A METHOD FOR CONCENTRATING SOLUTIONS OF HIGH MOLECULAR COMPOUNDS

Hans Palmstierna

Dept. of Bacteriology, Karolinska Institutet,
Stockholm 60, Sweden

Received November 23, 1959

In many instances solutions of biological, high molecular compounds cannot be concentrated by freeze-drying, vacuum distillation or similar methods without loss of biological activity. Methods that allow the salt concentration and the hydrogen ion concentration to be kept approximately constant in the solutions are most times to be preferred. Such a method is for example the widely used ultrafiltration method. Ultrafiltration is, however, time consuming. Rapid methods using the water absorbing potency of some carboxymethyl-cellulose derivatives will be described. Similar methods have been tried by other workers using a polyethylene glycol called Carbowax 2 M (Kohn,1959), a polyglucose called Dextran (Schneider and Wallenius,1951; Rossi and Schneider, 1953; Aly,1954) and a high polymer of sucrose called Ficoll (Holter and Møller,1958).

Methods: Cellugel Super (3000), kindly provided by Svenska Cellulosa Aktiebolaget, Kemiska Produkter, Essvik, Sweden, was rinsed by suspending it in 70 per cent (v/v) ethanol with the use of a high speed blendor. It was freed from ethanol on a Büchner funnel. The procedure was repeated twice or until tests for chloride were negative. The cellulose was then dried with ethanol and acetone, and left over night to dry. Ethyl ether in combination with heat seems to damage the product.

The solution to be concentrated may be enclosed in a dialysis bag, the bag put on a bed of dry Cellugel and covered by it. Slight rocking of the bed is to be recommended. It is

also possible to do it the other way round: By enclosing the Cellugel and dip the bag in the solution. A third way of doing it may be mentioned: Coarse filter paper is dipped in a 2 - 3 per cent solution of Cellugel. The paper covered with the gel is dried in a warm air current while rotating. Dialysis bags filled with the solution to be concentrated are wrapped in the treated paper and a weight is put on top to ensure good contact.

Results: 20 ml of a 20 per cent albumin solution was concentrated to 11 ml in an hour (bed method, diam of tubing 6.35 mm room temperature). Horse serum was concentrated over night to a semi solid solution that could be dissolved to a clear solution in 2 M NaCl. In order to test if the procedure would change the hydrogen ion concentration by means of ion exchange, 0.001 M NaCl was concentrated from 250 to 50 ml over night. The Cellugel was enclosed. The pH changed from 6.80 to 6.65. No correction was made for carbon dioxide. The chloride concentration was not changed as found by titration. A 2 per cent solution of Bordetella proteins in a 0.02 molar phosphate buffer pH 7.4 was concentrated to 6 per cent. The final pH was 7.3.

If the dialysis tubing is washed thoroughly before use in order to remove softening agents no residue is found after distilled water has been reduced in volume by the suction methods described. This was tested by reducing 2000 ml to 100 ml over night. No residue was found. The flask was washed in a few ml of water and tested for carbohydrate by means of alpha-naphtol after acid hydrolysis and the Dische carbazol method. No carbohydrate was found. All the substances described in the introduction gave residues.

More low molecular carboxy-methyl-celluloses have been tried. They are even better absorbers than the Cellugel but they tend to leak through the membranes. The method has been tried for concentrating suspensions of Escherichia coli, Salmonella typhi murium and phages. The yields approximated theory. The method has been shown to be valuable to the immunologists, since weak antisera can be improved by concentrating them with the methods described.

This work has in part been supported by the Research and Development Liaison Group, U.S. Department of Army, through its European Office.

Literature

Aly, F.W., Biochem Z. 325 (1954) 505

Holter, H., and Møller, K.M., Exptl Cell Research 15 (1958)631

Kohn, J., Nature 183 (1959) 1055

Rossi, G., and Schneider G., Klin. Wochsckr. 31 (1953)969

Schneider, G., and Wallenius G., Scand.J.Clin.& Lab.Invest. 3 (1951)145.