A Convenient Apparatus for Vertical Gel Electrophoresis

Samuel Raymond

An apparatus is described incorporating gel mold, electrode chambers, and direct-contact cooling plates. It eliminates several steps in preparing and handling the gel, and permits use of either rigid (starch) or flexible (acrylamide) gels. Electrophoretic analyses can be completed in 2–4 hr. Examples are given of serum and hemoglobin electrophoretic patterns obtained.

The introduction of starch gel as an electrophoresis medium by Smithies (4) marked a significant advance in the analysis of serum proteins. The increased resolving power afforded by this medium is indicated by the fact that as many as 30 distinct components can be electrophoretically separated; only 5 or 6 are usually observed on paper or in free-solution electrophoresis. Acrylamide gel (2, 3, 7) produces resolutions of serum protein qualitatively similar to those seen in starch gel.

In order to exploit fully the inherent advantages of these media in electrophoretic analysis, apparatus specially designed to accommodate their particular properties ought to be employed. Smithies (5) has demonstrated the desirability of supporting the gel in a vertical position to avoid distortions in the electrophoretic pattern due to electroderecital effects in the sample application slot. His apparatus cannot be used for acrylamide gel because the elastic and flexible gel cannot support its own weight.

The apparatus described herein is designed to utilize the superior resolving power of vertical-gel electrophoresis, either of starch or acrylamide gel, for analysis of serum, hemoglobin, and other proteins.

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From the William Pepper Laboratory of Clinical Medicine, Medical School, University of Pennsylvania, Philadelphia 4, Pa.

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mixtures. It incorporates several convenient design and operational features: (1) The gel solution is poured directly into the electrophoresis cell and is not transferred or manipulated in any way prior to the end of electrophoresis. (2) The gel is in direct contact with the buffer solution in the electrode chambers, eliminating wicks and salt bridges. (3) No petroleum jelly or other coating is required to protect the surface of the gel. (4) Temperature is adequately controlled by water-cooled plates in direct contact with the gel surfaces, permitting the application of higher voltages and consequently faster resolution times. (5) Evaporation of buffer from the gel is completely eliminated during electrophoresis, since the gel is nowhere open to a vapor space. (6) The sample is applied in liquid form directly to slots molded in the top edge of the gel slab, and no dialysis or other preparation of serum samples is required. (7) The design of the apparatus permits the use of either a rigid gel such as starch or a flexible one such as acrylamide.

**Design Principles**

The apparatus (Fig. 1) consists essentially of a vertical tube or channel connecting two electrode chambers, one above the other. The channel is completely filled with gel, which prevents flow of buffer from the upper to the lower chamber. For this purpose a gel impermeable to mass flow of liquid is required; starch gel (4) and acrylamide gel (2) are suitable. A medium such as filter paper or starch grains cannot be used, since leakage would occur through the body of the medium.

For efficient temperature control the channel is formed between two parallel water-cooled plates separated by gaskets 3 mm. thick. The gel slab is molded in place by pouring the liquid gel into the channel while it is in a horizontal position. Casting the gel directly within the channel ensures perfect contact and prevents leakage between gel and channel walls. The cooling plates are disassembled to remove the gel after electrophoresis.

The electrode chambers are attached directly to the cooling plates which form the gel channel. Contact between gel and buffer in the electrode chambers is direct at each end of the channel. No wicks are used, although a strip of cellulose sponge may be inserted into the channel at the lower end to provide a rigid support for the gel slab. This is essential for a flexible gel such as acrylamide but is not needed for the rigid gel prepared from starch by the Smithies technic (4).
The gel slab as molded in this apparatus is smooth-surfaced and has a uniform thickness. After clarification (1) the starch gels are suitable for transmission densitometry without slicing. Acrylamide and agar gels are intrinsically transparent.

**Fig. 1A.** (above) Cross-section of vertical gel electrophoresis cell: diagonal hatching, Plexiglas; wavy lines, buffer level; coarse stippling, gel slab; fine stippling, sponge block; positive and negative signs, position of electrodes. Cooling-water circulation channels are not shown. **B.** (right) Photograph of electrophoresis cell.

**Apparatus**

Dimensions of the apparatus may vary according to the scale of operation desired. For six samples of 0.05 ml. to be run simultaneously for a pattern 15 cm. long, the over-all dimensions of the apparatus are 15 cm. square by 20 cm. high. The base of the apparatus (Fig. 2A) consists of the outer cooling plate (A) and attached lower chamber (B) with platinum wire electrode (C) and connections for both cooling water and buffer supply. The cooling plate is a block of Plexiglas 1.5 cm. thick with internal channels (omitted from Fig. 2 for clarity) for circulation of cooling water, formed by milling grooves 3/8 in. wide and 1/8 in. deep on one face and cementing a cover plate over the grooves. Connection to the cooling channels is made through holes J and K. To the edge of the base cooling plate is cemented a low
wall \( (L) \), forming a shallow tray \( (D) \) into which liquid solution can be poured for gel formation. A rubber gasket \( (E) \) along each side of the tray provides a tight seal between the inner and outer cooling plates. The gasket seal does not extend across the ends of the cooling plate. The gel thus exposed at the ends makes contact with the buffer solutions in the chambers.

The lower chamber \( (B) \), with a capacity of approximately 1300 ml., is attached directly to the outer cooling plate, its side walls continuous with the wall of the gel tray. It provides support for the electrical connector \( (F) \) and to it are attached the clamps \( (G) \) which hold the two major parts of the apparatus together. These clamps consist of \( \frac{3}{4} \times 20 \times 1\frac{1}{2} \) stainless-steel bolts that project from the back of the lower chamber and pass through holes in an extension of the back wall of the upper chamber. The two parts are clamped by wing nuts on the bolts. The inner end of the bolt must be insulated from contact with buffer solution in the chamber. A hole \( (H) \) in the chamber wall provides entrance for a cooling-water tube (described below) and another \( (I) \) provides a drain for emptying the buffer.

![Diagram](image)

**Fig. 2.** A (left) and B (right). Base and top of apparatus, made of \( \frac{3}{8} \)-in. Plexiglas except where otherwise indicated. A. Parts of the base are as follows. A, outer cooling plate (\( \frac{3}{8} \)-in. Plexiglas); B, lower electrode chamber; C, polyvinyl bolt holding electrode wire; D, gel tray; E, rubber gasket; F, Cinch-Jones Plug P-2042 FHT; G, stainless-steel clamping bolts; H, hole for bulkhead union (Nylo-seal 4282-N); I, drain hole for lower chamber; J, cooling water inlet; K, cooling water outlet; and L, tray wall. B. Parts of the top are as follows. M, inner cooling plate (\( \frac{3}{8} \)-in. Plexiglas); N, upper electrode chamber; O, upper electrode contact; P, hole for bulkhead union (Nylo-seal 4282-N); Q, cooling-water inlet; R, cooling-water outlet; S, hole for clamping bolt; and T, drain hole.
The top (Fig. 2B) consists of the inner cooling plate (M), and attached upper chamber (N). The inner cooling plate has internal cooling-water channels (not shown) similar to those in the outer cooling plate. Connection to these channels is through ports Q and R. From upper port R a short loop of tubing passes through the wall of the chamber at P via a bulkhead union (Nylo seal 4282-N) to make external connection with the cooling-water supply. From lower port Q the tubing passes through the wall of the lower chamber via a hole (H), shown in Fig. 2.

During operation of the cell, cooling water flows in through J, out through K, then in through H and Q and out through R and P. Reversing the flow does not provide adequate cooling, because the channels do not fill. The upper chamber also has a platinum electrode (terminating in the brass contact, O) and drain port (T) for buffer. A dependent extension of the rear wall carries the two holes (S) with which the bolts (G) previously described engage. The top cooling plate fits into the tray of the base, pressing against the gaskets at each side and forming a horizontal channel 3 mm. thick within which the gel is formed.

Both faces of the gel block are in direct contact with the cooling plates, the sides are sealed by gaskets, and the ends are in direct contact with buffer solution; therefore, no evaporation is possible and adequate control of temperature is maintained within the gel even when operating on the open bench-top with ordinary tap water cooling. As much as 160 w of power (i.e., 800 v. at 200 ma) can be applied without exceeding the cooling capacity of the apparatus.

The electrodes are 5-mil platinum wire stretched across the back of the chambers. Electrical contact with the electrode in the lower chamber is made by leading the wire through the wall of the chamber into the connector housing (F) and connecting it directly to the lower pole of the connector (Cinch-Jones Plug P-2402-FHT). The electrode in the upper chamber is connected in a different manner, viz: the wire terminates in the brass contact O on the outside of the chamber wall in a position such that when the apparatus is assembled, contact is made with a spring-loaded pin inside connector F, and thence to the upper pole of the connector. The polarizing stud on connector F is removed to permit insertion of the socket (Cinch-Jones S-2402-FHT) from the power supply with either polarity, depending on the direction of migration of the sample.

The slot form (Fig. 3) is a rectangular plate with a projecting lip
along one edge. The lip may be divided by transverse cuts into a number of segments corresponding to the number of samples to be treated. This part is inserted into the gel tray (during the gelation process) at the upper end of the gel channel to form a series of short indentations or slots across the end of the gel which is exposed to the upper chamber buffer. Liquid samples placed in these slots enter the gel through electromigration without the use of wicks. For larger samples a single slot can be used extending across the width of the gel slab.

The power supply required for a 5% acrylamide gel in 0.1M TRIS buffer must furnish 500 v. at 200 ma. Higher current and voltage can be used without exceeding the cooling capacity of the apparatus. No attention need be paid to the problem of evaporation from the gel since evaporation is completely prevented by the design of the apparatus.

**Use**

The inside of the apparatus must be clean and dry to avoid dilution of the gel solution locally when it is poured into the gel tray; such dilution would cause local inhomogeneity resulting in distortion of the patterns of migration through these spots.

The outside must be clean and dry to avoid leakage paths of the electric current along the surface of the apparatus.

The apparatus is used in two positions consecutively, the horizontal and the vertical. In the horizontal position the cooling plates are horizontal and the electric connecting-plug points straight up, on the front of the apparatus. Rotation of the apparatus 90° to the rear brings it to the vertical position, with the cooling plates vertical and the electric plug pointing to the rear.

To assemble the apparatus, place the base in horizontal position,
bring the top close enough to permit the cooling water connection to be made between \( H \) and \( Q \). When the apparatus is fully assembled, this connection will be enclosed and inaccessible within the bottom chamber. The connecting tubing is long enough to make this connection before assembly of the two cooling plates, but short enough to coil up inside the the lower chamber without kinking after assembly of the two chambers.

Place a sponge strip transversely in the tray at the bottom edge where it will form a support across the gel slab. (This is essential for a flexible gel like acrylamide but is not necessary for a more rigid gel like starch.) Bring the two chambers together, compressing the sponge strip and rubber gaskets between the two cooling plates. Adequate pressure must be applied while locking the two plates together to ensure compression of the rubber gasket for wealing.

Insert a buffer connecting tube from the upper chamber drain port \( T \) to the lower chamber inlet \( I \). This tube is opened to fill the lower chamber but is clamped off during the electrophoresis.

Add the gel solution in liquid form to the tray and allow it to gel in place. Sufficient solution should be used to fill all the available space in the tray up to the top of the tray wall. In pouring, care must be taken to avoid trapping bubbles anywhere in the gel solution as these will cause local distortion and inhomogeneities in the resultant patterns. The transparent Plexiglas makes it easy to see any bubbles which may be trapped. Bubbles in the cooling water channels may cause some confusion unless these channels are completely filled with water before pouring the gel solution. When pouring hot gel solutions such as starch gel, it is advisable to have cooling water flowing through the plates to avoid heat-warping of the Plexiglas.

Before the gel solution solidifies, position the slot form vertically across the space at the top of the upper cooling plate. The lip of the form should project into the space between the two cooling plates. Note that the slot form is not intended to close off the gel space completely; sufficient looseness of fit is allowed to permit flow of the gel solution into all parts of the tray. The gel solution is allowed to stand undisturbed in the tray until it is completely gelled. Completeness of gelling can be tested by tilting the apparatus slightly and noting absence of liquid flow. Acrylamide gels also show a faint opalescence on complete gelling. When using acrylamide gel a part of the gel solution which is directly exposed to air will not gel, but this should be disregarded.
When the gel has completely solidified between the cooling plates and around the slot form, remove the latter carefully so as not to disturb the adherence of the gel to the surfaces of the cooling plates. Any small particles or threads of gelled material which may be left behind in the sample space should also be removed.

Rotate the apparatus into the vertical position. Close off the drain tube from the lower chamber and open the buffer connecting tube between upper and lower chambers. Pour buffer into the upper chamber and allow to drain into the lower to fill the latter to a level above the electrode. Then close off the connecting tube. Fill the upper chamber with buffer to a depth of 1 cm. above the upper edge of the gel slab.

At this point, observe carefully for leakage of buffer from upper to lower chamber. Leakage may occur for the following reasons:

1. Failure to clamp off completely the connecting tube between the two chambers (The remedy is to tighten the shut-off clamp.)

2. Failure to fill the gel compartment completely (A threadlike bubble may be trapped by surface tension in the gel compartment along one or both gaskets, forming a capillary channel which permits slow leakage.)

3. Failure of the gel to solidify completely (When using acrylamide gel this is most often due to air bubbles trapped within the gel solution.)

4. Dislodgment of the gel adhering to the cooling surfaces. (This is commonly due to failure to place the sponge strip properly at the bottom of the gel compartment when using flexible gels.)

The only remedy in the last three cases is to disassemble the apparatus, remove the defective gel, and begin again. With proper attention to the technic of preparing the gel this will seldom be necessary.

If no leakage is found, the next step is to add the liquid sample to each sample slot. Insert the tip of a pipet containing the sample beneath the surface of the buffer to a position just a few millimeters above the surface of the gel. Allow the sample to drain slowly into the sample slot. It is, of course, essential that the density of the sample be greater than that of the buffer solution. Protein solutions containing 2-5% protein have a higher density than dilute buffers (0.1M or less). If the density of a sample is too low, it may be increased by dissolving in it a few per cent of some nonionic compound such as urea or glucose.
The volume of sample to be used may vary, depending on the purpose of the experiment. For analytical patterns the minimum sample size which can be detected easily in the final pattern is preferred, as this gives the maximum sharpness of separation, although a sample which is too small may not reveal all the components present in minor amounts. Serum samples in the range of 20–50 µl. are suitable for qualitative and quantitative patterns. On the other hand, for sectioning and elution of patterns to obtain purified components, the sample size can be as much as 0.5 ml. The entire slot can be filled without excessive loss of resolution in the final pattern. With an appropriate modification of the slot form for preparative runs, a single slot can be molded across the entire width of the gel slab.

Before applying electric current to the cell the cooling water must be flowing. Maintain a flow of 2–5 L./min. if tap water is used. It is advisable to have a thermometer in the exit stream to check on the adequacy of the cooling. After the cooling water is properly adjusted, connect the electric power and adjust to deliver the desired current or voltage. At the end of the run, disconnect power and drain the cooling water. The buffer can be saved and reused if it is not significantly contaminated by electrolysis products. The pH should be checked after remixing the contents of the upper and lower chambers.

On removing the gel it is advisable to cut it into two equal pieces vertically for ease in handling, although with care the entire width of the gel slab can be handled as a unit. Processing of the gel pattern subsequent to its removal from the cooling plates will depend on the specific objectives of the experiment.

Results

Electrophoretic patterns of normal and abnormal hemoglobins and serum proteins were obtained with this apparatus, using as supporting medium the acrylamide gel previously described (3).

Serum protein patterns were stained with amidoblack and washed as previously described (3). Optical densities were recorded on a Photovolt Model 525* densitometer with Varicord Model 43* recorder. Because of the limited range of the recorder, peaks of high density were rerecorded using the next higher sensitivity range. The peaks were fitted to the main curve at the 1.0 optical density level.

Hemoglobin patterns were measured, without staining, using filters

*Photovolt Corporation, New York, N. Y.
of 525 and 420 m\(\mu\), in a technic described by Nakamichi (7). The 420-m\(\mu\) filter accentuated the minor components which were present in very small amounts, and the 525-m\(\mu\) filter permitted on-scale measurement of intense bands.

Although the supporting medium used is responsible for a major share of the resolution obtained, results may be unsatisfactory unless certain apparatus factors are optimized. These include total separating capacity, speed of development, symmetry of the electrophoretic pattern, reproducibility of pattern, and adequate resolution.

The total separating capacity of electrophoresis apparatus is a function of the size of the apparatus, but is best measured by the maximum electrical power which can be applied. Applied voltage is proportional to the rate of electrophoretic migration; current is proportional to the cross-sectional area of the migration path and, hence, to the weight of sample which can be applied on a given supporting medium. The product of the two—i.e., electrical power—provides a useful measure of capacity by which one apparatus can be compared with another. The present apparatus has been employed at power levels up to 160 w—i.e., 400 ma at 400 v. At this level, temperature within the gel reached 15\(^\circ\) with circulation of tap water at 12\(^\circ\) through the cooling plates.

The maximum speed of development of any specified pattern is directly related to the total separating capacity of the apparatus. For example, the serum protein pattern shown in Fig. 4 was obtained in this apparatus in 110 min. using a power level of 80 w. The prealbumin component had moved 13 cm. in this time.

The symmetry of the electrophoretic pattern obtained is dependent on uniformity of electric field within the gel. Figure 5 also demonstrates the uniformity of field obtained within this apparatus. Although the gel used was 12 cm. wide and 18 cm. long, each component moved uniformly across the entire width of the gel.

Figure 5 exhibits different types of serum protein patterns on acrylamide gel after staining with amidoblack 10B and washing as described (3). These samples were random specimens in which paper electrophoresis showed no abnormalities. In the figure the origin is at the left. All components, including the gamma globulins, move in the same direction in the gel at pH 9.0, because of the absence of electrosmosis in this gel. The relatively sharp albumin band at the right is also noteworthy in comparison with the broad diffuse albumin band often observed on starch gel. In most cases the albumin
Fig. 4. Normal serum protein pattern, stained with amidoblack 10B and photographed by transmitted light; acrylamide gel, 5% in 0.1 M Tris-EDTA-Borate, 24 v./cm. Twelve of the 16 separate components visible in the original gel are distinguishable in the photograph.
**Fig. 5** (A and B at side; C, D, E, and F on following pages.)

A–F. Optical density recording of different types of normal serum protein patterns, with electrophoresis conditions as in Fig. 4. The sharp peak at the left corresponds to the point of application. Other peaks correspond to visible separated components. Between the two major peaks at the left lie a series of closely spaced components which are not recorded separately by the recording equipment used but are distinguishable on visual inspection. The different numbers of components in the individual patterns is attributed to genetic variation (7). For the method employed to record the albumin peaks at the right, see text.
peak is so sharp as to run off scale, necessitating a rerecording of the peak area, using the next higher densitometer setting. The peak so recorded can then be matched with the original recording at the point corresponding to 1.0 optical density.

The resolutions obtained in acrylamide gel exceed the discrimination of both the recording densitometer and the photographic reproduction. Figure 4, for example, is a photograph of a gel in which 16 components were recognizable visually; only 12 appear in the photograph.

Discussion

The advantages of gel electrophoresis over paper electrophoresis, as described by Smithies, are increased resolving power and qualitatively different separation, the effect of a combination of electrophoretic mobility and molecular size or shape. The increased resolving power is presumably the result of the pore size in the gel, which approaches the molecular dimensions of proteins. In Smithies' original technic the sample was absorbed on paper or starch-grain wicks in slots formed in the gel, but such wicks decrease the resolution obtainable. A slot filled with liquid sample can be used only in a vertically oriented gel in which the electrophoretic mobility effect is absent.

The apparatus described herein permits application of the sample in liquid form to a slot in the upper edge of the vertical gel slab. This arrangement, although most convenient for application of the sample, does not permit observation of components which move upward. When observation of upward-moving components is essential an additional layer of gel can be applied above the sample slots after filling them with sample. An agar gel of 1% concentration can be used, as this liquefies at a temperature which does not denature protein samples. Alternatively, a block of preformed gel can be placed above the sample slot after addition of the sample. Another design of this apparatus, in which the sample is inserted into the gel through a slot in the underside of the base, has been worked out but proves to be more cumbersome than useful.

For many purposes, such as hemoglobin analysis, retrograde migration does not occur. It does occur in serum protein analysis. By the usual technic of paper or starch gel electrophoresis, the gamma globulin fraction moves retrograde with reference to the point of application, even in buffers of high pH where the resultant negative charge on the gamma globulin should result in a forward mobility.
The retrograde migration of this fraction is therefore due to electro-endosmotic flow of buffer through the supporting medium in the reverse direction, which retards the movement of all fractions and in particular results in a net reverse movement of the gamma globulin fraction.

The effect of endosmosis can be decreased by forces acting to oppose electro-osmotic flow. One such force is hydrostatic pressures applied to the ends of the gel in a net downward direction. The present apparatus makes one continuous liquid system of the gel column and both reservoirs, insofar as hydrostatic pressure is concerned. The net downward pressure applied to the gel is therefore equal to the vertical difference in height separating the liquid levels in the two reservoirs. The effect on retrograde migration appears to be substantial in that the gamma globulin fraction enters the gel as a narrow band just at the top of the gel. No additional fractionation of the gamma globulin is observed, however, as it is in customary designs of apparatus.

The chief advantage of this apparatus, in addition to the technical convenience of manipulation, is the rapidity with which electrophoretic analysis can be carried out. Whereas the original starch-gel apparatus of Smithies (4) requires 16–20 hr. for the development of a serum protein pattern, a pattern similar in all respects can be obtained in this apparatus in 3 hr. The increased speed is due to the efficient cooling, which allows the application of higher voltages and currents. A maximum of 40 v./cm. has been used in this apparatus.

References