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AN APPARATUS FOR THE DIRECT PREPARATION OF PORE GRADIENT GELS IN SLAB GEL ELECTROPHORETIC CELLS

H. FOISSY

Department of Dairy Research and Microbiology, Hochschule für Bodenkultur, Gregor Mendel-Strasse 33, A-1180 Vienna (Austria)

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SUMMARY

An apparatus is described that can be used to prepare homogeneous gradient gels even when using gel plates of a large size for use in slab gel electrophoretic cells. Complex microbial protein patterns were used in order to demonstrate the gradient layering ability.

INTRODUCTION

The preparation of polyacrylamide gels with increasing monomer and/or comonomer concentrations is a valuable procedure in electrophoresis. Linear or non-linear gradient profiles can be chosen, depending on the subject under consideration. Using gradient gels of polyacrylamide, very good results in the separation of sera have been reported[1–4]. With two-dimensional techniques, useful results are also obtainable, as has been shown with sera[3,5–9], other body fluids[8] and cell proteins of microorganisms[8]. The application of gradient gels in enzymatic studies[10], for the determination of the molecular weights of proteins[11,12] and for the separation of ribonucleic acids[13] has been successful.

The formation of gradient gels falls into two main parts, the preparation of the gradient gel solution and the layering of this mixture in the gel mould in such way that disturbance of the continuous gradient by turbulence or convection is avoided. The smaller and more regular the gel moulds, the better the layering succeeds. Gradient gel-forming procedures in capillary tubes were described recently[14,15]. However, for various reasons, the use of a slab gel apparatus is to be preferred. As the shape of the mould cross-section is unfavourable for forming gradient gels, it was suggested by Margolis and Kenrick[1] that one could compensate for it by preparing gel blocks in a square box and cutting these blocks into slabs of suitable dimensions. In later work, the same authors[3] preferred a method where a few glass plates with dimensions the same as those of the electrophoretic cell were fixed in an upright box with a square section and were flooded with gel solution. Direct formation of gel gradients in the fully assembled electrophoretic cell was described, using capillary tubes positioned against the cell wall above the expected height of the separation gel surface[7,16].

However, difficulties arise when using large gel plates, and a universal device was therefore constructed so as to permit a rapid and simple layering of gradient gels. The principle of the apparatus described here is based on a perforated tube acting as a spray nozzle that distributes the in-flowing gradient gel solution evenly along the whole length of the electrophoretic cell. At the same time, an elevator mechanism raises the spray nozzle with a speed equal to that by which the fluid level rises on the mould.

Applications are considered only insofar as they illustrate the method.

THE LAYERING DEVICE

The apparatus shown in Figs. 1 and 2 was constructed so as to be applicable to the home-made glass electrophoretic cell described elsewhere[17].

A metal section (a) that leaves the whole of the gel mould surface (240 × 3 mm) exposed is screwed down by wing nuts (b) and presses the fully assembled electrophoretic cell (c) firmly against the rubber cushion (d). Breakage at pressure points is

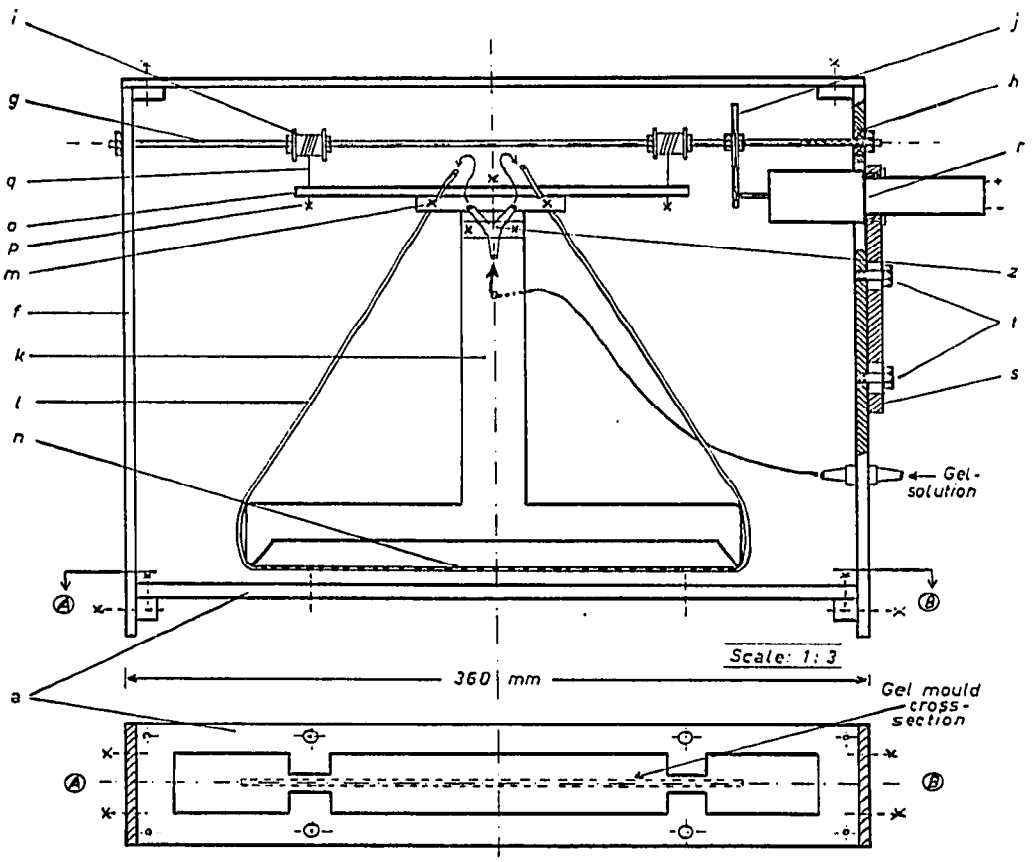

Fig. 1. Schematic diagram of the gel gradient layering device. The components are identified in the text.

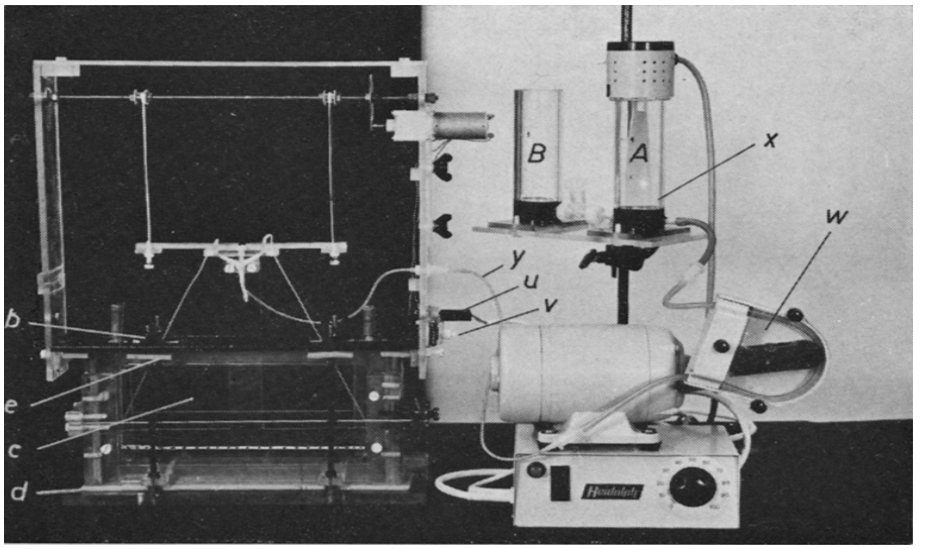

Fig. 2. Photograph of the complete apparatus assembled ready for the preparation of gradient gels. The components are identified in the text.

avoided by inserting vulcanite absorbers (e) between the metal and the glass. Fastened to the ends of the metal section are the vertical sides of a frame of Plexiglas acrylic material (Röhm, Darmstadt, G.F.R.) (f), these sides being 60 mm wide and 310 mm high, the latter measurement being taken from the top of the cell. At a distance of 40 mm from the top of the frame is a threaded spindle, 6 × 370 mm (g). Both ends are held by ball-bearings of the type EL 6 A-Z (SKF, Svenska Kullager Fabriken, Göteborg, Sweden) (h) so that the spindle rotates easily. The ball-bearings are firmly set in the plastic frame. Two small pieces of plastic tube of I.D. 6 mm, O.D. 10 mm and lenght 6 mm (i), which act as drums, and a brass cog-wheel of diameter 50 mm (j) can be fixed by means of nuts.

A T-shaped piece of 3-mm Plexiglas (k) of the dimensions shown in Fig. 1 is the support for a PTFE tube (l) of I.D. 1 mm, O.D. 2 mm and length 840 mm, which runs in a triangular shape around the arcs of the T-piece. The corners of the T-piece were notched with a small file so as to allow the tube to sit firmly. Both ends of the PTFE tube are clamped down by plastic screws (m). The part of the tube that runs horizontally was pierced with a blunt needle at regular intervals (n) along one of the sides that faces the electrophoretic cell.

At the top of the upright arm of the inverted T-piece, a horizontal bar (o) is attached with a screw. At both ends of this bar are plastic screws (p), which were drilled vertically through the centres. Through each hole, a 210-mm long thread (q) is passed and made fast. The other end of each thread is fixed to the drum opposite. By means of the screws (p), it is possible to adjust the position of the T-piece so as to allow the perforated tube to lie horizontally.

A 6-V Monoperm Special Super (Marx-Lüder, Gemmrigheim, G.F.R.) electric motor (r) with a Monoperm Pile gear box and a pinion moves the cog-wheel and thus the spindle and, as a result, the spray nozzle is raised. The motor itself is mounted on a separate piece of Plexiglas (s), which is attached to one side of the frame in such a

way that it can be adjusted. By loosening the screws (t), the motor can be lowered and the pinion detached from the cog-wheel.

The electrical equipment consists of a transformer and a rectifier, which are not shown in Fig. 2. The electrical connections are visible (u). It is, however, also possible to run the apparatus with a battery. The motor is started by means of a press-button (v).

The electronically controlled RGL 55 SP pump (Heidolph, Kelheim, G.F.R.) (w), to which a very narrow silicone tube (I.D. 2 mm, O.D. 6 mm) is attached, moves the liquid forward at the rate of 15 ml/min when in low gear. The gel solution drawn from the gradient mixer, a hand-made type (x), is now pumped through a connecting tube (y) and is divided into two equal streams by a plastic Y-branch (z). This Y-piece is screwed on to the top end of the inverted T-piece.

METHODS

The gel and electrode buffer systems were identical with those described elsewhere[18]. The cross-linkage with N,N'-methylenebisacrylamide was 1.33% with 10% acrylamide gel and 4% with 30% acrylamide gel. For both gel types, the catalyst used was a mixture of 0.045 ml of N,N,N',N'-tetramethylethylenediamine and 0.45 ml of a 1% solution of ammonium persulphate for each 50 ml of gel solution.

In order to prepare the gradient gel, 49 ml of the denser gel solution is poured into mixing cylinder A and 45 ml of the lighter solution into cylinder B of the gradient maker. Only then is the catalyst added. The gel solutions were continuously filtered. The surplus 4 ml in cylinder A serve to fill the tubes after starting the pump. When the levels in both cylinders are the same, the stopcock of the gradient mixer is opened and the elevator system set in motion. The perforated tube-line, first positioned near the bottom of the mould, is thus raised. The distance between the perforated tube and the T-shaped plastic support, which should be made as shown in Fig. 1, prevents disturbance of the gradient formation caused by adhesion between the glass and plastic surfaces. It is important to have extremely grease-free mould surfaces in order to ensure the formation of a film instead of irregular droplets. After the desired separation gel level is reached, the pump is stopped, the spray nozzle is removed from the mould, the remaining gel solution is pumped away and the whole tube system is washed thoroughly with water. The remainder of the gel preparation process is identical with that for single-pore gels.

At 15° and applying a voltage of 32 V per centimetre of separation gel, the separation time was 6 h for the single-pore gel and 12 h for the gradient gel. Amido black was used as a protein-specific dye.

RESULTS AND DISCUSSION

The gradient mixer should have a sufficiently large connecting tube between the cylinders to allow rapid levelling even with dense solutions. For the preparation of non-linear gel gradients, a simple and effective technique has been recommended[19].

Almost 6 min are needed to allow the 86.5 ml of separate gel solution to flow into the gel mould, giving a level of 120 mm. The resulting rate of rise of the level of the fluid in the cell is approximately 20 mm/min. The elevator system was set at this

speed using the transmission mechanism of gears, cog-wheels and drums. The time required to prepare the gradient gels is only slightly more than that using the usual method. The concentration of the catalyst was chosen so that the process of polymerization was finished about 10 min after filling the gel mould. This time represents a compromise in order to allow balancing of small inconstancies in the gradient that can arise as the solution flows in, but to avoid extended diffusion.

If the gradient is prepared in small layers, the heat is transferred efficiently and there is no reason to fear disturbances caused by convection during the polymerization process. The success of the gradient layering can be checked visually by the absence of marked refraction effects. Using a 2-mm T-piece for the spray-nozzle support, layering of the gradient gel in an even smaller film is possible.

Owing to the fact that 7.5 ml of gel solution remain in the apparatus after stopping the layering process, the effective concentration on the top of the gradient gel is approximately 12% when using 10% acrylamide gel solution for cylinder B.

The general considerations of gradient gels have been reported elsewhere[3,16,20], and the theoretical aspects have been discussed[16].

For example, this type of gel with a continuous acrylamide concentration and/or cross-linkage gradient, is of particular advantage in the separation of those protein mixtures whose components have considerably different mobilities. Microbial cell extracts are often such complex protein mixtures. Using a method described earlier[21], cell extracts of some Lactobacilli and Streptococci had been obtained and were, for instance, separated electrophoretically employing a linear acrylamide pore gradient of 12–30% with 1.50–4% cross-linkage.

Fig. 3 shows the separation in this gel of three identical groups of six samples, and the horizontal alignment of the bands throughout the gel demonstrates the uniformity of the gradient over a length of 240 mm. Fig. 4 shows the separation of four samples in a single-pore gel that was prepared from a 12% acrylamide solution alone (a) and the gradient gel (b). Although the faster bands are visible on the gradient

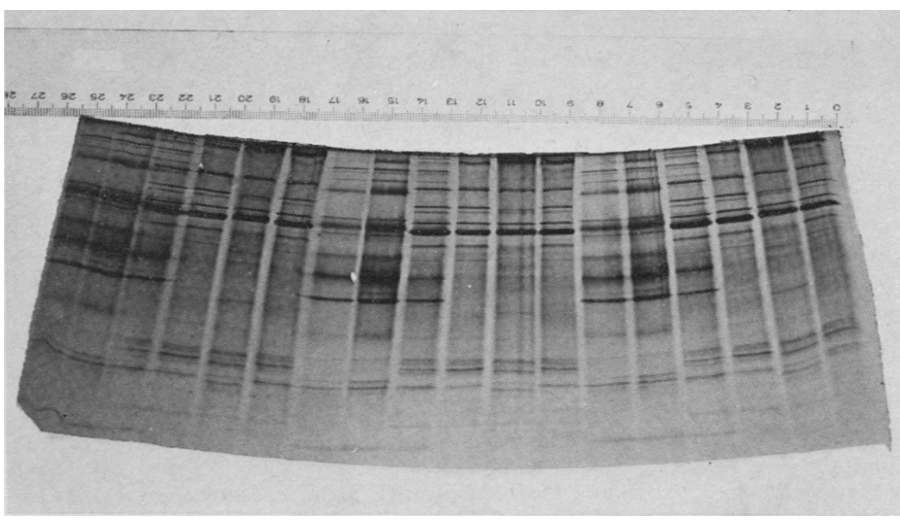

Fig. 3. Protein patterns of bacterial cell extracts in a 12–30% linear acrylamide gel gradient.

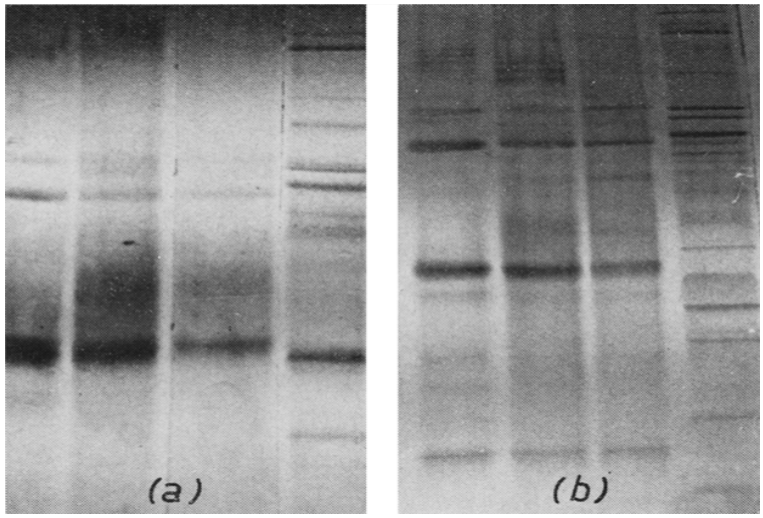

Fig. 4. Comparison of four bacterial cell extract samples separated in (a) a single pore gel and (b) a gradient gel.

gel, an improved quality of separation of slow-moving bands and a sharpening of the whole pattern is obtained. The reproducibility of gradient gel forming is good, provided, however, that constant conditions during the gel-forming process are maintained.

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