In the preparation of protein concentrates, particularly from sources dilute with respect to protein, it is useful to employ ultrafiltration. However, in order to achieve convenient filtration rates, it has heretofore been necessary to employ high pressures and especially prepared membranes (1). However, Schneider and Wallenius (3) concentrated the protein (0.02 to 0.03 %) in 4 to 9 ml of cerebrospinal fluid to 0.1 to 0.2 ml in 24 hours by suspending the fluid in a cellulose dialysis sac in 500 ml of a 10 to 20 % solution of dextran. As the dextran is too large to pass the pores of the membrane, the water, salts and other low molecular weight substances are osmotically removed from the interior of the sac. They cite Everbeck (2) as independently using this principle, while Slater and Kunkel (4) substituted polyvinylpyrrolidone. We have applied this principle to large scale concentrations with a slight modification in procedure and with the substitution of the less expensive polyethylene glycol (Carbowax 6000; Carbide and Carbon Chemicals Co., 30 East 42nd St., New York 17, New York) as the high molecular reagent. Thus, with only cheap chemicals and common laboratory equipment it is possible to concentrate the protein (and other colloidal materials) from large volumes of dilute solution. We propose the name, "osmotically forced dialysis," for this method of concentration. The essentials of our procedure are as follows:

Step 1. Load the liquid into small diameter cellulose dialysis tubing of any convenient length. Lay the tubing in a container between layers of dry Carbowax 6000, using a minimum weight in grams equal to one-fourth the volume in milliliters.

Step 2. When there is no more liquid in the tubes, transfer them to distilled water for dialysis. After a time, force the liquid to one end of the tube; rinse the remainder and discard it.

Step 3. Repeat concentration as in Step 1.

Step 4. Rinse concentrate from tubes and freeze dry. If necessary, any contamination of the dry product with Carbowax probably can be removed with dry methanol.

In one run, 3800 ml (11 % dry solids) of juice from apples rotted with Botryosphaeria ribis Gross. and Dug. were concentrated to 8.6 gm dry solids (0.23 %), a concentration of 440 fold over the original volume, or 50 fold over the original dry weight. Of this crude product, 13 % dissolved in dry methanol. In another run, 15,340 ml were processed, yielding 29.4 gm (0.19 %). The times involved were about: Step 1—18 hours; Step 2—12 hours; Step 3—12 hours. Steps 1 and 3 can be speeded by the use of more Carbowax.

The efficiency of any manipulation of proteins demands the avoidance of denaturation. The nature of the extracts used prohibits any generalizations on this matter, but there do not seem to be any fundamental difficulties (3, 4), as proteins are generally more stable when concentrated than when dilute. If difficulty is found, it may be wise to purify the Carbowax by precipitation from an organic solvent (e.g., acetone) as it is known to contain methyl hydroquinone (absorption maximum at 290 mμ), or substitute polyvinylpyrrolidone (General Aniline and Film Corp., 435 Hudson Street, New York 14, New York).

In this work all lengthy procedures were carried out in a cold room or refrigerator. Recovery of the one enzyme studied (a pectinase) was complete when the lyophilized powder was compared with the original juice. After washing with cold dry methanol and ether at room temperature, the recovery was only 75 %. The lyophilized powder was mostly polysaccharide, which may have helped prevent denaturation.

The choice of the high molecular reagent in this procedure is somewhat arbitrary. Carbowax 6000 has about the smallest feasible molecular weight (ca 6000), as Carbowax 4000 goes through cellulose tubing too easily. Besides being more economical, Carbowax 6000 is easier to handle than polyvinylpyrrolidone. The latter is a sticky powder while the former comes as brittle waxy flakes.

LITERATURE CITED


1 Received June 9, 1955.