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Autoradiography with Acrylamide Gel Slab Electrophoresis[1]

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Polyacrylamide disc electrophoresis (1) is a widely used technique for obtaining high resolution of the components of protein mixtures. Attempts have been made to measure radioisotope labeling in the many fine bands of an electrophoresis gel. The methods consist of mechanical extrusion (2) or slicing the gel crosswise into small segments (3–8) and subjecting them to one of the following treatments: solubilization (9–13) or combustion (14) of the gel, elution of protein by diffusion (15–18) or electrophoresis (4, 19), or replacement of water in the gel by scintillation solvent (20). Most of these methods are tedious and suffer some loss of resolution. Protein bands are usually much thinner than the sliced segments, and several bands can be closely stacked into a small region. Fairbanks *et al.* (21), however, have been able to retain the original electrophoretic resolution by slicing cylindrical gels lengthwise, drying them, and subjecting them to autoradiography. The use of gel slabs, rather than cylindrical gels, for autoradiography should obviate the necessity of slicing, and has the additional advantage of comparing multiple samples under virtually identical conditions in a single autoradiogram. Although electrophoresis with polyacrylamide gel slabs has been used frequently (22–28), its application for autoradiographic purposes has received only slight attention (28). In this paper we describe in detail a procedure for autoradiography with acrylamide gel slab electrophoresis.

METHODS

Electrophoresis Apparatus. Figure 1 shows the construction of the electrophoresis apparatus. The upper and lower buffer baths are con-

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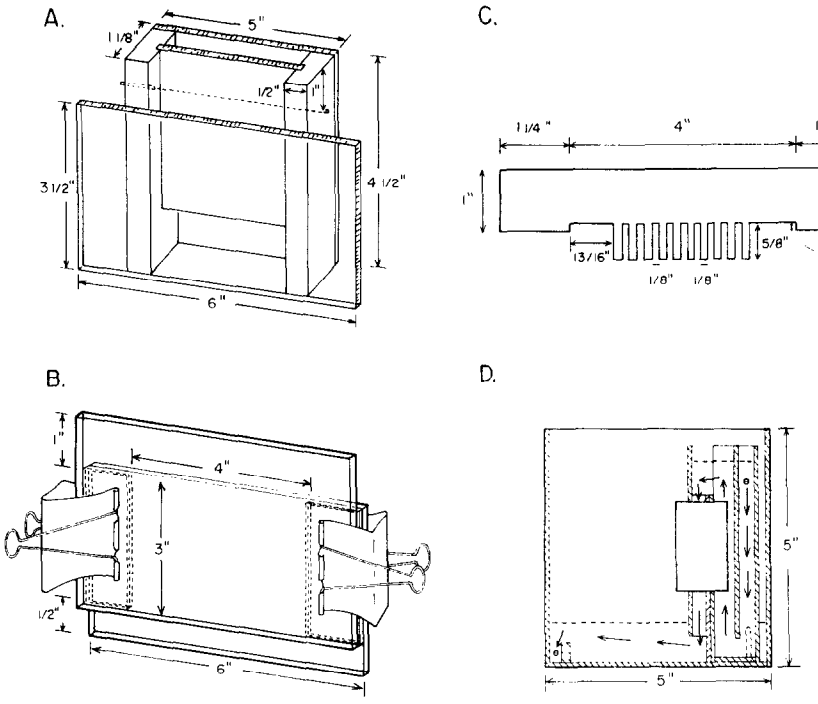


FIG. 1. Construction of electrophoresis apparatus. (A) Front view of upper bath or inner box. Broken line indicates a gauge 24 platinum electrode in the rear compartment. (B) Front view of gel chamber. In practice, clamping includes the front plate of the upper bath. The ears of the clamps are removed before electrophoresis. (C) Template for forming sample slots. (D) Side view of complete assembly, showing lower bath or outer box holding the upper bath and the gel chamber in place with two pins. The platinum electrode of the lower bath is shown on end at far left. Arrows indicate direction of flow of anions. Broken lines represent buffer levels. Dimensions of lower bath are $5'' \times 5'' \times 8\frac{1}{2}''$.

structed mainly from $\frac{1}{8}''$ and $\frac{3}{16}''$ thick Plexiglas (acrylic plastic), respectively, except where specified, and cemented with Cadco No. 94 monomer solvent (Cadillac Plastic and Chemical, Detroit, Mich.). During electrophoresis, the upper bath (inner box) is secured by means of two Plexiglas pins attached to the lower bath (outer box). The gel chamber is an all-glass assembly cut from two $\frac{1}{8}''$ thin-layer chromatography (TLC) plates (Brinkmann Instruments) and spaced by two layers of microscope slides. Glass is superior to Plexiglas for obtaining gels firmly attached to the walls. The template for sample slots is machined from a piece of 0.06″ (1.5 mm) thick Plexiglas with an $\frac{1}{8}''$ end mill and annealed for 0.5 hr at 62°C in a solution of Oakite Cleaner (Oakite Products, New York). It is coated with Siliclad (Clay-Adams) before the first use. As

discussed below, the thickness of the gel and of the template, and the gel concentration, are all critical for the successful electrophoresis and drying of the gel before autoradiography.

Preparation of Gel. Before each use, all surfaces that will be in contact with the gel are thoroughly cleaned with a suitable detergent such as 7X (Linbro Chemical Co., New Haven, Conn.) using tissue paper or a cotton swab. Brushing is to be avoided as this may cause scratches. The cleaned surfaces, except for the template, are then wetted with a 0.5% solution of Kodak Photo-Flo 200 and air dried. The gel chamber is then assembled by placing two pieces of ordinary glass microscope slides (1″ × 3″ × 0.04″) vertically on each side between the two TLC plates, the lower edge of the slides being aligned with that of the front plate of the gel chamber. In this way a space 0.08″ (2 mm) thick and 4″ long is created for the gel. Each side edge of the chamber is sealed by dipping into melted paraffin at about 70°C while the other edge is held clamped. The lower edge is sealed with Plasticine modeling clay. The gel chamber is then clamped to the front plate of the upper bath with a Swingline binder clip (#110) on each side. The junction between the upper bath and the gel chamber is sealed with 2 strips of Plasticine, preventing leakage of upper buffer during electrophoresis. During the preparation of the gel this bath is anchored by means of pins to a stand. By means of a Pasteur pipet a separation gel (small-pore gel) solution containing 7% monomer as prescribed by Davis (1) is introduced into the gel space to a height of 2⅛″ (5.3 cm). The solution is then carefully overlayered with water to a height of 3/16″ (0.5 cm) using a syringe and a gauge 26 needle having a blunt, slightly bent tip. Gelation starts in 10 to 15 min, but the gel is allowed to stand undisturbed for a total of 30 min. The water is poured off and the gel surface washed twice with the spacer gel (large-pore gel) solution (1). The template is then secured to the front glass plate of the gel chamber by several small clamps A. H. Thomas catalog #18). Six layers of Chef-foil regular aluminum wrap (Anaconda Aluminum, Louisville, Ky.), with a total thickness of 0.0045″ (approximately 0.1 mm), are placed between the template and the glass surface before clamping. The remainder of the space in the gel chamber is then filled with the spacer gel solution up to the top of the rear glass plate. Gelation is accomplished by irradiating for 45 min with a 15 W daylight-type fluorescent lamp placed in front of the gel chamber at a distance of 1.5″. Prolonged irradiation is to be avoided because this renders the template inseparable from the gel. The template is removed with care to avoid deforming the gel slots. It is advisable not to apply liquid to lubricate the template during removal as the liquid that goes into the slots is difficult to remove. However, small

amounts of ungelled liquid found in the slots may be taken up by a small wick made of tissue paper. Each slot is filled with 60 to 80 μl of sample gel solution (identical with spacer gel solution except for the presence of protein sample) and irradiated as before for 50 min. It is preferable that the viscosity of the sample gel be made similar to that of the spacer gel so as to avoid distortion in ionic flow. In the procedure described above, the presence of acrylamide and sucrose in both renders the presence of protein negligible as a contributing factor to viscosity difference. In instances where formation of sample gel is impossible or inconvenient, one should correct the viscosity difference by adding substances such as sucrose, urea, or acrylamide monomer into the sample solution.

Electrophoresis, Staining, and Destaining. Before securing the upper bath to the lower bath, the Plasticine seal at the bottom of the gel is completely removed. The two spaces at the upper corners of the gel (between the upper bath and the gel chamber), are sealed off with two pieces of glass, using Plasticine to fill the gaps and to hold them in place. Both buffer baths are filled with the same electrode buffer diluted 5-fold from the stock (1); 200 ml is needed for the upper bath and 400 ml for the lower in order to have the buffer level ½″ above the upper edge, and ¼″ above the lower edge of the gel. Bubbles trapped at the lower edge of the gel can easily be removed by suction, using a bent Pasteur pipet or a syringe with a bent needle. Tracking dye (bromphenol blue) can be added either to the sample gel before polymerization or to the upper buffer. Electrophoresis is carried out at 4°C with 25 mA for about 100 min with the lower electrode as the anode. The box is left uncovered in order to reduce heat accumulation. After electrophoresis, the gel is exposed by prying the two glass plates of the gel chamber apart through a notch previously made in one corner of the spacer plates (microscope slides). The gel is then removed and stained for 1 hr with 1% amido black in 7% acetic acid. Destaining is accomplished by three or four soakings in 7% acetic acid over a period of about 24 hr. If quick visualization of the band pattern is desired before destaining is complete, the gel can be viewed by holding the glass container directly above a bright Tensor lamp.

Drying and Storage of Gel. The gel is placed on a piece of wet dialysis membrane (cut from A. H. Thomas Co. catalog #4465-A2), which is in turn laid over two superimposed discs of porous hydrophilic linear polyethylene each 5/16″ thick (Bel-Art Products, Pequannock, N. J., catalog #F-1255). The smooth side of the plastic is in contact with the dialysis membrane, and the protein side of the gel is away from the membrane. The two plastic discs are sawed beforehand to fit the base

of a bell jar (New York Laboratory Supply Co.). With the bell jar in the upside-down position, the gel, the dialysis membrane, and the plastic discs are fixed to the jar with a piece of Saran Wrap tightened by rubber bands. Air bubbles trapped between the Saran Wrap and the gel are expelled by stroking gently with a finger. The jar is then mounted in an upright position and connected to a house vacuum line. The complete setup is shown in Figure 2. A reflector infrared lamp (250 W) is placed

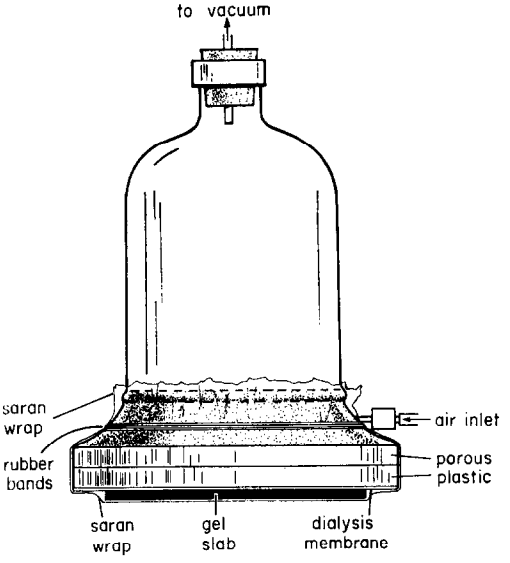


FIG. 2. Setup for drying the gel slab.

below, 1.5 ft from the gel. Complete drying takes 3–4 hr. Accumulation of moisture inside the jar slows down the drying process and is prevented by allowing a small stream of air to pass through continuously. Excessive vacuum bends the plastic discs and breaks the gel. The porous plastic discs can be reused many times, but the unavoidable slight bending after each use should be corrected by alternating the position of the two discs. After the drying procedure, the Saran Wrap can be removed with ease while the dried gel adheres tightly to the dialysis membrane. Owing to the transparency of the membrane, the protein patterns are clearly visible and densitometric tracing can be carried out at this stage. The dialysis membrane provides a firm support so that the dried gel does not curl up. The dried gel is not hygroscopic and can be stored indefinitely. For convenience, we "file" our dried gels in transparent photographic album sleeves. If projection of the protein patterns is desired, lantern slides can be made by directly mounting the dried gels, eliminating any loss of detail incurred by photography.

Autoradiography. Autoradiography is carried out by placing the dried gel in contact with Kodak No-Screen x-ray film between two pieces of Kodak projector slide cover glass ($3\frac{1}{4}'' \times 4''$). Pressure is applied with two pieces of plywood held together by screws. After exposure, the film is developed for 2 min with Kodak x-ray developer, rinsed briefly with water, and immersed for 7 min in Kodak x-ray fixer before a final rinsing with running water.

RESULTS AND DISCUSSION

Figure 3 shows the electropherogram of 10 identical samples in one piece of gel slab. Replicability from sample to sample in the same gel is good. The highly resolved patterns are well preserved, and individual bands are identifiable throughout the entire procedure. The relations between optical density and the amount of sample applied were determined for the protein stain and the autoradiogram over a 4-fold range of sample (Fig. 4) and were found to be reasonably linear. Variations in the dimensions of the teeth of the sample former could cause deviations from linearity, but each slot could be individually calibrated.

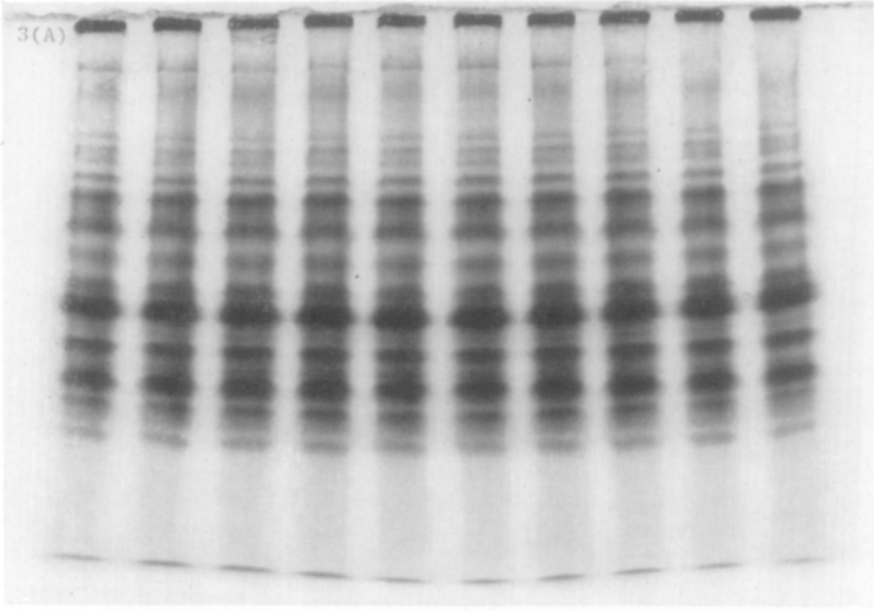
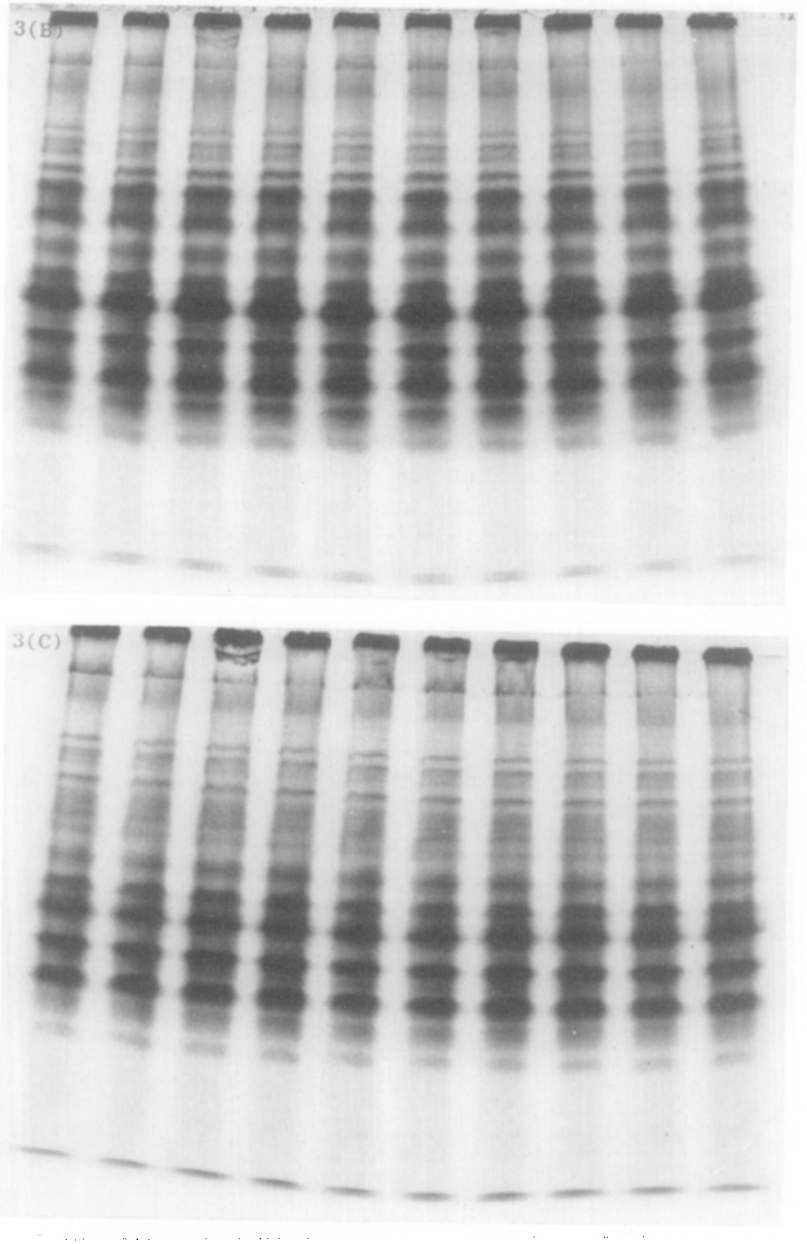


FIG. 3. (A) Electrophoresis of bacterial protein. L-Leucine-C^{14}, 40 μc, specific activity 180 mc/mmole, was added to 180 ml of an *Escherichia coli* B culture in minimum mineral medium at OD of 0.2 (590 mμ). The culture was further shaken at 37° until the OD reached 1.2. The harvested cells were suspended in water and



sonicated. The 100,000 *g* supernatant fraction, 4 ml in total volume, was used for electrophoresis studies. Each sample slot was filled with 0.06 ml of sample gel containing 0.025 ml of the supernatant (5×10^5 dpm). Photograph shows the protein pattern in the stained gel. (B) Photograph of same gel after drying. (C) Autoradiogram of the dried gel after 22 hr exposure.

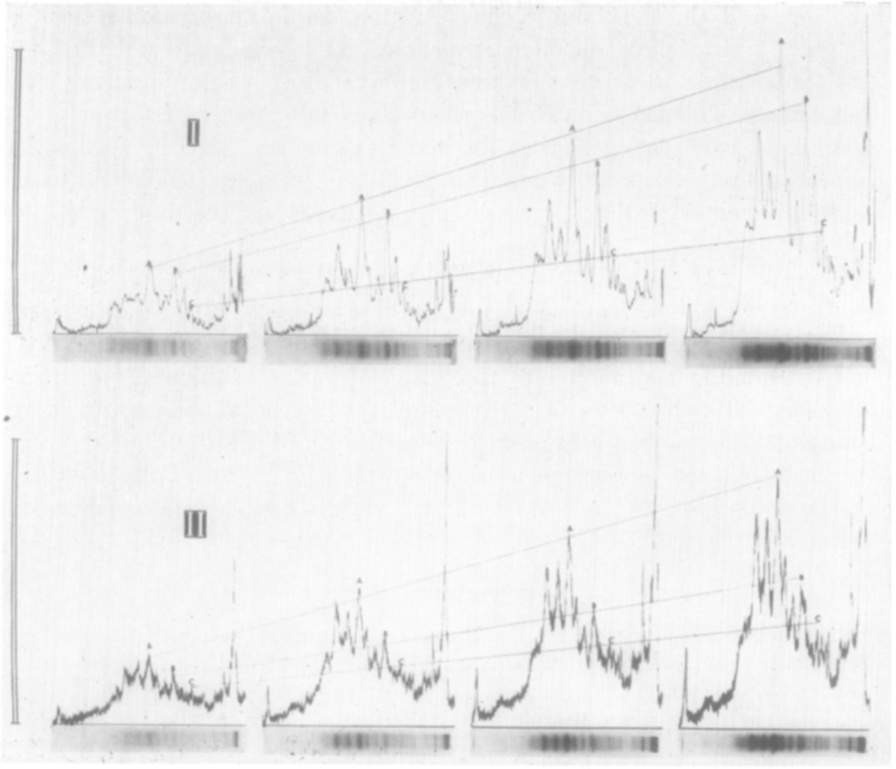


FIG. 4. Densitometric studies. Different volumes of the *E. coli* supernatant fraction (see Fig. 3A) were subject to electrophoresis in 4 adjacent slots. After drying and autoradiography (25 hr exposure), the dried gel as well as the film were cut out and subject to densitometric tracing at 540 mμ using a microspectrophotometer similar to that described by Allen and Jamieson (29). The volumes of sample used are 2.5, 5, 7.5, and 10 μl. (I) Tracing of protein bands in the dried gel. The vertical bar corresponds to OD 0.88. (II) Tracing of radioactive bands in the autoradiogram. The vertical bar corresponds to OD 1.5. Oblique lines drawn through some arbitrary peaks in the two tracings indicate the degree of linearity between optical density and protein concentration, and between optical density and radioactivity.

The method should be useful in many biochemical studies requiring the separation of proteins and the determination of their radioactivities. Minor protein fractions with high specific activities might be detected in the autoradiogram even though they may not appear with protein staining, provided that adequate time is allowed for exposure, and that the radioactivities are sufficiently higher than background. With minor modifications, the basic technique should be equally applicable to structural proteins and to nucleic acids.

Critical relations exist between the thickness of the template and of

the gel, and the acrylamide concentration. In forming sample slots, it is necessary to leave sufficient amount of gel in the front and the back for support and to prevent leaking and spreading of sample during electrophoresis. This necessitates the use of a gel slab above a certain minimal thickness. However, gels that are too thick or too high in acrylamide concentration are likely to break in the drying process. One should bear in mind such limitations when attempting to modify the procedure.

SUMMARY

A method for electrophoresis and autoradiography with a multisample gel slab system has been described. The method obviates slicing of the gel, retains the high degree of resolution of bands in the autoradiogram, and enables comparison of protein and radioactivity patterns of many samples under essentially identical conditions. Within a limited range, a linear relation is obtained between radioactivity and optical density in the autoradiogram, as well as between protein concentration and optical density in the gel.

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