A versatile gel casting cum electrophoresis apparatus

G. D. GHADGE, A. M. BODHE, S. T. DHUME, M. V. RELE and H. G. VARTAK

Biochemistry Division, National Chemical Laboratory, Poona 411 008, India

MS received 12 February 1983; revised 8 August 1983

Abstract. A simple apparatus for vertical, in situ, polyacrylamide or agarose gel casting as well as for the subsequent electrophoresis is described. The apparatus is completely leakproof and does not require any special device like clamps, O-rings, gaskets, grease etc. for sealing. Slab gels of various thickness (0.04 to 1.0 cm) can be made and the apparatus can be used for analytical or preparative purposes. Gel rods can also be cast and run in the device. Forward as well as reverse polarity electrophoresis of a sample can be run simultaneously in the apparatus.

Keywords. Polyacrylamide gel electrophoresis apparatus; gel electrophoresis simple apparatus; gel casting cum electrophoresis; simultaneous forward and reverse electrophoresis.

Introduction

Several models of vertical gel electrophoresis have been developed by different workers. Sophisticated commercial models are also available. In a recent comprehensive review on preparative gel electrophoresis, Chrambach and Nguyen (1979) have stressed the need for a simpler design for preparative gel electrophoresis which is free from the effects of mechanical and hydrostatic pressures on the gel. The equipment available including the commercial models possesses one or more of the following disadvantages: The gels are made separately in gel casting device and then transferred to the electrophoresis apparatus, thus developing mechanical stresses (Tichy, 1966; Akroyd, 1967; Studier, 1973; Bambeck and Black, 1981). Hydrostatic equilibrium is not attained (Tichy, 1966; Akroyd, 1967; Studier, 1973; Ogito and Market, 1979). Moreover to avoid leakage, the above mentioned models and also those described by Blatter (1969), Roberts and Jones (1972) and Andrew et al. (1979) require devices such as clamps, screws, O-rings, gaskets, melted agar, grease and plasticine clay.

This paper describes a simple and inexpensive, multipurpose apparatus for in situ gel making and subsequent electrophoresis which is free from the disadvantages mentioned above. An added advantage of this apparatus is that, with minor modifications, it

* NCL Communication No.: 3077.
Abbreviations used: PA, Polyacrylamide; PAGE, polyacrylamide gel electrophoresis; Bis, N,N'-methylenebis (acrylamide); TEMED, N,N,N',N'-tetramethylethylenediamine; UV, ultra-violet.
can also be used to obtain a total scan of biological extracts by running a simultaneous forward and reverse polarity electrophoresis.

Materials and methods

Chemicals

All common chemicals used were of the analytical reagent grade. The following chemicals and biochemicals were obtained from the sources indicated: myoglobin (from horse heart), trypsin (from bovine pancreas, Type III, EC 3.4.21.4), albumin (bovine serum, crystallized), amido black 10 B, ethidium bromide (crystalline) and ferritin (from horse spleen, Type 1) were from Sigma Chemical Co., St. Louis, Missouri, USA; and casein (Hammersten) was from E. Merck, Dermstadt, West Germany. The chemicals used for polyacrylamide gel electrophoresis (PAGE) such as acrylamide, N,N'-methylenebis acrylamide (Bis) and N,N,N',N'-tetramethylenediamine (TEMED) were obtained from Eastman Kodak Company, Rochester, USA. Glycine was from Kochlight Laboratories, Buckinghamshire, UK. Agarose (electrophoresis grade) was purchased from Sisco Research Laboratories, Bombay. DNA marker (Hind III digest of \( \lambda \) DNA) was a generous gift from Dr. S. Modak, Poona University, Poona. Culture filtrate from the fungus Conidiobolus (Srinivasan et al., 1983) was used for simultaneous forward and reverse electrophoresis. Perspex sheets were procured locally.

Solutions for gel electrophoresis of proteins and nucleic acids

Solutions for alkaline pH runs: These were prepared according to Davis (1964). Both the upper and lower electrode buffers contained 0·005 M Tris and 0·04 M glycine, pH 8·3. The gel composition was 0·38 M Tris and 0·06 M HCl, pH 8·9; 7% acrylamide, 0·18% Bis, 0·03% TEMED and 0·07% ammonium persulphate.

Solutions for electrophoresis at acidic pH values: These were prepared according to Reisfeld et al. (1962) with slight modification. The two electrode buffers contained 0·04 M glycine and 0·0035 M acetic acid, pH 4·0. The gel composition was 0·36 M acetic acid and 0·06 M KOH, pH 4·3, 7% acrylamide, 0·1% Bis and 0·14% ammonium persulphate.

Solutions for agarose gel electrophoresis: These were prepared according to Thomas and Davis (1975). Both the electrode buffers contained 0·089 M Tris, 0·089 M boric acid and 2·5 mM EDTA, pH 8·2. Agarose gel composition was 0·8% agarose in the above buffer.

Tracking dye: Basic fuchsin was used for acidic runs and bromophenol blue for other runs.

Composition of contact gel: Contact gel was made in the bath buffer composition of the respective runs (e.g. contact gel composition for alkaline runs was: 0·005 M Tris and 0·04 M glycine, pH 8·3, 7% acrylamide, 0·18% Bis, 0·03% TEMED and 0·07%
ammonium persulphate. Contact gel composition for the simultaneous forward and reverse runs was: 0·005 M KCl, 7% acrylamide, 0·18% Bis, 0·06% TEMED and 0·14% ammonium persulphate. Contact gel can be reused several times by preserving the gel in cold under a layer of the buffer used for making it.

**Staining and destaining:** The gels were stained with amido black (0·5 % in 7% acetic acid) and destained with 7% acetic acid. Agarose gel was stained with ethidium bromide (1 mg/litre H₂O) and the DNA markers were visualized over an ultra-violet (UV) transilluminator.

**Elution and assay of trypsin:** Trypsin was eluted from PAGE by the method of Bodhe et al. (1982) and estimated by the spectrophotometric assay of Kunitz using caseinolytic assay (Kunitz, 1947).

**Apparatus**

**Design principle:** The apparatus is designed for vertical gel electrophoresis. It is made from perspex sheets. Figures 1 and 2 show the photographs of the empty and the assembled unit. In addition, figures 3 and 4 give the dimensions of the unit.

The main apparatus consists of two vertical chambers which form the electrode compartments. These chambers are adjacent to each other and are separated by a common middle partition wall. A gap of 0·8 cm is kept at the base of the partition wall. One cm thick gel is cast at the base of the chambers which also closes the gap (0·8 cm) kept below the partition wall. Thus this basal ‘contact gel’ makes the two chambers leakproof (prevents the buffer flow from one chamber to the other), and also establishes an electrical contact between the two chambers.

**Figures 1 and 2.** 1. Photograph of the empty unit. 2. Photograph of the assembled unit.
Gel cassettes (molds for casting slab gels) are placed above the contact gel in one of the chambers (chamber ‘a’) and are secured in position by a wedge (figures 4h and 5). Polyarylamide (PA) slab gels are cast in the gel cassettes, both the chambers are filled with buffer and after loading the sample, \textit{in situ} electrophoresis is carried out since both the chambers also form the two electrode compartments. Thus out of the two chambers one chamber serves as a chamber for gel casting and both the chambers act as electrode compartments.

\textbf{Mode of construction:} In chamber ‘a’ where the gels are cast, the wall opposite the middle partition wall is made slanting for the operation of wedge. The middle partition wall is made of glass and sealed with araldyte against the walls of the equipment.

The wedge (figures 4h and 5) is made of perspex sheets, except for its surface facing the gel cassettes which is made of glass and sealed to the wedge with araldyte. The wedge is hollow and opens from the top only (see discussion).

Gel cassettes (glass, plates, 8.5×8.5 cm and 01 cm thickness) and well former for slab gel are made as in the Pharmacia apparatus (GE-2/4). Figure 6 shows the details regarding the slab and rod gel cassettes. For making a slab gel cassette, two spacer strips
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Figure 6. Gel cassettes, (a) Slab gel cassette; (b) Single well former for slab gel; (c) Multi well former for slab gel; (d) Two halves of the gel rod cassette (each 8.5×0.5×8.5 cm) exposing the half round grooves; (e) Gel rod cassette assembled, 8.5×1.0×8.5 cm.

are placed at the edges of a glass plate and the second glass plate is placed on them. The assembly is then held in position by two small strips of adhesive tape. The thickness of the gel can be varied as desired (0.04 to 1 cm) by using spacer strips of different thickness. For preparative type gel a ‘single well former’ is used. Spacers of different thickness and ‘well formers’ with different number of teeth can be easily cut from neoprene sheets.

Gel cassette for gel rod casting is made as shown in figure 6. It is made from two glass plates (8.5×0.5×8.5 cm) in which four half round grooves of 0.3 cm radius are made on each plate. When both the plates are placed on one another four hollow tubes are formed inside. The rod ‘well former’ is made of cylindrical perspex teeth.

Operating procedure

Forty ml of 7% ‘contact gel’ is polymerised at the base of the apparatus. This fills up the base and the basal gap between the two chambers and comes up to a 1 cm height and thus to the level of the support strips. One or more slab gel cassettes are placed in chamber ‘a’ at the base of the apparatus on the two support strips and above the contact gel. The cassettes are secured by the wedge (figure 4h). After polymerising the gel in the cassettes, both the chambers of the apparatus are filled with the bath buffer, sample is loaded, electrodes are positioned and the run is started by making electrical contacts. After completing the run the bath buffer in the chambers is poured out and after removing the wedge the gel slabs are taken out, stained, destained or if desired the proteins can be electrophoretically eluted (Bodhe et al., 1982). Gel rod is removed simply by opening the two halves of the rod casting cassette.
Simultaneous forward and reverse polarity electrophoresis

The above mentioned apparatus with a few modifications was used for simultaneous forward and reverse polarity electrophoresis. This design can be used for scanning biological extracts containing both acidic as well as basic proteins in a single run. The modification involves making chamber ‘b’ a replica of chamber ‘a’. Consequently the unit will have two slanting walls and two wedges. Figure 7 shows the photograph of the assembled unit. In this apparatus the gels are cast in both the compartments (one gel in each compartment), and the same sample is loaded in both the compartments. Electrophoresis is carried out as mentioned in the earlier unit. Thus in this apparatus both the chambers serve as chambers for gel making as well as, as electrode compartments.

Results

Electrophoresis of marker protein, trypsin, DNA markers and culture filtrate of Conidiobolus.

Electrophoresis under alkaline conditions

Normal size gels: Two slab gels (each 8.5×0.3×8.5 cm) were run together. In one gel a mixture of 400 µg each of ferritin, myoglobin, serum albumin and trypsin inhibitor were loaded. In the second gel the same marker mixture was loaded in three wells (15,30
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and 45 µg of each protein respectively). The band pattern of the proteins stained is shown in figure 8.

**Thin gels:** Three gels (8.5×0.04×8.5 cm) were run simultaneously in the apparatus. Fifteen per cent gels were made instead of 7%. In each gel, marker protein mixture (30 µg of each marker) was loaded. After electrophoresis, gels were stained for 5 min in amido black and destained in 10 min by suspending in 7% acetic acid (figure 9).

**Thick gel:** Preparative run in 1 cm thick gel. One gel (8.5×1.0×8.5 cm) was run. Marker mixture containing 7 mg of each marker protein (total 28 mg protein) was electrophoresed (figure 10).

**Gel rods of high PA concentration:** Four gel rods of 15% PA were cast in the cassette and a mixture of 20 to 40 µg of each marker protein was loaded in each gel rod. After the run the rods could be easily taken out by simply opening the cassette (figure 11).

**Electrophoresis under acidic conditions**

**A labile protein:** Trypsin was run in a cold room at 5 to 8°C. Two slab gels (8.5×0.3 × 8.5 cm) were run together. Two mg of trypsin was loaded in each gel. After the run, one gel slab was stained (figure 12) and the other gel was used for the electrophoretic elution of trypsin (Bodhe *et al.*, 1982), 78% of the trypsin activity was eluted.

**DNA markers in agarose gel**

One gel slab (8.5×0.3×8.5 cm) containing 0.8% agarose gel with wells was run after loading 0.1 µg of DNA marker (figure 13).

**Simultaneous forward and reverse polarity electrophoresis of fungal broth**

**Both forward and reverse runs at pH 8.9:** The gel and buffer compositions in both the chambers were those of pH 8.9 system. Broth sample (5 mg protein) was loaded in the gel of each chamber. Figure 14 shows the protein band pattern.

**Forward run at pH 8.9 and reverse run at pH 4.3:** In one chamber the gel and the buffer composition was that of pH 8.9 system. In the other chamber the gel and buffer composition was that of pH 4.3 system. Broth sample (5 mg protein) was loaded in the gel of each chamber. The band patterns of the stained gels is shown in figure 15.

**Discussion**

We report here a dual purpose simple and leakproof apparatus for vertical PA or agarose gel preparation and for the subsequent electrophoresis. Slab gels, as well as gel rods can be cast and run in the apparatus. Gel slabs of various thickness (0.04 cm to 1 cm) can be made and thus the apparatus can be used for analytical as well as for
Figures 8–12. 8. Two normal size gel slabs run together, each 8.5×0.3×8.5 cm. (a) Protein markers (ferritin, myoglobin, serum albumin and trypsin inhibitor), each 400 µg. Position of the tracking dye is shown with arrows; (b) Protein markers in three wells; 15, 30, 45 µg of each marker was loaded. Electrophoresis was at 90V, 25mA for 3 h. 9. Three thin gel slabs together, each 8.5×0.04×8.5 cm. Protein marker mixture containing 30 µg of each marker
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preparative purpose. More than one gel can be run in the apparatus. Gel rod cassette eliminates the troublesome removal of gels from the tubes. This is especially convenient for the gels of 15% or higher PA concentrations and does not require any rimming or tube breaking.

Hydrostatic balance (Chrambach and Nguyen, 1979) is automatically adjusted due to the position of the two buffer chambers. Mechanical stress (Chrambach and Nguyen, 1979) on the gel is also avoided by *in situ* polymerization of the gel and subsequent electrophoresis in the same apparatus. Contact gel is reusable. The band patterns obtained in the device described are as good as those obtained in other models.

The apparatus shows some resemblance with the Pharmacia gel making apparatus (Pharmacia Fine Chemicals, Sweden, Gel Slab Casting Apparatus GSC-8). However, the introduction of one of the main vital modifications—the gap kept below the central partition in our apparatus has made a vast difference and made the unit a ‘two in one’ unit *i.e.* the same apparatus is used for gel casting and subsequent electrophoresis also, for which M/s. Pharmacia had to develop a separate and costly electrophoresis apparatus. In our device the gel thickness can be varied from 0·4 mm to 10 mm, which is not possible in the Pharmacia electrophoresis apparatus. In the gel rod cassette of the present apparatus, gels of very high acrylamide concentration (30 to 40 % acrylamide) can be cast and the gel rods after the run can be easily removed from the cassette by just opening the two halves of the cassette like a book. Removal of high concentration gel rods is not possible in the commercial models. High concentration gel rods can be used for electrophoresing small molecular weight proteins and peptides. The present apparatus has also other advantages as mentioned earlier in ‘Introduction’.

The modified device (figure 7) can be used for a simultaneous forward and reverse polarity electrophoresis of a sample. Thus in a single run it will be possible to get a total protein pattern of a sample. In most of the reported models this facility is not available. This modified device can also be used for usual electrophoresis (unidirectional) as in our normal device (figure 2). However, the normal device is more compact, cheaper and easy to fabricate as compared to the modified design. Although simultaneous forward and reverse electrophoresis can be carried out only in the modified device, for routine runs the compact device is quite satisfactory.

Under normal experimental conditions the problem of heat dissipation—elimination of heat generated in the gels, is well taken care of in our device. Even during the preparative run the temperature rise in the bath buffer and in the gel proper, is not more than 3°C and 7°C respectively. In the case of labile proteins, runs can be carried out in a cold room. Since glass is a better conductor of heat than perspex, one surface of the wedge facing the cassette and the middle partition wall between chamber ‘a’ and ‘b’ are made of glass. When chamber ‘a’ is filled with bath buffer the hollow wedge also gets filled with it which cools the gel through the glass surface. Also the glass wall of middle partition offers cooling by the bath buffer of chamber ‘b’.

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was loaded. Electrophoresis at 100 V, 12 mA for 2 h. 10. One thick gel, 8·5×1×8·5 cm; preparative scale. Marker mixture, 7 mg of each marker (total 28 mg protein). Electrophoresis at 150 V, 30 mA, 4·5 h. 11. Gel rods of 15% PA concentration using a gel rod cassette 40,40,20 and 20 μg of each marker. Electrophoresis at 110 V, 15 mA for 4·5 h. 12. Electrophoresis of trypsin in two normal gel slabs in a cold room. 2 mg of trypsin in each gel. Electrophoresis at 120 V, 25 mA for 3 h.
Figures 13-15. 13. DNA marker in 0.8% agarose gel, normal size gel 0.1 μg of DNA marker loaded in a well. Electrophoresis at 60 V, 15 mA for 2.5 h. 14. Simultaneous forward and reverse electrophoresis of fungal broth, (a) Forward run at pH 8.9; (b) Reverse run at pH 8.9. Broth sample (5 mg protein) in the gel of each chamber. Electrophoresis at 120 V, 20 mA for 5 h. 15. Simultaneous forward and reverse electrophoresis of fungal broth (a) Forward run at pH 8.9; (b) Reverse run at pH 4.3. Broth sample (5 mg protein) in the gel of each chamber. Electrophoresis at 120 V, 20 mA for 3.5 h.
The present construction is very simple and thus allows reproduction in a poorly equipped workshop. The design will be of help to those who have only rather small resources.

It is possible that when very high current densities are used, as in a few cases, the present apparatus may not be able to solve the heat dissipation problem, unless some additional cooling arrangement is introduced. This problem can however be easily solved by sandwiching the gel cassettes or the gel between cooling plates as described by Tichy (1966).

The hollow wedge can also be used as one of the cooling plates.

Acknowledgements

The authors are thankful to Dr. C. Siva Raman and Dr. V. Jagannathan for their encouragement and keen interest in this work.

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