A CUVETTE FOR MICROELECTROPHORESIS ON AGAR AND STARCH GEL AND FOR MICROIMMUNOELECTROPHORESIS

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Agar and starch gel are being used ever more frequently as a stabilizing medium for electrophoresis of protein mixtures. When using these gels the resolving power of the method of separation is very high, adsorption of proteins on the carrier is minimal, the electrophorograms do not require later clarification for a densitometric analysis and are readily subject to elution.

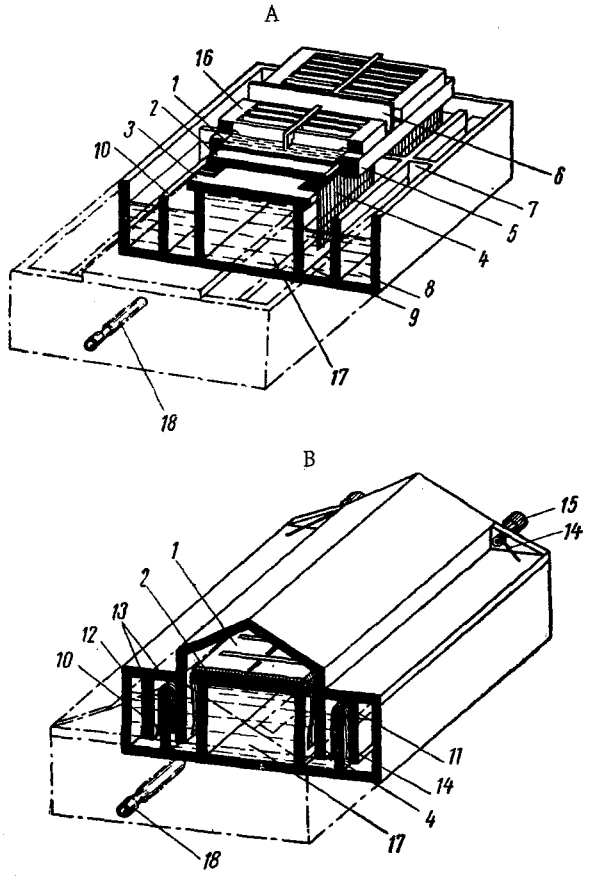

Fig. 1. Schematic section of cuvette. A) Pouring the blocks for immune microelectrophoresis on agar gel; B) during electrophoresis. Explanation in text.

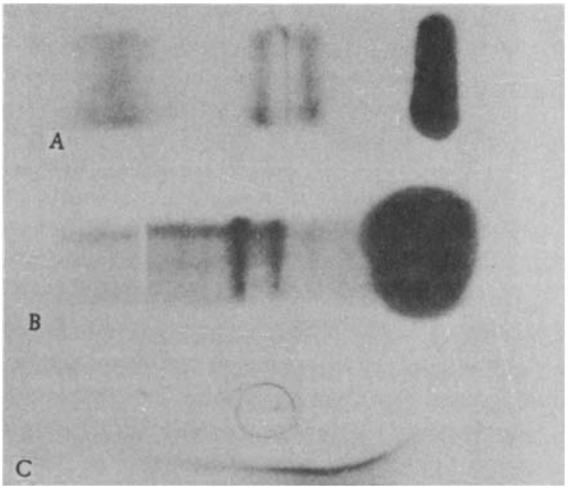

Fig. 2. Samples of electrophorograms obtained on the cuvette. A) Microelectrophoresis on agar gel. Blood serum of tuberculosis patient. Barbiturate buffer, pH 8.6, ionic strength 0.05, agar 1%, layer thickness 2 mm, 6 V/cm 1 h 30 min, staining with Amido Schwarz 10B; B) microelectrophoresis on starch gel. Blood serum of tuberculosis patient. Borate buffer, pH 8.2, starch gel 15%, layer thickness 5 mm, 6 V/cm, 3 h 30 min, thickness of sections 2 mm, staining with Amido Schwarz 10B; C) immune microelectrophoresis on agar. Blood serum of rabbit immunized with lung antigen (section material). Barbiturate buffer, pH 8.6, ionic strength 0.05, agar 1%, layer thickness 2 mm, 6 V/cm, 1 h. Precipitation against lung antigen, 24 h at room temperature.

The method of electrophoresis of proteins in agar gel proposed by Gordon and cohorts [8] was later modified to a micromethod [4, 13, 20, 21] in which the agar blocks were placed on slides used in microscopy. Electrophoretic separation of proteins in starch gel, developed by Smithies [19] and perfected by other authors [6, 7, et al.] can also be carried out in a micromodification [5, 15] in which the length of separation does not exceed 5-6 cm with practically no drop in the resolving power of the method.

Graber and Williams [9, 11] proposed a method of immunoelectrophoretic analysis which has become widely used in recent years and is based on combining the separation of proteins by sign and magnitude of their charge in an electrical field with the antigen-antibody reaction performed by double diffusion in agar after the manner of Ouchterlony [14]. Among the further developments of the method it is necessary to note immune electrophoresis on slides described by several authors [2, 16, 17].

These micromethods are not inferior with respect to results to macromethods, furthermore they require much smaller quantities of protein mixtures to say nothing of the savings in reagents. Furthermore, in immune microelectrophoresis the time of the formation of the precipitates is appreciably reduced. All this makes the micromodifications especially convenient when analyzing a large number of samples.

The rather simple cuvette we fabricated is intended for performing serial investigations by somewhat modified methods of microelectrophoresis on agar and starch gel and by the method of immune microelectrophoresis. Underlying the development of the instrument were the works of the separation of proteins in agar gel [4], on electrophoresis in starch gel [5, 15, 19], and on immunoelectrophoresis [2, 16].

The Plexiglas cuvette permits performing electrophoresis on blocks of gel 75 mm long and 95 mm wide (on 1, 2, 3, or 4 blocks simultaneously). Each block 1 (Fig. 1A) is placed on a Plexiglas (or glass) plate 2, 3 mm thick. Before pouring the gel connectors 4 made of four layers of filter paper are placed on stand 3 of the cuvette which is placed directly horizontally and they are clamped by frame 5 divided into four sections by partitions 6. Each section of the frame corresponds to a section of the electrode portion of the cuvette and is isolated from adjacent sections by partitions 7 in order to economize the buffer solution with incomplete loading of the cuvette. Plate 2 which serves as the base for the gel is inserted into the section of the frame. The slits between plate 2 and frame 5 are carefully filled with a hot solution of agar (or soluble starch) from a pipette so that the solution soaks the paper connectors 4. After the solution gelatinizes hot agar (starch) is poured on plate 2 in the amount needed to form a block of a prescribed thickness. Thus, to produce a block 2 mm thick, 14.25 ml of hot solution is poured into each section. When the gel solidifies, frame 5 is carefully removed; if necessary the edges of the block abutting the paper connector are additionally filled with hot solution to ensure a reliable electrical contact of the connector with the gel.

The buffer is poured into the cuvette (into the electrode 8 and intermediate portions 9) into as many sections as blocks used for a given experiment. Connectors 11 made of four layers of filter paper are laid on partitions 10 (Fig. 1B). The cuvette is closed with a cover 12 with vertical partitions 13 which serve to prevent the direct contact between the paper connectors 4 and 11 and platinum electrodes 14 attached on the outer partitions and also to lengthen the pathway of the current for a more complete removal of the polarization products. Terminals 15 connected with the electrodes 14 are also attached on the cover. The gable shape of the cover is needed for the runoff of water condensing on it as a consequence of evaporation during electrophoresis.

The samples of the protein mixture are applied differently depending on the type of electrophoresis.

In the case of analytical separation on agar gel the protein is applied on small strips of filter paper (1 · 10 mm) which are superposed on the surface of the solidified gel before the cuvette is covered. For this purpose it is best to take chromatographic paper, for example, No. 2B or No. 2M produced by the Volodarskii Plant. The protein diffuses into the gel from the strip [4]. With this method of application 5-6 samples of the protein mixture can be separated on each block. Figure 2A shows a specimen of the electrophorogram obtained by the indicated method. To introduce the protein into a block of starch gel a depression measuring 1 · 10 mm is cut in the gel with a razor blade and about 0.03 ml of the investigated solution is poured into it. Figure 2B illustrates the result of separation in starch gel.

In immune microelectrophoresis a comb 16 (see Fig. 1A) having projections for forming eight depressions in the gel for the protein mixture being separated and seven depressions for the precipitating antiserum is installed on frame 5 before the gel solidifies. This comb is needed to produce a constant shape of the depressions and a constant distance between them [2] which is important for standardization of the experimental conditions [12, 18]. After the

gel solidifies the comb is carefully removed (without damaging the edges of the depression!). The investigated sample, which is preliminarily mixed with agar of double concentration, is placed into the 2 · 5 mm depressions thus formed [1, 3]. When performing immune electrophoresis on two blocks or more simultaneously, it is desirable to use for each block the same comb, forming one block after the other, since under laboratory conditions it is difficult to manufacture four combs identical in shape and mutual arrangement of the projections. A specimen of the immunoelectrophorogram is shown in Fig. 2C.

After the cuvette is prepared for electrophoresis by one of the above-described methods, it is cooled by water. The water circulates in chamber 17 equipped with pipe 18 which connects with the water line (see Fig. 1).

The cuvette is easily made with the simplest tools.

SUMMARY

A simple cuvette is described which is to be used for serial analyses by means of modified micromethods of electrophoresis on agar and starch gel immune electrophoresis on agar. The instrument can be made in any laboratory.

LITERATURE CITED

1. G. I. Abelev and V. S. Tsvetkov, Vopr. Onkol., No. 6 (1960), p. 62.
2. S. S. Vasileiskii, Byull. Éksper. Biol., No. 10 (1959), p. 97.
3. L. A. Zil'ber and G. I. Abelev, Virology and Immunology of Cancer [in Russian], Moscow (1962), p. 374.
4. R. Portokale'and V. Boeru, Vopr. Med. Khimii, No. 4 (1959), p. 310.
5. G. Bundschuh, Dtsch. Gesundh. Wes., Bd. 15, S. 2103 (1960).
6. J. M. Fine and J. Loeb, Rev. Hémat., Vol. 13 (1958), p. 449.
7. J. M. Fine et al., Ibid., Vol. 12 (1957), p. 698.
8. A. H. Gordon et al., Collect. Czechosl. Chem. Commun., No. 15 (1950), p. 1.
9. P. Grabar and C. A. Williams, Biochim. Biophys. Acta, Vol. 10 (1953), p. 193.
10. P. Grabar, Biokhimiya, Vols. 1-2 (1957), p. 49.
11. P. Grabar and P. Burton, Immunoelectrophoretic Analysis, Use for Investigating Human Biological Fluids [in Russian], Moscow (1963).
12. H. I. Keutel, In book: Moderne Chemische Methoden in der Klinik, S. 542, Leipzig (1961).
13. I. Monnier and R. Fischer, Rev. Hemat., Vol. 13 (1958), p. 458.
14. O. Ouchterlony, Acta Path. Microbiol. Scand., Vol. 25 (1948), p. 186.
15. H. J. Rubinstein et al., Clin. Chim. Acta, Vol. 5 (1960), p. 583.
16. J. J. Scheidegger, Int. Arch. Allergy, Vol. 7 (1955), p. 103.
17. G. Schwick, Laborbl. Hoechst, Bd. 8, S. 11, Lit. [18] (1958).
18. Idem, In book: Moderne Chemische Methoden in der Klinik, S. 532, Leipzig (1961).
19. O. Smithies, Biochem J., Vol. 61 (1955), p. 629.
20. R. J. Wieme, Clin. Chim. Acta, Vol. 4 (1959), p. 317.
21. R. J. Wieme and M. Rabaey, Naturwissenschaften, Bd. 44, S. 112 (1957).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
