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A Simple Apparatus for Protein Fractionation by Polyacrylamide Gel Disc Electrophoresis

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A simple apparatus has been designed for polyacrylamide gel disc electrophoresis of proteins. Buffer economy and the absence of gaskets used in commerical models to hold the tubes are special features of the apparatus.

COMMERCIAL models available for polyacrylamide gel electrophoresis with tris buffer[1-3] need large quantities of the buffer, and gaskets to hold the tubes. A simple home-made apparatus is described in this paper.

The apparatus is of the vertical type made of perspex for a simultaneous run in 6 tubes (Fig. 1). A tank at the bottom with 100 ml buffer forms the anode vessel. Another tank at the top which can also hold 100 ml buffer forms the cathode vessel. Six holes of about 2 mm diam. are drilled 1.5 cm apart in the front wall of the cathode vessel, 1 mm above the buffer level. The distance between these holes and a platform 1 cm below the buffer line in the anode vessel is 10 cm. Pyrex glass cylindrical tubes of 7 mm inner diam. and 10 cm length with the edges ground using a sandpaper, cleaned well with chromic acid and dried, are filled with the gel and placed vertically passing through holes of 1 cm diam. at the top and bottom platforms of the apparatus. Platinum electrodes resting at the bottom of the two buffer vessels covering the entire length are connected to a power supply. The assembly is placed on a glass plate and enclosed in a perspex chamber with a base hole for the wires to pass through.

Reagents (Barka[4]) — (1) Tris (6.05 g), EDTA (0.6 g) and boric acid (0.46 g) dissolved in water and made up to 100 ml served as the stock tris buffer, *p*H 8.8. It was diluted to 10 times its volume for use in buffer vessels; (2) acrylamide (30g) and N, N′-methylene-*bis*-acrylamide (0.8 g) were dissolved in water and made up to 100 ml; (3) dimethylaminopropionitrile (1.6% vol./vol.) in stock buffer; (4) potassium ferricyanide (0.03% wt./vol.) in water; and (5) ammonium persulphate (0.48% wt/vol.) in water.

Ammonium persulphate is stored in small sealed polythene packets in a desiccator. After use from each packet, the excess is discarded. The clean and dry glass tubes are placed vertically with the bottom ends tightly kept in ampoule caps. Equal quantities of reagents (2) to (5) are mixed without air bubbles and 2 ml of the mixture poured into each tube. A space of 1 in is left at the top of the gel surface in the tubes. The tubes are allowed to stand at room temperature till polymerization is complete. This takes about 40 min. The top portion in contact with air is not polymerized, since polymerization takes place only in the absence of oxygen. When polymerization is complete, a thin discrete line of demarcation is visible between the unpolymerized and polymerized gels. The top unpolymerized gel is now discarded and the inner walls of the tubes are washed with dilute buffer. The bottom ampoule caps are removed.

The serum to be analyzed is stained with a little bromophenol blue as a marker. The tubes are kept in position in the apparatus. Exactly 10 μl of the stained serum is transferred with a micropipette to the gel-buffer interface to form a thin disc. Connections are given between the buffer in the tubes and the buffer in the cathode vessel by means of cotton wicks which pass through the holes in the vessel. The bottom gel in the tube is in direct contact with the buffer in the anode vessel. The assembly is now closed. Connections are made to an electrophoresis power supply at 200 V, which gives a current strength of about 5 mA per tube. A good resolution is obtained in 2 hr. At the end of the run, the cotton wicks are folded up and the tubes removed. The gel

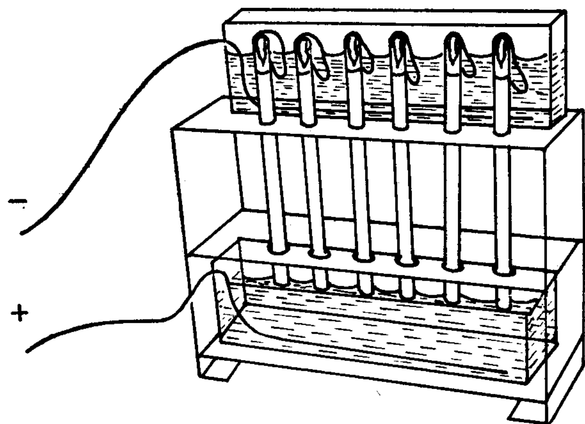

Fig. 1 — The gelelectrophoresis apparatus

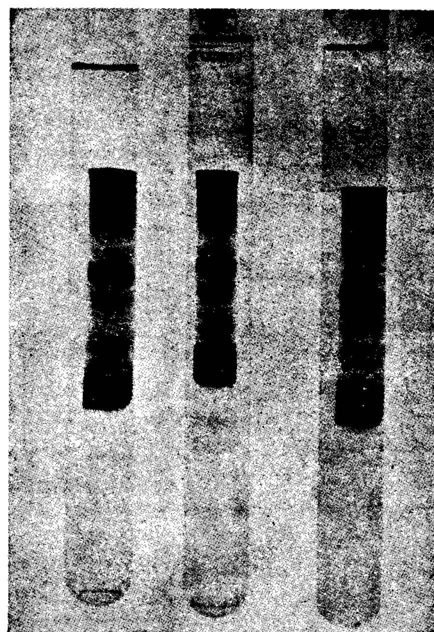

Fig. 2 — The serum protein patterns

is forced out on to a petri dish by a water jet from a syringe.

The gel is stained with 0.7% amidoblack prepared in 7% acetic acid for 10 min. Excess stain is washed off from the gel with dilute acetic acid till the pattern is clear.

The patterns obtained with this apparatus are of recommended standard. The normal serum protein patterns obtained are shown in Fig. 2. Any protein specimen can be fractionated using this apparatus. However, if the sample is not viscous like serum, it may be made so by dissolving a few crystals of sucrose for easy application as a thin disc. Prealbumin in serum is separated distinctly. The order of resolution is: prealbumin, albumin postalbumins (3-5 fractions), ceruloplasmin, transferrin, post-transferrin fractions (7-10) and γ-globulin. Haptoglobins, formed in the post-transferrin region as a thin disc, are difficult to identify by photography. However, if only haptoglobins are to be studied, benzidine or o-toluidine saturated in glacial acetic acid is used with a few drops of fresh hydrogen peroxide. The fractions of haptoglobins are very clear and need immediate photography. The apparatus can be used for lipoprotein separation and for studies on isoenzymes. This apparatus was used in an earlier study on prealbumin in health and diseases[5].

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