Fractionation by free electrophoresis

The procedure of removing samples from a Tiselius electrophoresis cell by means of a long thin tube is common practice and particularly when the electrophoretic mobility of a biologically active component is to be determined. A number of devices or techniques to accomplish this have been described which either minimize stirring, increase the precision of sampling or facilitate the introduction and removal of the sampling tube[1–3]. The number of samples obtained by any of these methods is limited by the stirring or mixing of the contents of the electrophoresis cells and by the time required to take each sample. The precision with which electrophoretic mobility of a biologically active component can be determined depends to a considerable extent upon taking samples which define the boundaries. By the procedure to be described here the entire contents of each limb of the Tiselius cell can be separated easily into a large number of fractions and hence the boundary of any component can be determined rather precisely.

An Aminco electrophoresis apparatus with the conventional Tiselius center section (3 × 25 × 80 mm) was used for these studies. Fig. 1 shows the arrangement used for collecting fractions from the descending (left) limb. The essential features are the 18-gauge stainless-steel tube (A) extending into the center section and a method of forcing the solution in the descending column up the steel sampling tube. This was

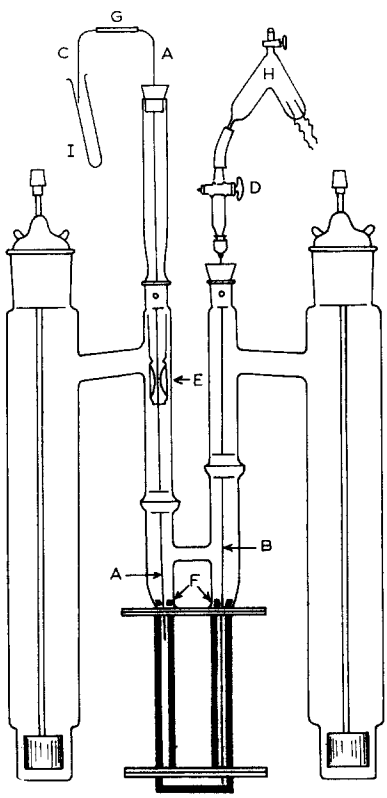

Fig. 1. A diagrammatic section of the assembly for fractionating the descending (left) limb after a conventional electrophoretic separation.

accomplished, without moving the ascending boundaries, by introducing a liquid, more dense than the solution being fractionated, into the bottom section with a long 22-gauge stainless-steel tube (B).

A typical experiment will be described, in which normal rabbit serum was fractionated, to illustrate the steps involved in the procedure. After conventional electrophoretic separation the 22-gauge stainless-steel tubing (B) was filled with 5 % NaCl in buffer and inserted as shown in Fig. 1. The electrolytic compensator (H), which also was filled with the saline solution, was attached and all openings on the ascending side were closed.

Next, the assembly containing the springs (E) for holding the sampling tube, which has been described by HECKLY[4], was inserted and filled with buffer. The sampling tube (A) was pushed nearly into position before seating the rubber stopper at the top. The tube was centered in the cell by a piece of 7 mm thick plastic which was fitted loosely into the bottom of the upper section. The hole through the center of the plastic was tapered to facilitate insertion of the sampling tube (A). The delivery tip (C), which was held in position by a clamp (not shown), was connected to the sampling tube by a flexible polyethylene tube (G) so that slight movements of the delivery tip would not be transmitted to the sampling tube which would mix the cell contents.

The current through the electrolytic compensator was set at 10 mA, the collecting tube was changed at 1-min intervals, and photographs were taken at 5-min intervals. The photographic record was used to determine the position of the sampling tube, the time of entry of each component into the tube, and to relate the fractions to cell position. The rate of movement of the albumin peak, determined from the photographic record, indicated that the volume delivered to each collecting tube was approximately 0.104 ml. Obviously, lower feed rates and a larger number of fractions can be taken to improve the resolution. It is important, particularly with smaller fractions, that the delivery tip be made to touch the wall of the collecting tube at all times to minimize variations in flow rate due to pressure differences produced by surface tension. The maximum allowable delivery rate was not determined, but the 18-gauge tubing did not produce excessive back pressure at a flow rate of 0.3 ml/min. If the pressure becomes appreciable, there is a danger of unseating a joint or a part of the Tiselius cell. It was found that the hold up in the sampling-tube assembly was approximately 0.4 ml, and, therefore, the contents of collecting tube No. 5 correspond to the material which was at the lower end of the sampling tube when fractionation was started.

The procedure for fractionating the ascending limb was comparable to that described above, except that buffer was introduced in the upper section of the descending limb. The 5 % NaCl previously introduced into the bottom section was adequate to stabilize the cell contents.

Fig. 2 shows the results of an experiment in which the components of normal rabbit serum in 0.1 *M* veronal buffer, pH 8.5, were separated electrophoretically. The typical schlieren pattern, which is a measure of the refractive-index gradients in the column, is shown below the curve indicating the protein concentration of the fractions. The two are presented on a common scale to facilitate direct comparisons, each fraction representing 1.4 mm of cell height.

The protein content was determined by the method of LOWRY *et al.*[5], using bovine

serum albumin as the standard. A comparison of the two curves shows that each step in the protein curve corresponds to one of the protein peaks of the schlieren pattern. It is also apparent that the concentration of each component can be estimated from the protein curve. For example, the protein concentration of fraction No. 42 was 5 mg/ml, which represents the globulins, and the protein concentration increased from 5 to 13.5 mg/ml between fractions No. 43 and 53 (the albumin component); hence, the preparation contained approximately 5 mg/ml globulin and 8.5 mg/ml albumin.

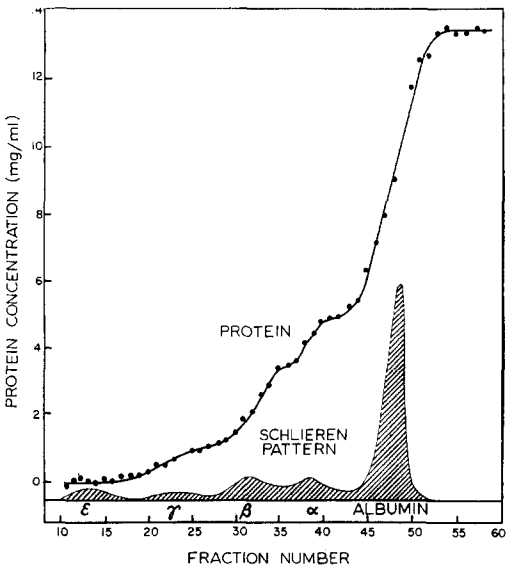

Fig. 2. Electrophoretic patterns of a normal rabbit serum in 0.1 *M* veronal buffer, pH 8.6 after 140 min at 7.4 V/cm. The upper curve represents the protein concentration of each fraction obtained from the descending limb, and the lower is a tracing of the usual schlieren pattern as it appeared before fractionation. The flow rate was 0.1 ml/min, and each fraction represented about 1.4 mm of cell height.

The described method makes practical the measurement of the electrophoretic mobilities and concentrations of biologically active components independent of the schlieren pattern and is of great value when working with highly biologically active materials whose concentration is too low to be detected by the optical methods. A significant advantage of free boundary electrophoresis over paper electrophoresis is that one need not be concerned with adsorption and the mobilities can be measured directly without comparison with a substance of known mobility. Thus, fractionation of the entire contents of both limbs facilitates electrophoretic studies of active materials and increases the precision with which the mobility can be determined.

This work was sponsored by the Office of Naval Research, U.S. Navy, under a contract between the Office of Naval Research and the Regents of the University of California. Opinions expressed in this report are not to be construed as reflecting the views of the Navy Department or of the naval service at large.

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Received July 31st, 1959