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A. Chrambach[a]; J. Pickett[a]; M. L. Schlam[a]; G. Kapadia[a]; N. A. Holtzman[b]
[a] REPRODUCTION RESEARCH BRANCH, NATIONAL INSTITUTE FOR CHILD HEALTH AND HUMAN DEVELOPMENT NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND [b] DEPARTMENT OF PEDIATRICS, THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE, BALTIMORE, MARYLAND

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# A Partitioned Slab Apparatus for One-Dimensional Polyacrylamide Gel Electrophoresis in Multiphasic Buffer Systems under a Wide Range of Conditions

A. CHRAMBACH, J. PICKETT, M. L. SCHLAM, G. KAPADIA

REPRODUCTION RESEARCH BRANCH
NATIONAL INSTITUTE FOR CHILD HEALTH AND HUMAN DEVELOPMENT
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

N. A. HOLTZMAN

DEPARTMENT OF PEDIATRICS
THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE
BALTIMORE, MARYLAND 21205



## Summary

An apparatus for one-dimensional polyacrylamide gel electrophoresis in multiphasic buffer systems of multiple samples on a gel slab has been constructed. This apparatus is made of Pyrex, providing satisfactory wall adherence of polyacrylamide at any pH, 0 and 25°C. The apparatus provides permanent sample partitions and can, therefore, be used with mechanically labile, stacking gels. Temperature control for polymerization and electrophoresis is provided. Procedures and applications are described for use of the apparatus with uniform gel concentration or with pore gradient gels. A gradientmaker for the preparation of polyacrylamide pore gradient gels is described.

## INTRODUCTION

The use of a gel slab for polyacrylamide gel electrophoresis (PAGE) (*1*) has several advantages when compared with the use of multiple gel

tubes, at least in certain applications. Since the entire slab is polymmerized at one time, one may expect better uniformity and reproducibility of gel pore size, pH, ionic strength, temperature, and electrical field, than for separate tubes.

Data analysis (*2*) has shown that the standard deviation of relative mobility ($R_f$) values of proteins in duplicate gels in the same experiment (*3*) is significantly smaller than the standard deviation of $R_f$ for gels in separate experiments. It is expected that the standard deviation of $R_f$ (or of difference in $R_f$) would be even smaller for components analyzed in a single gel, than in "split tubes" (*4*) or in slabs. Slabs provide convenience, since the number of pipettings is reduced. All samples are exposed to a gel of the same height. All of these advantages for the use of a slab apparatus are especially important when pore-gradient-electrophoresis (P-G-E) (*5*) is used—it has proven to be impossible to generate identical pore gradients in separate tubes, due to minute differences in flow rates through a manifold. Gel slabs have also been exploited for two-dimensional fractionation involving a variety of techniques, and for transverse gel gradients (*6–10*). However, the present report is concerned with providing a tool for fractionation problems that require (*2, 7*) simultaneous one-dimensional fractionation of multiple samples.

Among the numerous methods and apparatus for PAGE on gel slabs that have been reported (*6–10*) and used extensively, however, none provide all of the features which we deem desirable:

1. Provision of temperature control during polymerization and electrophoresis.
2. Construction of Pyrex glass, to provide adequate wall adherence over the wide range of gel concentration (%T, %C), pH, and temperature required for many fractionation problems.
3. Suitability for use with multiphasic (discontinuous) buffer systems (*18*). These buffer systems are essential for the fractionation of "dilute" samples which otherwise would require excessively large sample zones or preliminary concentration by some other technique. These buffer systems have several other advantages and can be designed or selected specifically for any fractionation problem (*1*). However, they impose several constraints on apparatus design: (a) use of upper (stacking) gels; (b) use of vertical alignment; and (c) inability to use conventional sample slot-formers due to the mechanical lability–fragility of the

minimally restrictive* and, therefore, soft, low %T, high %C (*11*), open pore stacking gels (particularly those formed at acid or neutral pH).

4. Suitability for use with pore-gradient electrophoresis.
5. Relative absence of improvisation from design and procedure.

An apparatus meeting the first four criteria and to some extent the fifth has been designed, tested, and found satisfactory.

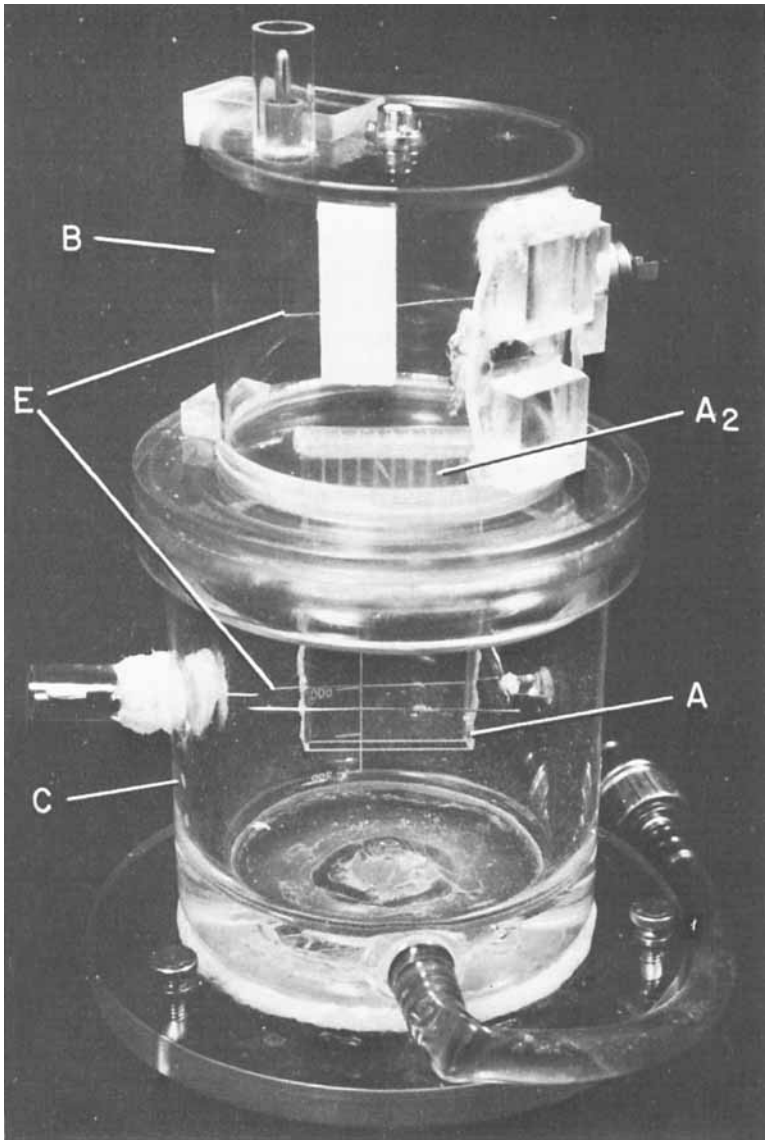
Although numerous two-chamber gradientmakers are available commercially, none of these was found to provide the following five desirable features for generating polyacrylamide gel (pore) gradients: (a) temperature control, required for reproducible polymerization; (b) construction from black plastic to prevent a premature onset of photopolymerization; (c) design of the channel between the two chambers to prevent formation of an air-lock, and avoid the need to transfer liquid between the two chambers: (d) constant position of the stirrer, to achieve reproducible stirring; and (e) conical gradient chambers, allowing for volumes between 5 and 100 ml of polymerization mixture [this feature has been previously used by Hoefer Instruments (Cat. No. SG-201) and Buchler Instruments Div. (Cat. No. 2-5102)]. A gradientmaker meeting these requirements was, therefore, constructed.



## APPARATUS DESIGN

A slab, 9.5 × 4.9 × 0.4 cm, was constructed by fusion (*19*) of 0.125-in. thick Pyrex plates, using Pyrex rods of square cross section (0.4 × 0.4 cm) (A of Fig. 1). The slab is open at the upper and lower faces. The front is permanently bonded to the sides only across the upper 3.5 cm of its length. The lower 6 cm of the front face is composed of a "window": a removable Pyrex plate (A1 of Fig. 1b) is inserted along guide strips to cover this section of the front plate. It is secured in place by a thin band of Vaseline–paraffin sealing mixture. Eight partitions (A2 of Fig.

* The presently available output of multiphasic buffer systems (*1*, *18*) was generated by selection of constituents and concentrations that would minimize the value of the lower stacking limit, RM (1, 4), at any given pH. Nonetheless, the mobility values obtained for constituent 1 in the stacking phase (ZETA or 4) are almost as high as those for most proteins, particularly in neutral buffer systems. Thus, any significant molecular sieving will decrease the mobility of the protein to a value less than constituent 1 and thereby unstack the protein.



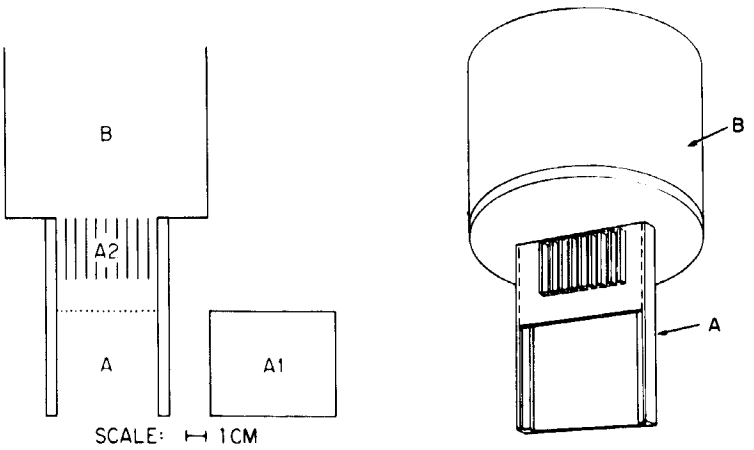



FIG. 1. (a) Assembled partitioned gel slab apparatus—(A) Partitioned Pyrex slab; (A2) partitions; (B) upper buffer reservoir; (C) lower buffer reservoir; (E) platinum electrodes. (b) Schematic diagram of partitioned Pyrex slab attached to upper buffer reservoir— (A) partitioned Pyrex slab; (B) upper buffer reservoir; (A1) window; (A2) partitions.



1) of 2 cm length and 0.1 cm thickness are permanently fused into the top of the slab at 0.4-cm intervals resulting in ten square openings for samples.

The slab is bonded by RTV-adhesive into an upper buffer reservoir (B of Fig. 1). The removable cover of this reservoir contains a linear platinum electrode the same length as the gel.

The water-jacketed, temperature-controlled lower buffer reservoir (C of Fig. 1a) is similar to that used for apparatus employing gel tubes (*12*, *13*) except for use of a rectangular platinum electrode symmetrically surrounding the slab.

To assemble the apparatus, during both polymerization and electrophoresis, the gel slab (A) is completely immersed in the lower buffer, with the upper buffer reservoir supported on the upper rim, or on a ledge within the lower buffer reservoir (the latter to provide hydrostatic equilibration). Blueprints of the apparatus (JWH 1-8-68, BEIB, NIH) are available upon request.

## PROCEDURE

The procedure for polymerization and electrophoresis previously described (*13*) is followed except as stated below. The upper buffer

reservoir with attached slab (A-B of Fig. 1) is positioned horizontally on a cork ring with the window of the slab turned upward. The window plate (A1 of Fig. 1b) is inserted along the guide strips as far as possible into the slab (A). The edges of the window (A1) are then sealed by application of a continuous fine stream of liquid paraffin–vaseline (1:3) with a Pasteur pipet. The upper unit (A-B) is then inverted, and the bottom of the slab is sealed with Parafilm, which is held in place with two rubber bands. After testing that the Parafilm seal is water-tight, the apparatus is inserted into the filled lower buffer reservoir (C of Fig. 1a and is leveled. The polymerization mixture is pipetted into the slab. The volume of lower gel is selected such that its upper surface extends just above the upper edge of the window. Pore-gradient gels are formed by pumping the polymerization mixture from the gradient-maker into the slab through three channels of 0.015-in. tubing, at a rate of 0.3 ml/min/tube, using a Technicon proportioning pump. The three channels discharge into the central and the two peripheral partitions of the slab *via* capillary tubes which are positioned against the front face of the slab slightly above the top edge of the window. Water for overlayering the gels is introduced in the same way. During introduction of the polymerization mixture, the pump, all tubing connections, and the slab apparatus are covered by black cloth. After the slab is filled, the black cloth is removed, and photopolymerization is allowed to proceed for 30 min as previously described (*5*, *13*). Then the gel surface is washed, the Parafilm seal is removed, the upper buffer reservoir is filled with water or buffer to a height of a few centimeters, reinserted into the thermostated lower buffer reservoir (C of Fig. 1a) and realigned vertically. The polymerization mixture for the formation of stacking gel, in 25% sucrose, is then pumped at a rate of 0.32 ml/min through a 50-$\mu$l Drummond micropipet inserted into the central slot onto the surface of the separation gel ("underlayering" the water), using one 0.030-in. i.d. vein of the proportioning pump. Pumping is discontinued when the partitions (A2) are half-filled with upper gel polymerization mixture. Then the upper gel is photopolymerized, the upper buffer reservoir is drained, leveled, and filled with upper buffer.



### Electrophoresis

The samples are introduced into each partition slot as described previously (*13*) using a Kirk micropipet and Pipet Control (Microchemical Specialties Co. # M1001), directing the stream of sample

against an upper corner of each partition to prevent damage to the stacking gel. Electrophoresis is allowed to proceed at a constant current density of 6–10 mA/1.6 cm² at 0°C or 12–20 mA/1.6 cm² at 25°C. After electrophoresis the gel slab is rimmed with needle and water jet as described for tubes (*13*). The paraffin–Vaseline seal is removed with spatula and the window (A1) is lifted. The stacking gel is severed by spatula below the partitions, the upper buffer reservoir is filled with water and the gel slab is again rimmed until the gel slab is freed. It is collected in a petri dish, photographed, and stained. Fixation and staining are carried out as described previously (*14*). The apparatus is readily cleaned with pipet brush and concentrated detergent (e.g., undiluted Wisk) or by acid washing.

## PERFORMANCE

Figure 2 shows a PAGE fractionation (system B, 0°C, 7.7%T, 2.6%C) of human serum. (Load—2.0 μl serum/channel in 25 μl 25% sucrose containing bromphenol blue; current—10 mA/1.6 cm². Alternate channels are used.)

Figure 3 shows a fractionation of chick oviduct protein extract (*15*) using P-G-E (system B, 0°C, 4.2%T, 5%C to 10.2%T, 2%C) (*5*, *13*). (Load—200 μg extract/channel, containing 25% sucrose and bromphenol blue; current—10 mA/1.6 cm².) Other applications have been reported previously (Experiment I of Ref. *5*; Fig. 5 of Ref. *16*).

## GRADIENTMAKER

Figure 4 shows a cross section of the polyacrylamide gel gradientmaker. The apparatus is water-jacketed (J). The external surface is made entirely of black Plexiglas to prevent premature polymerization of the solutions (L and H) contained in the two chambers and in the connecting channel. The presence of the water jacket prevents the mounting of the stopcock (C) at the bottom of the apparatus. Accordingly, a stem extending to the top of the apparatus is used. Provision was made to displace air in the channel between the chambers into the hollow stem of the valve control (C), using a three-way stopcock control (via valve openings 01, 02, and 03) following the procedure outlined previously (*5*) and in Appendix I (provided upon request)—

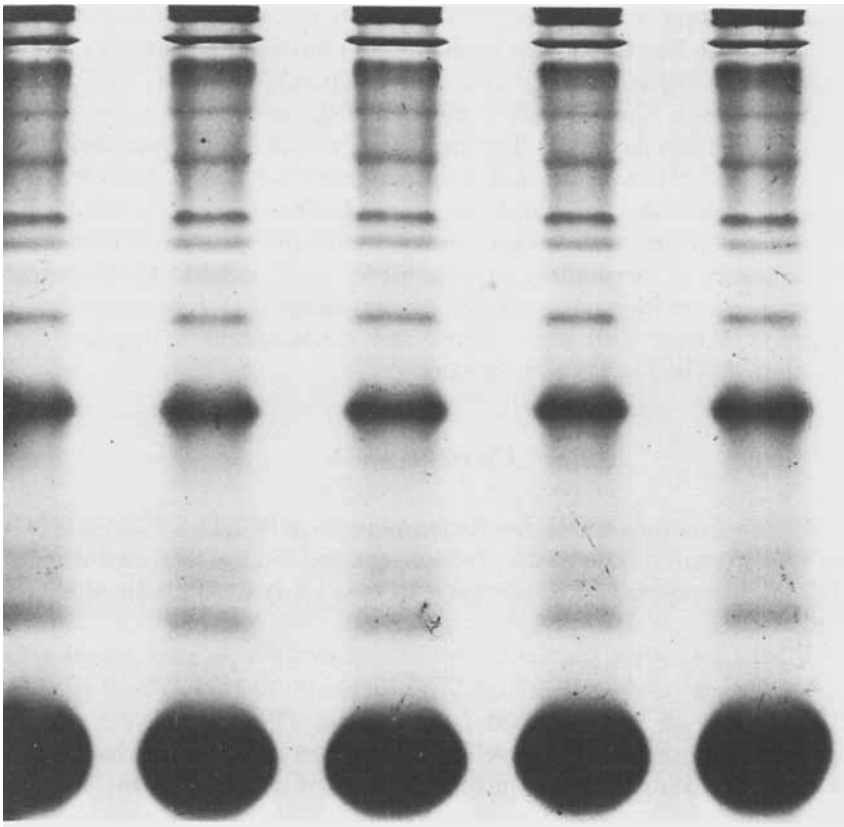


FIG. 2. PAGE (system B, 7.7%T, 2.6%C) fractionation pattern of human human serum. Current—10 mA; load—2 μl/channel.

(this step avoids the procedure of returning liquid passed through the channel). A helical mechanical stirrer (S) (Hoefer Instruments, No. SG-104) and dummy-stirring helix (D) are positioned within the narrowly circumferential opening in the covers of the two gradient chambers. [A blueprint of the gradient maker (J.O. 726268, BEIB, NIH) is available upon request.]

The performance of the gel gradientmaker was tested by a spectrophotometric recording of a linear gradient formed with dye. It has also been used in the formation of linear pH gradients between buffers in a polyacrylamide gel matrix (*17*).

## DISCUSSION

Prototypes of the partitioned slab apparatus had been constructed by epoxy-bonding. After repeated use at 0°C, these models developed leaks along the bonded surfaces. Also, polyacrylamide separated from the walls at the points of contact with epoxy adhesive. The present mode of construction of the slab by Pyrex fusion (*19*) has avoided these problems. The use of Pyrex fusion on a carbon matrix (template) also allows for the production of multiple units from a single template and thereby reduces substantially the cost of manufacture.

Our aim to eliminate completely the "element of art" from the procedure of operation of the apparatus has only been partially fulfilled. The present apparatus still does not permit one to affix in one operation

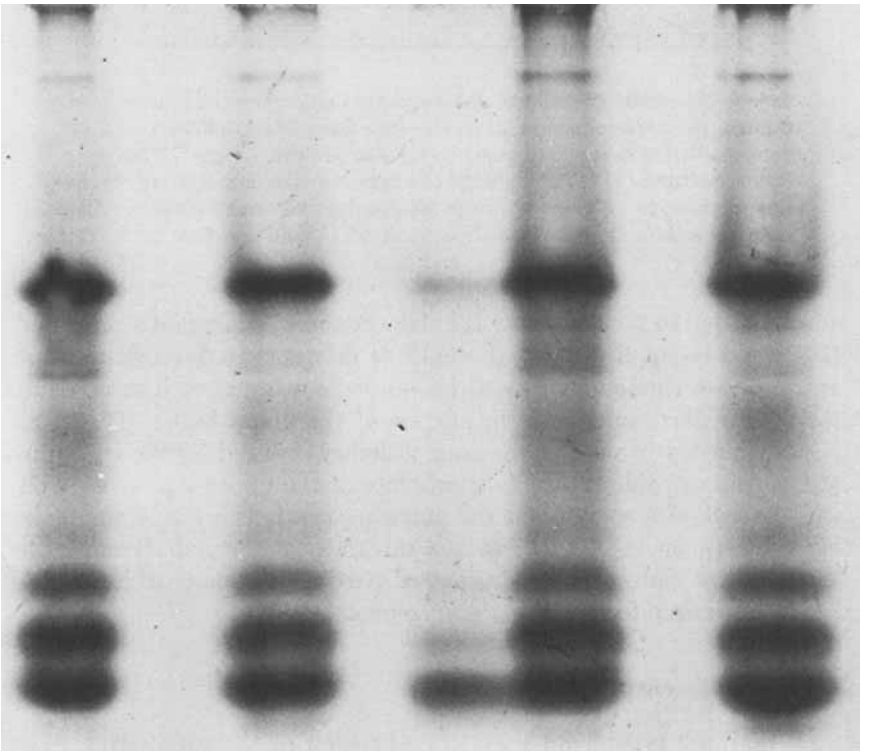


FIG. 3. P-G-E (system B, 4.2%T, 5%C to 10.2%T, 2%C) fractionation pattern of chick oviduct extract. Current—10 mA; load—200 μl/channel.

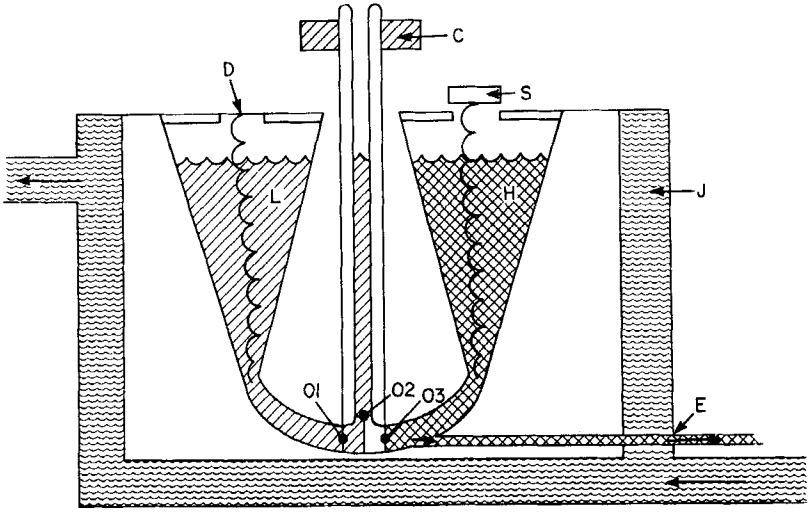



FIG. 4. Schematic diagram of the polyacrylamide gel gradientmaker. (C) Control for triple stopcock 01-02-03; (the stem has a hollow core); (D) dummy stirring helix; (E) exit port; (H) heavy solution, high %T polymerization mixture; (J) coolant jacket; (L) light solution, low %T polymerization mixture; (01, 02, and 03) stopcock openings allowing for selective flow from either L or H into the hollow stem of C, and for flow between L and H.



a reliable seal to the bottom of the slab. Possibly, sealing on a "cushion" (*10*) could be applied. Also, it would be desirable to develop a sample applicator to eliminate the need for micropipetting of each sample into the proper partition under the surface of the upper buffer. This is especially needed in view of the poor visibility induced by the curvature of the reservoir,the opaque bottom plate of the upper buffer reservoir, and the lack of a support for the pipetting hand. The $4 \times 4$ mm$^2$ cross section between the partitions, the thickness of the slab (4 mm), the length of the slab, and the number of partitions remain arbitrary and can be optimized for any particular application.

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BEIB, NIH) built several prototypes and constructed the first gel slab apparatus by Pyrex fusion. Mrs. G. Sherman typed the manuscript into the WYLBUR text-editing system.

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