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AN APPARATUS TO FACILITATE THE PREPARATION OF CONTINUOUS AND GRADIENT DISC ACRYLAMIDE GELS

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SUMMARY

A compact, rugged, versatile plexiglass apparatus of single-unit construction is described that speeds and facilitates the preparation of either continuous or gradient disc acrylamide gels of consistent quality.

INTRODUCTION

Since the introduction of disc electrophoresis by ORNSTEIN[1] and DAVIS[2] numerous improvements and modifications have been introduced to the technique of casting acrylamide gels. With the gel tube stand described by DAVIS[2] it was necessary to adjust visually the cylindrical glass columns to a vertical position. By fitting the rack with an upper-column guide* this time-consuming and empirical procedure was eliminated[3]. The gel surface, in contact with the rubber serum cap, often failed to polymerize leaving a cavity. Thus it was necessary to place a hanging drop of buffer on the bottom of each gel tube to prevent trapping of air bubbles. According to NIELSON AND HALE[3], the failure to polymerize at the base of the gel was caused by contamination from used rubber serum caps and replacement with "Parafilm" as a closure remedied this imperfection.

It has been argued that it is preferable to apply a number of samples to a single gel slab rather than to individual gels because in a slab the samples are migrating through a more homogeneous gel[2]. Also the technique of treating the gels as individuals is tedious and time consuming[4,5]. Consequently effort has been put into the design of apparatus which casts a number of gels in a single batch[4–6]. The drawback of these systems is that each gel has one or more laterally adjacent gels contributing ohmic heat whereas gels cast in individual glass cylinders dissipate the heat more symmetrically and efficiently. As a result, lower currents must be used with the batch systems to prevent overheating and the time saved in casting the gels is offset by longer electrophoretic runs. In one of these batch systems[4], hydrostatic principles have been ignored, resulting in gels of various lengths.

A rugged, easy to construct apparatus is now described that incorporates the time-saving features of the batch system with the preparation of gels cast in

* Loading rack and viewer; Canal Industrial Corporation, 5635 Fisher Lane, Rockland, Md. 20852, U.S.A.

individual glass cylinders. The cylinders are held in a vertical position and any number of gels of uniform length may be cast without problems of polymerization failure.

The same apparatus may also be used for the formation of acrylamide continuous-gradient molecular sieve gels. Preliminary work confirms the earlier observation[7,8] that continuous-gradient molecular sieve systems usually result in better separation and resolution of serum proteins than that obtained with 7 % acrylamide gels.

MATERIALS AND METHODS

The apparatus is constructed of five pieces cut from 1/4-in. plexiglass (Fig. 1A) and assembled as shown in Fig. 1B using a glue made from plexiglass trimmings dissolved in ethylene dichloride. A sheet of 10-to-the-inch graph paper is glued to one of the side pieces to provide a background against which the heights of the gels

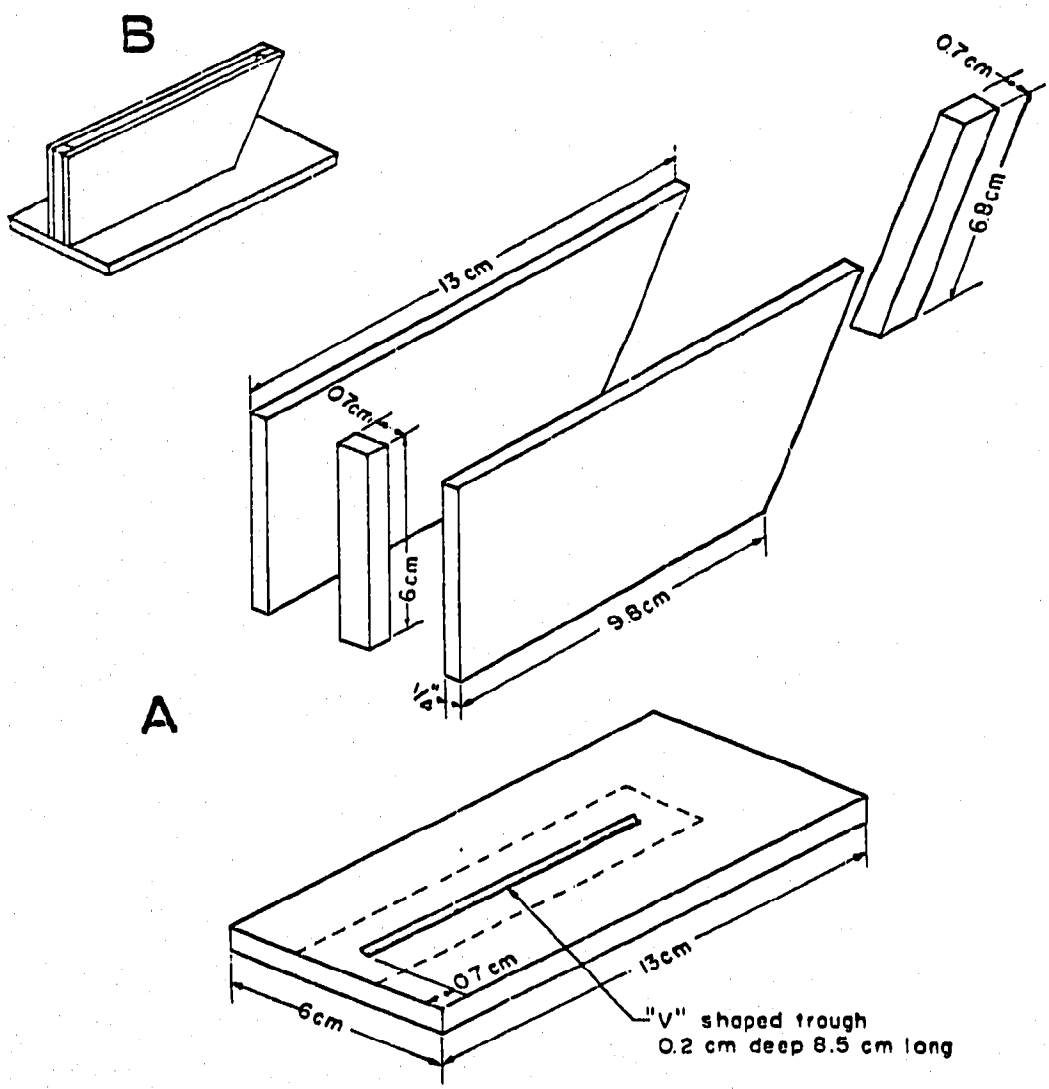

Fig. 1. Construction details.

can be adjusted. If using the refractive index method of water layering, described below, it is advisable to trace over the horizontal lines of the graph paper with India ink prior to gluing. The completed apparatus accomodates twelve 7 mm O.D. glass cylinders but may be modified in dimensions to handle any number of cylinders of any size.

The glass cylinders are lined up in the apparatus and held against the vertical end with an elastic band (Fig. 2). A second elastic band is placed at the opposite end to maintain the apparatus at level. Small-pore acrylamide mixture (running gel) is poured into the space at the sloped end using a glass rod to direct the flow until a pre-chosen horizontal line in the background is reached. The surface of each gel is water layered by one of two alternative systems and polymerized. In the first system water is slowly added down the size of each tube observing the water–acrylamide refractive index pattern formed by the horizontal line closest to the interface (Fig. 2). Additional water is added to those gels of greater length until the refractive index pattern is uniform. The alternative system is to add 50 μl of water down the side of each tube sequentially in one direction and the same volume on returning in the opposite direction.

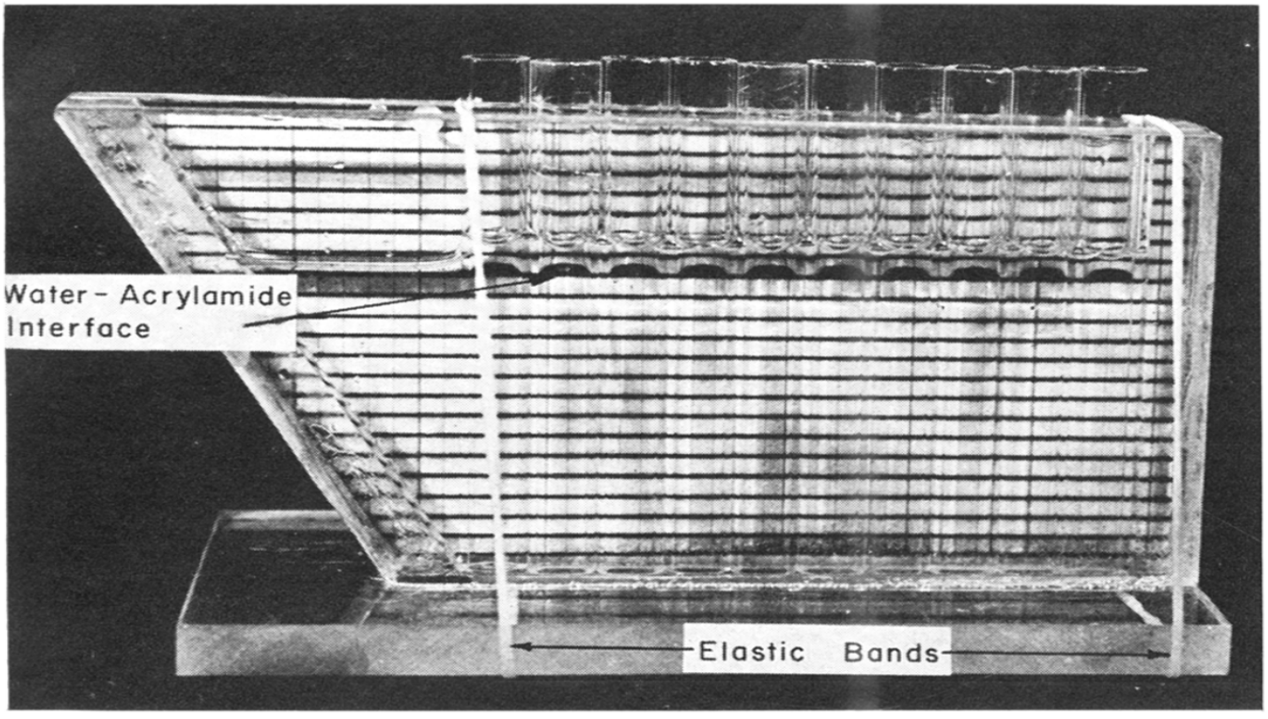

Fig. 2. Apparatus in use, showing refractive index pattern formed by water–acrylamide interface.

When the acrylamide mixture has polymerized the water is poured off and the gel surfaces rinsed as described by DAVIS[2]. Large pore gel is added to each tube to a level determined by a second horizontal line displayed on the background. Water is layered over the surface and the acrylamide mixture is photopolymerized. To remove the glass cylinders from the apparatus the triangular piece of gel at the sloped end is removed using a spatula and the resulting space filled with distilled

water. The water acts as a lubricant for the glass cylinders as they are individually tipped toward the sloped end and lifted from the apparatus. The bottoms of the gels will have chisel-shaped protrusions.

To prepare twelve 3.5 to 10 % continuous acrylamide gradient gels the A and C solutions as described by DAVIS[2] are used as well as the following two ammonium persulfate solutions: 59 mg in 100 ml water and 210 mg in 100 ml of 40 % sucrose solution (*i.e.* solution F, DAVIS[2]). Gradients are formed utilizing the system shown in Fig. 3 and the mixtures given in Table I. All tubes are disconnected from the mixing chamber (25-ml beaker) and 10 ml of the 3.5 % mixture is added. The 10 % acrylamide delivery tubing (A) is filled by use of the peristaltic pump and all tubing (A) is positioned as in the diagram. The magnetic stirrer and the peristaltic pump are switched on and the system allowed to run until air bubbles approach the tip of the gradient delivery tubing (B). The gradient delivery tube is removed from the plexiglass apparatus and the gels are overlaid with water as previously described. To prevent blockage the gradient-forming system should be rinsed with distilled water prior to acrylamide polymerization. The acrylamide in the plexiglass apparatus should gel within 1 h and then the previously described procedure is followed to form the spacer gel.

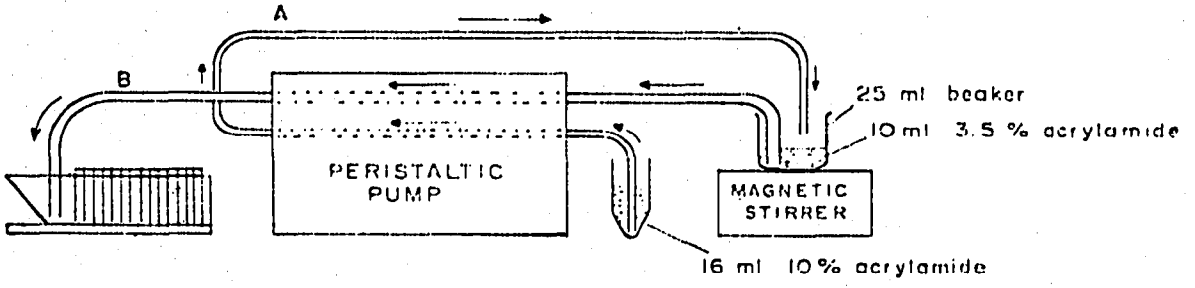

Fig. 3. Gradient-forming system. Flow rate: tubing A, 1 ml/min; tubing B, 2 ml/min.

TABLE I

MIXTURES FOR GRADIENT-FORMING SYSTEM

	3.5 % mixture (ml)	*10 % mixture (ml)*
Solution A	2	2
Solution C	2	6
Persulfate solution 59 mg	12	—
210 mg	—	8

To determine the linearity of the gradient and its uniformity between gels a lot of eight gels were cast. The glass cylinders were removed from the apparatus in sequence and numbered. As each gel was removed from its glass cylinder, it was sliced into portions using a Canalco 1802 lateral gel slicer. The concentration of the acrylamide–sucrose mixture in each slice was determined using the sucrose scale of a Carl Zeiss refractometer.

In the work to be reported the solutions used, the conditions of electrophoresis and the staining procedure were as described by DAVIS[2].

RESULTS

Five lots of eight gels per lot showed variation in length of gel of about ± 0.5 mm. Aliquots of 2 μl from a single sample of human blood serum gave identical 21-band patterns when electrophoretically separated over the 47-mm lengths of 7 % acrylamide gel (Fig. 4).

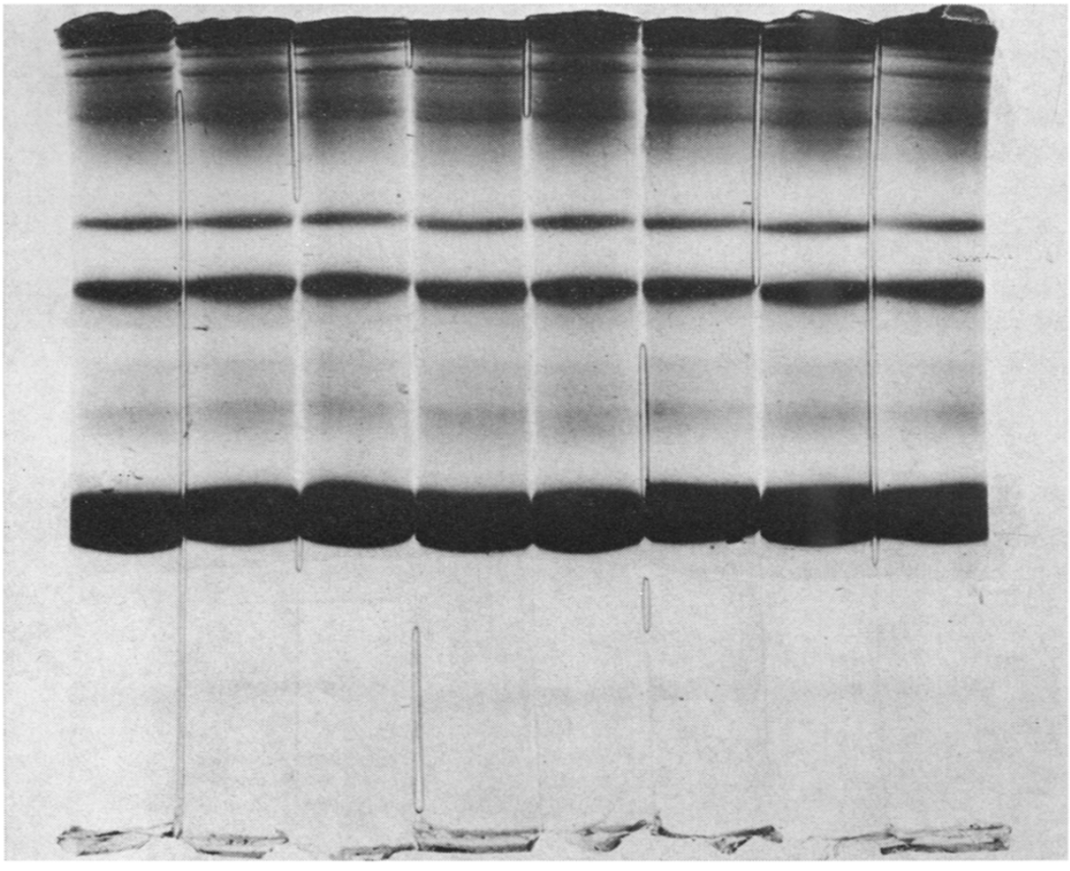

Fig. 4. Separation of human blood serum by 7% acrylamide disc electrophoresis.

The refractometer readings of the sliced gradient molecular sieve gels are shown in Table II. Some of the variation within a gel may be attributed to the failure of the lateral gel slicer to cut equal portions. To compensate for this, conclusions are drawn from averages and totals. From the average values it will be seen that the gradient is linear whereas the total values indicate that the gels furthest from where the gradient delivery tube was placed are slightly lower in acrylamide concentration. Despite this, the electrophoresis pattern of eight aliquots from a single sample of human blood serum is relatively uniform (Fig. 5).

Comparing the two systems (Figs. 4 and 5) the Amido Schwartz staining bands of human blood serum are more sharply defined in the gradient sieve system than in the 7% continuous acrylamide system. This is especially evident in the globulin region. A total of 28 bands were resolved by the gradient system and 21 bands by the continuous system.

TABLE II

REFRACTOMETER READINGS ON GEL SLICES FROM GRADIENT MOLECULAR SIEVE GELS

Gel number								*Average*
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	
13.4	13.3	13.4	13.2	13.3	13.1	12.5	12.7	13.1
15.4	15.1	15.0	14.9	15.0	14.5	13.8	13.9	14.7
16.4	17.3	16.5	16.6	16.5	15.3	15.0	15.3	16.2
19.1	19.0	18.7	17.9	17.2	17.2	16.9	17.6	17.9
21.0	20.5	20.6	20.0	19.4	19.2	18.8	19.0	19.8
23.1	22.4	22.7	22.3	21.7	21.0	20.9	20.8	21.9
24.8	24.0	24.5	24.3	24.0	23.2	23.0	23.2	23.9
26.8	26.3	26.6	25.8	25.6	24.5	24.2	25.7	25.7
28.6	28.7	28.9	28.8	28.6	27.0	27.0	27.5	28.1
30.1	30.7	30.7	31.1	29.8	28.1	28.3	28.7	29.3
31.9	32.2	31.7	33.4	32.5	31.0	30.8	30.7	31.8
33.1	33.4	33.7	34.0	33.0	32.8	32.0	32.0	33.0
283.7	283.5	283.0	282.3	276.6	267.5	263.2	262.1	Totals

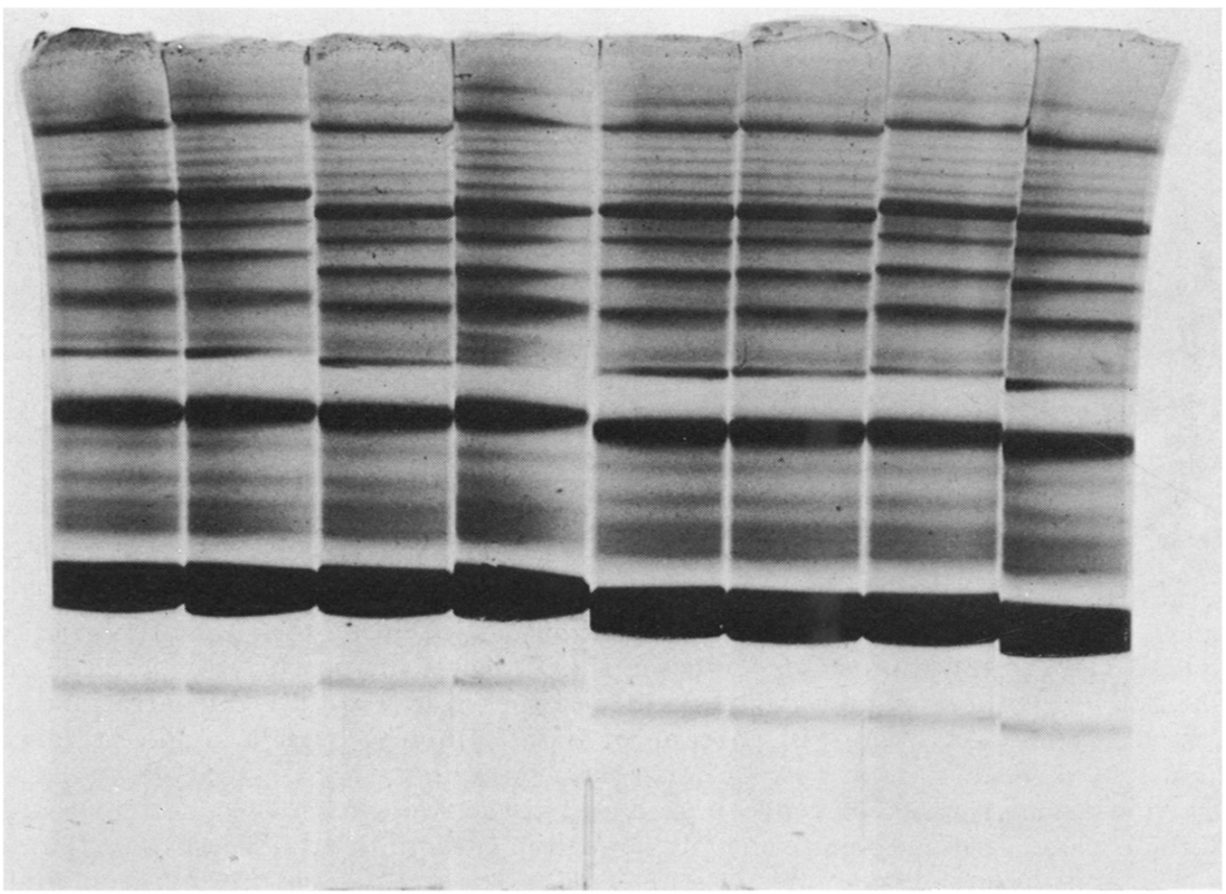

Fig. 5. Separation of human blood serum by 3.4 to 10% acrylamide continuous gradient disc electrophoresis.

DISCUSSION

The plexiglass apparatus described combines the most desirable features of both slab and disc electrophoresis. The apparatus is of single-unit construction eliminating time-consuming assembly–disassembly and the many precautions required to prevent leakage[4–6]. In this respect disc acrylamide gels are cast with greater ease than the slabs. Polymerization failures mentioned by NIELSON AND HALE[3] were not encountered. Thus the observed ± 0.5-mm variation in gel length can be attributed to the water-layering technique of the operator. Of the two alternative water-layering techniques the refractive index method has the advantage that "bombing" is detected by the appearance of a diffuse line at the water–acrylamide interface.

The investigator is able to perform both continuous and gradient disc electrophoresis by mixing different ratios of the same solutions, except persulfate. Since the ionic strength of the buffer is identical in the continuous and the gradient systems, both can be run side by side in the same electrophoresis unit. Other investigators[7–12] found it necessary to make up special solutions when forming gradient sieve gels.

Attempts were made to introduce water into the apparatus prior to adding the acrylamide mixture as reported by others[4,9–11] to facilitate water layering. This resulted in bands which sagged in the middle, giving rise to overlapping U-shaped zones. MARGOLIS AND KENRICK[10], experiencing the same band distortion, resorted to slicing the gels longitudinally to improve resolution. The water-layering techniques described in this paper do not result in distorted bands.

Our apparatus enables linear gradients to be cast in lots as compared to the recently proposed system[12] of casting each gel individually. The peristaltic pump system (Fig. 3) is preferred over the two-compartment gravity flow system[10,11] as a means of producing linear gradients. When the stopcock joining the two compartments is first opened there may be a sudden rush of the more dense into the less dense solution. This apparently is the reason why MARGOLIS AND KENRICK[9,10] found it necessary to use 2 % acrylamide solution to obtain a 4 to 20 % gradient. In a later paper[11] these authors introduced a complicated modification to remedy this situation.

The presently described apparatus casts gels with a "chisel-shaped" protrusion extending beyond the lower end of the glass cylinder. This design does not appear to affect the electrical performance of the system but does prevent air bubbles from being trapped. These protrusions also designate the anode end of the gel throughout subsequent treatment.

Unless the ionic concentration of the sample solutions are similar in an electrophoretic run, the time taken for the marker dye to travel a designated distance will vary. BERKING[4] uses a layer of heavy sucrose solution to break the current flow through the faster gels. If the interval between breaking the current and staining is appreciable, the protein bands will diffuse and decrease resolution. Using the apparatus described by DAVIS[2] it is possible to use an empty glass cylinder to force the faster gel down and out so that it may be stained immediately. The empty glass cylinder seals the vacated grommet and leakage from the upper to the lower chamber will not occur if the glass cylinder is long enough to protrude above the buffer level in the upper chamber.

SLATER[7] separated a sample of human blood serum into thirty bands over a

11.7-cm path length of 4–22 % gradient polyacrylamide slab. Conditions of electrophoresis were 400–700 V at 40 mA for 24 h. The disc electrophoretic pattern in Fig. 5 contains 28 bands over a 4.7-cm path length of 3.5–10.0 % gradient disc polyacrylamide gel. Conditions of electrophoresis were 400 V at 35 mA for 36 min. Although different samples of human blood serum were used, comparable results were obtained using shorter gels, a narrower gradient and a briefer period of electrophoresis.

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