COUNTERCURRENT ELECTROPHORESIS ON PAPER
PART VI. ELECTROPHORETIC DISTRIBUTION ISOTHERMS*

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INTRODUCTION

When we tried to describe quantitatively the separation process in countercurrent electrophoresis on paper we realised that the opinions about the nature of separation processes in paper electrophoresis so far published are neither complete nor generally valid.

There are three groups of opinions concerning these processes. The authors of the first group[1–12] accept the original opinion of KUNKEL AND TISELIUS[13] and regard paper electrophoresis as a method analogous to classical electrophoresis. They consider that the only difference between these two methods consists in the fact that in paper electrophoresis the path of the travelling particle is prolonged due to the geometrical structure of the paper. They suppose that the separation of components of the mixture being analysed occurs only as a result of the different mobilities of the components in the electrical field. The prolongation of the path is characterized by the tortuosity factor or by another factor defined similarly.

The second group of views is represented by the work of MCDONALD *et al.*[14–18], who formulate the idea of "barrier effect". The only acceptable feature of this conception is that it shows the possibility of interaction of the compounds being analysed with the paper. Attempting to formulate their opinions quantitatively these authors introduce the term "thermodynamic activity". We shall show elsewhere that this quantity because of the way in which it is measured again more probably describes the prolongation of the path of the substance rather than its interaction with the paper. It corresponds therefore to the tortuosity factor. Only the equivalent hydrodynamic scheme of the porous medium differs in this case from the previous one.

The authors of the third group[19–23] consider the interaction of the compounds being analysed with the paper as a form of adsorption; they do not, however, make quantitative calculations. They regard the sorption effects mostly as disturbing and they try to eliminate them. Only a few authors[10,14] mention both the geometrical arrangement of the support and the interaction with paper.

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* For Part V, see ref.[25].

These opinions are for the most part acceptable. They are, however, not complete. We suppose that paper electrophoresis includes both the features of classical electrophoresis in free solution and those of paper chromatography. In paper electrophoresis the separation principles of both these methods occur simultaneously. The difference in the electrophoretic mobilities on paper of two components is the result of the differences in their electrophoretic mobilities in the free solution and of the differences in their chromatographic distribution isotherms.

It is therefore desirable to find out the relationship between the electrophoretic mobility in free solution U_0 and the macroscopic electrophoretic mobility U of the substance on paper. The substance moving under the influence of the electric field of a unit potential gradient in the free solution with the velocity U_0 is slowed down on the paper by the tortuosity of the path. If there were no interaction with the immobile phase the substance would move on paper with the velocity u_0:

$$u_0 = \frac{U_0}{f} \tag{1}$$

Here f is a correction factor which can be obtained from conductivity measurements. The quantities U, U_0 and u_0 have the dimension $cm^2 sec^{-1} V^{-1}$, f is a dimensionless number.

The velocity u_0 is analogous to the linear macroscopic velocity of the solvent system in chromatography. This velocity is reduced in paper electrophoresis as well as in chromatography to the value observed directly, U (corresponding to the motion of the substance analysed). U is the electrophoretic resulting mobility on paper which is measured directly. The slowing down of the motion of the substances from the velocity u_0 to U can be described as in chromatography, by the factor R_F:

$$R_F = \frac{U}{u_0} \tag{2}$$

It is possible to define the factor R_F in this way only for substances with linear distribution isotherms. In this case R_F is independent of the concentration. If we use the factor R_F for a substance with a non-linear distribution isotherm we must take into account the dependence of this factor on the concentration. Similarly, the quantity U_0 is independent of the concentration only if the current is transported chiefly by the electrolyte and only negligibly by the substance analysed and if this substance does not participate in any chemical equilibrium in solution. In the opposite case it is not possible to neglect the concentration dependence of U_0. The paper electrophoretic distribution isotherms are therefore generally different from the chromatographic ones.

From eqns. (1) and (2) it can be derived that:

$$U(c) = u_0(c) R_F(c) = \frac{U_0(c)}{f} R_F(c) \tag{3}$$

If the potential gradient is constant along the whole paper strip including the place of the zone, the form of the function $U(c)$ is the same as that of the function $R_F(c)$. The functions $U_0(c)$ and $R_F(c)$ are unknown. However, we should like to point out that the dependence of the mobility U on the concentration will make itself manifest as a non-linear time dependence of the path travelled by the zone in paper electrophoresis. Some authors (see *e.g.* refs.[17,18]) regard this non-linearity to be due to the disturbing effects of the flow of electrolyte in the paper. They attempt to obtain linearity by a suitable experimental arrangement. It is obvious from eqn. (3) that except for the linear paper electrophoretic distribution isotherms such attempts are unlikely to be successful. Further it should be noted that during the mobility measurements on paper only the suction flow due to evaporation from the paper can be satisfactorily eliminated but not the electro-osmotic flow. The effect of electro-osmotic flow must be taken into account in eqn. (3) by introducing an additional term. Let us designate the macroscopic linear velocity of the electro-osmotic flow under the influence of the unit potential gradient as u_e ($u_e \gtrless 0$). Then:

$$U''(c) = [u_0(c) + u_e]R_F(c) = \left[\frac{U_0(c)}{f} + u_e\right] R_F(c) \qquad (4)$$

where $U''(c)$ is the mobility measured on the paper when electro-osmotic flow is present. The term u_e is additive to the velocity u_0. Corrections for electro-osmosis made by means of a substance that does not travel in an electrical field are not entirely correct. Let us consider a substance for which $U_0^* = u_0^* = 0$ and $R_F^* < 1$. This substance will move on paper under unit potential gradient with the velocity $U^* = u_e R_F^*$. The following relationship obviously refers to the true electrophoretic mobility of the substance in question, the correction for electro-osmosis having been made:

$$U(c) = U''(c) - u_e R_F(c) = U''(c) - U^*(c^*)\frac{R_F(c)}{R_F^*(c^*)} \qquad (5)$$

The aim of this paper is to apply GLUECKAUF's method of measurement of the distribution isotherms[26,27] to electrophoretic separations on paper and thus verify the suggestions mentioned above, to show the connection between chromatography and electrophoresis on paper and to show the possibility of correlating electrophoretic and chromatographic measurements.

EXPERIMENTAL

Materials

Whatman No. 4 chromatographic paper was used. The electrolyte was 0.1 *N* acetic acid prepared from the concentrated acid of purity "pro analysi". To facilitate indication of the zones the following organic dyes were used: Orange G ($C_{16}H_{11}N_2NaO_4S$), Anthosin 3BN ($C_{24}H_{14}Cl_2N_2O_8S_2$), Neptunblau Bb Extra ($C_{74}H_{70}CaN_4O_{14}S_4$) and Naphthol Black 10B ($C_{36}H_{16}N_4Na_4O_{14}S_4$). These dyes were selected from a large set because

they differ markedly from each other as regards their mobility and interaction with paper. They were homogenous from the electrophoretic point of view.

The apparatus for measuring the concentration profiles of the zones

The apparatus should permit the measurement and recording of the concentration profile of the zones during the test at any distance from the starting point and at any moment after beginning the experiment, as well as the measurement of the potential gradient on the paper during the experiment. The suction flows should be eliminated as much as possible.

The apparatus itself was a wet chamber with a cooler (Fig. 1). The cuvettes were

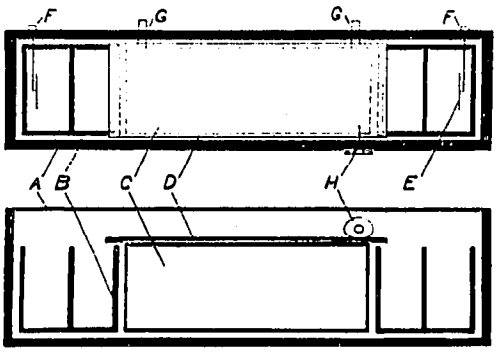

Fig. 1. Schematic diagram of the device for the measurement of the concentration profiles of the zones. A = trough of vinidur (10 × 12 × 55 cm); B = cuvettes of umaplex (8 × 11 × 65 cm); C = brass cooler (7 × 11.5 × 40 cm); D = glass plate (40 × 11.5 cm); E = platinum electrodes (1 × 9 cm); F = supply lead jacks; G = inlet and outlet of the cooler; H = platinum contact for measuring the potential gradient (the second contact was located on the sliding lid).

divided into two in order to separate the space of the electrodes from the space in which the end of the paper is immersed. The connection of both spaces was achieved by a paper bridge. The sliding lid of the device was supplied with a scale serving for adjustment of the position of the detector.

The detector. For detecting the amount of substance on the paper the reflection method was used. The arrangement of the detector, which was built on the sliding cover, is evident from Fig. 2.

The recording device was a mirror galvanometer (sensitivity $2.8 \cdot 10^{-9}$ A/mm.m) and a rotary cylinder with photographic paper. To avoid permanent illumination of

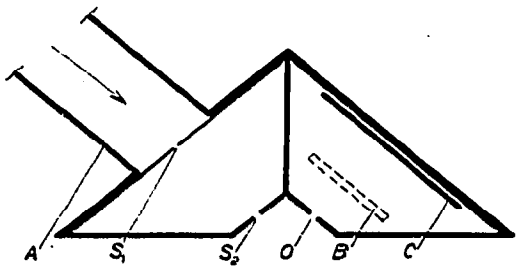

Fig. 2. Schematic diagram of the detector. A = illuminating lamp; B = colour filter; C = selenium photo-cell; S_1 and S_2 outlet apertures of the lamp (1.5 × 20 mm); O = entrance aperture of the photo-cell (3 × 20 mm). The light trace on the paper had an area of about 40 mm².

the chromatographic paper and of the photo-cell, which would hamper the stability of the whole system in tests lasting several hours, the following arrangement was used. The motor that served for turning the cylinder switched on the lamp of the detector at minute intervals for about 25 sec, and (with some lag) the lamp of the

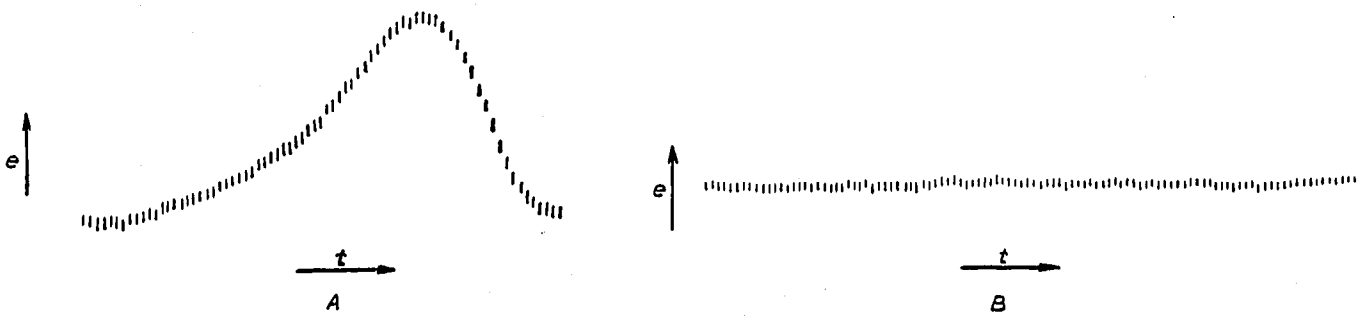

Fig. 3. Example of the original record. A = registration of the passage of the strip through a given place on the paper, B = stability of the recording for clean paper. *e* is the deflection, *t* the time.

galvanometer for 3 sec. An example of the resulting records is given in Fig. 3A. The lamp of the cell was supplied with stabilised A.C. of 6 V.

An ordinary two-way rectifier (output 0–1000 V) supplied with stabilised current was used.

Measurements of the potential gradient. A valve voltmeter of usual type was used (measuring region 1–500 V, entrance resistance 10 MΩ and 20–10,000 V, 200 MΩ, precision 3 and 5% of the whole deflection, current sensitivity 0.1 mA). The voltage between the fixed contact and the sliding contact in the lid of the device was measured.

Testing the device

Sampling was carried out by means of a divided haematological pipette of 0.1 ml, ending in a capillary.

The stability of recording is apparent from Fig. 3B.

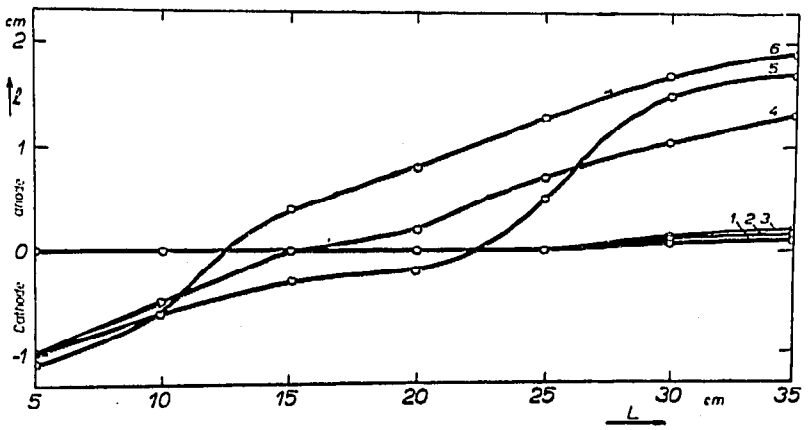

Fig. 4. Degree of elimination of the suction flow under different conditions. L is the starting position of the spot, *l* the distance reached by the spot in the time interval of the test. Curves 1–4 were obtained after tightening the apparatus and using cellophane membranes (time interval 2, 4, 6 and 22 h); curve 5 – with cellophane membranes but without tightening (15 h), curve 6 – without membranes and tightening (22 h).

The suction flow of the electrolyte was eliminated satisfactorily (Fig. 4) by cooling the paper (16°), by tightening the sliding lid with vaseline and by wrapping cellophane membranes round the ends of the paper (see Fig. 5).

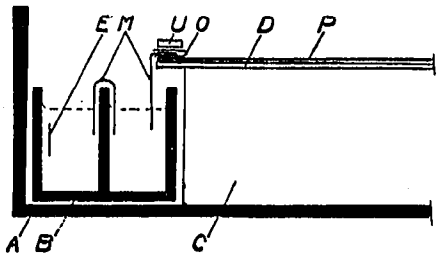

Fig. 5. Arrangement of the cuvettes. A = trough of the apparatus; B = cuvette; C = cooler; D = glass plate; E = electrode; M = paper bridges; O = cellophane membrane; P = chromatographic paper; U = fixing plate of umaplex.

Fig. 4 was obtained in the following way. At distances of 5, 10, . . . 35 cm from the edge 0.08 ml of a 0.1% solution of the dye was applied and the alternating voltage was switched on (current 1 mA). Other conditions were the same as in the other runs. After the time interval given in Fig. 4 the distance was measured that the particular dye spots had travelled under the influence of the electrolyte flow.

The potential gradient in the cooled and tightened apparatus with cellophane membranes was constant along the paper and independent of time. In a period from 30 min after closing the apparatus up to 24 h, the potential gradient oscillated around an average value ± 3.0% (standard deviation, 85 measurements). In an untightened apparatus it did not reach a steady value even after 76 h and oscillated even as much as 100%.

The electro-osmotic flow was measured by means of glucose, using different potential gradients (range 10 to 28 V/cm). Its value was $u_e = 3.0 \cdot 10^{-6} \pm 0.1 \cdot 10^{-6}$ $cm^2\ sec^{-1}V^{-1}$ (standard deviation, 10 measurements).

The content of water in the paper was measured after each experiment by weighing, four samples being taken from the paper. A value of 2.35 ± 0.05 g of water in 1 g of dry paper was found (standard deviation, 80 measurements).

For the calibration on paper strips with a surface of 12 cm^2, 0.26 ml solution of a known concentration was applied, always using six strips for one concentration and one dye. The amount of 0.26 ml corresponds to the content of water in the paper mentioned above. These strips together with four strips moistened with pure electrolyte were inserted into the device and after 30 min a record was taken.

Other measurements

The static measurements of the distribution isotherms were carried out in closed vessels in the following way:

Four grams of dry paper were brought into equilibrium with 20 ml of the solution of the particular concentration (always three times). After 60 h the change in the concentration was determined colorimetrically.

The measurements of the mobility in free solution were carried out with the Tiselius electrophoretic apparatus LKB Produkter Stockholm, Type 3017. The mobility was estimated from the change of the position of the boundary between the 0.1% solution of the dye in 0.1 N acetic acid and the pure solution of 0.1 N acetic acid, five measurements always being made.

The chromatographic measurements were only preliminary (vertical arrangement, 0.1 N acetic acid, atmosphere of the vapours of this solution). After 105 min the R_F value was determined.

RESULTS

Measurement of the electrophoretic distribution isotherms

A strip of chromatographic paper was pulled through a solution of pure electrolyte, inserted in the device and the device closed. After 30 min the device was opened for a short time and a known amount of the solution of the dye applied. The device was then closed again and the potential gradient E was adjusted. The detector was located at a chosen distance x from the starting point and the recorder switched on (t = 0). In this way the curves shown as example in Fig. 6 were obtained. The same

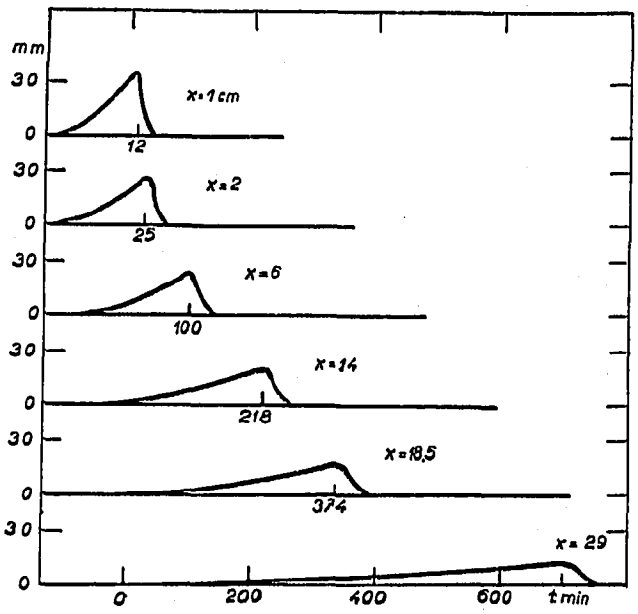

Fig. 6. Example of dependence of the concentration of the dye (Orange) on the time t for different distances x. The deviation of the galvanometer is plotted on the vertical axis. The curves were obtained by tracing the original records. E = 20 V/cm.

operation was performed with different potential gradients and in one case for various amounts of dye applied. From the curves the time t was read when the maximum of the concentration passed through the point x. When the values of x were high a correction was made for the suction flow and in the case of the dye Naphthol Black also for the electro-osmotic flow. With the other more mobile dyes the electro-osmotic influence was negligible. The dependence of the distance x on the product

Et was plotted. An example of this dependence is presented in Fig. 7. In other cases similar curves were obtained. The dependence of x on *Et* for different amounts of dyes applied is plotted in Fig. 8.

Results of the static measurements are given in Fig. 9. The values of the amounts of the substance in the immobile phase q (number of grams of the dye in 1 g of dry

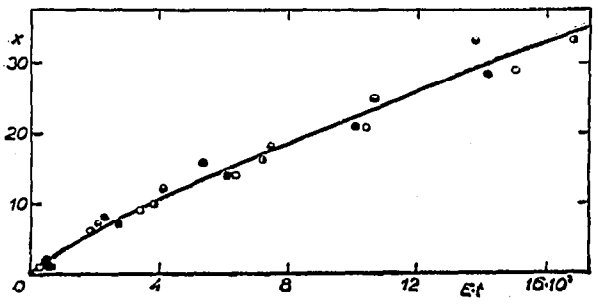

Fig. 7. Example of dependence of the distance x (cm) reached by the maximum of the strip (Neptunblau) on the product of time t (min) and potential gradient E (V/cm) at different potential gradients. (◒) 10.00; (○) 14.28; (◑) 20.00; (●) 28.57 V/cm. The dashed part of the curve is corrected for suction flow.

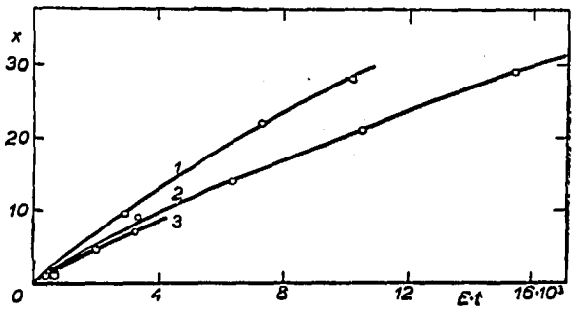

Fig. 8. Dependence of the distance x reached by the concentration maximum (Neptunblau) on the product of time t (min) and potential gradient E (V/cm) with different amounts of dye applied: Curve 1 – 0.10, curve 2 – 0.05, curve 3 – 0.02 ml of 0.1% solution.

paper) in relation to the swelling-water b (number of ml of water in 1 g of dry paper) were calculated with the equation:

$$q = \frac{V}{n}(c_0 - c) + bc \qquad (6)$$

In this equation V is the volume of the solution (cm^3), n the weight of the dry paper (g), c_0 the original and c the equilibrium concentration ($g.cm^{-3}$). For b 0.65 was taken.

The mobilities in free solution and the preliminary values of R_F are given in Table I.

Calculations. For the calculation of paper electrophoretic distribution isotherms GLUECKAUF's method[26,27] was used. GLUECKAUF based his calculation on DE VAULT's equation:

$$q' = \frac{w}{x} - \alpha \qquad (7)$$

where $q' = dq/dc$ (cm^3g^{-1}), x is the length of the column (expressed in grams of the immobile phase), α the pore space (cm^3g^{-1}), w the volume of the solvent used for elution (cm^3). By integrating this equation he obtained an expression which can be written in the form:

$$m = q_x x - (w - \alpha x)c_x \qquad (8)$$

Here x is the distance travelled by the sharp front of the zone after elution by the volume w, m is the whole amount of substance analysed in the column (g). This

equation for given m, w and x represents the linear dependence of the variables q and c and is the tangent to the distribution isotherm. Therefore:

$$c_{q=0} = -\frac{m}{w - \alpha x}; \quad q_{c=0} = \frac{m}{x} \tag{9}$$

By measuring the dependence of x on w the tangents may be constructed, the envelope of which is the distribution isotherm sought.

In this study we obtained from the measured curves the values q' and m/x. Since we measure the length of the column in cm and not in grams of swelled immobile

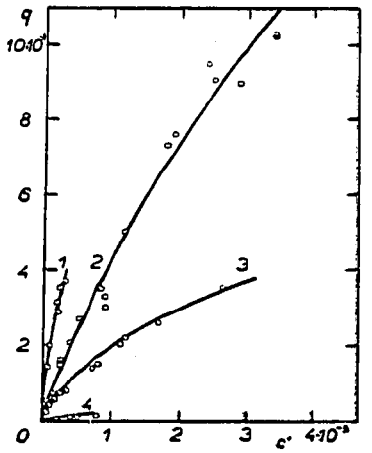

Fig. 9. Comparison of distribution isotherms from electrophoretic (lines) and static (rings) measurements. Curve 1 – Naphthol Black; curve 2 – Orange; curve 3 – Anthosin, curve 4 – Neptunblau.

phase and our amount q refers to the dry immobile phase it is necessary to correct these values adequately. The necessary data were taken from the previous papers[24,25]. The distance of the maximum of the concentration in the zone from the starting point was regarded as the length x.

In chromatography the quantity w has a clear physical meaning. It means the volume of the pores in the column, through which the substance being analysed would have passed during the experiment if no interaction with the immobile phase

TABLE I

VALUES OF ELECTROPHORETIC MOBILITIES ($cm^2 sec^{-1} V^{-1}$) OBTAINED BY DIRECT MEASUREMENT AND BY CALCULATION

Dye	R_F direct measur.	$U_0 \times 10^4$		$u_0 \times 10^4$	
		direct measur.	calc. from u_0 obtained electrophor.	electr. measur.	direct measur.
Neptunblau Bb extra	0.9	0.57	0.59	0.45	0.44
Anthosin 3BN	0.3	1.6	1.7	1.3	1.2
Orange G	0.2	1.4	1.8	1.4	1.1
Naphthol Black 10B	0.07	2.2	2.1	1.6	1.7

occurred. According to what was said in the introduction this quantity in electrophoresis has an analogous meaning. This analogy is more obvious if we use the volume velocities. For the volume electrophoretic mobility on paper v_0 (in $cm^4\ sec^{-1}V^{-1}$) the following equation holds:

$$Au_0Et = v_0Et = w \qquad (10)$$

Here A is the area of pores on the cross section of the column.

In the calculation we select the value of v_0 such that the envelope of the tangents is identical with the statically determined distribution isotherm. An example of such a calculation is shown in Table II, the results being given in Figs. 9 and 10. The values of v_0 are collected in Table I.

TABLE II

EXAMPLE OF THE MEASURED AND CALCULATED VALUES FOR THE CONSTRUCTION OF THE DISTRIBUTION ISOTHERM

(Anthosin 3BN; $m = 2.4 \cdot 10^{-4}g$; $v_0 = 8.15 \cdot 10^{-4}cm^4min^{-1}V^{-1}$)

x cm	$Et \times 10^{-3}$ $cm^{-1}min\ V$	x g	αx cm^3	$\frac{m}{x} \times 10^3$	dq/dc cm^3g^{-1}
1	1.8	0.0566	0.100	4.23	0.07
2	3.4	0.113	0.201	2.12	0.55
3	5.4	0.169	0.302	1.41	0.8
6	13.4	0.340	0.604	0.706	1.3
12	32.4	0.679	1.21	0.353	2.0
16	44.6	0.906	1.61	0.265	2.2
18	52.5	1.02	1.81	0.235	2.3
27	88.0	1.53	2.72	0.156	2.7
31	107.5	1.75	3.12	0.136	2.8

DISCUSSION

From these results it follows that there is a relation between paper chromatography and paper electrophoresis. This is proved by the fact that the distribution isotherms obtained by GLUECKAUF's method based on electrophoretic measurements are identical with isotherms obtained by the static method. This is true in cases where the electrophoretic mobility U_0 in free solution is practically not dependent on the concentration. Both isotherms may be made equal by an adequate choice of u_0. If both isotherms were different in shape, no u_0 could be selected that would make them identical. Therefore it is clear that the quantity u_0 has the meaning of the linear macroscopic velocity defined in the introduction by equations (1) and (2). The value of u_0 obtained by this method may be compared with the value of U_0 calculated from equation (1) using direct measurement of the electrophoretic mobility in free solution. This comparison is shown in Table I.

Table I and Fig. 10a–c show that the values u_0 obtained by both methods agree satisfactorily and that the isotherms are equal both in shape and in absolute values. Only the substances with R_F values close to unity (*e.g.* Neptunblau, Fig. 10d) inter-

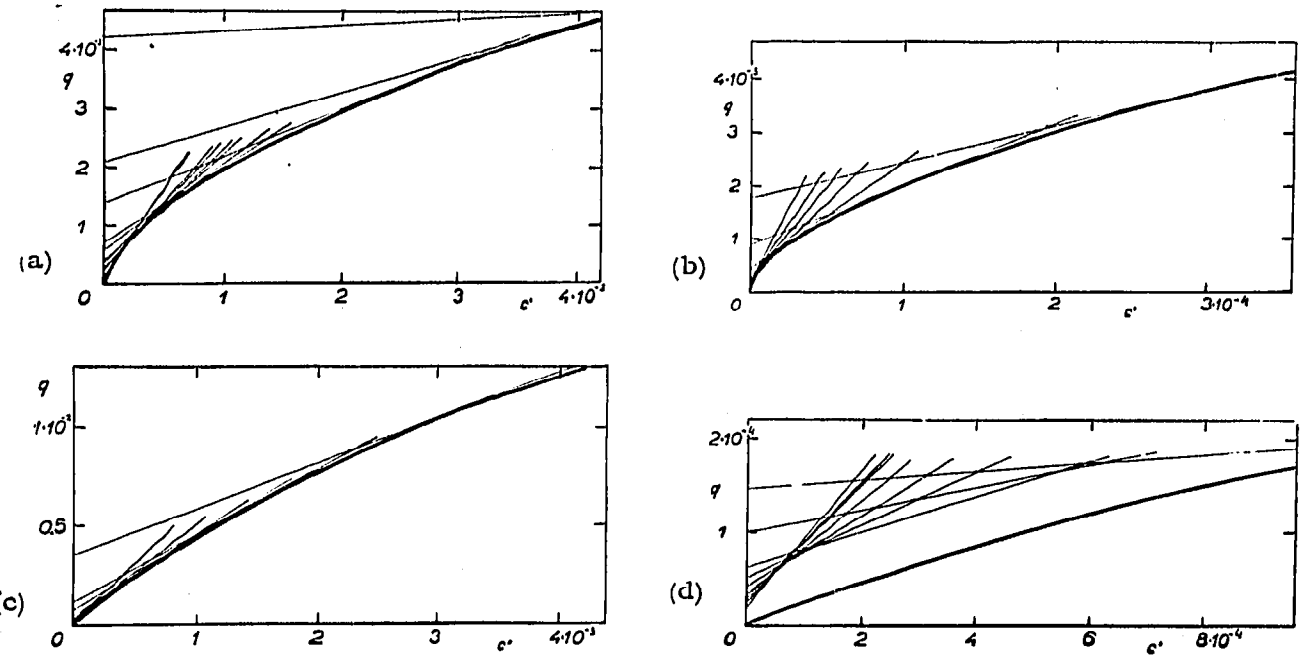

Fig. 10. Construction of tangents and envelope. a = Orange, b = Anthosin, c = Naphthol Black, d = Neptunblau.

act with the immobile phase so weakly that their electrophoretic mobility on paper may be influenced by the concentration dependence of the electrophoretic mobility U_0.

The mobility u_0 was calculated from the linear macroscopic velocity for the potential gradient equal to unity. The experiments were performed with different potential gradients in a sufficiently large region. This proves that the electric field has so far no influence on substance distribution between the immobile and mobile phase. This confirms that there is no fundamental difference between electrophoresis and chromatography on paper as regards the nature of the interaction of the substances with the immobile phase. Chromatographic and electrophoretic separation processes differ mainly in the fact that in electrophoresis the different substances have different mobilities u_0, whereas in chromatography the corresponding quantity, the velocity of the solvent, is the same for all substances. (For a kinetic model for diffusion in an electrical field see ref.[28]). From this fact certain conclusions can be drawn.

Let us define the separation factors for chromatography, F_c, for electrophoresis on paper, F_{ep}, and electrophoresis in free solution, F_{ef}, as follows:

$$F_c = \frac{(R_F)_1 - (R_F)_2}{(R_F)_1 + (R_F)_2}; \quad F_{ep} = \frac{U_1 - U_2}{U_1 + U_2}; \quad F_{ef} = \frac{(U_0)_1 - (U_0)_2}{(U_0)_1 + (U_0)_2} \qquad (11)$$

The indices 1 and 2 correspond to the two different components of the mixture being analysed. For the sake of simplicity the dependence on concentration is not considered. It is evident that the factors F may reach values between -1 and $+1$ for substances travelling in the electrical field in the same direction. (In the opposite case the choice between electrophoresis and chromatography is evident *a priori* without any cal-

culations.) The separation will be the better, the greater the absolute value of the separation factor. From eqns. (3) and (11) it follows that:

$$\frac{1 + F_{ep}}{1 - F_{ep}} \cdot \frac{1 - F_{ef}}{1 + F_{ef}} = \frac{1 + F_c}{1 - F_c} \tag{12}$$

Let us designate the fractions $(1 + F_c)/(1 - F_c) = (R_F)_1/(R_F)_2 = \varphi_c$, $(1 + F_{ep})/(1 - F_{ep}) = U_1/U_2 = \varphi_{ep}$ and $(1 + F_{ef})/(1 - F_{ef}) = (U_0)_1/(U_0)_2 = \varphi_{ef}$. The quantities φ have also the meaning of separation factors. Equation (12) can then be written in a simple form:

$$\varphi_c \varphi_{ef} = \varphi_{ep} \tag{13}$$

It is evident that $\varphi = 1$ when $F = 0$, $\varphi > 1$ for $F > 0$ and $0 < \varphi < 1$ for $F < 0$.

By means of these separation factors we can determine which method is more effective for the separation: paper electrophoresis or paper chromatography or if both methods are equal. (A comparison is shown here for a "one phase" system, *i.e.* under conditions not usually used in chromatography. These conditions may, however, have more general validity.) The chromatographic method is more advantageous than paper electrophoresis ($|F_c| > |F_{ep}|$) when, *e.g.*, $1/\varphi_c > \varphi_{ef} > 1/\varphi_c^2$ for $F_c > 0$, $F_{ep} < 0$, as in the case of the analysis of the mixture of Neptunblau and Orange, where $F_c = 0.51$, $F_{ep} = -0.06$ (concentration 0.1%, see Fig. 11b, c) and $F_{ef} = -0.42$; $\varphi_c = 3.09$, $\varphi_{ep} = 0.89$ and $\varphi_{ef} = 0.41$. Separation by free electrophoresis is better than the use of paper electrophoresis ($|F_{ef}| > |F_{ep}|$) when, *e.g.*, $1 < \varphi_c < 1/\varphi_{ef}$ for $F_{ep} < 0$, $F_{ef} < 0$. This would occur when analysing the dyes mentioned above.

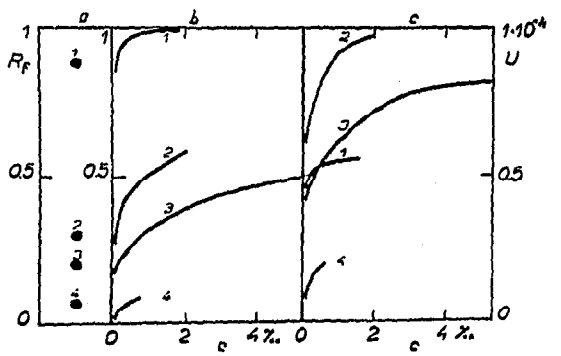

Fig. 11. Measured values of R_F (a) and the concentration dependencies of R_F (b) and U (c) calculated from electrophoretic measurements. Curve 1 – Neptunblau; curve 2 – Anthosin; curve 3 – Orange; curve 4 – Naphthol Black.

The use of paper electrophoresis is on the contrary advantageous ($|F_{ep}| > |F_{ef}|$) when, *e.g.*, $\varphi_c > 1/\varphi_{ef}^2$ for $F_{ef} < 0$, $F_{ep} > 0$. This would occur in the analysis of a mixture of Anthosin and Naphthol Black, where $F_{ep} = 0.73$ (concentration 0.05%) and $F_{ef} = -0.16$; $\varphi_{ep} = 6.43$, $\varphi_{ef} = 0.73$; similary in other cases.

DE VAULT's equation was derived under the following assumptions: (1) The equilibrium of the distribution process is very quickly reached; the rate of the diffusion of the substances to the surface is much greater then the rate of motion of the

zones. (2) The process is reversible. (3) The linear velocity of the movement is so great that it is practically not influenced by longitudinal diffusion. (4) The column is a homogeneous medium as regards all its properties; additional diffusion and gradients other than longitudinal concentration gradient do not occur. From the results of this study it follows that these assumptions are fulfilled with sufficient accuracy in electrophoresis on paper under the experimental conditions used.

Only the assumption of longitudinal diffusion is not exactly fulfilled, because the frontal boundary is slightly "diffuse". For the calculation, the velocity of the concentration maximum was used, and for m the amount actually applied was taken. An informative calculation with a correction for the diffusion was carried out and it was found that this correction results in a maximum deviation of 5% in the value of u_0. This deviation is lower than the errors of the other measurements. It is obvious that longitudinal diffusion does not influence the results to a greater extent than the results of analogous calculations in chromatography.

From the electrophoretic measurements both the value of the chromatographic R_F and its dependence on concentration may be calculated. From the value of the electrophoretic mobility in free solution it is possible (the correction factor f and the dependence on concentration of R_F being known) to calculate the electrophoretic mobility of the substance in the paper and the dependence of the mobility on the concentration. In Fig. 11a the chromatographic R_F obtained directly is shown. Fig. 11b shows the dependence of R_F on the concentration calculated from electrophoretic measurements. Fig. 11c shows the dependence of the electrophoretic mobility on paper on the concentration calculated from the former measurements and from the values of the electrophoretic mobility in free solution (with the assumption that it is independent of concentration). Fig. 11 confirms the qualitative agreement of calculated and measured values of R_F and illustrates the considerations concerning the separation factors introduced.

The results given in the Table I and Fig. 11 may be taken as proof of the validity of the ideas presented in the introduction of this paper.

SUMMARY

Assumptions concerning the similarity of paper chromatography and paper electrophoresis as regards the nature of the interaction of the substances analysed with the immobile phase were verified.

GLUECKAUF's method for constructing distribution isotherms from chromatographic measurements was applied to electrophoretic measurements. It was proved that the isotherms obtained in this way are identical with the isotherms obtained by the direct method, when the dependence of the mobility in free solution on the concentration can be neglected.

The values of R_F, the electrophoretic mobility in free solution U_0 and the velocity u_0 obtained both by direct measurement and by calculation were proved to be identical.

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