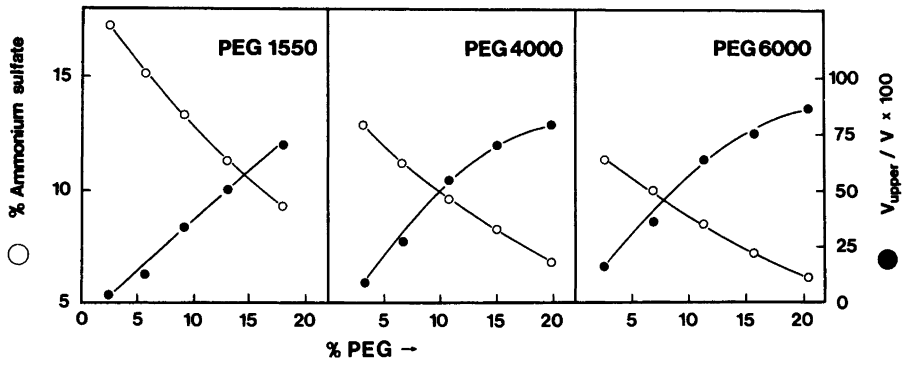

Fig. 1. To 5 ml PEG-solution (PEG 1550, 4000 and 6000) in water is added 3 M ammonium sulfate until onset of turbidity. ○=% ammonium sulfate in final solution, ●=volume of upper phase/total volume ×100 after phase separation. % PEG relates to the final solution.

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Table 1. Phase separation of serum albumin/PEG-solutions by ammonium sulfate.

PEG	% PEG in initial solution	% PEG in final solution	% ammonium sulfate in final solution	$V_{upper}/V \times 100$	% of total amounts in the lower phase		
					PEG	ammonium sulfate	albumin
	4.5	2.1	18.6	3	93	98	67
	9.1	5.0	15.1	7	70	94	83
1550	13.0	6.8	16.1	16	57	93	86
	16.6	precipitation					
	19.9	precipitation					
	4.5	2.8	13.8	11	81	99	98
	9.1	6.3	10.2	18	40	88	90
4000	13.0	8.3	12.0	43	9	67	90
	16.6	12.3	8.7	66	14	47	85
	19.9	15.2	8.1	83	2	28	89
	4.5	3.1	11.9	10	57	94	99
	9.1	6.1	10.8	26	55	74	95
6000	13.0	9.3	9.6	54	20	54	91
	16.6	12.4	8.5	56	2	68	75
	19.9	no visible phase separation					

House. Bovine Serum albumin, crystallized and lyophilized (No. A.4348) was from the Sigma Chemical Company. Ammonium sulfate concentrations were determined by ammonium determination, either by Kjeldahl analysis or by the indophenol method.[2] A Cary 16 spectrophotometer was used to determine protein. Absorbance at 280 nm, in protein solutions without PEG, was determined as an index of protein concentration.

Phase separation in water solutions of pure PEG by addition of ammonium sulfate. To a 5 ml solution of PEG in water, 3.0 M ammonium sulfate is added from a burette until the onset of turbidity. After standing overnight (maximum 20 h) or centrifugation the solution separates into two phases.

Phase separation in water solutions of protein and PEG by addition of ammonium sulfate. To a solution of protein and PEG (3 ml solution of bovine serum albumin plus 3 ml solution of PEG) 3.0 M ammonium sulfate is added from a burette until the onset of turbidity. After centrifugation (3000 G, 30–60 min) the solution separates into two phases. Protein concentration in the lower phase is determined after removal of the PEG: 0.3 ml of the lower phase is applied to a Sephadex G-25 fine column (dimensions: 1×18 cm) and the column is eluted by 0.4 M sodium chloride. (PEG in the concentrations in the lower phase does not clog the column.) Fractions (2–4 ml) were collected and protein determined. Total weights of protein, ammonium sulfate and PEG in upper and lower phase were determined by weighing after lyophilization. Ammonium sulfate concentrations in the upper and the lower phase were determined by ammonia determination on the lyophilized products (corrected for protein in the phases). PEG concentrations in the upper and the lower phase were determined by "difference computing".

Results and discussion. In the experiment shown in Fig. 1 3.0 M ammonium sulfate was added to samples containing 0.25–1.27 g PEG (PEG 1550, 4000 and 6000) in 5 ml water at 22 °C until the onset of turbidity. After standing, the solutions split up into two phases. The figure illustrates that all three kinds of PEG show the same pattern of phase separation. Increasing concentrations of PEG result in decreasing amounts of ammonium sulfate necessary to obtain phase separation. By increasing the PEG concentrations all three kinds of PEG show increasing ratios of upper volumes to lower volumes in the phase separation.

Similar experiments were undertaken for samples containing bovine serum albumin besides PEG. To a mixture of 30 mg serum albumin in 3 ml water and 0.3–1.5 g PEG in 3 ml water, at 22 °C, 3.0 M ammonium sulfate was added until the onset of turbidity. After phase separation the phases were analyzed for protein, PEG and

ammonium sulfate as described above. Table 1 illustrates typical experiments. Addition of ammonium sulfate results in high concentrations of PEG 1550 in the formation of precipitates, whereas no phase separation occurs for PEG 6000 in high concentration. Apart from that, the results are qualitatively similar to those from the experiments without protein, although not reproducible to the same degree.

The table shows that in most experiments nearly all the protein is in lower phase. The recovery may vary from experiment to experiment, by very careful manipulation the protein amount in lower phase can be brought up to about 90–99 % of total protein for all the three PEG's at the different concentrations. The amounts of PEG in the lower phase range from about 2–93 % of total PEG. It is remarkable that a small amount of PEG (2–20 %) in the lower phase is found for PEG 4000 and 6000 in initial concentrations about 13–20 (17) %. Under these conditions the volume of the lower phase is about 25–85 % of the original protein-PEG volume. Thus, separation of protein from PEG 4000 and 6000 by means of ammonium sulfate seems to be a practical tool in protein purification procedures in which PEG has initially been introduced to cause protein precipitation.

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1. Gambal, D. *Biochim. Biophys. Acta 251* (1971) 54.
2. Marczenko, A. *Spectrophotometric Determination of Elements,* Ellis Harwood Ltd., Chichester, England 1976.

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