Apparatus for Vertical Polyacrylamide Gel Electrophoresis[1]

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ABSTRACT

A description is given of the construction and use of an apparatus for vertical electrophoresis of protein solutions through polyacrylamide gels. It is versatile and rather simple to build. It is cooled by circulating water and does not require use of filter-paper connections to the electrode chambers. As many as six different samples can be subjected to electrophoresis in the same run. The design of the gel mold allows the starting point for migration of the samples to be near the middle of the gel strips, as well as near the ends.

Zone electrophoresis with starch (1) or polyacrylamide (2) as the supporting medium makes possible resolution of complex mixtures of proteins not otherwise attainable. Each medium has advantages. Polyacrylamide gels can be prepared more readily, and with a wider range of pore size. Their transparency is an obvious advantage where quantitative results are sought. Hjertén et al. (3) have shown that equally good patterns can be obtained with continuous and discontinuous buffer systems with polyacrylamide gels. One can adjust the pore size, pH, and composition of the buffers more easily with the continuous system.

Apparatus for the latter system ranges from simple cylindrical tubes to adaptations of molds designed for starch gels (4,5) to the more sophisticated equipment described by Raymond (6). Provision must be made to minimize contact of the polyacrylamide solution with air while it is setting, and to provide adequate support, since polyacrylamide gels are not as nearly rigid as starch gels.

The apparatus described here is used in a vertical position, allowing use of a measured liquid sample without interference by electrodecantation effects. Efficient cooling permits use of high voltages and consequent shortened running times. Filter-paper wicks or buffer bridges are avoided. There are two optional

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positions for the starting slot, either for situations where all proteins are expected to migrate in the same direction (downward), or where simultaneous migration in both directions is to be expected. We have used the apparatus for a quantitative study of wheat proteins (7). For this purpose, the origin was located near one end, as the pH used resulted in migration in only one direction.

DESCRIPTION OF APPARATUS

The apparatus is constructed of Plexiglas[4] or similar material. Figure 1

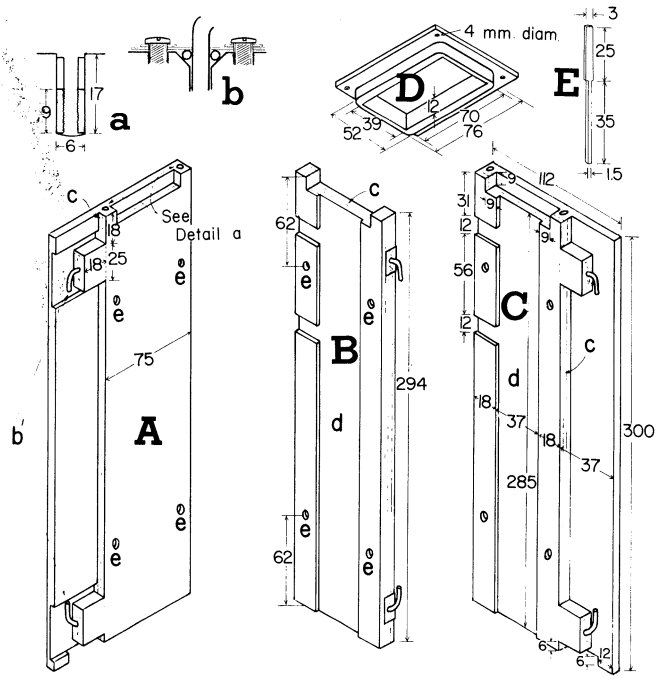


Fig. 1. Part A is a side section made up of one piece of Plexiglas 1/4 in. thick and two pieces 1/8 in. thick cemented together. Part B is a center section made up of four layers of Plexiglas 1/8 in. thick cemented together. Five center sections are used. Part C is the other side section made up of one piece 1/4 in. thick and three layers of Plexiglas 1/8 in. thick cemented together. The rails in parts B and C in which are the openings for the slot-formers should each be made from a single piece of Plexiglas, since pieces vary slightly in thickness. The pieces marked "c" have the center cut out for circulation of cooling water. The cut-out area coincides with area "d" (which holds the gel), except for 12 mm. at each end. In part C, the holes for the inlet and outlet of water to the cut-out area must be drilled at an angle.Parts A, B (five), and C are held firmly together with 1/4-in. nylon bolts 10 cm. long, through holes "e." Part D is the top section (see text). Part E is a slot-former made of teflon 1/16 in. thick. Six of these are needed. Detail "a" shows how threads are set in to receive the screws by which the top section and electrode chamber are attached. A stainless-steel insert knurled and tapped with 6/32 threads is cemented in the bottom of the hole with a light push fit. It is held in with a cemented-in Plexiglas tube drilled out with a No. 28 drill. Detail "b" shows the method of attachment for 1/8-in. o.d. stainless-steel tubes b' (Fig. 2) for cooling water. They are held by a stainless-steel plate against an O-ring seal. All dimensions in the figure are given in millimeters.

[4]Trademark of Rohm and Haas Company. Plexiglas is a thermoplastic acrylic resin.

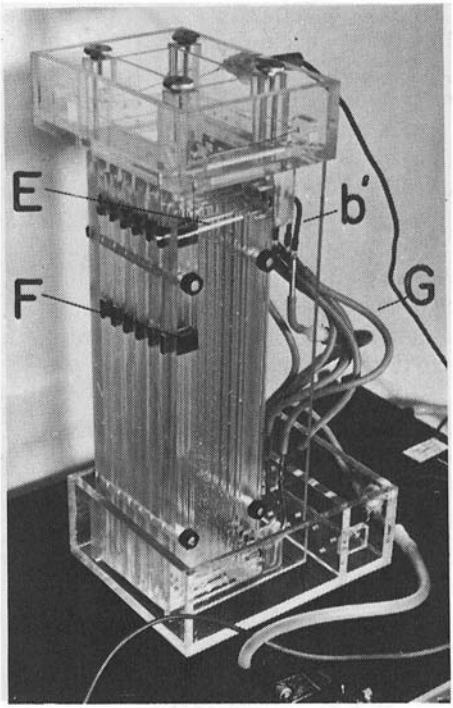


Fig. 2. Gel mold in position for electrophoresis, but without buffers in the electrode chambers or gels in the gel mold. The slot-formers are shown at E, and the optional position for them is closed off by a rubber plug F. The cooling water circulates in channels on both sides of the gels, with connections through the stainless-steel tubes b′ and rubber tubes G.

illustrates the component parts and construction details of the gel mold. Figure 2 shows the assembled apparatus with the electrode chambers in position, but with no gels in the gel mold. The first step in the assembly of the apparatus is to position the slot-formers (E, Figs. 1 and 2) in the openings provided for them in parts B and C. The large end of each slot-former is held in place in the opening by two wedges of rubber gasket material approximately 3 by 6 by 24 mm. The small end extends into the gel space. Except for the point of entry, no part of the slot-former should come closer than about 0.5 mm. from any outside surface of the gel. The alternative openings for slot-formers are closed off with larger pieces of rubber gasket material. The two side sections and the five center sections are then assembled so that there are chambers for circulating water on both sides of each gel, and the sections are clamped together by firmly tightening the nuts on the nylon bolts. Short lengths of rubber tubing (G, Fig. 2) are kept attached to the stainless-steel connections (b′, Figs. 1 and 2) to the cooling water chambers so that the water flows through each of the chambers in series. Part D of Fig. 1 is then attached to the upper end of the mold with stainless-steel screws about 19 mm. long, which need not be particularly tight. The object is to provide a flat surface for attachment of the upper electrode chamber after the gel has set.

The assembled mold is now placed in a Plexiglas box (not shown) in a horizontal position with water connections up, to receive the unset gel solution. A rectangle of ¼-in. Plexiglas with an area cut out to receive and protect the protruding ends of the slot-formers is first laid in the bottom of the box. To conserve gel ingredients, spacers with holes cut out for the nuts that hold the mold together are placed along the sides to fill void spaces. Another spacer fills the void at the opposite end from part D. The depth of the box is such that the mold in the position described can be immersed to just below the point of attachment of the water connections. The box is then filled with unpolymerized polyacrylamide gel solution, care being taken that no air bubbles are trapped in the interior of the gel mold. Exclusion of air bubbles is facilitated if one end of the box is raised slightly during most of the filling operation. Occasional crisp tapping of the box at some point during the filling may be necessary to dislodge a bubble.

When the gel has set, one slides a spatula along the sides of the box to loosen the gel mold, which is then removed. It is set upright (part D on top) for attachment of the electrode chamber. It will be necessary to trim the gel on top to the size of the

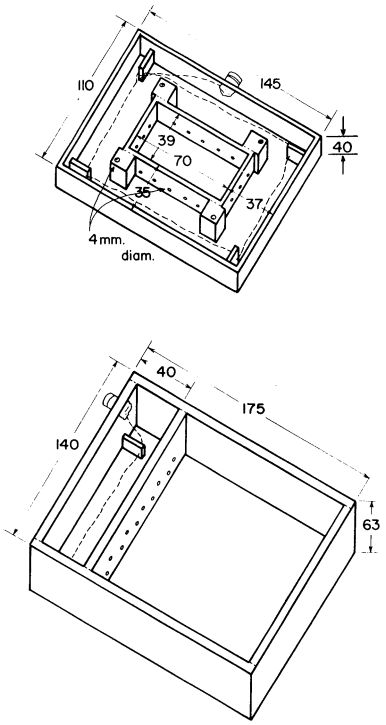


Fig. 3. Upper electrode chamber (above) and lower electrode chamber (below). Dashed lines represent the platinum wire electrodes to which connection is made by way of banana jacks, with the connection itself protected against corrosion with a silicone rubber adhesive sealant. Communication between the compartments with the electrodes and the compartments in contact with the gels is by way of holes 4 mm. in diameter. The inner compartment of the upper chamber is open at the bottom, fitting over the gel pad at the top end of the gel-electrophoresis strips. Dimensions are given in millimeters.

opening in D. The screws holding D in place are removed, with care not to disturb the position of D. A soft rubber gasket is placed on D with the electrode chamber fitted over it (Fig. 3). These parts are held in place by stainless-steel screws 65 mm. long which fit through the electrode chamber, the gasket, and then into the holes from which smaller screws were removed. The mold is now placed on its side with the opening to the sample slots upward. The rubber wedges and slot-formers are carefully pulled out. With practice, the sample solutions can be introduced into the slots with a small hypodermic syringe. It may be desirable to place a short piece of small-diameter polyethylene tubing over the sharp end of the needle to prevent any accidental tearing of the gel. One can more surely introduce an accurately measured volume from a micrometer syringe clamped in position over the slot. By raising the mold on a lab-jack, the syringe needle can be made to enter the slot smoothly. Each slot will usually hold from 100 to 150 λ of sample. Because the gel is transparent, one can readily observe the operation from the end nearest the sample slots, and stop just before the slot is full. The samples are then sealed in by pouring melted paraffin into the space previously occupied by the rubber wedges.

The lower electrode chamber is placed on the base of an adequate safety enclosure (8), the appropriate buffer is added, and the gel mold is placed in the larger compartment in an upright position as shown in Fig. 2. Buffer is added to the upper electrode compartment, electrical connections are made from the power supply to the electrode compartments, inlet and outlet water connections are made to the gel mold, the cover is placed on the safety enclosure, and the power is turned on. It has been found wise to connect the gel mold cooling system to the water line by way of a small overflow vessel placed about a meter above the gel mold to provide constant water pressure.

On completion of a run, the mold is disassembled, and the gel strips are transferred on a wet plastic ruler from their channels to trays containing dye.

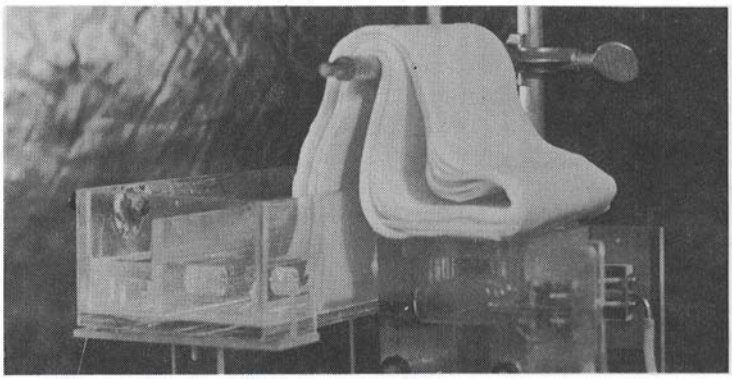
DISCUSSION

Clearly, the dimensions of the mold can be tailored to suit the individual investigator. In our earlier work, we used a mold in which the gel strips were 18 mm. wide rather than 37 mm. The wider gel, however, allows more satisfactory densitometry, since three scans can be made with the densitometer, one down the middle and one toward each side. A modification for preparative work is described in the last section of this report.

A separate electrode compartment connected to the gel pad at the upper end of the gels by a filter-paper wick draped over a rod, as illustrated in Fig. 4, can be substituted for the upper electrode chamber, although the bands will not be so reliably straight. This arrangement has the advantage that it allows one to stop the electrophoresis at any time, disassemble the gel mold, remove one of the gel strips for dyeing, reassemble, and continue the electrophoresis in the others.

Having the initial placement of the sample in the interior of the gel rather than at the electrode end has the advantage that in a properly formed slot, there is no possibility of the protein sample migrating along the surface of the gel strip, thus eliminating a major source of streaky patterns. Even if leakage does occur from the opening to the sample slot (if the slot is overfilled, for example), that edge of the gel can easily be trimmed away leaving a presentable pattern.

Our principal use for the apparatus has been in the electrophoresis of wheat flour proteins at pH 3.2 (7). At this pH the gel does not set readily, and it was



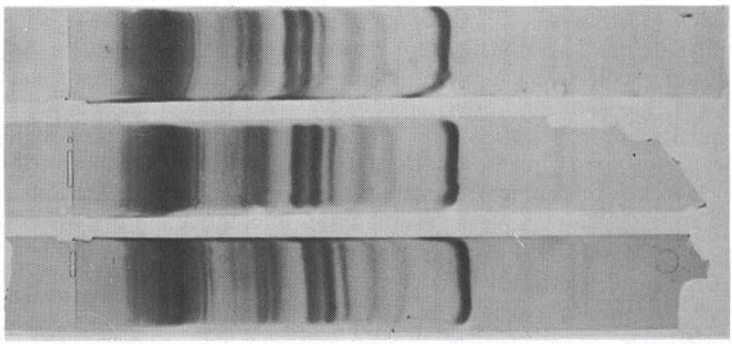


Fig. 4 (top). Method of connecting the electrode chamber and the upper end of the gel strips through a filter-paper wick.

Fig. 5 (bottom). Protein pattern obtained by electrophoresis of an aluminum lactate-urea buffer (pH 3.2) extract of Federation wheat flour in 4% polyacrylamide-2.7M urea gel for 90 min. at 30 ma. and approximately 17 v. per cm. across the gel. The positive pole was at left and the negative on the right (top and bottom, respectively, as positioned during electrophoresis). The polarity was the opposite for preliminary equilibration at 6 v. per cm. (at the start). About 2.5 mg. of protein was placed in each sample slot.

found advantageous to make the gel with water in place of buffer, and to equilibrate the gel (9) by overnight electrophoresis with the appropriate buffer in the electrode chambers. Under these circumstances, the slot-formers were left in place throughout the equilibration step, after which they were removed and the sample added. A typical electrophoretic pattern of wheat flour proteins is shown in Fig. 5.

PREPARATIVE SCALE APPARATUS

Using the same construction principles, a gel mold has been built to accommodate a gel 75 by 225 by 9 mm. thick. The slot holds almost 0.9 ml. of sample, as compared with approximately 0.1 ml. for the regular analytical apparatus. The apparatus is illustrated in Fig. 6. It is designed for recovery of the proteins by extraction from sections of the gel. The problem is to locate the bands in the undyed gel. This is commonly done by cutting strips from the sides and

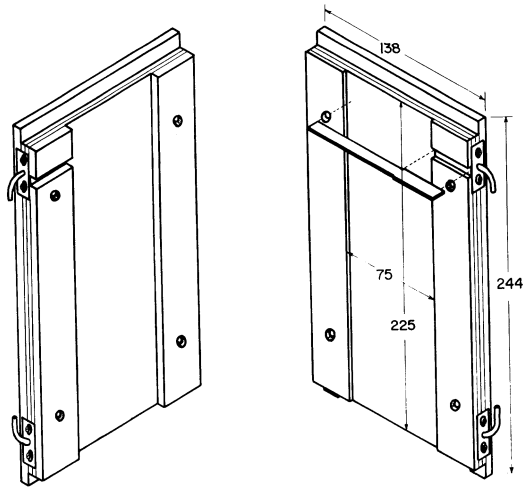


Fig. 6. Preparative polyacrylamide gel electrophoresis apparatus. The orientation of the slot-former is indicated. Dimensions are given in millimeters. Baffles are present in the cooling water sections to ensure good circulation. A handle cemented on one of the parts, on the same side as the slot entrance, helps in withdrawing the gel mold from the box after the gel has set.

sometimes also the center as guides and dyeing them. Any irregularities in the shape of the bands will introduce uncertainties in this method. A more accurate guide is obtained by slicing the gel horizontally, i.e., in the same plane as the major gel dimensions. This is frequently done with starch gels, and can be done with the less rigid polyacrylamide gel using the mold described here as follows:

At the completion of the electrophoresis, the bolts are removed, and a taut wire is passed between the two sections of the gel mold. Thus a layer of gel that is one-third of the original thickness can be stained as a guide gel. When both portions are dyed, the patterns turn out to be identical. In use the thicker, undyed portion can be laid directly on the dyed part (separated by an intervening plastic film, if desired), and the cuts made accurately.

This gel mold is placed in its box for pouring in the gel-forming ingredients with the slot opening and the water connections at the top. We have used it with a filter-paper wick to make connection with the upper electrode chamber, but a chamber making direct contact such as that described for the analytical mold could readily be designed.

Acknowledgment

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