

CHROM. 545I

High-voltage paper electrophoresis at various proportions of liquid and solid phases

The electrophoretic system containing paper as a supporting medium may be considered as a mixture of solid phase, *i.e.* paper, and liquid phase, *i.e.* electrolyte.

Three factors indicate that the amount of liquid phase ought to be controlled, at least in high-voltage electrophoresis. First, the proportion of electrolyte to the non-conducting solid phase determines the total conductivity and thus the amount of Joule heat produced as well. Secondly, the proportion of solid and liquid phases affects stabilization of the latter. Thirdly, in the plate chamber, the most useful in high-voltage electrophoresis, a pressure on the supporting medium must not cause the electrolyte to be pressed out of the paper.

Little attention has been paid in the literature to the significance of the amount of aqueous phase in paper^{1,2}. This lack of interest was probably due to the fact that the natural saturation of paper by electrolyte solution was quite sufficient for the low-voltage technique.

It is therefore necessary to determine the influence of the quantity of aqueous phase in paper on the current and relative ionic mobility under conditions of high-voltage electrophoresis.

Experimental

A glass plate chamber with two-sided cooling was used³. The strip of paper was separated from the electrode vessel by a Cellophane membrane. No changes in moisture of the strip were observed for 30 min and stable conditions within the electrophoresis were assured⁴. Pressure on the strip was 150 g/cm². In all the experiments, the size of the paper strips, Whatman No. 1, was exactly the same. The strips were moistened for 10 min in a suitable buffer. Excess water was removed by passing the paper between two rubber rollers under regulated pressure. The strip was weighed before and after moistening and the remaining water was calculated as a percentage of dry paper weight.

The changes in current were measured according to the proportions between

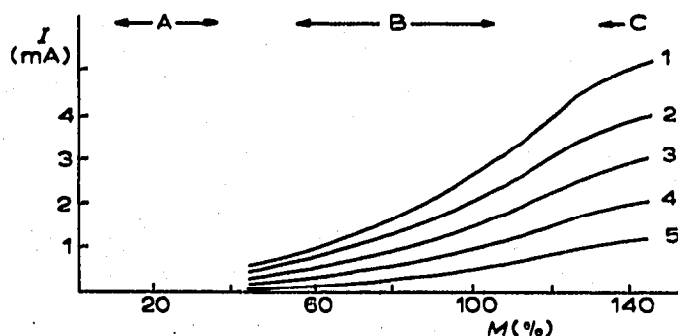


Fig. 1. Changes in current, I , according to amount of aqueous phase, M , in the supporting medium expressed as percent of dry paper weight. The changes were observed at five different potential gradients: (1) 227; (2) 181; (3) 136; (4) 91; (5) 45 V/cm. Temperature, 11°. Electrolyte solution: acetic and formic acids, 0.05 M . Segment A, two-phase system: solid and air. Segment B, three-phase system: solid, water and air. Segment C, two-phase system: solid and water.

TABLE I

INFLUENCE OF AQUEOUS PHASE EXPRESSED AS PERCENT OF DRY PAPER WEIGHT, ON THE POWER PRODUCTION (W/cm^2) IN THE PAPER DURING THE ELECTROPHORETIC RUN

Potential gradient (V/cm)	Water (%)				
	60	80	100	120	140
227	0.48	0.77	1.2	1.7	2.1
181	0.27	0.47	0.74	1.1	1.4
136	0.16	0.27	0.42	0.58	0.77
91	0.064	0.11	0.18	0.26	0.35

the liquid and solid phases (Fig. 1). Various kinds of paper and buffers were used and the results obtained were similar. In one series of measurements, the moisture of the strip was varied and the current measured at constant external voltage. All the measurements on the same plot were carried out at the same temperature, or a correction was made if the temperature of the strip was higher than that of the coolant³. The power produced per cm^2 is shown in Table I.

Two mixtures of compounds were used to study the influence of the aqueous phase in paper on the apparent mobility of the migrating ions. These were amino acids (Fig. 2) and organic acids (Fig. 3); the quantities of substances applied on the 3-cm path of the paper were 5 and 30 μg , respectively.

Discussion

The curves in Fig. 1 indicate that there is a very close relationship between

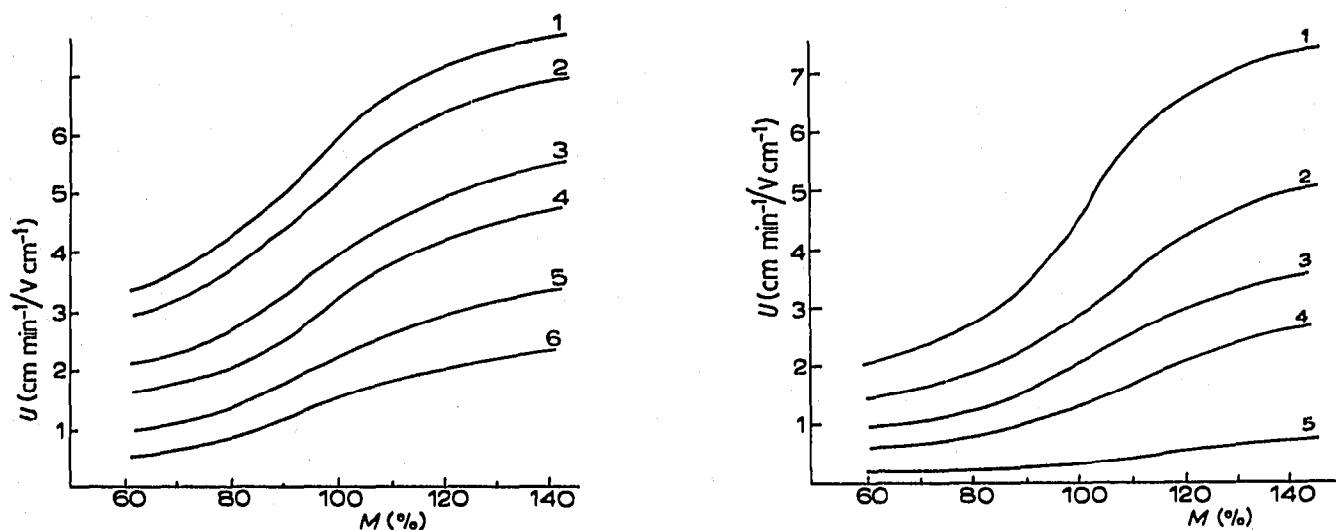


Fig. 2. Changes in apparent mobility, U , of amino acids according to the amount of aqueous phase, M , in the supporting medium expressed as percent of dry paper weight. (1) Lysine; (2) histidine; (3) glycine; (4) alanine; (5) methionine; (6) hydroxyproline. Electrolyte solution: acetic and formic acids, pH 1.9. Specific conductivity, $3.2 \cdot 10^{-3}$ S/cm. Potential gradient, 234 V/cm. Temperature, 11°.

Fig. 3. Changes in apparent electrophoretic mobility, U , of organic acids according to the amount of aqueous phase, M , in paper expressed as percent of dry paper weight. (1) oxalic acid; (2) malic acid; (3) maleic acid; (4) pyruvic acid; (5) fumaric acid. Electrolyte solution: formic acid, pH 2.0, 0.75 M . Potential gradient, 234 V/cm. Temperature, 5°.

the quantity of moisture and the conductivity of the system. There are at least three reasons for this. The first is the decreased amount of the conducting fraction. The second is shown by the segment A of the curves in Fig. 1, *i.e.* below about 40% moisture. This is due to the presence of the microporous system of microfibrils in cellulose which have absorbed the water. The ions in this system show a very great retardation coefficient and do not take part in conductivity.

Information concerning segment B of the curves in Fig. 1, above 40% moisture, is given in Figs. 2 and 3. These show that the apparent electrophoretic mobility also decreases greatly with the decrease in moisture, and this is the third reason for decrease in conductivity. Thus removal of water lowers the effect of the voltage increase, but it should be remembered that the apparent mobility is observed here which depends on both the actual mobility and porosity of the supporting medium.

GIDDINGS AND BOYACK⁵ suggested a theoretical model of migrant wandering in the medium, which is characterized by the retardation coefficient due to swelling of the supporting medium, the constriction coefficient characterizing the macro-structure of the supporting medium, and the path-length parameter (tortuosity coefficient). It seems that the last factor is responsible for the above changes in mobility. It is commonly assumed that the entire space of the conductor created by a porous solid and electrolyte, not occupied by a solid, is filled by a liquid phase. The amount of water in the paper corresponding to the full saturation ability of the micro and macro systems is about 130–150% of the dry weight of the paper. As the removal of the water does not cause changes in swelling or changes in macro-structure air must take the place of the removed water. The assumption that there is also a gas phase in paper with a lower moisture content satisfactorily explains the experimental observations. Thus, in segment B of the curves in Fig. 1, a three-phase system exists. For the wandering ion, the bubbles of air are as much an obstacle as a solid would be and the actual path of the ion is therefore lengthened. The change in the water content necessitates a change in the porosity, and this may be useful in modifying the conditions of the electrophoresis. As a consequence of decreasing the moisture in the paper, we may expect a separation on a shorter macroscopic path and at a lower power production.

*Department of Clinical Biochemistry,
Medical Academy, Gdańsk (Poland)*

T. BADZIC

- 1 R. H. HACKMAN AND M. GOLDBERG, *Anal. Chem.*, 36 (1964) 1221.
- 2 G. N. ATTFIELD AND C. J. O. R. MORRIS, *Biochem. J.*, 81 (1961) 606.
- 3 T. BADZIO AND T. POMPOWSKI, *J. Chromatogr.*, 39 (1969) 344.
- 4 T. BADZIO AND T. POMPOWSKI, *Chem. Anal. (Warsaw)*, 15 (1970) 411.
- 5 J. C. GIDDINGS AND J. R. BOYACK, *Anal. Chem.*, 36 (1964) 1229.

Received May 14th, 1971

J. Chromatogr., 60 (1971) 150–152