

Concentration of dilute haemoglobin solutions with Sephadex¹

B. DEUTSCH, R. D. LEVERE, and J. LEVINE *From the Department of Medicine, State University of New York, Downstate Medical Center, New York*

Sephadex provides a rapid and efficient means of concentrating haemoglobin solutions without significantly altering the original pH or causing denaturation of the protein. Sephadex consists of small grains of a cross-linked polysaccharide (dextran) which is hydrophilic and has polar properties due to a high content of hydroxyl groups. The degree of cross-linkage determines the porosity, with these properties bearing an inverse relationship. As a result of its hydrophilic character, Sephadex has a great affinity for water and swells when placed in aqueous solutions. The amount of water held is related to the cross-linkage. The quantity of captured water is expressed as water regain and recorded as grams of water per gram of dry gel. The solute of salt solutions can diffuse relatively freely through the gel grains, while large molecules, *e.g.*, haemoglobin, are completely excluded. These properties of Sephadex have been most extensively utilized for gel filtration and chromatography although they lend themselves well to the concentration of solutions. Flodin, Gelotte, and Porath (1960) have described the use of Sephadex in concentrating the high molecular weight solute, cellulose.

TECHNICAL

To concentrate haemoglobin solutions of an initial volume greater than 10 ml., 1 g. of Sephadex G-25 (water regain 2.5 g. H₂O/g. dry gel) is used for each 5 ml. of solution. With volumes smaller than 10 ml. 1 g. of Sephadex is used for each 6 ml. of solution. The Sephadex is added to the haemoglobin solution and the mixture stirred for 10 minutes. Following this the slurry is poured into a scintered glass funnel and collected under vacuum. Some of the water and salts are now 'bound' by the Sephadex whereas haemoglobin is not and passes into the collecting flask. The collecting vessel is then changed and, if a red colour persists in the gel, an additional 1 to 2 ml. of the original haemoglobin solvent is added to the funnel to clear the slurry of any remaining haemoglobin.

This technique will increase the concentration of the original solution approximately three-fold and can be repeated as many times as desired to obtain the required concentration. With each concentration new gel grains should be used. The grains can be regenerated after use by washing with acetone and then allowing them to dry. With haemoglobin solutions of 120 mg. %, obtained after elution from a starch block, a concentration to 1.8 g. % has been accomplished after three passages through

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Book reviews

FLUORESCENT PROTEIN TRACING Edited by R. C. Nairn. (Pp. xv + 279; illustrated. 42s.) Edinburgh and London: E. & S. Livingstone Ltd. 1962.

The idea of using the specificity of antibodies for identifying antigens at the cellular level goes back at least to 1934, when J. R. Marrack showed that anti-typhoid antibody conjugated with an azo-dye would specifically bind to and stain the bacilli. The intensity of the staining, however, was insufficient to be of practical use.

In 1942 A. H. Coons, with the help of the experience of Creech in conjugating serum proteins with fluorescent carcinogenic hydrocarbons, succeeded in conjugating antibody with fluorescein and demonstrating that by means of it pneumococci could be specifically and beautifully identified in fixed tissue sections. The significance of this work must have been obvious, but the technique at the time appeared to be limited to demonstrating antigens which would withstand conventional fixation and embedding procedures; adequate fluorescence microscopes were uncommon in biological laboratories; fluorescein isocyanate for conjugation was not easy to prepare; and the idea that antibodies could be chemically manipulated with comparative ease and without loss of their specificity was unfamiliar to those most likely to benefit from their use. Ten years later Coons and his colleagues showed that fluorescent antibody methods could be applied to cryostat sections of frozen tissue, fixed sufficiently gently to retain the antigenic properties even of proteins; they had overcome many of the difficulties due to non-specific staining; and, most exciting of all, by means of the now familiar 'sandwich' technique they could even identify specific antibodies within individual cells. From this time onwards doubts and inhibitions on the part of others were thrown overboard, cryostat microtomes and suitable optical equipment began to appear in research, and even in routine laboratories, fluorochromes became available which were simpler to use, and a great harvest of theoretically and practically important information began to be reaped in many fields. The bibliography of 'Fluorescent Protein Tracing' con-

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Sephadex. Recovery of haemoglobin, based on the original weight, has ranged between 85 and 90% with this method. Little to no change (0 to 0.05) in the pH has been noted and haemoglobin denaturation, as evidenced by electrophoretic mobility, has not been apparent.

REFERENCE

Flodin, P., Gelotte, B., and Porath, J. (1960). *Nature (Lond.)*, **188**, 493