APPARATUS FOR PREPARATIVE SEPARATION OF SUBSTANCES BY DISK ELECTROPHORESIS IN POLYACRYLAMIDE GEL

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An apparatus for preparative electrophoresis in polyacrylamide gel with a system for cooling the gel on both sides is suggested. As much as 150-180 mg of serum proteins can be simultaneously fractionated in this apparatus.

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Analytical electrophoresis in polyacrylamide gel (PAG) is one of the best methods of analysis and fractionation of protein mixtures [2, 4-7]. Several different types of apparatus have recently been suggested for preparative fractionation of substances in PAG [1, 3], but they are very complex in design, small in capacity, and are made by special firms.

This paper describes an apparatus for preparative electrophoresis in PAG capable of separating up to 150-180 mg protein, and simple to manufacture and operate.

The scheme and general appearance of the apparatus are shown in Figs. 1 and 2. The main parts of the apparatus consist of two glass cylinders: an inner cylinder with external diameter 60 mm and an outer cylinder with internal diameter 70 mm. The length of the outer cylinder is 80 mm and of the inner 155 mm. The narrow cylinder is fitted into the wide one. They are fixed together and centered relative to each other by means of guide-supports, so that a circular space 5 mm in width is formed between the centered cylinders. This is the working chamber of the instrument. The cylinders are made from ordinary thick-walled centrifuge jars, having walls of strictly uniform thickness (± 1 mm).

Firmly fixed to the upper end of the outer cylinder (Fig. 1), and separated from it by a thin rubber washer, is a Plexiglas ring into which the three stainless steel guide supports are mounted. Plexiglas bushes are fitted onto these supports, their lower end being glued to the Plexiglas ring. The bushes support the electrode, maintain the inner cylinder at the specified height and, in addition, insulate the stainless steel from the buffer. A Plexiglas cylinder (the cooling chamber) is glued to the lower surface of the Plexiglas ring. There are two connecting pipes in the Plexiglas cylinder through which the cooling fluid circulating between the cylinders enters and leaves.

The inner cylinder of the apparatus consists of a complete centrifuge jar. A Plexiglas collar is mounted on its upper part. Seven holes are drilled in it: three for the guide-supports, and the rest, equidistant from one another, are for pouring in the reagents and PAG and for introducing the specimen.

The electrode jars, made from Plexiglas (Figs. 1 and 2), have a capacity of not less than 1 liter. A Plexiglas disk, on the upper surface of which a circular groove 1 mm deep and 0.6 mm wide is cut, is glued to the center of the floor of the lower electrode jar. Along the whole length of the circular groove runs a platinum wire 0.5 mm thick and the diameter of the circular groove must be 1-2 mm greater than the diameter of the outer glass cylinder. By dividing the electrode in this manner a uniform electric field is created, and gases formed during electrophoresis do not come in contact with the lower surface of the gel.

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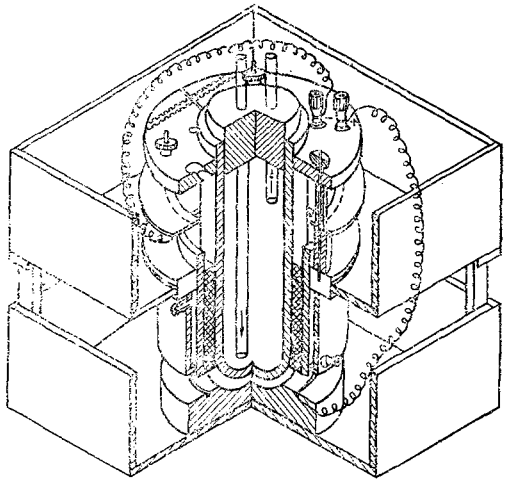

Fig. 1. Scheme of apparatus for preparative electrophoresis in PAG.

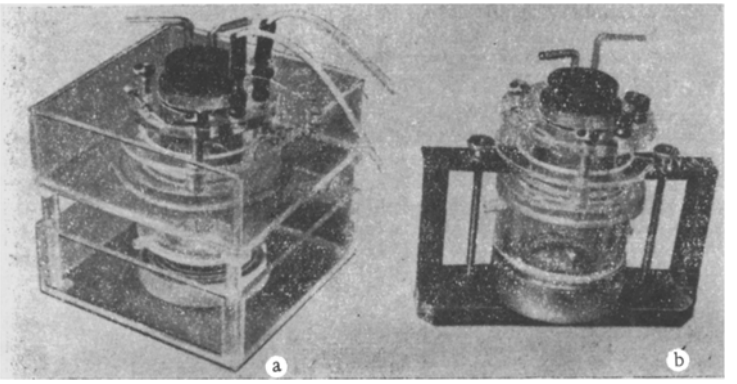

Fig. 2. General view of apparatus: a) apparatus prepared for experiments; b) apparatus on stand for polymerization.

For reasons of economy in platinum, the platinum wire is welded to a copper wire of the same thickness in a thin tongue of flame from a gas blow lamp. The burned-off end of the insulation on the copper wire is replaced by the same sort of insulating material, which easily fuses on gentle heating with the insulation material present on the wire. Plexiglas supports 35 mm high are glued in the corners of the lower electrode jar.

A hole is drilled in the center of the floor of the upper electrode jar, its diameter 3 mm greater than that of the Plexiglas ring of the outer glass cylinder. A lining made from rubber tube is fitted over the edge of this hole. When the apparatus is inserted into the hole, the lining ensures an airtight junction between the apparatus and the upper electrode jar.

Stopping plates are glued to the lower surface of the floor of the upper electrode jar, so that the upper electrode jar always sits on the supports in the same position relative to the lower jar.

The stand for polymerization of the gel in the apparatus consists of the stand itself, supports with clamps, and the Plexiglas ring (Fig. 2b). A groove for supporting the lower edge of the outer glass cylinder is cut in the Plexiglas ring fitted to the stand. An airtight rubber lining, 3-5 mm thick, the upper part of which must be perfectly smooth, is fitted into the groove. The internal diameter of the rubber lining must be 2 mm less than external diameter of the inner glass cylinder. The conical part of the inner cylinder fits into this hole, and the lower surface of the outer cylinder rests on the rubber lining.

Clamps fitted to each support rest on side-pieces, the length of which is 2 mm shorter than that of the outer cylinder.

Assembly of the apparatus: the Plexiglas ring with the platinum electrode in the groove on its lower surface is fitted over the guide supports of the Plexiglas ring of the outer cylinder. The electrode lies at a distance of 2 cm from the upper part of the working chamber of the apparatus. Next, the ring supporting the inner cylinder is placed over the same supports. The cylinders are fixed together by means of three thumbscrews and are at the same time centered, forming a gap of 5 mm between them.

The apparatus is placed on the stand for gel polymerization. So that the inner cylinder easily fits into the hole in the rubber lining, this is slightly moistened with distilled water. One of the ends of the clamps is placed over the upper edge of the outer cylinder, and the side-pieces placed under the other ends. When the thumbscrews located on the stand are turned, the clamps press the cylinder evenly against the surface of the rubber lining, making an airtight junction in the lower part of the working chamber of the apparatus, which is filled with solutions of fine- and coarse-grain gel [8]. Next, 55 ml of 7% PAG solution is poured into it. Above this 3 ml of distilled water is carefully poured, insulating the solution from the atmospheric air, which inhibits polymerization, and the meniscus is abolished. Polymerization begins after 10-15 min. The water is poured off and remains of it removed with filter paper, after which 10 ml of 4% PAG solution is poured in, and a layer of distilled water above it. The gel is formed after 10-15 min. The ap-

paratus is fitted into the hole in the upper electrode jar. Into each electrode jar is poured 1 liter of triglycine buffer, pH 8.3. The upper electrode jar with the apparatus is placed on the lower electrode jar (Fig. 2a).

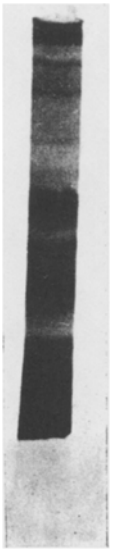

Fig. 3. Electrophoresis of human fetal serum proteins.

Better separation of the substances is obtained by diluting the serum with electrode buffer in the ratio 1:1. Bromphenol blue, in the smallest possible amounts is used as indicator. Next, for every milliliter of material to be fractionated, 0.08 ml of a dry biogel preparation of R-150 is added, to act as supporting medium for the fractionated serum. The resulting mass is carefully and very slowly layered beneath buffer directly on the large-pour gel by means of a syringe with No. 15-M needle through the holes in the Plexiglas collar. When the specimen is uniformly distributed over the surface of the gel (care must be taken to ensure that the apparatus is placed horizontally, or otherwise the specimen may flow to one side, resulting in differences in concentration of the substances in different parts of the apparatus), the apparatus is connected to a type UIP-1 rectifier, and tap water is admitted to the cooling system.

Electrophoresis is carried out at 200 V and 80-100 mA for 2-2.5 h.

To remove the gel from the working chamber, a thin steel wire is taken around it on the inner and outer sides, after which it can be easily removed as a cylinder from the working chamber.

The removed gel is cut with a knife and spread out on glass. Several strips, 1.5-2 mm wide, are cut from it perpendicularly to the zones of migration of the substances and immersed for 5 min in dye (a 1% solution of amido black 10B in 7% acetic acid). The free dye is removed in 7% acetic acid by electrophoresis for 5-10 min.

Elution of proteins from gel: the stained strip of gel, washed to remove free dye, is replaced where it was cut from the main layer of gel. The stained fractions on this strip indicate the location of each particular substance in the main layer. The strips of gel are cut out with a sharp knife, and the substances subsequently eluted from them. To do this, each cut-out strip is transferred to a Potter's homogenizer, treated with 4 to 5 times its volume of physiological NaCl solution, and homogenized for 2-5 min. The gel suspension is transferred to glass jars and agitated on a magnetic mixer for 1 h. The gel is then removed by centrifugation or by filtration through filter paper. After dialysis, the eluate is lyophilized.

Individual specimens of human and animal serum proteins were isolated in the apparatus described. The results of one experiment to fractionate 2.1 ml of human fetal serum are illustrated in Fig. 3. Fourteen separate, discrete zones can be seen.

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