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Note

A simple apparatus for preparative polyacrylamide gel electrophoresis

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On searching for a method for the preparative separation of proteins, we found no commercial apparatus that combined simplicity of handling, purity of the prepared protein fractions and a reasonable price.

Most problems in using preparative polyacrylamide gel electrophoresis (PAGE) arise from a lack of tightness between the two electrode chambers, especially between the lower buffer-filled electrode chamber and the elution chamber. Traditionally the chambers have to be tested for leaks, commonly with dyes, but the dyes cannot be removed from the PAGE system completely, even with careful and prolonged pre-electrophoresis. Thus the remaining dye may be bound to the protein fractions, possibly affecting their chemical properties. Therefore, we have developed the following device, which is a modification of Raymond *et al.*'s apparatus[1,2] for analytical PAGE and an extension to the preparative PAGE systems according to Maizel[3] and Stegemann[4].

APPARATUS

The material of construction is mainly of acrylic glass, 5 and 10 mm thick. The side-walls of the chamber (2) (Fig. 1) are made of glass for better adherence of the polyacrylamide gel, which might otherwise slip into the elution chamber (7). The gasket (8), which is cut from 1-mm silicone sheets, at same time forms the lateral limitation of the elution chamber (7).

The frame (11) is fastened with commercial Macrolon® screws, and the elution chamber is tightened carefully. No check with dye is then necessary. The diaphragm (9) is easily cut from membrane tubes (e.g., Visking 36/32). The whole apparatus is set into the lower buffer chamber, which is a common refrigerator box of dimensions 200 × 200 × 75 mm.

This apparatus is suitable for use with photo-polymerized and catalytically polymerized gels. For this purpose, the silicone gasket (8) and the frame (11) of the elution chamber have to be exchanged for modified parts without a trench in the middle. The screws (diameter 4 mm) are tightened and freshly prepared gel solution (7.2 g of acrylamide, 0.3 g of N,N'-methylenebisacrylamide, 1.4 ml of 0.045% riboflavin solution, 1.4 ml of 1.8% sodium sulphite solution and 150 ml of buffer) is poured into the gel chamber, emerging air bubbles being removed. The apparatus is then

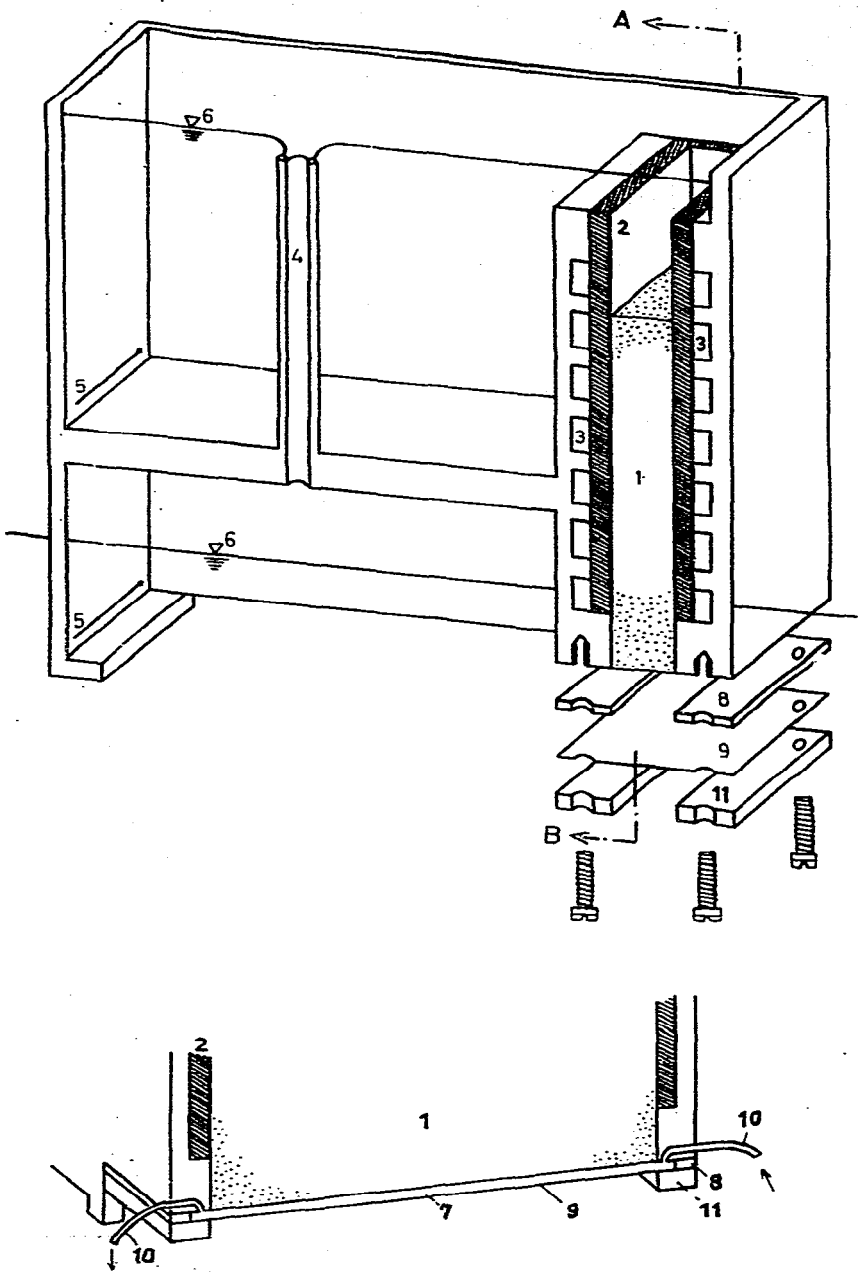

Fig. 1. Cross-section through the apparatus for preparative PAGE. 1, Gel; 2, gel chamber sides (glass); 3, cooling coils; 4, overflow tube; 5, electrodes (platinum); 6, buffer level; 7, elution chamber; 8, silicone packing; 9, dialysis membrane; 10, inlet and outlet of the elution chamber; 11, frame.

placed on a neon-lit table for photo-polymerization and parts 8, 9 and 11 are rearranged as described above in order to form the elution chamber. The silicone packing (8) has to be lubricated lightly with Vaseline.

Firm adherence of the gel on to the glass is of great importance. Therefore, the side-walls of the gel chamber and especially their edges have to be cleaned very carefully and must be completely free of any greasy substances. The buffer used for the gel, chambers and elution was 0.3 *M* Tris–borate buffer of pH 7.9. The lower chamber, in which the apparatus is set, has to be filled with 800 ml of buffer while the upper chamber is filled to the top.

A schematic diagram of the apparatus is shown in Fig. 2.

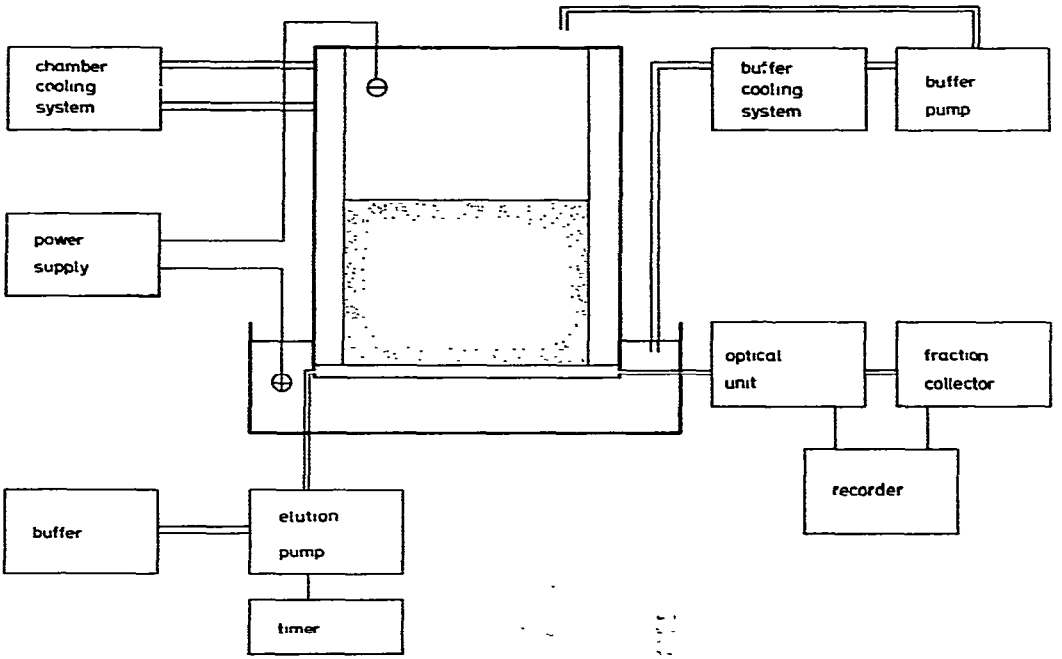

Fig. 2. Schematic diagram of the apparatus.

ELECTROPHORESIS PROCEDURE

After pre-electrophoresis overnight (50 V, 10 mA), the buffer solution is changed. The sample, charged with 10% sucrose and concentrated to 5 ml, if needed, is slowly laid on the gel with a syringe, connected with a polyethylene tube. Usually we take a sample of 0.5 g of protein, the maximum load being 1 g. The electrophoresis is carried out at 150 V and 25 mA; 4 W should not be exceeded in order to prevent curved zones. The buffer is revolved by an Eheim 381 pump, to lower the electrolytic products and to compensate for temperature differences. The cooling coils work efficiently. The temperature difference between the gel and the elution chamber can be eliminated by use of a buffer cooling system. We usually use a temperature between 4° and 10°, depending on the purpose of the protein fraction. We use a discon-

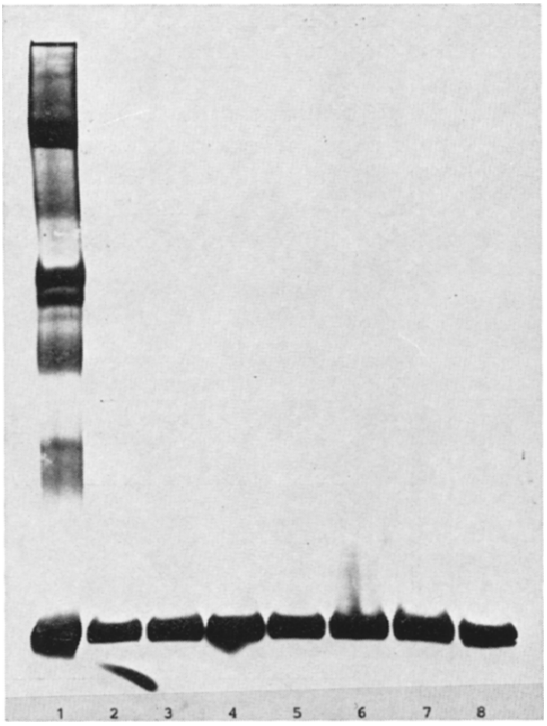

Fig. 3. Analytical electrophoresis of isolated albumin fractions from mouse serum. 1, Mouse serum; 2–8, mouse albumin from several preparative electrophoreses.

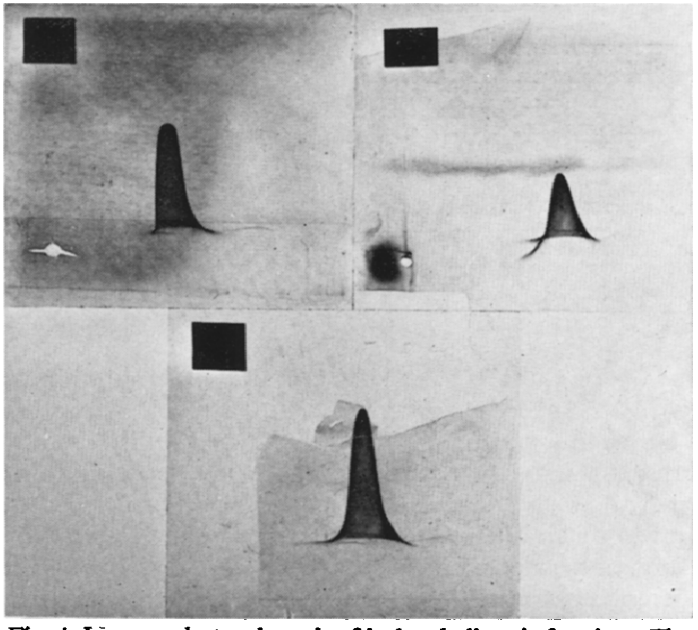

Fig. 4. Immunoelectrophoresis of isolated albumin fractions. Three fractions from a preparative electrophoresis of rat serum (3.5 ml of serum, each fraction 5 ml, containing 0.3–0.5% of albumin).

tinuous elution system for better efficiency and lower dilution. We start with an interval of 15 min followed by a 3-min elution period, produced by a Meredos-SP-GS pump regulated by a Theben-276 0003 timer. The volume eluted in 3 min is 5 ml. At the end of this period, 99% of the fraction in the elution chamber is exchanged and only 0.25% remains after a further period. The eluted sample is pumped through the optical unit of a printing recorder (ISCO UA 5 absorbance monitor), recording the extinction at 280 nm. The fractions are collected in an ISCO Golden Retriever 328. The dilution of the sample during electrophoresis is 1:10. Re-concentration is possible by using collodion bags (Sartorius SM 13200) in a glass apparatus (Sartorius SM 16304)[5].

We separated albumin, for example, from serum from various animal species for binding studies. The purity was controlled by analytical PAGE and by immunoelectrophoresis according to Clarke and Freeman[6]. The analytical PAGE did not show any impurities and the immunoelectrophoresis showed only slight impurities (Figs. 3 and 4).

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