NEW METHODS

MODERNIZED MODEL OF ELECTROPHORESIS APPARATUS OF THE EXPERIMENTAL FACTORY OF THE ACADEMY OF MEDICAL SCIENCES, USSR

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Much of the electrophoresis apparatus produced in the Soviet Union has been described in the literature (Troitsky [3], Bresler and Finogenov [2], and Balandin and Rozenberg [1]). The model described in this paper is an improved form of Balandin and Rozenberg's model.

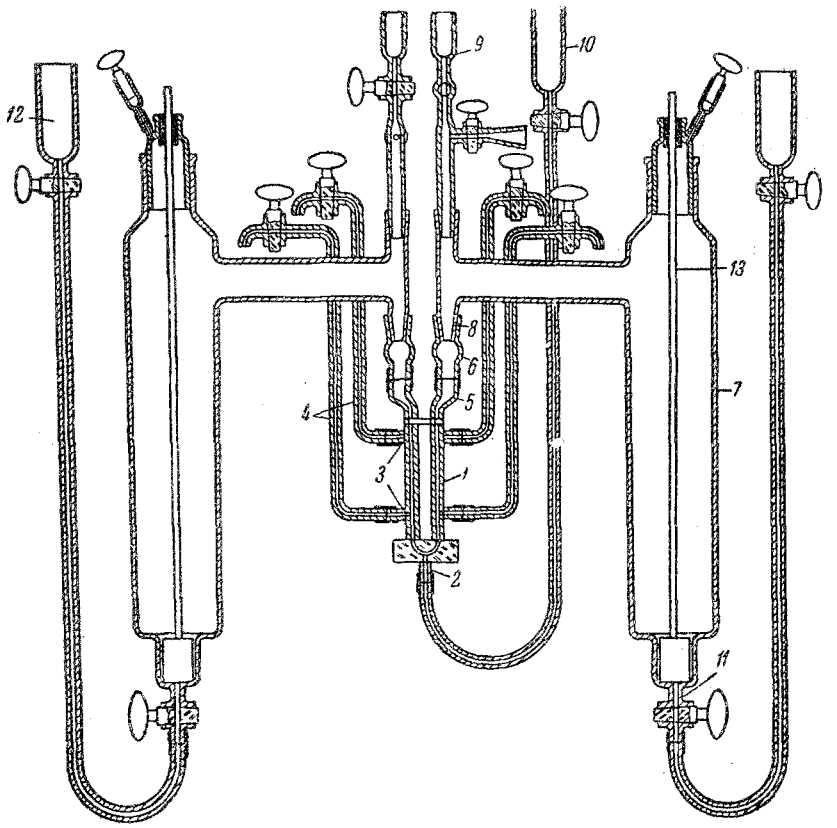

Diagram of a modernized model of electrophoresis apparatus. For explanation see text.

The optical and electrical details of the apparatus are identical with those of Balandin and Rozenberg's model, and will not be described further in this paper. The analytical section and the compensator have been radically redesigned. For this purpose we made use of a number of principles elaborated by Svensson [4] and S. E. Bresler [2].

The most important part of the analytical section, to a large extent determining the quality of the working of the apparatus, is the cuvette (marked 1 in the diagram). This is made of optical glass joined by sintering (this eliminates the possibility of its being attacked by acids, alkalis, and other solvents); in this respect it is greatly superior to plexiglass cuvettes used by Bresler and Troitsky. The lumen of the cuvette is a U-shaped channel, and is filled with 2.2 ml of the solution under examination.

The actual expenditure of protein involved in performing the experiment is somewhat greater. The cross section of the channel is equal to 2 × 15 mm. The cuvette is provided with a capillary at the bottom, 2, through which protein solution enters from the reservoir, 10, and with 4 side capillaries, 3, two on each side. The distance between the axes of the side capillaries is 45 mm, this distance being determined by the parameters of the main objective. The side capillaries are joined to tubes, 4, and serve for: a) rapid adjustment of the boundaries in the initial position, without using a compensator, b) washing out the cuvette without the necessity. of dismantling the section, and c) removing the individual protein fractions. This last function allows of the use of the analytical cuvette for preparative electrophoretic separation of small amounts of protein solutions.

At the top of the cuvette are two sockets, 5, joined by rubber tubing to the ground glass joints, 6. The buffer solution vessels, 7, are connected through the ground glass joints, 8, with the cuvette. This permits of the easy dismantling of the section far more conveniently than in the earlier models [1, 2, 3].

Three-way pieces, 9, provided with funnels and ground glass side sockets are fitted into ground glass sockets at the top of the columns. They serve for washing out the cuvette and capillaries, and for connecting with the compensator.

The electrodes, 13, are mounted in glass stoppers. At the bottom of the buffer solution vessels are taps, 11, for the introduction and discharge of potassium chloride solution. The whole section is mounted in a special frame, which facilitates rapid assembly. The compensator, serving for more complete separation of protein fractions, consists of a glass flask filled with buffer solution, and communicates through one tube with the hydrolyzer, and through another with the ground glass socket of the three-way attachment to the buffer solution vessel.

The gas evolved during electrolysis of 0.1 N NaOH solution exerts pressure on the buffer solution, forcing it into the cuvette. Since the amount of current can be changed smoothly, this permits of varying the rate of flow of the compensating solution as desired. A further difference between this and the earlier models is that the possibility of measuring the outlet current is envisaged. All the controls of the instrument are located on a special panel.

Use of the Instrument

As stated above, the new model of electrophoresis apparatus differs from the old one chiefly in the design of the analytical section and the compensator. We shall therefore confine our description to the manipulations concerned in the use of the redesigned sections.

The special construction of the rack, and the provision of ground glass joints to the cuvette and the buffer solution vessels facilitates the rapid assembly of these sections. The assembled sections are filled with buffer solutions before the beginning of the experiment.

The funnels, 12, are first connected to the lower taps of the buffer solution vessels. Filling with buffer solution is effected through the three-way attachments, 9.

Air bubbles in any part of the section can easily be removed by opening the taps to the side capillaries of the reservoir, funnels, and buffer solution vessels, and the whole can be filled with buffer solution. Potassium chloride solution is delivered from funnels, 12, to the electrodes, after which the funnels are detached from the section, which is transferred to a thermostat, the water in which is maintained at 0.5-1° by addition of ice.

The compensator is put into operation before the beginning of the experiment, should it be necessary to separate proteins having closely approaching velocities of transport.

The protein solution, prepared in the usual way, is introduced into the reservoir,10. In order to bring out the protein fronts the taps to the reservoir and the lower side capillary are opened, giving the lower protein boundary. The tap of the opposite upper side capillary is then opened, giving the upper protein boundary.

It is thus possible, without the use of compensator, rapidly to establish sharp protein boundaries in the initial positions, after which electrophoretic analysis is performed [1]. On the basis of our experience with Svensson's and Bresler's models, we have grounds for belief that our analytical section can be applied to the preparative separation of protein mixtures. Since the capacity of the cuvette is small, the capacity of the side capillaries, through which the contents of the cuvette may be passed, should first be accurately measured.

Our modification permits of the rapid establishment of the protein boundaries, and of the performance of a number of electrophoretic analyses without dismantling the apparatus or removing it from the thermostat. This greatly facilitates work with the apparatus, and economizes expenditure of buffer solution.

LITERATURE CITED

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