UNIVERSAL CUVETTE FOR ELECTROPHORESIS

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In experimental and clinical biochemistry in recent years the use of paper for electrophoresis of proteins has been increasingly supplemented by the use of other inert carriers: gels (agar, starch, polyacrylamide, etc.), starch and other blocks, and so on. In addition, certain special types of electrophoresis, especially immuno-electrophoresis, have continued to gain popularity.

The proposed cuvette is intended for analytic and preparative electrophoresis of proteins and other macromolecular compounds. Fractionation can be carried out by many different methods, including immunoelectrophoresis.

The cuvette is made of Plexiglas. It can be taken to pieces and consists of the following principle parts (see figure): base (1), electrode vessels (4), their lids (7) with the electrodes (8), and interchangeable analytic sections (11) with lids (15).

The base (1) has three screws (2) for levelling the instrument and a flange (3) on its upper surface for preventing possible displacement of the electrode vessels. The electrode vessels (4), measuring 170 × 70 × 55 mm, rest on the base (1). Each has a longitudinal partition (5), 30 mm high, for creating a zigzag flow path, and two nipples (6) on opposite walls of the vessel: one is placed at the bottom of the wall, the other 45 mm above the bottom (the level of buffer solution). The nipples are used for providing a continuous-flow buffer system when required. Otherwise they may be used either for siphoning buffer between the anode and cathode vessel or to prevent differences in its levels, or they may simply be covered with rubber caps. The lid (7) of the electrode vessel (170 × 48 mm) carries a platinum electrode (8), fixed to the plate (9) glued to the lid. A terminal (10) for connection to the rectifier is fixed on the upper surface of the lid. The П-shaped analytic section (11) is placed on the electrode vessels. It has a double floor (12) and is used for cooling the unit circulating water through the nipples (13). The dimensions of the working surface of the analytic section given in the figure (250 × 140 mm) may be taken as suitable for use in most cases of electrophoresis of protein mixtures. However, for some methods other dimensions are more suitable. For example, for preparative electrophoresis of heterogeneous protein mixtures in a starch block [5, 14] and in certain other cases a unit 400 mm long is more convenient. For such purposes it is advisable to have a spare analytic section with a working area of 400 × 140 mm.

When working with micromodifications of electrophoresis, a replacement section with a unit measuring 100 × 60 mm is suggested. For electrophoresis of hemoglobins, a section with a unit measuring 110 × 140 mm is suitable. The interchangeable sections are of identical design, including measurements (apart from those mentioned above). The saddle roof (15) of the analytic section is suitable for most methods. It rests on the outer ledge of the wall of the analytic section, providing a reasonably air-tight chamber.

For electrophoresis on paper (23) the component (16) is placed inside the analytic section. Strips of chromatographic paper, soaked in buffer and squeezed out, are placed on the sharp edges of the prisms (17). The ends of the strips hang freely in the vertical portions (14) of the analytic section. The electrode vessels are filled with buffer and connected by a rubber siphon tube attached to the nipples (6). The cuvette is then assembled: the electrode vessels are placed on the base (1), made level, and the analytic section (11), covered by the lid (15), is placed on them. Before adding the test samples, it is advisable to connect the instrument to the assigned voltage for 40-60 min to ensure constant humidity, which is essential during electrophoresis, in the chamber. Next the samples of protein mixture are applied in the usual manner. After the end of electrophoresis the component (16) together with the strips of paper is removed. The subsequent procedure depends on the material and the purpose of the investigation.

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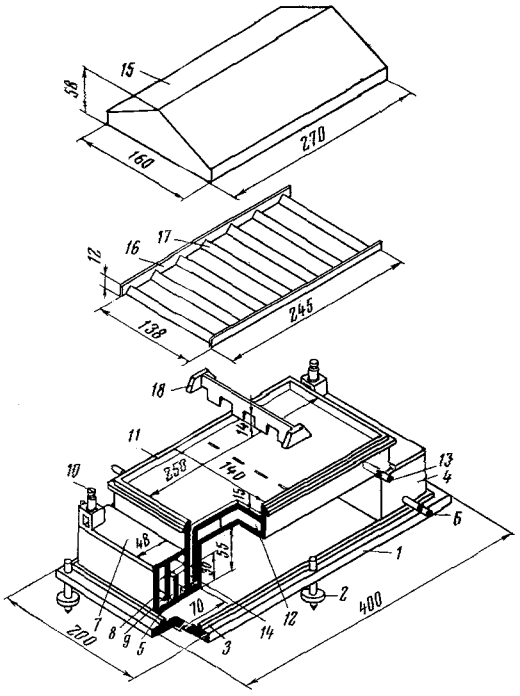

Diagram of cuvette. Analytic section is filled with agar gel. Explanation in text.

For electrophoresis on cellulose acetate [14], the preparation of the cuvette is similar to that described above for electrophoresis on paper.

For electrophoresis in agar gel [3, 10], the cuvette is prepared as follows. Two strips of sheet rubber 1-3 mm in thickness are placed on the base (1) and the ends (14) of the analytic section rest on them. A level is placed on the surface of the analytic section and the horizontal position is obtained by adjusting the screws (2). The hot agar is introduced by a pipet into the vertical divisions (14), at first to a height of 4-5 mm, and allowed to solidify to form a plug in the lower part of the vertical portions of the analytic section. The vertical portions are then filled to the top with hot agar, and a measured volume of agar is poured into the horizontal division of the analytic section (the volume of agar is calculated from the area of the working surface and from the thickness of the block desired). To form wells for introducing the samples of protein mixture, the rack (18) is used. The lower edges of its teeth are sharpened and they extend down to within 1 mm of the floor of the analytic section so that the protein will not escape beneath the agar. After the agar has solidified, the rack is carefully removed, taking care not to injure the edges of the wells. To improve the stabilization of the gel structure, the cuvette filled with agar should be left overnight in a refrigerator. Buffer is then poured into the electrode vessels (250 ml into each), and these are placed on the base, the analytic section is fitted in position, the samples of protein mixture are applied, and the section is covered with the lid (15). Other methods of applying protein may also be used, for example, on strips of filter paper without cutting wells with the rack (18). The nipples of the analytic section (13) are connected to the water cooling system and the voltage is applied to the terminals. If a continuous-flow buffer system is not used, the anode and cathode electrode vessels should be connected by a siphon tube fixed to the nipples (6). After the end of electrophoresis the agar block is cut out around the walls of the analytic section; the section is taken from the electrode vessels and overturned above the cuvette with the fixing fluid (usually 2-5% acetic acid solution in 10% glycerol). The block of 1-2% agar gel readily falls out without breaking. It will be clear from the design of the cuvette that a piece of glass of suitable size may be inserted into the analytic section, and the agar or other liquified gel poured on it. In this case the gel block is removed together with the glass after electrophoresis. These and similar modifications have no effect on the result of fractionation.

The cuvette can be used for preparative electrophoresis in agar or other gels [1], or for a micromodification of analytic separation [3]. In these cases the cuvette is prepared for the experiment as described above.

For electrophoresis in starch gel the preparation of the cuvette differs from the manner described above only in that the vertical portions (14) of the analytic section are filled with agar gel prepared in the same buffer as the starch gel, while the horizontal portion is filled with starch gel. Otherwise, the method is almost identical with the original [18]. This applies also to the method of electrophoresis in a starch block [5, 14], for which a lid with a water cooling system is used, compressing the block from above.

The cuvette may also be used for immunoelectrophoresis, with modifications either in agar gel [1, 10, 17] or on cellulose acetate [13]. Electrophoresis may also be performed with the use of practically any medium suggested for horizontal blocks: Sephadex [8], semiliquid films [16], and so on.

When describing the cuvette I attempted to keep the advantages and avoid the disadvantages of the universal cuvettes described in the literature [2, 4, 6, 7, 9, 11, 15]. These advantages are simplicity of construction, obviation of the need to use paper bridges, the existence of interchangeable analytic sections, and the use of water cooling. Furthermore, the apparatus is easily disassembled, so that it is easier to use.

Trials of the cuvette have shown that the results of fractionation with its use are equal to those obtained with other, nonuniversal, cuvettes.

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