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Note

Apparatus for electrophoresis in microcapillary polyacrylamide gels

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The established techniques for electrophoresis in gels in microcapillaries involve the positioning of individual capillaries with the upper end in one container of buffer and the lower end in another, with an electrode in each container (see, *e.g.*, refs. 1 and 2). This is convenient when only one or two capillaries are to be run, but presents problems when several are to be run simultaneously. The apparatus described below enables electrophoresis to be carried out simultaneously on up to 20 microcapillary gels.

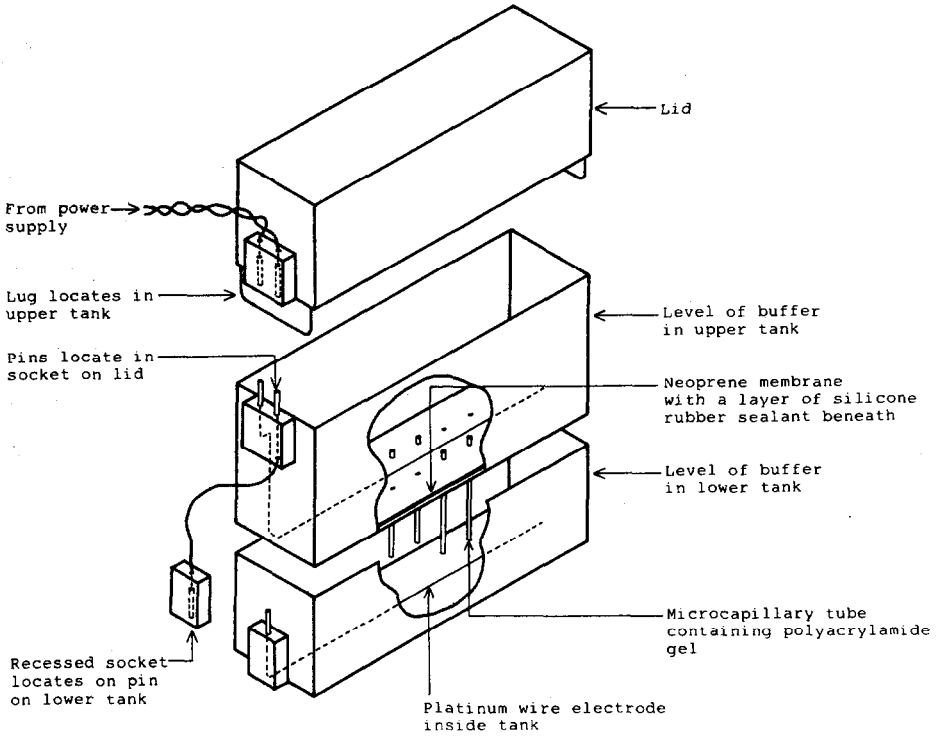

Fig. 1. Microcapillary electrophoresis apparatus. The plastic frame that holds the upper tank in position above the lower tank is not shown, but appears in Fig. 2.

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APPARATUS

The apparatus consists of an upper tank with a lid, supported above an open-topped lower tank. The upper tank consists of a transparent polystyrene box, 45 mm high by 30 × 108 mm. The lid is similar, but 36 mm high, with a lug across the inside of each end to locate onto the base (Fig. 1). The box used in this case was one in which small adhesive bandages were purchased, but any similar box could be used. The lower tank consists of a "Kodak" photographic slide box with the central divider removed, but again any similar polystyrene box would be suitable. A plastic frame (not shown in Fig. 1 for clarity) holds the upper tank in position. This frame was fabricated from transparent plastic sheet and angle, recovered from boxes similar to the upper tank, and consists of a rectangular box shape, with no top or bottom, which is a close fit over the lower tank (Fig. 2). A ledge glued inside each end supports the upper tank the appropriate distance above the lower tank (10 mm when using 32-mm capillaries). Twenty holes of 4 mm diameter were drilled in the base of the upper tank. A 1-mm layer of silicone rubber sealant (*e.g.*, Dow Corning Bathtub Caulk) was then spread over the bottom surface of a 27 × 104 mm piece of 1 mm thick neoprene sheet which was then pressed onto the bottom inside surface of the tank before the sealant had dried. After the sealant had set (24 h), 20 holes were made with a needle through the rubber and sealant, in the centre of the holes drilled through the bottom of the tank. A microcapillary tube can be pushed from below through each of these holes. When no tube is inserted, the holes are self sealing.

Electrical connections are made via gold-plated turned pins, 1 mm diameter, and matching spring-loaded sockets of the type used in multipole connectors, which are cemented into small Perspex blocks thus acting as miniature plugs. The gold plating provides the required resistance to corrosion. The sockets are recessed 2 mm below the surface of the Perspex so that they cannot be accidentally touched while connected to a power supply. The electrodes consist of 28 standard wire gauge (0.376 mm diameter) platinum wire, fixed with spots of polystyrene cement along the bottom

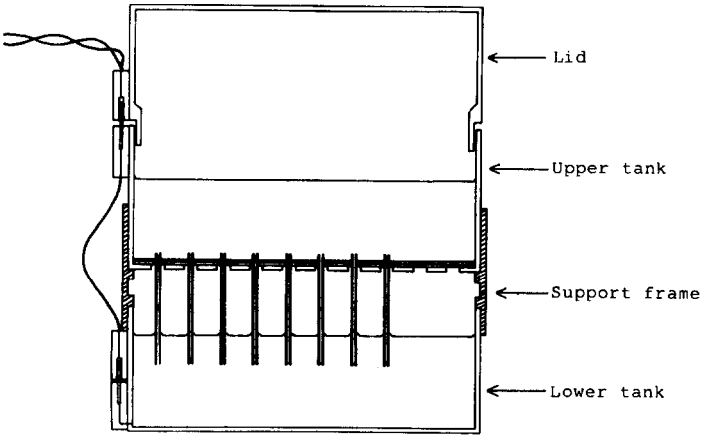

Fig. 2. Section of microcapillary electrophoresis apparatus, with 16 microcapillaries in position. The platinum wire electrodes inside the upper and lower tanks are not shown, but appear in Fig. 1.

of both tanks. The upper tank electrode wire continues up the end wall of the tank, through a hole 10 mm from the top edge, and is soft soldered to one of two pins fixed in a 15 × 15 × 15 mm block of Perspex glued to the outside of the end of the tank. The second pin is connected by a 55 mm length of high-flexibility insulated wire to a socket held in a 10 × 15 × 15 mm block of Perspex. This socket locates onto a pin in a similar block glued to the end of the lower tank. The electrode wire in the lower tank passes through a hole in the end wall and connects to the pin on the end of the tank. Two sockets in a Perspex block glued to the end wall of the lid of the upper tank are wired to the power supply. These sockets connect with the two pins on the upper tank end wall when the lid is placed in position.

PROCEDURE

Buffer is poured into the upper and lower tanks to a depth of about 25 mm. The microcapillary tubes containing the polyacrylamide gels are then topped up with buffer, and inserted into the upper tank from below, through the holes in the neoprene. This procedure reduces the possibility of trapping air bubbles in the capillaries. Because the holes in the base of the upper tank are self-sealing, any number of microcapillary tubes between 1 and 20 can be accommodated. The plastic frame that holds the upper tank in position is fitted, and the socket attached by the insulated wire to the upper tank is located on the pin of the lower tank. The length of the insulated wire is such that this connection can be made only when the upper tank is in position above the lower tank. To improve stability, when in use the apparatus is held in a small clamp attached to a retort stand. The power supply connects to the plugs supplying the electrodes when the lid is placed on the upper tank. Thus it is

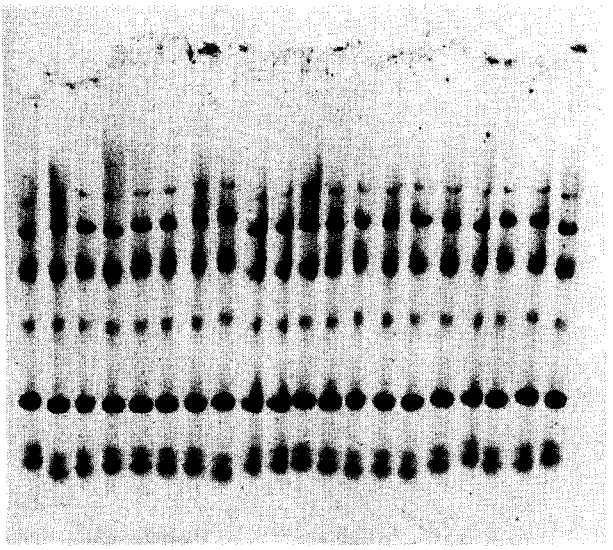

Fig. 3. Twenty concentration-gradient polyacrylamide gels; each gel was loaded with an aliquot consisting of 50 ng of each of seven standard proteins and then stained with Coomassie Blue R250 after electrophoresis in the apparatus.

not possible to touch the buffer in the upper or lower tank when the power supply is connected. Although the equipment has been designed with electrical safety in mind, it is recommended that a power supply with earth leakage-sensing circuitry is used for maximum operator safety. During the course of an experiment, individual tubes may be readily added or removed with only a brief interruption of the power supply.

Thus this apparatus can be constructed from readily available inexpensive components, and allows simultaneous electrophoresis of up to 20 microcapillary gels, while avoiding the electrical risks associated with more *ad hoc* arrangements for microcapillary electrophoresis.

The tank has been used routinely for electrophoresis on homogeneous, concentration gradient, and isoelectric focusing gels, all run under conditions of constant voltage. The separations achieved are consistent from gel to gel within the limitations of gel reproducibility. This may be as good as ±2% with homogeneous gels but is more difficult to achieve with gradient gels[1], being typically ±4% (Fig. 3).

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2 V. Grossbach, in R. C. Allen and H. R. Maurer (Editors), *Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel*, Walter de Gruyter, Berlin, 1974, p. 207.