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A new apparatus for polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis is now widely used in protein analysis. The sieving characteristics of the gel together with the different mobilities of the constituent ions along a voltage gradient result in the well-known electrophoretic patterns.

Gel columns are commonly prepared in cylindrical glass tubes of appropriate sizes. However, the preparation of a large number of these columns, freeing of the gels from the columns and subsequent treatment of the individual gel rods for staining and destaining are rather tedious and time-consuming. Attempts have been made to simplify this procedure. RAYMOND[1,2] has described a vertical slab technique, which allows a simultaneous analysis of different samples in the same apparatus, and WOODWORTH AND CLARK[3] have recently presented a modification of this apparatus, allowing the casting of two parallel gel slabs with several sample slots.

This paper describes a new type of vertical gel electrophoresis cell with gel mould, cooling chambers, buffer reservoirs and electrodes all in the same unit. It combines the advantages of the cylindrical gel technique with the simplicity and time-saving of the slab method.

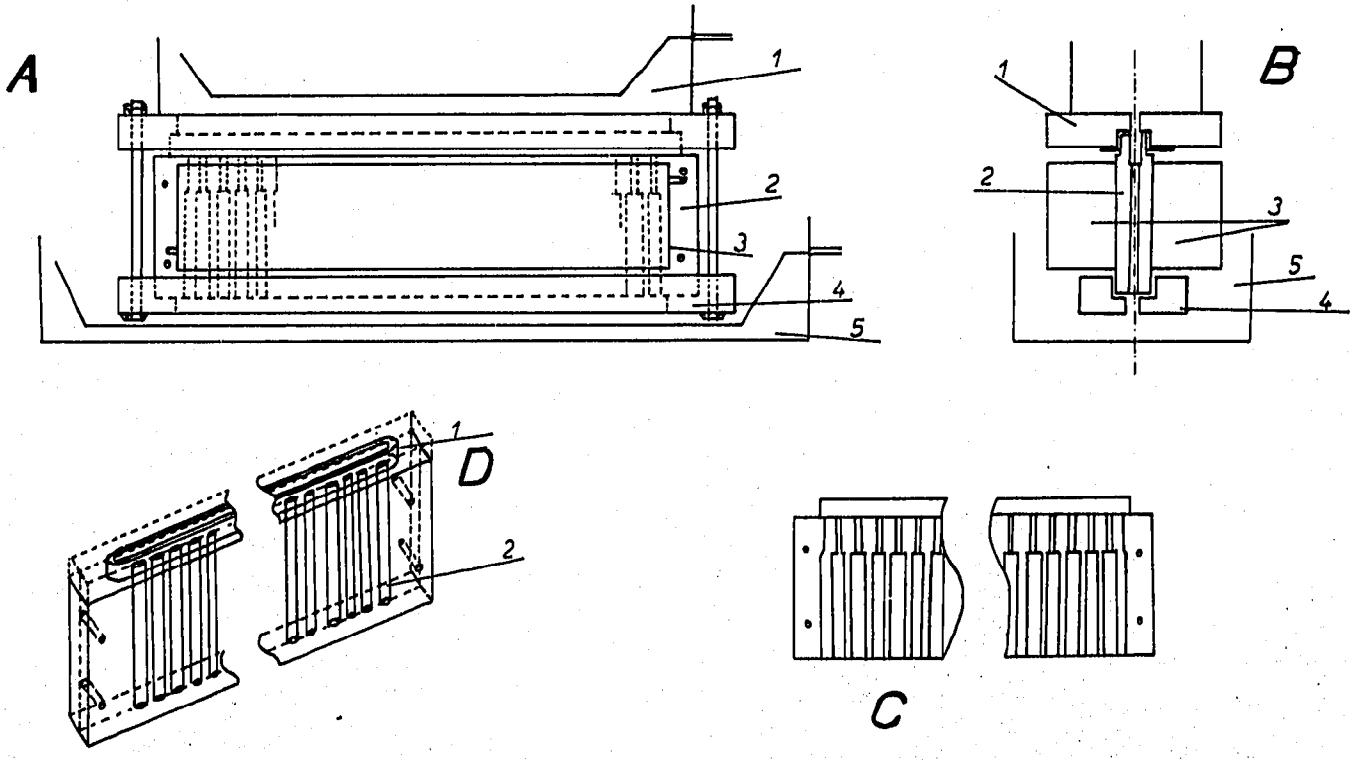

Fig. 1. Apparatus for continuous gel electrophoresis. (A) General view of the assembled unit with buffer tanks and electrodes (1 and 5), separation compartment (2) with cooling chambers (3), and base (4). (B) Section of the apparatus with upper buffer tank (1), separation cell (2), cooling chambers (3), base (4) and lower buffer tank (5). (C) View of the inner side of one part of the separation cell. (D) Separation cell with edge (1) and separation channels (2).

Apparatus

Fig. 1 depicts the principles of this apparatus. All parts are fabricated from clear plexiglas. Two plexiglas pieces (27 × 9 × 1 cm) are clamped together along their greatest surface and 25 holes of 6 mm I.D. are drilled through the width of the two pieces, just where the sides touch. A 3 mm interspace is kept between the holes. Then a tray of 7 mm width and 1 cm depth is made at the upper edge. This tray will contain the water layer on the top of the acrylamide mixture during polymerisation. An edge of about 3 mm thickness and 1 cm height is formed around this tray by cutting away the plexiglas. The upper buffer reservoir is mounted around this edge. Finally, two holes are drilled at each side to clamp the unit together during operation (Fig. 1D). The two parts are separated and the interspace between the half cylinders is milled away over 1 mm depth and 6 cm height, starting from the bottom. In this way, a continuous gel slab will be obtained, while individual sample slots of about 2 cm depth still exist at the top, when the unit is assembled for operation (Fig. 1C). Cooling chambers with connection ports are mounted on the outer side of each part of the separation unit.

A piece of plexiglas of 30 × 8 × 2 cm serves as the bottom of the upper buffer reservoir. Suitable grooves are milled into this piece to fit it around the edge of the separation unit and to make contact between the buffer and the gel. The reservoir is built up with appropriate plexiglas pieces. A silicon rubber joint ensures a watertight fit between the buffer tank and separation unit. Another piece of plexiglas (30 × 5 × 2 cm) serves as the base for the separation unit. Grooves are milled into this piece to hold the separation unit and to make contact between the buffer and the gel. Four holes are drilled into the bottom of the upper reservoir (out of the buffer compartment) and the base piece for finally mounting of the cell (Figs. 1A and B). The lower electrode chamber is made from appropriate pieces of plexiglas.

Electrodes consist of stainless steel wire and are mounted into a wall of the buffer tanks. All nuts and screws are made of stainless steel.

Fig. 2 is a photograph of the assembled unit.

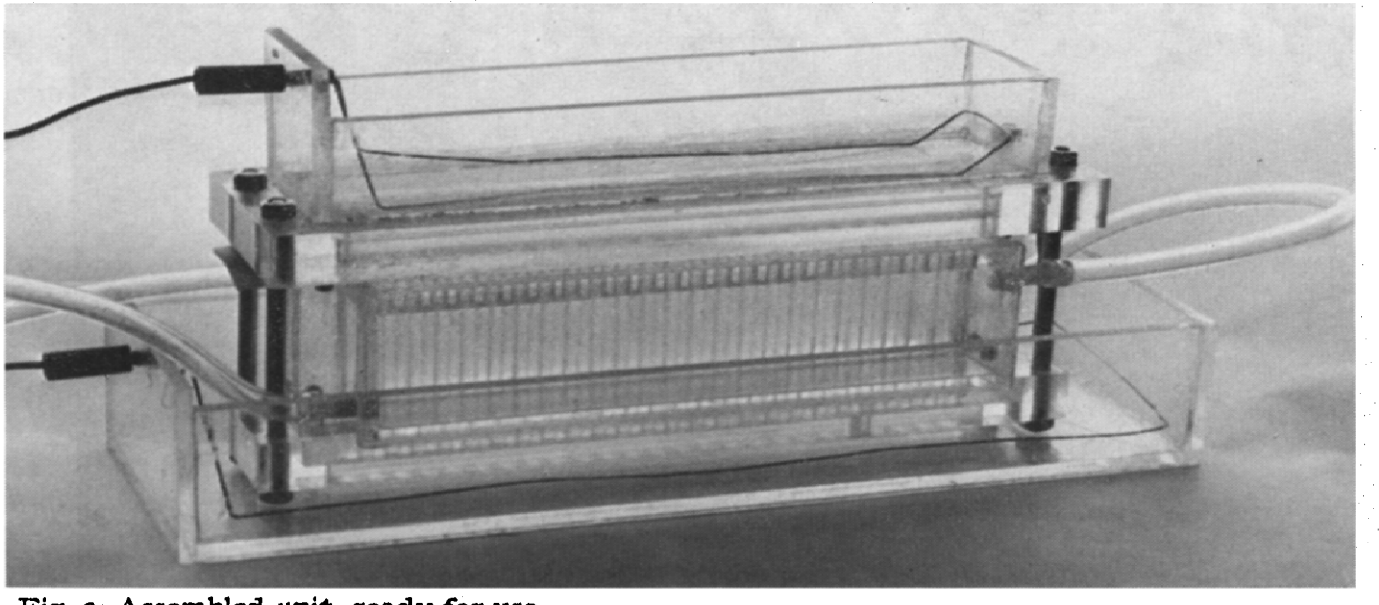

Fig. 2. Assembled unit, ready for use.

Reagents

All chemicals are P.A. or puriss. grade. Solutions are prepared using deionized water.

Solution A. 7.5 g acrylamide and 0.2 g N,N'-methylenebisacrylamide are dissolved in 25 ml water.

Solution B. 1.6 ml dimethylaminopropionitrile in 100 ml Tris-buffer (60.5 g trishydroxymethylaminomethane, 7.82 g EDTA and 4.64 g boric acid in 1 l water).

Solution C. 0.03% potassium ferricyanide in water.

Solution D. 0.48% ammonium persulfate in water.

The gels are fixed and stained for 2 h in 7% acetic acid containing 0.1% amidoblack, and destained in 7% acetic acid.

Gel preparation and electrophoresis

The separation cell is mounted by clamping the two parts together with four screws and nuts. The bottom of the cell is covered with adhesive tape (Tesa-band 651, Beiersdorf, Hamburg). Equal amounts (20 ml) of solutions A, B, C and D are mixed and this mixture is poured slowly into the separation chamber to about 2 mm from the top of the sample slots. Care is taken to avoid trapping air bubbles within the gel solution. Water is then layered above the gel solution. The cell is covered and polymerisation is achieved at room temperature overnight or at 37° for 2 h. The sealing tape and the water layer are discarded and the upper buffer reservoir and the base are mounted on the separation cell with appropriate screws and nuts. The gel surface is washed with 25% Tris-buffer and the unit is placed in the lower buffer tank. Connections for cooling water are made and both electrode chambers are filled with 25% Tris-buffer (two 0.5 l portions). The samples are pipetted under the buffer into the different slots. The electrodes are connected to a d.c. power supply and the

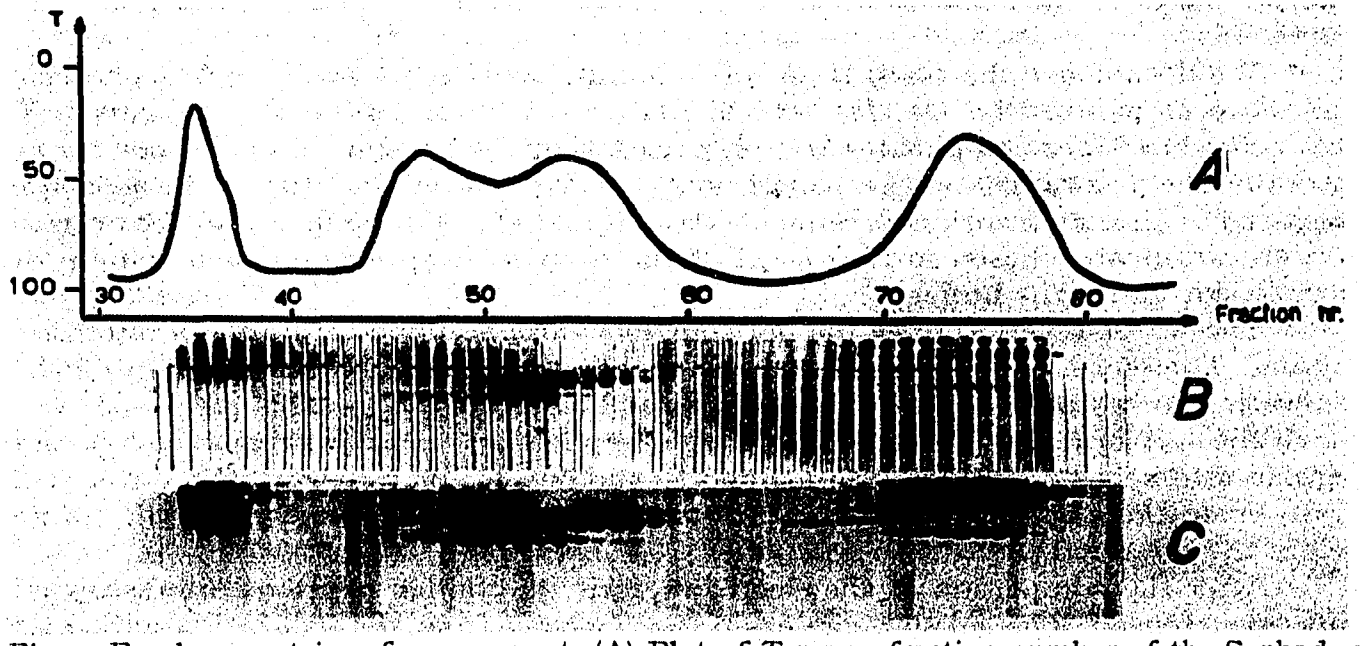

Fig. 3. Eye lens proteins of a young cat. (A) Plot of *T versus* fraction number of the Sephadex G-100 column effluent. (B) Polyacrylamide electrophoresis of the protein fractions, obtained with the continuous gel apparatus. (C) Electrophoresis of the same fractions on individual gel rods.

electrophoresis is started at 120 mA and about 80 V. After 10 min, a 170 mA current is applied at 110–130 V. The analysis is completed in about 90 min, as determined by the position of the marker dye. The separation cell is disconnected from the upper buffer tank and the base, and separated into its two parts. The gel slab is removed carefully with a spatula and is now ready for staining. With some experience, freeing the gel from the separation cell can easily be done without breaking it.

Results and discussion

Results obtained with this apparatus can be seen in Fig. 3. Eye lens proteins of a young cat are separated on a 200 × 1.1 cm Sephadex G-100 column and eluted with 25% Tris-buffer at 20°. Fractions of 1.6 ml are collected at flow rates of about 10 ml/h and weighed. The column effluent is monitored continuously using a LKB Uvicord II photometer with UV light of 280 mμ. The results are plotted as *T versus* fraction number (Fig. 3A). Dextran, about 2%, was added to the samples to ensure better layering under the buffer. One drop of a 0.015% bromophenol blue solution is also added to each sample.

Electrophoresis was carried out using the continuous gel apparatus and also on 7 × 0.5 cm gel rods in glass columns. The results are depicted in Fig. 3B and C.

As can be seen from these figures, both methods give reliable electrophoretic patterns. The resolution seems even to be better with the continuous gel technique. However, this is due to a difference in running distance between the two methods. Relative distances of migration of the protein fractions are the same for both versions of gel electrophoresis.

Fig. 3B demonstrates that the protein bands diffuse in the interspace between the separation areas. This "tailing" however does not influence the separation itself. In preliminary experiments, protein mixtures of totally different composition were pipetted in adjacent sample slots and analysed. Tailing occurs, but fractions from one sample have never been found to diffuse up into the adjacent separation compartments.

We do not use the three layer gel columns (sample gel, spacer gel, separation gel) as recommended by ORNSTEIN[4] and DAVIS[5]. We found that, for our purposes, this procedure only complicates the preparation of the columns and did not really improve the resolution of the constituents of the protein mixture. The gels are prepared as already mentioned. Some dextran is added to the samples and the protein concentration was chosen so that 50 μl of the solution is applied on each column or sample slot under the buffer.

The water layering over the gel solution must be done carefully to minimize mixing. Therefore, a filter paper strip of 4 mm width is placed on the top of the separating unit, covering half the surfaces of the sample slots. This strip does not touch the acrylamide mixture. Water is then applied to this paper strip at a slow and regular rate using a syringe with a hypodermic needle. In this way, the water will drain on to the surface of the gel solution and flat smooth gel surfaces of the same height are obtained.

After the completion of the electrophoresis, the buffer solutions are discarded and never reused. The gel slab can easily be removed from the separation unit, and sticking on the plexiglas walls does not occur. Slicing of the gels can easily be done, if desired.

For photography, the gels are rinsed with water and placed on a clean glass plate. They can be stored in plastic boxes in diluted acetic acid, or dried as described by WOODWORTH AND CLARK[3].

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Chromatography on ion exchange papers

XXIV. The adsorption of chlorocomplexes of metal ions on a carboxylic resin from HCl solutions

In this series of papers we have dealt repeatedly with adsorption effects other than actual "ion-exchange" on various resin and cellulose exchangers. While the work of NELSON *et al.*[1] shows adsorption of metal ions on a sulphonic acid resin from both HCl and $HClO_4$ solutions we were not aware of any data on the adsorption of chlorocomplexes on a carboxylic acid resin from relatively concentrated HCl solution *i.e.* under conditions where the carboxylic group must be considered as completely unionised.

Experimental

The metal ions were chromatographed, in the ascending manner, on washed Amberlite WA-2 paper strips in the H^+ form.

We found considerable variation of R_F values with the amount of the metal ion chromatographed, *i.e.*, a rather low capacity as would be expected in absence of ionised exchange groups. The R_F values are thus in most instances given as a range rather than precise values for an exact concentration. The results obtained with a number of chlorocomplexes is shown in Table I and compared with the adsorption on pure cellulose paper (second last column).

Strong adsorption was observed with Au(III), Bi(III), Pt(IV) and Tl(III). Only in the case of Bi(III) is the general trend of increased adsorption with increased HCl concentration reversed and Bi(III) is more strongly adsorbed in 2 *N* HCl than