An easily-constructed apparatus for two-dimensional paper electrophoresis

Mixtures of peptides, too complex to be resolved by single dimensional methods, can often be satisfactorily fractionated on square sheets of filter paper by successive electrophoresis in two orthogonal directions using buffers of different pH values, or by electrophoresis in one direction followed by partition chromatography at right angles[1–4]. For satisfactory electrophoretic separations high voltage gradients must be employed together with efficient removal of heat from the paper. The type of apparatus originally described by MICHL[5], in which heat is effectively removed by immersing the paper in a large bath of inert oil, is widely used but is inconvenient because large volumes of buffers and cooling oils are involved and the procedure is messy. Apparatuses that employ water-cooled metal plates for supporting the paper[6–8] are superior for single-dimensional applications but not easily constructed for two-dimensional use owing to the difficulty in making accurately flat metal plates of large dimensions. The apparatus described in this paper embodies a novel design to overcome this latter difficulty and permit the construction of a large apparatus relatively cheaply.

The apparatus

Electrophoresis is carried out on a square sheet of filter paper sandwiched between a water-cooled metal plate and the flexible bottom of a tank of water. In this way heat is effectively removed from both surfaces of the paper during electrophoresis and at the same time a uniform film of buffer is maintained in the paper by the hydrostatic pressure of the water in the tank. The lower plate (A, Fig. 1) consists of an

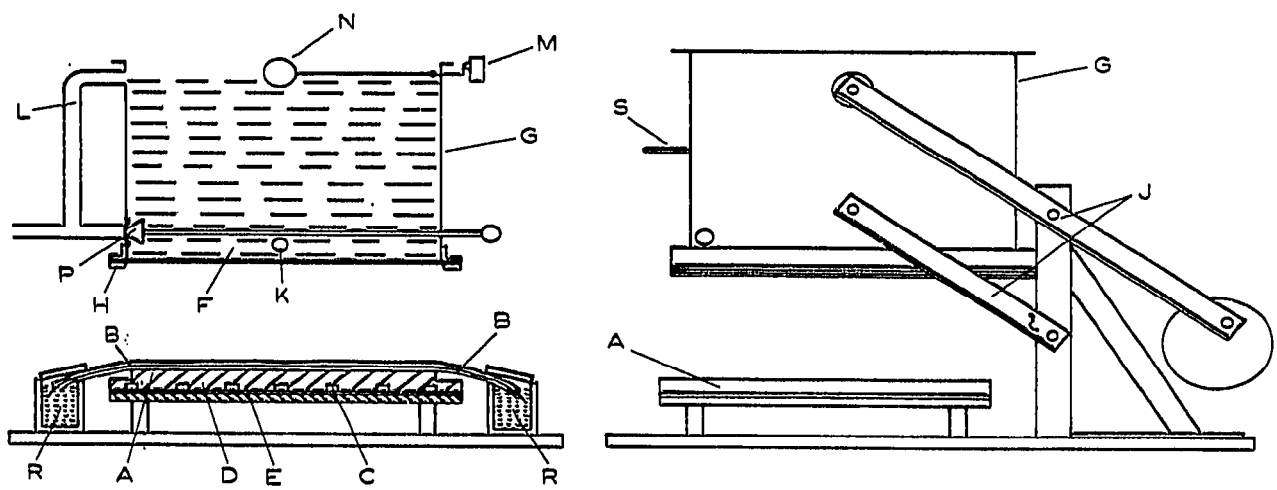

Fig. 1. Sketch of the apparatus. A = lower plate; B = step; C = groove; D = rubber gasket; E = retaining plate; F = upper cooling surface; G = copper tank; H = flange; J = lever arms; K = water inlet; L = water outlet; M = microswitch; N = float; P = stopper; R = electrode compartment; S = handle.

aluminium alloy block, 50 × 50 × 3 cm the top surface of which is reasonably flat but not necessarily planar. A step, B, 1 cm deep by 3 cm wide, is machined in each of two opposite edges of the plate to allow space for the wicks that connect the paper to the electrode vessels. To provide a channel for cooling water a continuous groove, C, 6 mm wide by 20 mm deep, is milled in the underside of the plate, A, and closed by means

of a rubber gasket, D, and retaining plate E, held in place by bolts. Inlet and outlet ports are connected at the edge of the plate, A.

The upper cooling surface, F, consists of a sheet of 400 gauge Melinex* sealed to the bottom of a rectangular copper tank, G, 46 × 52 × 36 cm deep, by means of a rubber gasket, bolted in place through a square metal flange, H. The flexibility of the Melinex enables it to conform closely to the upper surface of the lower plate provided there are no regions of sharply rounded curvature. The copper tank can be raised or lowered on two pairs of levers, J, which are counterpoised to balance the weight of the tank when empty. A small ratchet holds the tank in the raised position for the insertion and removal of papers. Water emerging from the lower plate passes into an inlet tube, K, to the centre of the bottom of the copper tank and during electrophoresis flows out to waste through a wide-bore tube, L, fitted near the top of one side of the tank. A microswitch, M, controlled by a small float, N, actuates a solenoid-operated valve in the water-inlet line in the event of too rapid a flow and prevents the possibility of the tank overflowing. The tank is emptied completely through a drain hole at the bottom of one side by removal of a rubber stopper, P. Filling and emptying of the tank each take about three minutes.

The electrode compartments, R, consist of perspex boxes, 51 × 6 × 6 cm, fitted with lids and platinum wire electrodes. Covers fitted to the sides of the copper tank surround these compartments completely when the tank is lowered. Accidental contact with the electrode compartments while the voltage is being applied is prevented by means of a microswitch, actuated by one of the lever arms of the tank, which disconnects the power supply unless the tank is lowered.

Experimental method

Electrophoresis is carried out on sheets of Whatman No. 3MM filter paper, 48 cm long and up to 48 cm wide. Several sheets in contact may be used when necessary to separate large amounts of material. For preparative experiments one sheet is stained to reveal separated components and used as a template to locate the same components on the unstained sheets. The papers are wetted with their own weight of buffer solution by immersion and passage through a rubber-roller wringer set to the appropriate pressure and the load is applied as a spot or line as required. The papers are placed between two sheets of polythene, 51 × 51 × 0.1 cm, and positioned on the plate, A, for connection to the electrode compartments through paper wicks. The wicks are made from Whatman No. 3 MM paper folded as shown in Fig. 2, and are encased in a short length of Layflat polythene tubing, 200 gauge, 18 in. in width**. To prevent direct transfer of buffer from the wicks to the paper a piece of Visking cellophane tubing***, cut as shown in Fig. 2, is wrapped round each edge of the paper before insertion into the wick. The tank, G, is lowered and electrophoresis started when the cooling water is running to waste. With the wicks connected in the manner described the current remains constant during electrophoresis at a fixed voltage and reproducible mobilities are obtained.

* Manufactured by Imperial Chemical Industries Ltd., Temple Chambers, 32, Brazennose Street, Manchester, 2, England.

** Supplied by Kingsmead Manufacturing & Merchandising Ltd., 123, Barlby Road, London, W. 10.

*** Supplied by Scientific Instrument Centre Ltd., 52, Gloucester Place, London, W.1.

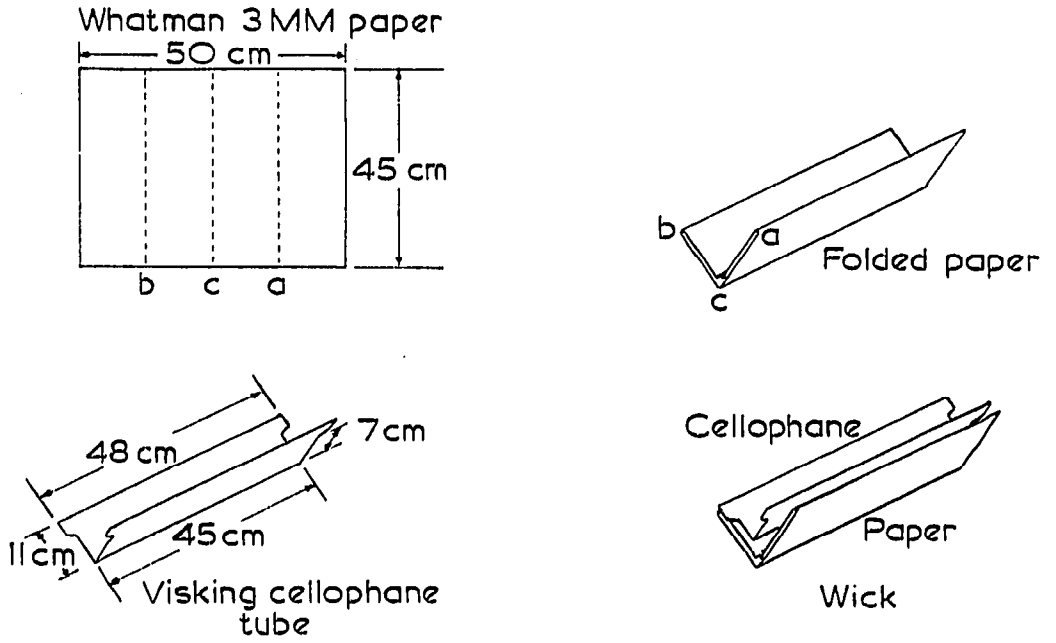

Fig. 2. Construction of wicks. Wicks constructed from paper and cellophane as shown are encased in Layflat polythene tubing, 5 cm long and 46 cm diameter, and inserted into the electrode compartments. The cellophane encloses the edges of the elctrophoresis paper and prevents direct diffusion of electrolyte.

Results

Fig. 3 shows the separation obtained with a mixture of amino acids subjected to electrophoresis in pH 1.85 buffer (21 ml of 98 % formic acid and 74.2 ml of acetic acid per litre) at 2.1 kV (50 V/cm) for 70 min. The load was applied 7.5 cm from the long edge and 15 cm from the anode edge of a sheet of paper 48 × 43 cm. Cooling water flowed at 2 litres/min. After electrophoresis the paper was dried in a stream of warm air and developed with *n*-butanol–acetic acid–water (4:1:5 by vol.) by descending paper chromatography for 16 h. After drying, amino acids were revealed by treatment with cadmium–ninhydrin reagent[9].

Fig. 4 shows the separation obtained with a mixture of dyes[10] subjected to electrophoresis under the conditions described above except that the load was applied as a line across the middle of the paper. It is evident from the migration of the dyes that uniform mobilities are obtained across the full width of the paper and that there is no appreciable distortion of the electric field at the edges. Non-uniform performance occurs if the buffer solution is not evenly applied to the papers. Should this happen it can usually be traced to a fault in the wringer.

The apparatus is particularly convenient for performing the diagonal electrophoretic procedure for locating components whose charges can be changed by chemical reactions[11–14]. The procedure requires a strip of paper cut from the electrophorogram obtained in the first direction to be sewn into another sheet of paper for electrophoresis in the perpendicular direction. With the present apparatus the strip is simply laid onto an intact sheet of paper and stitching is unnecessary. This also obviates the possibility of distortion of the bands by the stitches.

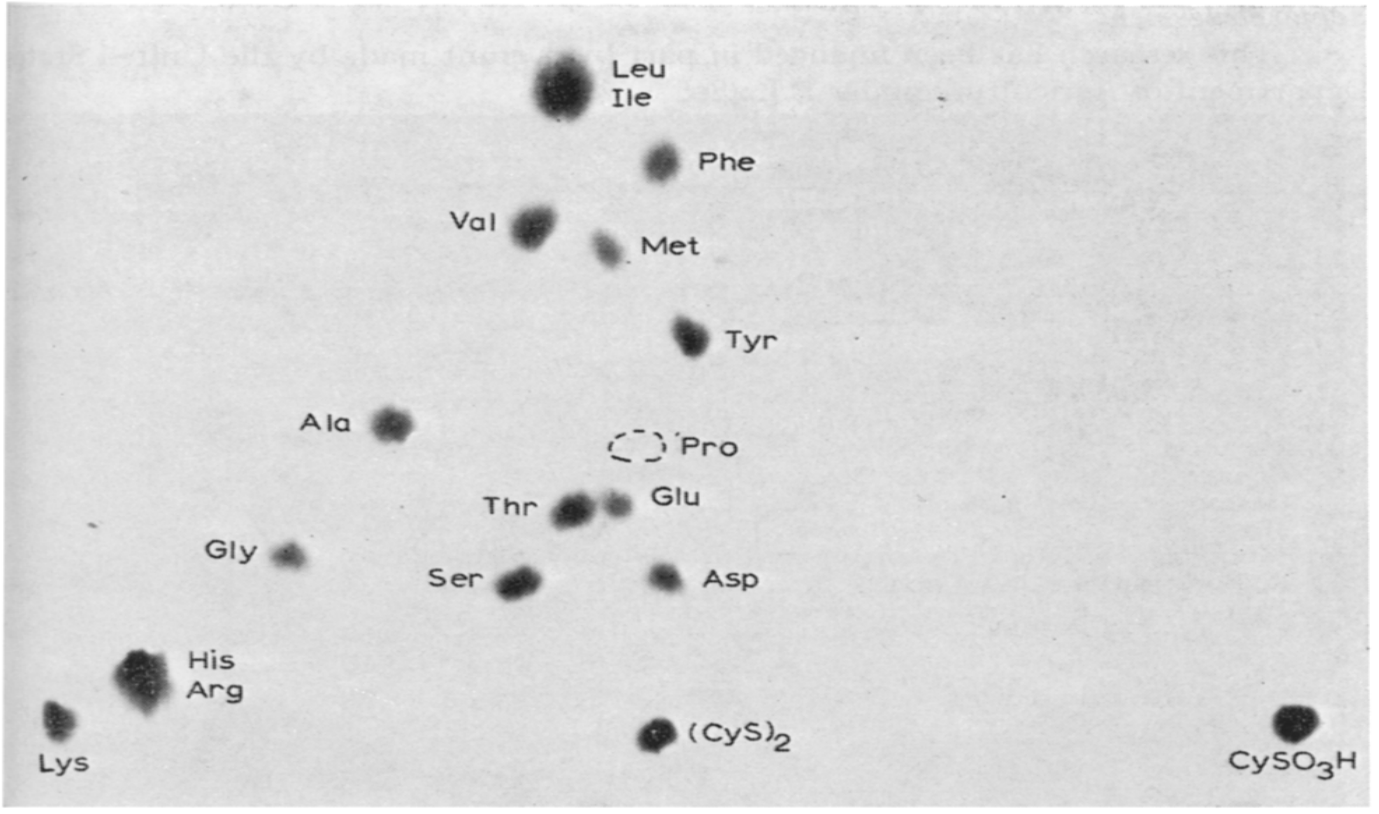

Fig. 3. Separation of a mixture of amino acids. The separation was achieved by electrophoresis in pH 1.85 buffer in one direction followed by chromatography in *n*-butanol-acetic acid–water (4 : 1 : 5, by vol.) in the direction at right angles. For experimental details see text.

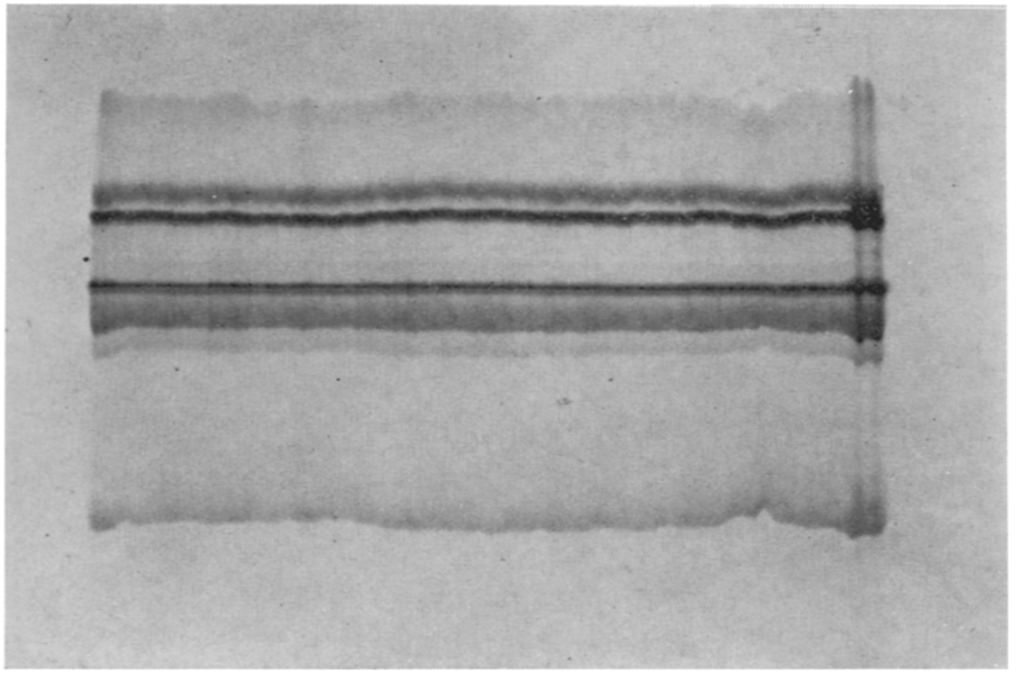

Fig. 4. Separation of a mixture of dyes. Electrophoresis was in pH 1.85 buffer. For experimental details see text.

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