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Discontinuous Potential in Planar Electrophoresis

Hugh J. McDonald[a]; George G. McDonald[ab]
[a] Department of Biochemistry and Biophysics, Loyola University Stritch School of Medicine and Graduate School, Chicago, Illinois [b] Department of Physiological Chemistry, Johns Hopkins University, Baltimore, Maryland

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NOTE

Discontinuous Potential in Planar Electrophoresis*

HUGH J. McDONALD and GEORGE G. McDONALD†
DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS, LOYOLA UNIVERSITY STRITCH SCHOOL OF MEDICINE AND GRADUATE SCHOOL, CHICAGO, ILLINOIS

Summary

Direct experimental evidence has been obtained to show that in planar electrophoresis, as opposed to curtain electrophoresis, the electric and centrifugal forces act together in a synergistic fashion to yield an enhanced shift of the electromigration path of a migrant. For a mixture, this means an enhanced separation of its components, under otherwise similar experimental conditions. At the same time, it is evident that a discontinuous potential can also be utilized in planar electrophoresis, thus reducing heat production in the wet paper sheet and therefore obviating the many complicating factors that are involved.

In earlier publications (*1*) the basic principles of centrifugally accelerated paper electrochromatography or planar electrophoresis were described and the technique was applied to the discontinuous separation of lower-molecular-weight materials such as indicator dyes, amino acids, etc. In a later paper the principles were developed to a greater extent, contrasted with those of curtain electrophoresis, and the use of the technique in the continuous separation of serum proteins was described (*2*). It was pointed out

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†Present Address: Department of Physiological Chemistry, Johns Hopkins University, Baltimore, Maryland.

that in planar electrophoresis as opposed to curtain electrophoresis, the electric and centrifugal forces act together in a synergistic fashion to yield an enhanced separation of the components of a mixture. In this paper, the authors explore this idea further and present experimental evidence to support it. They also explore the merit and feasibility of a discontinuous potential across the electrodes versus a continuous potential.

APPARATUS AND EXPERIMENTAL TECHNIQUE

The apparatus used in these experiments was similar in its main features to that described earlier; except for the fact that it was equipped with a built-in refrigeration unit to control the temperature at any level between room temperature and 0°C.

In planar electrophoresis, the radial flow of the background electrolyte is accelerated by the action of centrifugal rather than gravitational force. The horizontally rotating moist chamber or rotor consists of two shallow rectangular trays between which the separation sheet is clamped in a horizontal position. The paper sheet has parallel sides, 60 cm in length, to make contact with the two platinum electrodes, also 60 cm in length, which are arranged in parallel, 56 cm apart. Both ends of the paper sheet are rounded, the distance being 40 cm from the center of rotation to any point on the arc representing the beginning of the dual set of 39 drip points. A series of 39 collecting tubes (i.d., 8 mm; length, 6 cm) are positioned at each end of the rotor to correspond to the drip points of the paper sheet. The other details of the apparatus and experimental procedure are essentially the same as described earlier (2).

As test substances, Bromophenol Blue and bovine hemoglobin were used. Other experimental conditions were: paper, Whatmann 3 MM; potential gradient, 17.9 volts/cm, that is, a total impressed voltage (across the paper sheet) of 1000 volts; rotor speed, 230 rpm; temperature, 25°C. In preparation for an experiment, the rotor was run for about 0.5 hr with an applied potential, the background electrolyte being added at the same rate as was used later on during the run. The background electrolyte (veronal; pH, 8.6; ionic strength, 0.05) was added at the usual rate, namely, about 0.8 ml/min. Once a steady state was reached, as determined by constancy of amperage and voltage, the Bromophenol Blue or hemo-

globin solution, both made up to saturation in the veronal buffer solution, were added to the cup without stopping the rotor and at a rate of approximately 1.0 to 1.5 ml/hr.

Figure 1 represents a portion, approximately one quarter, of the paper sheet, including the center of rotation, a part of one electrode, and the path of the migrant, which in this case was bovine hemo-

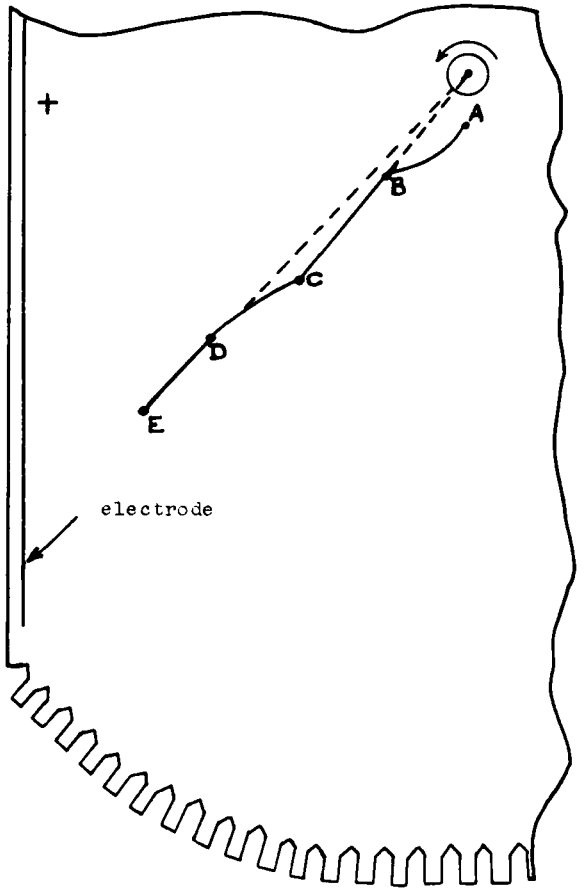

FIG. 1. A portion, approximately one quarter, of the paper sheet, including the center of rotation, a part of one electrode, several drip points, and the path of the migrant.

globin. The point A represents the spot at which the hemoglobin solution was fed to the rotating paper sheet through the wick from the feeder cup (2). During the time, 5 min, required to trace out the path AB, both the centrifugal and electric fields were in operation. When the path reached point B, the potential impressed across the electrodes was shut off for 10 min, and the migrant traced out the path represented by BC. It will be noticed that this section of the path lies on a line extending radially from the center of rotation of the paper sheet through point B. When the front of the migrant spot had reached point C, the electrical potential was reestablished across the electrodes for 5 min, and the path represented by CD therefore again represents the joint effects of both the centrifugal and electric fields. At point D, the electric current was again turned off for 10 min. The path ED therefore represents the movement of the migrant under the influence of the centrifugal field only. It is apparent that this portion of the path again lies on a line extending radially from the center of rotation of the paper through point D.

The shapes of paths AB and CD are not identical. This fact is readily explained because the centrifugal force increases as the distance from the center of rotation increases. During the time when path AB was being traced out, the centrifugal force was quite small, and the electric field exerted the greater influence on the direction of progress of the migrant. On the other hand, when path CD was being laid down, the centrifugal force was much greater, whereas the electrical force remained the same as before. The resultant curve CD was therefore not identical to AB. Similar results were obtained with Bromophenol Blue as a migrant.

The important point brought out by this work is that an interrupted electric current can be used satisfactorily in planar electrophoresis without any important sacrifice of resolving power as regards separation of components of a mixture. This cannot be said for curtain electrophoresis. The benefit of the application of a discontinuous potential is simply that at a given electric potential much less energy is expended in the wet paper sheet in the form of heat. The development of heat has always been a concern in any form of electrophoresis in stabilized media. For planar electrophoresis it would appear now not to be a serious complicating factor.

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