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ZONE-SHARPENING IN PAPER ELECTROPHORESIS — A METHOD ALLOWING APPLICATION OF DILUTE PROTEIN SOLUTIONS

STELLAN HJERTÉN

Institute of Biochemistry, University of Uppsala, Uppsala (Sweden)

(Received June 12th, 1958)

SUMMARY

To obtain good results in ordinary paper electrophoresis it is necessary to apply small volumes (about 0.01 ml) of highly concentrated solutions. Thus, very often the samples to be analysed must be concentrated prior to the electrophoresis. To avoid this preliminary concentration and to obtain a narrow starting zone a method has been developed that allows application of comparatively large volumes (0.1–0.2 ml) of dilute protein solutions (as low as 0.02 %).

INTRODUCTION

Paper electrophoresis is very convenient as a routine method for the characterization of different fractions obtained in protein chromatography. This procedure has been used by, amongst others, the author in a previous work on chromatography of serum proteins[1]. The chromatographic fractions obtained were, however, rather dilute and

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required considerable concentration before application to the paper. In the very first experiments this concentration was carried out with the aid of pervaporation. This method was often ineffective because the proteins showed a tendency to adhere to the wall of the bag. This disadvantage was also pointed out by SOBER *et al.*[2]. The risk of denaturation of the proteins is another obvious disadvantage of this method.

THEORETICAL

Because of these drawbacks of pervaporation another method for concentration appeared desirable and preferably one which would permit comparatively large volumes of dilute protein solutions to be applied directly to the paper without preliminary concentration. An application of large volumes of protein solutions must, of course, be combined with some kind of zone-sharpening. This can be effected by causing an abrupt decrease in the electrical field strength in the paper. This can be done in different ways. The method outlined in Fig. 1 has given satisfactory results. A cellophane dialysis tube, filled with buffer solution, is inserted between the paper strip and its support, perpendicularly to the direction of the electric field. As the buffer ions will migrate through the bag a "local shunt" is thus provided, causing a considerable reduction in potential gradient in the narrow contact zone. The protein solution to be concentrated is applied at *A*. When the protein molecules, owing to their electrophoretic migration, which in the following is supposed to be directed towards the anode, come to the line *L*, they will meet a region of low field strength, which effectively reduces their migration and thus gives rise to a zone-sharpening (= concentration). The protein molecules migrate under the influence of only that component of the field strength that lies in the plane of the paper, since they are mechanically prevented from entering the dialysis bag.

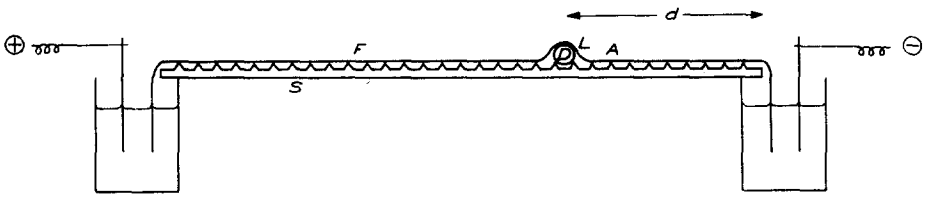

Fig. 1. Method for zone-sharpening in paper electrophoresis. *A*: the place for application of the dilute protein solution; *D*: dialysis bag, filled with buffer; *F*: filter paper; *L*: boundary line for the surface of contact between the dialysis bag and the filter paper; *S*: supporting plate for the filter paper. When the protein molecules have migrated to the line *L*, they will enter a region where the field strength in the paper is comparatively very low. Thus the migration of the proteins will be very effectively decreased, *i.e.* a zone-sharpening is attained.

PRACTICAL TECHNIQUE

The practical performance of the zone-sharpening is accomplished in the following way. A dialysis bag of about 5 cm in circumference is filled up with the same buffer as will be used for the electrophoresis and is placed on the supporting plate *S* at such a distance from the cathode that the distance *d* will be about $^1/_3$ of the length of the plate *S*. The filter paper *F* is moistened in the usual way with buffer and is blotted between two other filter papers. The part of the paper that—according to Fig. 1—touches the bag should once more be carefully moistened with the buffer.

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The paper is then put down on the plate *S*, which supports the bag *D*. It is very important to establish a good contact between the paper and the bag. Air bubbles should be eliminated. After about 20 min the paper is in equilibrium with the buffer and the sample is applied at *A*, preferably on a small glass rod (0.5 × 1.4 cm), placed on the paper. The glass rod, which is to be seen in Fig. 2, prevents the protein solution from spreading too much during the application. The current is then turned on. When the proteins have migrated to the line *L* and have collected there in a narrow zone, the current is turned off. (In order to determine the time required for this zone-sharpening it has been found practical always to use a reference spot of a colored

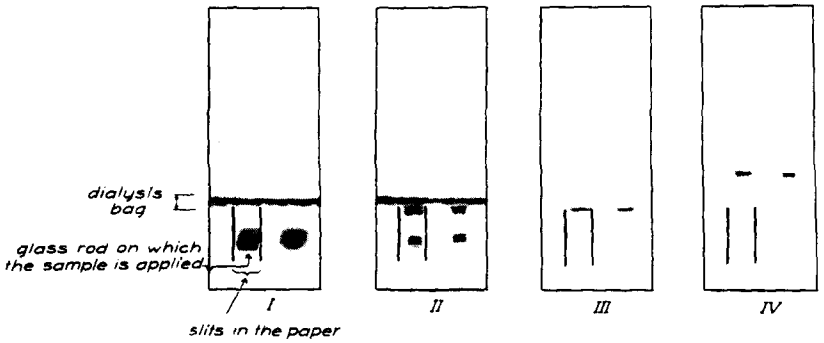

Fig. 2. The different phases of the zone-sharpening. In I 0.1 ml of a solution of phycoerythrin has just been applied. It is so dilute that it can only partly be reproduced photographically. In II the front of the protein spot has reached the dialysis bag and the zone-sharpening has started. III shows the appearance of the protein spot when the zone-sharpening is finished and the bag and the glass rods have been taken away. The last photograph, IV, was taken after 2.5 hours' electrophoresis. The electrophoresis was carried out on Whatman paper No. 1 in veronal buffer, pH 8.6 and $\Gamma/2 = 0.05$. The current was adjusted to 1.5 mA. The time required for the zone-sharpening was 3 hours at the same current.

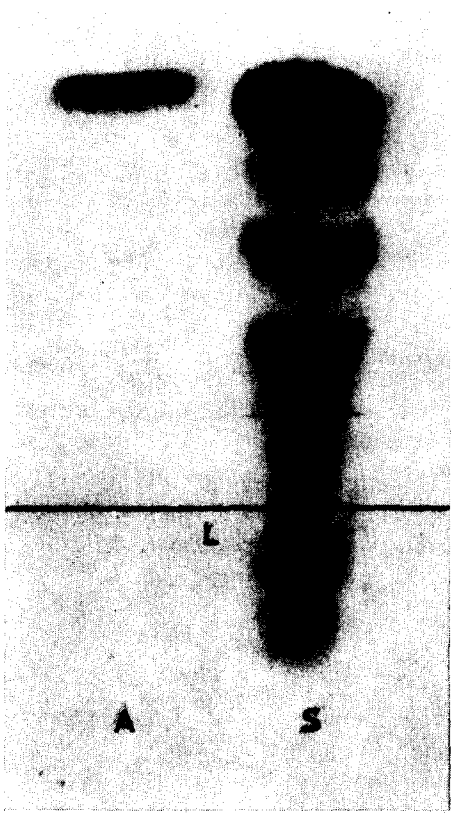

Fig. 3. Electrophoresis (after zone-sharpening) of a very dilute solution of human serum albumin (0.02 %). Volume applied: 0.2 ml. For comparison a sample of 0.01 ml of undiluted human whole serum (S) was applied simultaneously with the albumin (A). The sites of the zone-sharpening are along the line L. The experiment was carried out in veronal buffer, pH 8.6, $\Gamma/2 = 0.05$ on Whatman paper No. 1. The time required for the zone-sharpening of the albumin was 3 h at 2.0 mA. To show that prolonged zone-sharpening does not destroy the narrow zone obtained, the zone-sharpening was continued for 3 hours more. During these 6 h all the serum proteins, except the γ-globulin, reached the line *L*. After the zone-sharpening ordinary electrophoresis was performed at 2.0 mA.

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protein, having a mobility of the same order of magnitude as the sample.) The paper is lifted out of the cathode vessel and is raised up carefully so that it is separated from the bag, which is then removed. The paper is replaced in the cathode vessel. After 10 min the current is turned on and the electrophoresis is performed in the usual manner. It has been found that the effectiveness of the zone-sharpening is determined to a great extent by the following two factors:

1. *The point at which the sample is applied.* The rate of flow of liquid as a consequence of the evaporation of the water increases with the distance from the centre of the paper. Near the electrode vessels the rate of flow is relatively very high. This flow, of course, also causes the proteins to migrate towards the dialysis bag, but does not give an effective zone-sharpening, probably because most of the liquid seems to stream through the paper and not through the bag. In contrast to this flow of liquid, the electrical current passes very easily through the bag. These observations are in accordance with those discussed by WEBER[3] in connection with the use of dialysis membranes to reduce the water content of the filter paper in high-voltage electrophoresis. An effective zone-sharpening is thus obtained when the migration caused by the electrical field is fast compared with that resulting from the flow of liquid. Consequently the flow of liquid seems to be a factor that should be reduced as much as possible.

2. *The voltage used* must not be too high, since this gives rise to a strong flow of liquid in the paper, which results in a rather ineffective zone-sharpening. There is no risk, however, in using the same voltage as is allowed in the succeeding electrophoresis.

If the applied solution contains proteins that migrate in opposite directions, one can place the protein solution between two bags.

In Fig. 2 the different phases of the zone-sharpening are shown. If the protein solution has a tendency to spread laterally during the zone-sharpening this can be remedied by cutting the paper as shown for the left spot in Fig. 2. The concentration of the protein solution applied to the paper can be as low as 0.02 %, and still be easily detected by the staining procedure, as is concluded from Fig. 3. All runs have been carried out with an electrophoresis apparatus of the horizontal, moist-chamber type (manufactured by LKB, Sweden). To learn the technique of this method of zone-sharpening, the use of colored proteins is recommended.

ACKNOWLEDGEMENTS

The author is indebted to Prof. A. TISELIUS for his kind interest in this work.

The work is part of a program of research on electrophoretic and chromatographic methods financially supported by the Rockefeller and Wallenberg Foundations and by the Swedish Cancer Society.

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