

The direct calculation of  $E$  involves dividing the voltage difference between the ends of the migration path by the path length. In the usual type of free-solution electrophoresis cell, the direct measurement of voltages in the migration path is inconvenient and one may then resort to an indirect method based on the equation  $E = I/KA$ , which assumes that the conductivity  $K$  is constant and the cross-sectional area  $A$  is uniform. With these conditions satisfied, a constant-current supply insures the required electric field.

The situation is not the same in paper electrophoresis. Here we must distinguish between (i) apparatus in which the paper is exposed to an atmosphere and (ii) apparatus in which it is pressed between two solid surfaces.

When the paper is exposed to an atmosphere, whether saturated or not, evaporation from the paper occurs because of heat generated by the electric current. The quantity of heat  $H$  is calculated for a constant-current supply from the equation  $H$  (cal/min) =  $14.3 RI^2$ , and for a constant voltage supply from the equation  $H$  (cal/min) =  $14.3 V^2/R$ . Calculations based on a paper strip carrying 7 ma at 500 v indicate that as much as 0.1 ml of water may be evaporated per minute. A sequence of events is thereby initiated (concentration of buffer, influx of water from electrode compartments, and decreased resistance), which, with a constant-voltage supply, results in increased current density, increased heat production, and still more evaporation. At higher voltages, the cycle may proceed to the point of igniting the paper. This occurred in one case while operating at room temperature with 1000 v across a 30-cm strip of paper using 0.1M barbital buffer.

On the other hand, a constant-current supply, while it does not prevent the change in conductivity, does control the heat production, but at the cost of a constantly decreasing applied voltage as the resistance decreases. In this case, therefore, a choice must be made between constant-voltage supply with the danger of overheating and constant-current supply with the disadvantage of varying migration rate.

When the paper is compressed between two solid surfaces—for example, glass plates—evaporation is completely prevented and the temperature rise is controlled by the temperature of the contact surfaces. If no other disturbing factors are present, either constant-current or constant-voltage supply can be used. However, it is found experimentally that even with constant pressure the conductance of the paper-buffer system increases during the run, although not as much as in the exposed strip, apparently because of electro-osmotic influx of buffer. The constant-voltage supply is therefore preferred, since it has the advantage of producing constant field strength. The field strength is calculated by dividing applied voltage by paper length (measured between buffer surfaces).

The subject is treated more fully by the writer in a forthcoming monograph, "Paper and zone electrophoresis manual" (E-C Apparatus Co., New York, 1954).

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## Glass-Fiber Paper Impregnated with Silicic Acid as a New Chromatographic Tool

In attempting to separate saturated mono-, di-, and triglycerides by paper chromatography, it was found difficult to locate the spots, because the paper is destroyed by the drastic tests necessary to locate these compounds. To overcome this difficulty, a fine glass-fiber filter paper was obtained, which, when impregnated with silicic acid, was found to have chromatographic properties similar to that of silicic acid columns.

D. L. Fillerup and J. F. Mead [*Proc. Soc. Exptl. Biol. Med.* 83, 574 (1953)] were able to separate a mixture of triglycerides, fatty acids, cholesterol, and cholesterol esters on a silicic acid column by using increasing amounts of ethyl ether in petroleum ether as eluting solvents. It was found in this laboratory that mono-, di-, and tripalmitin, cholesterol, and cholesterol acetate can be separated on glass paper impregnated with silicic acid using a developing solvent consisting of a 2-percent ethyl ether in isooctane. Typical  $R_f$  values obtained were as follows: 1-mono-palmitin, 0.05; dipalmitin, 0.27; cholesterol, 0.41; tripalmitin, 0.79; cholesterol acetate, 1.0.

The location of the sterol spots was accomplished by spraying one side of the chromatogram with the Liebermann-Burchard reagent followed by heating over an electric pot plate with exposed heating element. Cholesterol and cholesterol acetate appeared as bright pinkish-red spots on a white background. All areas containing carbon compounds were located by spraying the reverse side of the chromatogram with a dichromate-sulfuric acid-water solution followed by heating over the hot plate. All areas containing carbon compounds appeared as light to dark grayish-black spots on a yellowish-orange background. The color of the spot depended in large measure upon the amount of carbon present.

This technique is being adapted to the separation of other groups of compounds. The details of the procedure will be published later.

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