COUNTERCURRENT ELECTROPHORESIS ON PAPER

VII. MEASUREMENT OF ELECTROPHORETIC MOBILITIES ON PAPER BY MEANS OF THE FRONTAL METHOD*

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INTRODUCTION

In a number of studies attempts have been made to work out an exact method for measuring electrophoretic mobilities on paper (for reviews of these methods see the various monographs, *e.g.* ref. 1). The mobilities are measured by the rate of migration of the zones of the given substances. We can call this technique the "elution technique", because we consider that the main difference between chromatography and electrophoresis on paper lies only in the nature of the force which causes the motion of the substance being studied: in chromatography it is the flow of the solvent, in paper electrophoresis the electric field. The authors of the papers mentioned above try to diminish the flow of the electrolyte in the paper. Under conditions where elimination of the electrolyte flow is impossible they introduce appropriate corrections. Some authors consider that if the migration rate of the zone moving along the strip remains constant this proves that the disturbing flow has been eliminated. In the preceding paper[2], however, we have shown that the path can be a linear function of time only when the expression for the distribution equilibrium which results from the electrophoretic process on the paper, is a linear equation. We shall call this expression the "electrophoretic distribution isotherm". A constant migration rate can occur in spite of a nonlinear isotherm, but only by the fortuitous compensation of various factors.

When the distribution isotherm is nonlinear the movement of the zone depends on the concentration. From a single experiment it is quite impossible to conclude whether the change of velocity is caused by the nonlinearity of the distribution isotherm or by other factors. The dependence of the mobility on the concentration is another reason for considering the measurement of the zone migration rate unsuitable for the required purpose. To give values of mobilities without the corresponding values of the concentration is therefore meaningless. It is not easy to determine the concentration when the "elution" method is used, and it is also difficult to determine theoretically which point on the concentration profile of the zone is most suitable for measurement.

The purpose of this paper is to show that the frontal technique applied to the measurement of electrophoretic mobilities is able to give correct values at any concentration.

* For Part VI see ref. 2.

The frontal method has been theoretically worked out for free electrophoresis (see *e.g.* ref. 3). The free mobility of a substance U_0 ($cm^2 \cdot sec^{-1} \cdot V^{-1}$) can be calculated from the relation

$$u_0 = U_0 \kappa / i \tag{1}$$

where u_0 ($cm \cdot sec^{-1}$) is the measured linear velocity of the moving boundary, κ ($\Omega^{-1} \cdot cm^{-1}$) the specific conductivity and i ($A \cdot cm^{-2}$) the current density. κ corresponds to the solution on that side of the boundary where the substance being investigated is present.

Similarly the theory of frontal chromatography has been thoroughly worked out. For the equilibrium case the following equation is valid

$$(\delta x / \delta t)_c = u_s / [1 + q'(c)] \tag{2}$$

where x (cm) is the distance from the upper end of the column, t the time (sec), u_s the linear velocity of the solvent ($cm \cdot sec^{-1}$) and c ($g \cdot cm^{-3}$) the concentration of the substance under consideration in the mobile phase. The distribution isotherm $q(c)$ expresses the amount of the substance in the stationary phase ($g \cdot cm^{-3}$); $q'(c) = dq(c)/dc$.

For the nonequilibrium stationary case and for a distribution isotherm with $q''(c) < 0$ the equilibrium equation is valid in the point $\bar{x}$ defined by the relation (see ref. 4; x and $\bar{x}$ are distances from the end of the column):

$$\int_0^{q(c^0)} x \cdot dq(c) = \bar{x} \cdot q(c^0) \tag{3}$$

When the path travelled is expressed as a distance between two points in both of which the front was already in the stationary state, any point on the concentration profile of the front (any concentration) can be used for this expression. The distance must then be determined always for this point. The integration may be carried out graphically, the concentration profile of the front being known. The value of c^0 is the value corresponding to the values (*e.g.* of R_F) determined by the frontal method. It is the concentration of the solution entering the chromatographic column.

The velocity u_s is a constant for a homogeneous chromatographic column and the value of $q'(c)$ depends on the concentration only. Therefore the velocity $(\partial x / \partial t)$ is also a function of the concentration only. The distance x_f travelled by the front of the solute in the equilibrium case and the distance $\bar{x}$ in the nonequilibrium stationary case, are then linear functions of time.

The frontal method makes it possible to determine the distribution isotherm of the given substance from measurements at different concentrations c^0. In this case the equation of the mass balance takes the following form: $c^0(x_s - x_f) = x_f q(c^0)$. Here the indices f and s refer to the solute and the solvent respectively. The distance x is expressed in ml of the stationary phase corresponding to the length unit of the column. The following equation is evidently valid

$$q(c^0) = \alpha c^0 \left(\frac{u_s}{u_f} - 1 \right) \tag{4}$$

Here α is the porosity. (For calculation of the term α the swelling of the supporting medium must be considered. This question will be treated in another paper.)

By means of the equation given in the previous paper we can apply the equations mentioned above to electrophoresis on paper. We have shown that $U_s = u_s/E = U_0/f$, where E is the potential gradient ($V \cdot cm^{-1}$) and f the tortuosity factor. When all kinds of liquid flow, except the electro-osmotic flow, are eliminated and the observed macroscopic velocity of the front is corrected for electro-osmosis the following equation is valid

$$q_e\,(c^0) = \alpha\, c^0 \left(\frac{U_0}{U_{cor.} f} - 1\right) \qquad (5)$$

Here q_e is the electrophoretic distribution isotherm, $U_{cor.}$ the mobility of the front corrected for electro-osmosis. The values $U_{cor.}$, q_e and U_0 correspond to the concentration c^0.

EXPERIMENTAL

Materials

Whatman No. 4 chromatographic paper was used; its structural parameters were taken from ref. 5.

Acetic acid solutions prepared from the concentrated acid (analytical reagent purity) were used as electrolyte.

The acidic azodye Kashmir Blue T.G. Extra (from Farbenfabriken F. Beyer, Elberfeld, Germany) was used as a sample for the study. The solubility of this dye is 0.092 % in 1 N acetic acid. The contribution of the dye to the conductivity is negligible in comparison with the conductivity of 1 N acetic acid.

Apparatus

The apparatus for measuring the concentration profile of the front was essentially the same as the apparatus described in the previous paper[2], but with the following differences: the vessels were connected by a glass tube with taps to bring the levels to the same height. This permitted exchange of the whole volume from one vessel without opening the wet chamber. The ends of the paper strip were dipped directly into the solution contained in the vessels, and were not wrapped in cellophane membranes.

The detector was moved along the strip and the galvanometer deflections were read. The temperature of the paper strip was measured by a thermistor thermometer. The potential gradient was determined as the voltage between two movable contacts 1 cm apart.

In this method calibration of the detecting arrangement is not necessary. Under the conditions employed the electro-osmotic flow was negligible, as was established by experiments with glucose. The constant velocity of the dye front (see Fig. 3) was evidence of the absence of all other liquid flow. Fig. 1 shows that the zones of the dye in different sections of the strip remained at their original positions after the alternating current was switched on. They broaden only by diffusion (symmetrically).

Experimental technique

After the apparatus and surrounding space had been thermostated the vessels were filled with acetic acid solution and leveled. When leveling was accomplished the

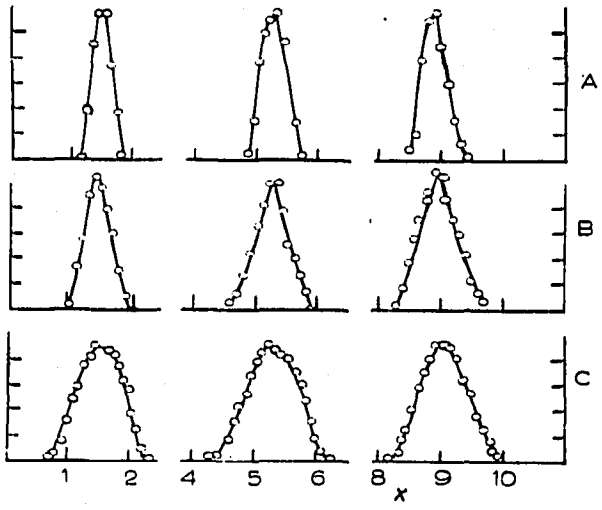

Fig. 1. Change of zone shape with time (alternating current). A = original shapes; B = after 4 h; C = after 8 h.

connecting tube was disconnected. The surface of the defatted glass plate (in the horizontal position) was moistened with 3 ml of acetic acid solution.

The dry chromatographic paper strip from which the impurities had been eluted by 25 ml acetic acid was moistened by dipping it into the solution. Then it was put onto the glass plate.

After the wet chamber had been closed direct current of about 2.40 mA was switched on for 12 hours to establish equilibrium conditions. After this period the acetic acid solution in one vessel was replaced by the same volume of a solution of the dye of known concentration. During the experiment the galvanometer deflection and the potential gradient were measured. An example of the concentration profile thus obtained is shown in Fig. 2. It is evident that this is not the equilibrium but the stationary non-equilibrium case. The shape of the profile does not alter during the movements of the front.

The dependence of the distance travelled on the time is linear as can be seen in Fig. 3. The potential gradient reached a constant value along the strip with an ac-

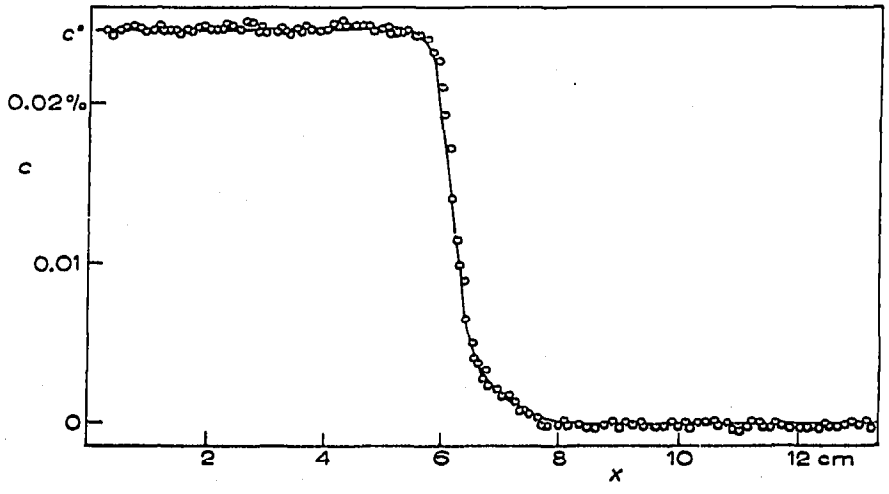

Fig. 2. Example of concentration profile of the front.

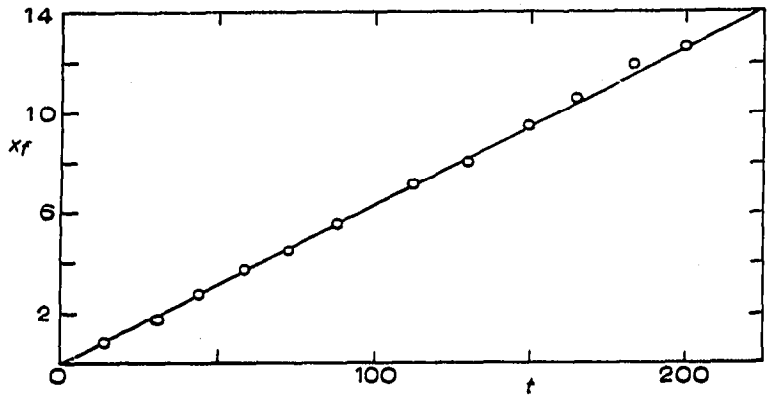

Fig. 3. Relationship between the distance x_f (cm) travelled by the front of the dye and the time t (min) for 0.025 % Kashmir Blue in 1.0 N acetic acid.

curacy of ±2 %. Under the conditions employed the chromatographic paper contained 2.52 g of water per 1 g dry paper. A value of 0.65 was taken for swelling water. The tortuosity factor was taken equal to 1.30. The temperature of the paper strip was 20°, the temperature of the surrounding space of the apparatus was kept at about 27°.

All measurements were carried out at least three times. Even for the lowest concentrations the experimental error was not greater than 5 %.

All measurements were performed in 1.0 N acetic acid. As has already been stated the contribution of the dye to the overall conductivity is negligible. Several experiments were performed with a dye that is a better conductor, *viz.* Guinea Red 4B, in 0.1 N acetic acid. In this case the potential gradient changes simultaneously with the concentration (see Fig. 4). These changes could be used to indicate the position of the front for colourless substances.

The mobility in free solution was measured in the same way as in the previous paper[2]. The experimental error was ±5 %.

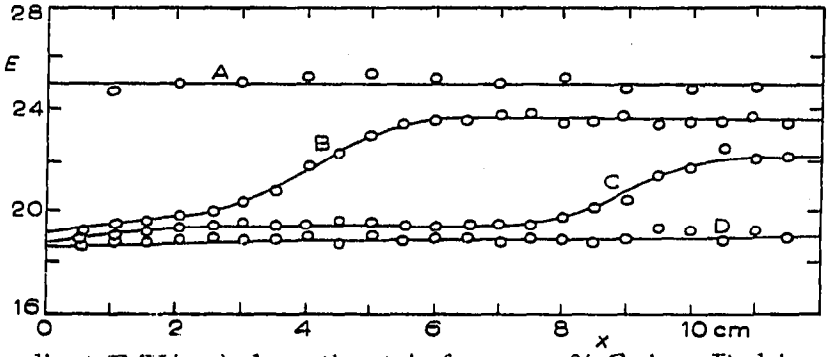

Fig. 4. Potential gradient E (V/cm) along the strip for 0.025 % Guinea Red in 0.1 N acetic acid. The curves A, B, C and D were measured after 0, 2, 4 and 8 h respectively.

RESULTS

With the arrangement described above the dependence of the linear velocity of the front U on the potential gradient E give a straight line going through the origin (Fig. 5). The electrophoretic mobility of the dye on paper, $U = u/E$ (under the conditions employed no correction for any flow in the paper need be made and $U = U_{cor.}$),

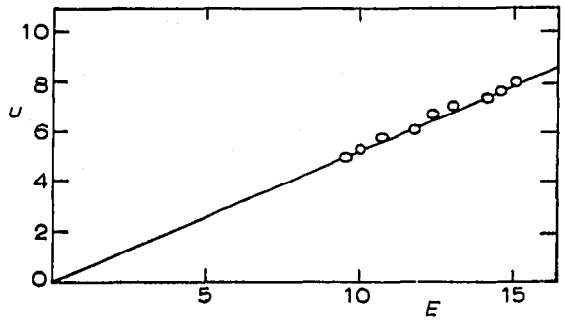

Fig. 5. Relationship between the front velocity u (cm/min) and the potential gradient E (V/cm) for 0.025 % Kashmir Blue in 1.0 N acetic acid.

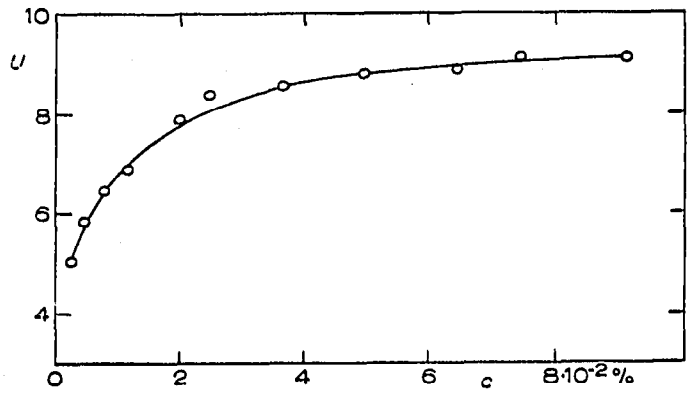

Fig. 6. Relationship between the mobility U (10^{-5} cm^2/V. sec) of Kashmir Blue and its concentration c in 1.0 N acetic acid.

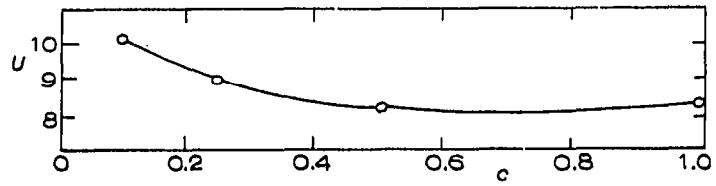

Fig. 7. Relationship between the mobility U (10^{-5} cm^2/V. sec) of the dye and the concentration c (mole/liter) of acetic acid for 0.025 % Kashmir Blue.

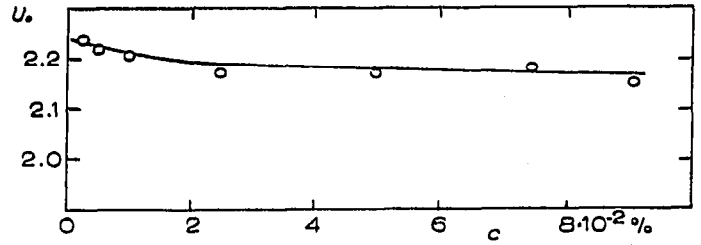

Fig. 8. Relationship between the mobility in free solution U_0 (10^{-5} cm^2/V. sec) of Kashmir Blue and its concentration c (g/liter) in 1.0 N acetic acid.

depends as we expected on the concentration of the dye (Fig. 6). This mobility also depends on the concentration of acetic acid (Fig. 7). The free mobility U_0 is slightly dependent on the concentration (Fig. 8).

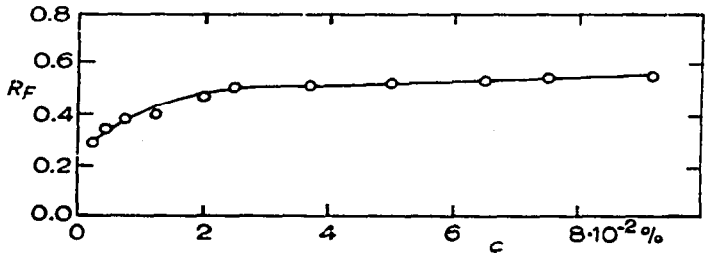

Fig. 9. Relationship between the R_F of Kashmir Blue and its concentration c (g/l) in 1.0 N acetic acid.

It is possible to calculate the factor R_F from the measured values of U and U_0 by means of the relation $R_F = Uf/U_0$. The dependence of R_F on the concentration is plotted in Fig. 9. By means of eqn. (5) the electrophoretic distribution isotherm, $q_e(c)$, can be determined. The function obtained is plotted in Fig. 10.

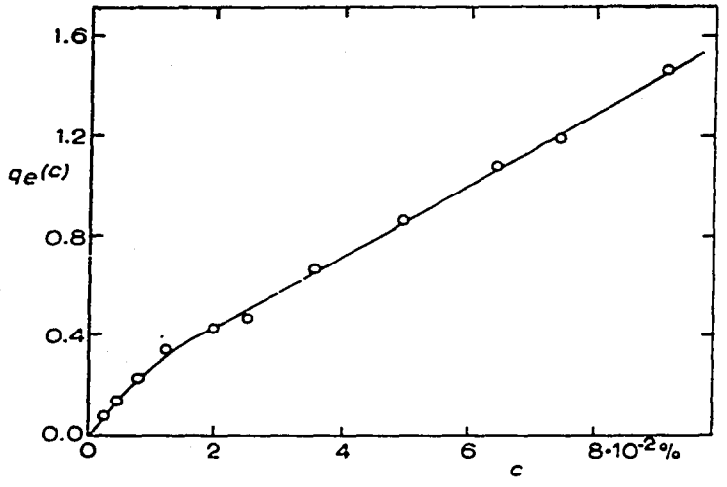

Fig. 10. Electrophoretic distribution isotherm q_e (c) of Kashmir Blue in 1.0 N acetic acid.

CONCLUSIONS

The results obtained show that the frontal method has the expected advantages for the measurement of electrophoretic mobilities on paper. The time dependence of the distance travelled by the front is linear even in the case of a nonlinear distribution isotherm and a nonequilibrium process. Therefore the changes of the migration rate of the front can serve as a sensitive indicator of errors in the experimental arrangement. This method permits the determination of the dependence of the mobility on concentration.

The value of R_F may be calculated from the results obtained. It is possible to compare the values obtained in this way with the values that were determined directly. Agreement of these values would be a further proof of the concepts given in the preceding communication of this series[2]. The dependence of the chromatographic

coefficient R_F on the concentration causes complications similar to those occurring in the case of the electrophoretic mobility and therefore this problem will be studied in the next paper in this series.

The mobility measurements give also the values of the electrophoretic distribution isotherms. Application of the GLUECKAUF method[6] to electrophoresis[2] also gives the values of this function. Thus these methods supplement each other. The GLUECKAUF method is suitable for smaller concentrations, while the frontal method is better for higher concentrations. The fact that the mobilities are independent of the potential gradient provides evidence that the electrophoretic distribution isotherm does not depend on the potential gradient in the range of concentrations and potential gradients employed.

Therefore the frontal method is important for the precise measurement of the true mobilities and their dependence on the concentration and for the verification of various opinions about the nature of the separation processes in electrophoresis on paper.

SUMMARY

The frontal method of measuring chromatographic distribution isotherms was applied to paper electrophoresis. Measurements were performed for a model coloured substance. Concentration dependence of free electrophoretic mobility, of electrophoretic mobility on paper, and of R_F were obtained.

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