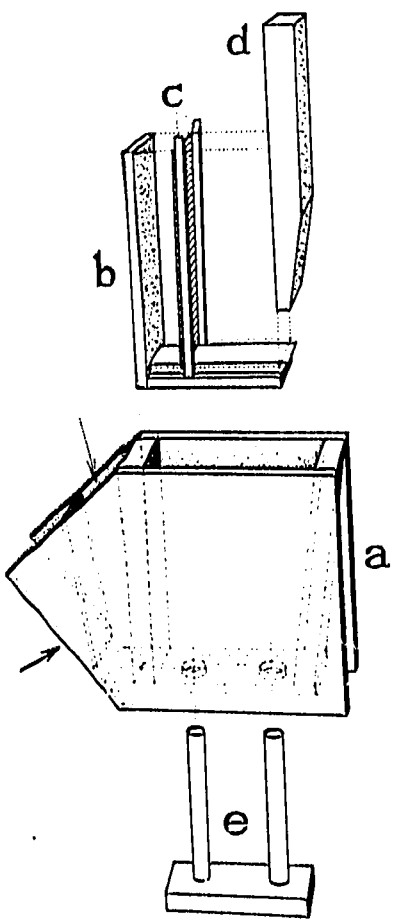
CHROM. 5774

Polyacrylamide electrophoresis of peroxidases on microscope slides and evaluation with the aid of reflecting light

As an alternative to polyacrylamide electrophoresis, described by DAVIS[1], ORNSTEIN[2], TOMBS AND AKROYD[3] and many others, some authors have suggested replacing the gel tube with a plate. However, this introduces a disturbing influence from the oxygen of the air. HOLMES[4] suggested the use of a gel plate (3.25 × 4 in.), which is cast in a nitrogen atmosphere in a plexiglass chamber with a casting time of 14–16 h. The electrophoresis is carried out in the horizontal plane. GÖTZ *et al.*[5] described a method for casting polyacrylamide plates on microscope slides, but an arrangement which prevents the admittance of air was not mentioned. If polymerisation takes place under these conditions, one must expect a very long polymerisation time, because oxygen has a restrictive influence on polymerisation[6]. Only if the polymerisation is rapid (10–30 min) are reproducible gels obtained.

In our laboratory, a rapid and safe method of producing polyacrylamide gel plates on slides has been developed. The gel plates show superior reproducibility,



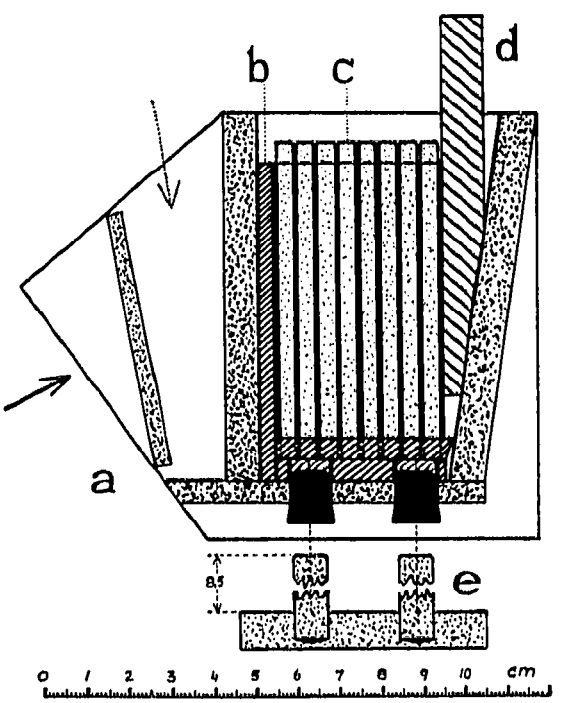


Fig. 1. Apparatus for casting gel plates, front view. a = Cell; b = chair; c = spacer pieces; d = wedge; e = ejector. Scale as in Fig. 2.

Fig. 2. Apparatus for casting gel plates, top view. Parts a–e as in Fig. 1.

demonstrated with a Zeiss TLC spectrophotometer, which fortunately could be used efficiently beyond its normal range of activity.

The main parameters (the gel filtration effect and the Kohlrausch effect) are the same as in disc electrophoresis. It is, however, unnecessary to use a spacer gel, because the application, as with starch gel plates, is performed by placing a small piece of filter paper, moistened with the extract, in a small cut in the surface of the gel plate. If it seems that the use of a spacer gel would give a better separation, however, it is possible to include the technique of ordinary tube casting.

For the casting of the gel plates, 7.5% acrylamide is used, and the casting is carried out in a gel mould made of plexiglass (Figs. 1 and 2).

The mould is designed for making eight 4-mm gel plates at once. It consists of a cell, a chair, spacer pieces, a wedge and an ejector. The mould is made ready for casting as follows. The two holes in the bottom of the cell are closed with stoppers. The chair is placed in the cell with its "back" along the vertical end plate, and the cell is placed in a slanted position on its angled bottom edges (Figs. 1 and 2, solid arrows). The "seat" of the chair is made of two plates. The upper plate is 6 mm narrower than the lower one and therefore there are grooves, slightly larger than 3 mm wide, between the upper plate and each of the side plates of the cell. In these grooves, *i.e.*, on the lower plate of the seat of the chair, is placed one spacer piece, along each edge of the microscope slide. A microscope slide is placed on these spacer pieces. The same procedure, with two spacer pieces and one microscope slide, is repeated seven times. The loading is finished with an additional slide which is pinched by the wedge (Fig. 2) with a light pressure. The mould is now turned up in such way that the edges of the mould opening are horizontal and filled with the 7.5% acrylamide.

After the polymerisation is complete (*ca.* 10 min), the wedge and the stoppers in the bottom of the cell are removed and a block consisting of the chair, microscope slides, spacer pieces and gel plates can be pressed out of the cell with the help of the ejector. By a little twisting of a scalpel, placed in between the slide and the gel, a unit consisting of a gel plate on a slide is relaxed. In order to prevent the chair from sticking to the end plate of the cell and the first microscope slide, it is an advantage to have the back of the chair roughened on both sides by shallow saw-traces. A similar treatment is suggested for the flat side of the wedge. A reservoir for spacer pieces not in use, is indicated by dotted arrows in Figs. 1 and 2.

The electrophoresis is carried out in the horizontal plane with a Boskamp

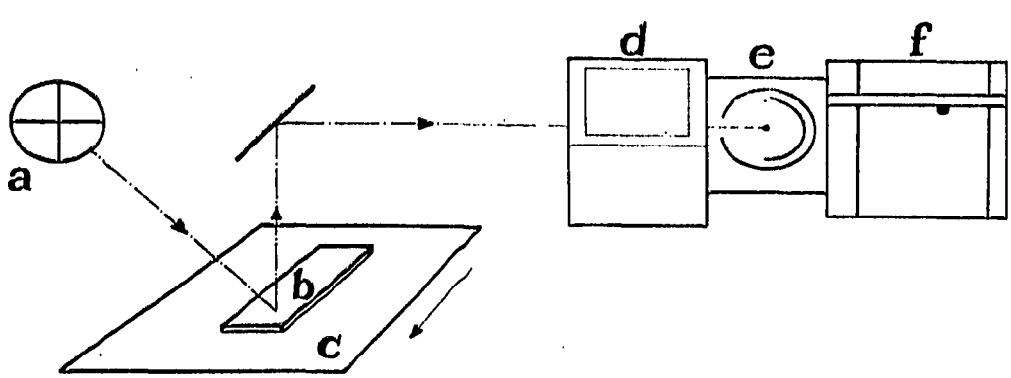


Fig. 3. Apparatus for evaluating zymograms (schematic). a = Glow-lamp; b = zymogram; c = white pad; d = monochromator; e = photomultiplier; f = recorder.

licrophor apparatus. The power supply is a VoKam, SAE 2761 instrument. The urrent used was 35 mA for eight plates during 3.5 h. Electrical contact to the plates s established using a commercial window-cleaner paper (Enka, G.F.R.). When arefully moistened with buffer, this paper gives a much better contact than the louble layer of different filter-papers often used. The electrode buffer was diethyl-arbituric acid/Tris, pH 7.4 (5.52 g of diethylbarbituric acid and 1.0 g of Tris liluted with water to 1 l). The dyeing of peroxidases was carried out with equal arts of the following two solutions: (a) 6 g of benzidine chloride, 54 ml of acetic acid nd 216 ml of water; and (b) 18 ml of hydrogen peroxide and 162 ml of water.

The zymograms were dyed for 7 min, and then carefully washed with 10% cetic acid solution. To begin with the bands were bluish violet. The colour changed o reddish violet in 10–15 min. The evaluation of the bands on the zymogram was arried out with a Zeiss TLC spectrophotometer. The principle of the measuring levice—sample–monochromator—is shown in Fig. 3.

The zymogram, placed on a white pad, is exposed to a glow-lamp. The reflecting ight passes through a monochromator adjusted to 580 nm and is amplified with a hotomultiplier from which the impulses are recorded.

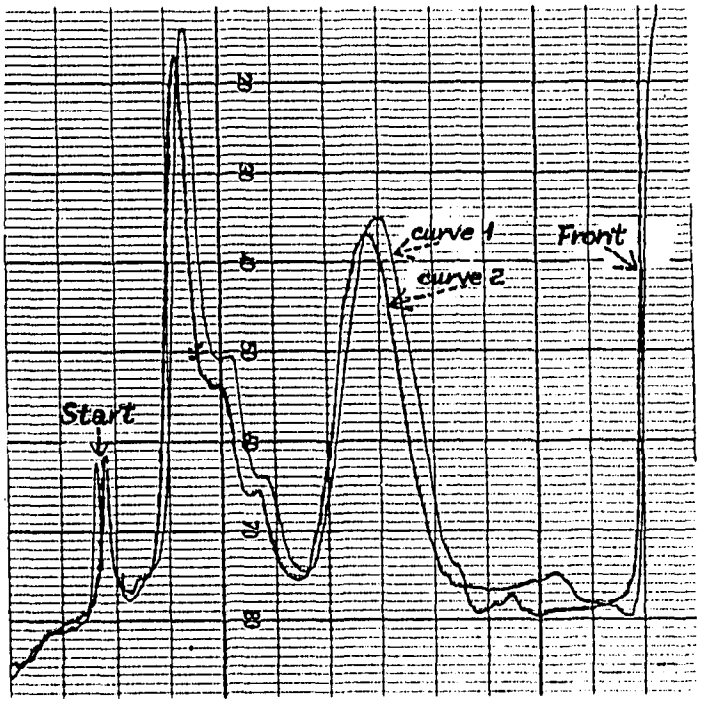


Fig. 4. Curves 1 and 2 indicate the colour intensity curves from a pair of replicated zymograms.

Colour intensity curves from a pair of replicated zymograms are shown in Fig. 4.

By careful application of the measuring device, deviations in the colour intensity are in the range 5–7%. The reproduction in this respect is satisfactory but, since the colouring represents an unfinished chemical reaction, one should always bear in mind that the analysis is only semi-quantitative.

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