CHROM. 6100

PEAK BROADENING IN PAPER CHROMATOGRAPHY AND RELATED TECHNIQUES

VIII. PEAK BROADENING IN THIN-LAYER ELECTROPHORESIS

C. L. DE LIGNY AND N. G. VAN DER VEEN

Laboratory for Analytical Chemistry, University of Utrecht, Croesestraat 77A, Utrecht (The Netherlands)

(Received March 17th, 1972)

SUMMARY

Peak dispersion (*i.e.* the separation efficiency) in thin-layer electrophoresis was investigated and compared for six different cellulose layers.

The relative importance of longitudinal diffusion and of macroscopic inhomogeneities in the electric field and in the electroosmotic and sucking flow have been assessed.

INTRODUCTION

In a series of investigations on peak broadening in paper (PC) and thin-layer chromatography (TLC) the following mechanisms of peak dispersion have been demonstrated:

(1) Longitudinal diffusion in the mobile phase[1,2].

(2) Longitudinal diffusion in the stationary phase[1,2].

(3) Slow mass transfer between the mobile and the stationary phase (caused mainly by slow diffusion in the mobile phase)[2-4].

(4) The velocity profile of the sample components, caused by the macroscopic mobile phase velocity profile[5].

In paper and thin-layer electrophoresis the situation is much simpler, as in this case there is only a solvent and the inert support for it. Thus peak dispersion can only be caused by:

(1) Longitudinal diffusion in the solvent.

(2) The velocity profile of the sample components, due to macroscopic inhomogeneities in the electric field, and in the electro-osmotic and sucking flow (the latter caused by evaporation of the solvent).

So, peak dispersion in paper and thin-layer electrophoresis is governed by the simple equation:

$$H = \frac{\sigma^2}{|\mu|} = \frac{B}{|u|} + C_F(u_{eph}, u_{eo})L^2 \cdot |u| \qquad (1)$$

or

$$\sigma^2 = Bt_{diff} + C_F(u_{eph},u_{eo})L^2u^2t_{eph} \quad (1a)$$

or

$$\sigma^2/t_{eph} = B\frac{t_{diff}}{t_{eph}} + C'_F(u_{eph},u_{eo})L^2 \quad (1b)$$

where

H = height equivalent to a theoretical plate,
σ = standard deviation of the solute distribution in the medium used for electrophoresis,
μ = distance travelled by the solute,
u = velocity of the solute,
B = $2\gamma D$,
γ = tortuosity factor,
D = diffusion coefficient of the solute,
$C_F(u_{eph},u_{eo})$ = function, describing the effect of macroscopic inhomogeneities*,
$C'_F(u_{eph},u_{eo})$ = $C_F(u_{eph},u_{eo})u^2$,
u_{eph} = velocity of the electrophoretic transport of the solute,
u_{eo} = velocity of the transport by the electro-osmotic and sucking flow,
L = dimension, characteristic for the velocity profile (either the breadth of the paper or thin-layer strip scanned by the densitometer, or the mean distance between maxima or minima in the velocity profile, whichever is the smaller),
t_{diff} = diffusion time,
t_{eph} = electrophoresis time.

It follows from eqns. 1, 1a and 1b that the value of $C_F(u_{eph}, u_{eo})$ can be determined in two ways:

(1) By measuring peak dispersion as a function of u (in contrast to the case of PC or TLC, u can be varied easily, *viz.* by varying the field strength).

(2) By measuring peak dispersion as a function of L (the method of choice in the case of PC or TLC[5]).

In this paper, peak dispersion in thin-layer electrophoresis is investigated for three amino acids on six different cellulose layers. Our aims were to assess the relative importance of the B/u and $C_F(u_{eph},u_{eo})u$ terms and to compare the peak dispersion (*i.e.*, the separation efficiency) for the six different cellulose layers. Further, the magnitude of the electro-osmotic and sucking flow was determined.

EXPERIMENTAL

Materials

L-Leucine, L-glutamic acid, L-aspartic acid (Fluka). D(+)-Xylose (Hoffmann-La Roche).

Camag DS-0, Merck, Schleicher and Schüll 1440 prefabricated cellulose thin-layer plates; Macherey and Nagel 300, Macherey and Nagel 300/starch (50:1) and Whatman CC 41 home-made cellulose thin-layer plates (layer thickness 0.25 mm).

* The exact functional form of $C_F(u_{eph},u_{eo})$ is derived in the APPENDIX.

Apparatus

Shandon SAE 2525 electrophoresis apparatus and a Vitatron Model 705 densitometer.

Procedure

The variance of the solute distribution, originating from the application procedure and the equilibration period (σ_o^2), was determined as follows: by removing part of the cellulose, each layer was divided into four parallel strips, separated by *ca.* 1-cm wide strips of bare glass.

Streaks of the amino acids were applied as described before[6]. The plate was then sprayed with buffer solution (1 ml of pyridine and 10 ml of acetic acid in 500 ml of water, pH = 3.5) and placed in the electrophoresis apparatus.

Each strip was connected individually with the buffer reservoirs by means of paper strips, an uncoated glass plate was placed upon the paper strips to decrease evaporation, and the apparatus was left to equilibrate for an hour, all the while being cooled by tap-water (temperature of the cellulose layer 15 °). The plates were then dried, stained and scanned (using a slit length L of 0.4 cm) and σ_o^2 was determined as described before[6].

The variance of the solute distribution, originating from the application procedure, the equilibration period and diffusion (σ_{oV}^2), was determined as follows: after the equilibration period, the apparatus was left for another 5½ h. The variance, originating from the diffusion (σ^2_{diff}), and the tortuosity factor were calculated from the equation:

$$\sigma_{oV}^2 - \sigma_o^2 = \sigma_{diff}^2 = 2\gamma D t_{diff} \qquad (2)$$

D was calculated according to the equation proposed by WILKE AND CHANG[7], as modified by BIDSTRUP AND GEANKOPLIS[8].

The variance of the solute distribution after electrophoresis (σ_{xV}^2) was determined as follows: streaks of the amino acid mixture were applied instead of streaks of the individual amino acids. After the equilibration period, the connections between the cellulose strips and the buffer reservoirs were removed, except for one strip, and electrophoresis on this strip was carried out, applying a potential difference of 300 V for 3 h to the electrodes.

Then these connections were removed, and another strip was connected up and electrophoresis carried out on this by applying 600 V for 1½ h. Electrophoresis on the third and fourth strips was carried out simultaneously by applying 900 V for 1 h. The plate was then removed from the apparatus. Four to eight plates of each kind were used.

It follows from eqn. 1a that the $C_F(u_{eph},u_{eo})$ term can be calculated by:

$$\sigma_{xV}^2 - \sigma_{oV}^2 = C_F(u_{eph},u_{eo})L^2u^2t_{eph} \qquad (3)$$

σ^2, holding for electrophoresis at 900 V on Camag DS-0 plates ,was determined as a function of the streak length L scanned by the densitometer as described below. L ranged from 0.4 to 2.8 cm; five plates were used.

According to eqn. 1b, the increase of σ^2/t_{eph} over its value at L = 0.4 cm, $\Delta\sigma^2/t_{eph}$, is equal to:

$$\Delta\sigma^2/t_{eph} = C'_F(u_{eph},u_{eo})\Delta L^2 \qquad (4)$$

where ΔL^2 is the increase of L^2 over 0.16 cm^2. C_F' and C_F can be calculated with this equation.

Determination of σ^2 as a function of L. After staining, each streak was divided in q parts of length a, so that $q \cdot a = b$, where a is the applied slit length of the densitometer and b is the total length of the streak. The variance, σ^2, and the position of the maximum, μ, of the solute distribution were determined for each part.

As a result of the inhomogeneities in the electric field and in the electro-osmotic and sucking flow over the distance b, each part of the streak has its own individual value of σ^2 and μ. So, the result of the measurements on one streak is:

$$\mu_1, \mu_2, \ldots \mu_i \ldots \mu_q$$

$$\sigma_1^2, \sigma_2^2, \ldots \sigma_i^2 \ldots \sigma_q^2$$

σ_p^2, for the variance of a composite part of the streak composed of p neighbouring strips (breadth equal to $L = p \cdot a$; $p = 1,2,3 \ldots . q$) is now introduced. It follows from the principle of additivity of independent variances that the total variance of the solute distribution in the considered composite part of the streak is equal to the sum of the variance within a single strip and the variance between strips:

$$\sigma_p^2 = \overline{\sigma_i^2} + \overline{\frac{1}{p} \sum_1^p (\mu_i - \mu_p)^2} = \frac{1}{q} \sum_1^q \sigma_i^2 + \frac{1}{rp} \sum_1^r \sum_1^p (\mu_i - \mu_p)^2 \qquad (5)$$

where

$$\mu_p = \frac{1}{p} \sum_1^p \mu_i \qquad (6)$$

and r is the largest entire number $\leq q/p$.

The osmotic and sucking flow was determined by applying spots of D(+)-xylose on to the plates at regular intervals and applying a potential difference of 900 V for 1 h to the electrodes. The spots were stained with aniline–phthalic acid reagent and the distances travelled were measured.

RESULTS AND DISCUSSION

Table I shows that, as regards the variance of the solute distribution originating from the application procedure and the equilibration period, the plates can be divided into a "good" group, consisting of Schleicher and Schüll 1440, Merck

TABLE I

VARIANCE OF THE SOLUTE DISTRIBUTION, ORIGINATING FROM THE APPLICATION PROCEDURE AND THE EQUILIBRATION PERIOD (σ_0^2, cm^2) AND ITS STANDARD ERROR

Type of plate	σ_0^2			
	Leucine	*Glumatic acid*	*Aspartic acid*	n[a]
Schleicher and Schüll 1440	0.055 ± 0.004	0.055 ± 0.008	0.048 ± 0.004	4
Merck	0.067 ± 0.003	0.055 ± 0.003	0.047 ± 0.004	5
Macherey and Nagel 300/starch	0.037 ± 0.002	0.039 ± 0.002	0.039 ± 0.002	14
Macherey and Nagel 300	0.068 ± 0.003	0.065 ± 0.005	0.059 ± 0.005	5
Camag DS-0	0.125 ± 0.009	0.098 ± 0.007	0.073 ± 0.007	5
Whatman CC 41	0.077 ± 0.007	0.062 ± 0.007	0.069 ± 0.004	4

[a] n = number of determinations.

and Macherey and Nagel 300/starch, and a "poor" group, consisting of Macherey and Nagel 300, Camag DS-0 and Whatman CC 41.

Table II shows that, as regards peak dispersion by diffusion (which is characterised by the values of γ) the same two groups can be distinguished. The improvement on addition of 2 wt% starch in the case of Macherey and Nagel 300 is remarkable. For comparison, the γ-values that hold for (chromatography of) amino acids in a *n*-butanol–acetic acid–water mixture (4:1:5)[3] are given in the last column of Table II. They appear to be smaller than the γ-values that hold (for electrophoresis) in an aqueous buffer, presumably because the cellulose grains are more swollen in the latter medium.

It appeared that the values of the left-hand side of eqn. 3 did not differ significantly from zero, neither for the whole set of data, nor for various sub-sets that may be envisaged. So, the $C_F(u_{eph},u_{eo})$ term is too small to be detected in this way, or, in other words, the application of up to 900 V in the Shandon SAE 2525 electrophoresis apparatus has no measurable adverse effect on peak dispersion and resolution. On the other hand, it has the very desirable effect of decreasing separation time, and hence of decreasing peak dispersion by diffusion and improving the resolution of mixtures.

Fig. 1 shows that $\Delta(\sigma^2/t_{eph})/\Delta L^2$ decreases with increasing L and finally becomes equal to 0. Probably, the mean distance between maxima and minima in the velocity profile is equal to only a few times the densitometer slit length a. When L exceeds this mean distance, σ^2/t becomes independent of L. Therefore

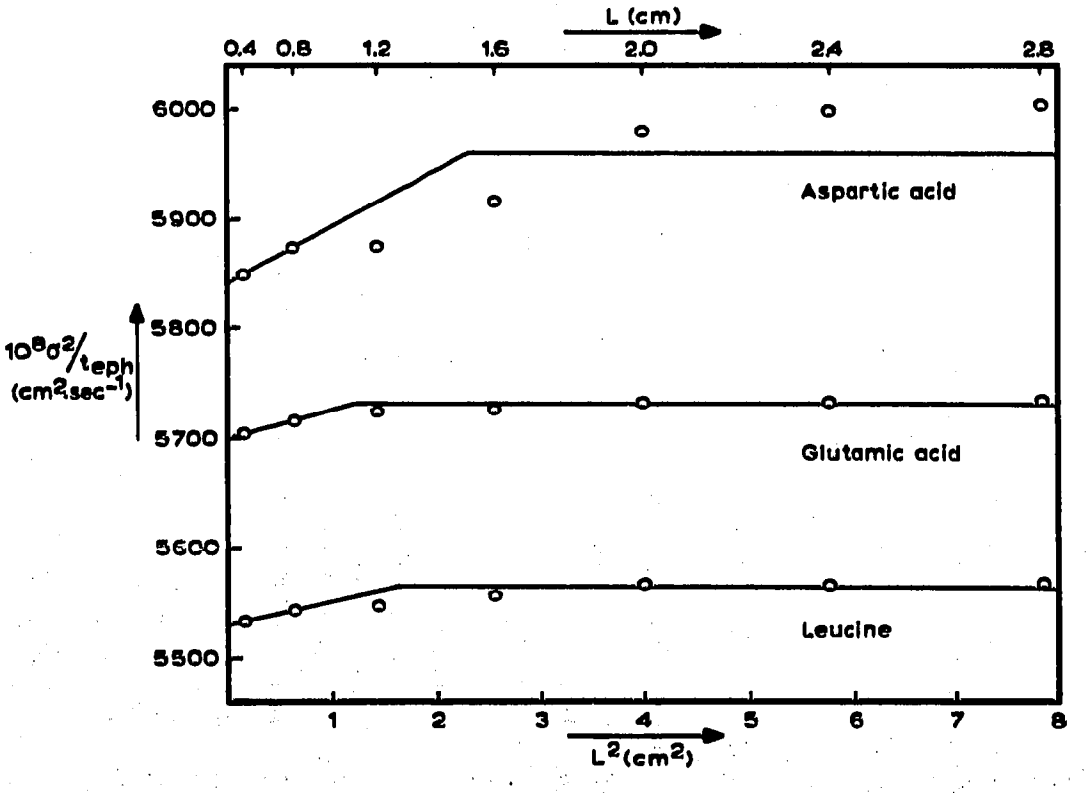

Fig. 1. σ^2/t_{eph} as a function of L (streak length, scanned by the densitometer).

TABLE II

VARIANCE OF THE SOLUTE DISTRIBUTION, ORIGINATING FROM THE APPLICATION PROCEDURE, THE EQUILIBRATION PERIOD AND DIFFUSION (σ^2_{ov}, cm^2), THE TORTUOSITY FACTOR γ, AND THEIR STANDARD ERRORS

Type of plate	*Leucine*[a]		*Glutamic acid*[b]		*Aspartic acid*[c]		γ	γ_{chrom}[e]
	σ^2_{ov}	n[d]	σ^2_{ov}	n	σ^2_{ov}	n		
Schleicher and Schüll 1440	0.206 ± 0.014	3	0.218 ± 0.008	3	0.189 ± 0.007	3	0.58 ± 0.02	0.37 ± 0.10
Merck	0.225 ± 0.015	6	0.179 ± 0.005	4	0.196 ± 0.018	6	0.55 ± 0.02	
Macherey and Nagel 300/starch	0.184 ± 0.007	3	0.162 ± 0.010	3	0.184 ± 0.020	3	0.53 ± 0.02	
Macherey and Nagel 300	0.306 ± 0.024	7	0.261 ± 0.015	7	0.274 ± 0.016	7	0.82 ± 0.03	0.52 ± 0.33
Camag DS-0	0.267 ± 0.022	3	0.306 ± 0.012	3	0.275 ± 0.009	3	0.71 ± 0.02	0.42 ± 0.29
Whatman CC 41	0.319 ± 0.017	5	0.271 ± 0.027	3	0.266 ± 0.016	5	0.82 ± 0.03	0.40 ± 0.08

[a] D (leucine, 15°) = 6.0 × 10^{-6} cm^2·sec^{-1}.

[b] D (glutamic acid, 15°) = 5.5 × 10^{-6} cm^2·sec^{-1}.

[c] D (aspartic acid, 15°) = 5.3 × 10^{-6} cm^2·sec^{-1}.

[d] n = number of determinations.

[e] γ_{chrom} = tortuosity factor, holding for chromatography of amino acids by an *n*-butanol–acetic acid– water mixture (4:1:5) (ref. 3).

TABLE III

CALCULATION OF $C_F(u_{eph}, u_{eo})$ AND K FOR CAMAG DS-0 PLATES FROM PEAK DISPERSION DATA (HOLDING FOR ELECTROPHORESIS AT 900 V) AS A FUNCTION OF THE SCANNED STREAK LENGTH

Accuracy is given in terms of standard deviation. λ_R, d_p, u', u'', C_L', C_L'' and K are defined in the APPENDIX.

	Leucine	*Glutamic acid*	*Aspartic acid*
$\Delta(\sigma^2/t_{eph})/\Delta L^2$ (cm^{-1})	$(21 \pm 3) \times 10^{-8}$	$(23 \pm 6) \times 10^{-8}$	$(49 \pm 14) \times 10^{-8}$
u($cm \cdot sec^{-1}$)	-0.70×10^{-3}	0.17×10^{-3}	1.27×10^{-3}
$C_F(u_{eph}, u_{eo})$ ($cm^{-2} \cdot sec$)	0.43	8.0	0.30
$\lambda_R d_p$ (cm) (ref. 5)	0.26×10^{-3}	0.26×10^{-3}	0.26×10^{-3}
γD ($cm^2 \cdot sec^{-1}$)	4.3×10^{-6}	3.9×10^{-6}	3.3×10^{-6}
$(u'C_L'/C_L''+u'')^2K$ ($cm^2 \cdot sec^{-2}$)	$(47 \pm 7) \times 10^{-14}$	$(45 \pm 12) \times 10^{-14}$	$(88 \pm 25) \times 10^{-14}$
u'($cm \cdot sec^{-1}$)	-0.39×10^{-3}	0.48×10^{-3}	1.59×10^{-3}
u''($cm \cdot sec^{-1}$)	-0.31×10^{-3}	-0.31×10^{-3}	-0.31×10^{-3}
K	$5 \pm 1 \times 10^{-6}$		
C_L'/C_L''	-0.04 ± 0.03		

$\Delta(\sigma^2/t_{eph})/\Delta L^2$ was calculated from the data at $L = 0.4$ and 0.8 cm. The results are shown in Table III. It appears that the values of $C_F(u_{eph},u_{eo})$ are about an order of magnitude larger than the corresponding values, holding for TLC[5]. They are further commented upon in the APPENDIX.

In the case of leucine, the following equation holds for the plate height:

$$\frac{\sigma_{xV}^2 - \sigma_0^2}{|\mu|} = \frac{B}{|u|} + C_F(u_{eph},u_{eo})L^2 \cdot |u| =$$

$$= \frac{8.5 \times 10^{-6}\ cm^2 \cdot sec^{-1}}{|u|} + (0.4\ cm^{-2} \cdot sec)L^2 \cdot |u| \qquad (7)$$

For $L = 0.4$ cm, the right-hand side has a minimum value of 1.5×10^{-3} cm for $|u| = 1.15 \times 10^{-2}$ $cm \cdot sec^{-1}$, corresponding to an applied voltage of 15000 V, or a field strength of 700 V/cm.

APPENDIX

Derivation of the functional form of C_F (u_{eph},u_{eo})

This derivation is analogous to that given earlier for longitudinal and radial dispersion of matter by fluid flow through packed beds[9].

KLINKENBERG AND SJENITZER[10] have shown that when the molecules of a sample must choose a number of times n between two situations P and Q, and where in the former they do a step p backward and in the latter they do a step q forward relative to the mean position of the sample, a distribution is generated with a variance equal to:

$$\sigma^2 = npq \qquad (8)$$

We suppose that the bed is an arrangement of equal numbers of nearly cylindrical volumes P and Q that are passed by the sample molecules in a random

way. The velocity of the sample in the volumes P and Q shows small deviations (negative and positive, respectively) from the mean velocity in the column, *i.e.*, the volumes P and Q are characterised* by a

longitudinal velocity $u'(1 - C'_L) + u''(1 - C''_L)$ and $u'(1 + C'_L) + u''(1 + C''_L)$, respectively,

radial velocity $-u'C'_R - u''C''_R$ and $u'C'_R + u''C''_R$, respectively,

length $C_1 \dfrac{u'(1 - C'_L) + u''(1 - C''_L)}{u' + u''} d_p$ and $C_1 \dfrac{u'(1 + C'_L) + u''(1 + C''_L)}{u' + u''} d_p$, respectively,

radius $C_2 d_p$,

where the C's are constants. The primed quantities are related to the transport by electrophoresis, the doubly primed quantities to the transport by the electro-osmotic and sucking flow. d_p is the mean diameter of the support particles.

The mean longitudinal and radial velocities are then $u' + u'' = u$ and o, respectively, and the mean length of a volume is $C_1 d_p$. So, the number of volumes which a molecule would pass (in the absence of a radial velocity component and in the absence of diffusion) when it traverses a length l of the bed, *i.e.*, the number of times the components of its velocity may change, is:

$$n_1 = \frac{|l|}{C_1 d_p} \tag{9}$$

The mean time τ_f needed to reach a neighbouring volume by radial flow is:

$$\tau_f = \frac{C_3 d_p}{|u'C'_R + u''C''_R|} \tag{10}$$

The time t spent in traversing the bed length l is equal to l/u. The number of volumes which the molecule passes in this time, *i.e.*, the number of times its velocity components may change, is:

$$n_2 = \frac{t}{\tau_f} = \frac{l}{C_3 d_p} \frac{|u'C'_R + u''C''_R|}{u} \tag{11}$$

The mean time τ_d needed to reach a neighbouring volume by lateral diffusion is equal to:

$$\tau_d = \frac{C_4 d_p^2}{D} \tag{12}$$

* It is immaterial whether or not the longitudinal and radial velocities are correlated, as supposed here for ease of formulation.

The number of volumes which the diffusing molecule passes in the time $t = l/u$, *i.e.* the number of times its velocity components may change, is:

$$n_3 = \frac{t}{\tau_d} = \frac{lD}{C_4 d_p^2 u} \tag{13}$$

So, the total number of times the velocity components of a molecule may change, is:

$$n = n_1 + n_2 + n_3 = |l| \cdot \left(\frac{1}{d_p} \frac{|C_5 u' + C_6 u''|}{|u|} + \frac{D}{C_4 d_p^2 \cdot |u|} \right) \tag{14}$$

The mean residence time in a volume is $t/n = l/un$. The backward and forward steps p and q during this residence time are:

$$p,q = \frac{l}{n} \frac{C'_L u' + C''_L u''}{u} \tag{15}$$

So, the resulting variance is:

$$\sigma^2 = npq = \frac{l^2}{n} \left(\frac{C'_L u' + C''_L u''}{u} \right)^2 = |l| \left(\frac{C'_L u' + C''_L u''}{u} \right)^2 \cdot$$

$$\cdot \frac{d_p^2}{d_p(|C_5 u' + C_6 u''|) + D/C_4} \cdot |u| \tag{16}$$

When u' and u'' are o, we get the results for electroosmosis and electrophoresis only:

$$\frac{\sigma^2}{|l|} = \frac{(C''_L)^2 d_p^2}{C_6 d_p \cdot |u| + D/C_4} |u| \text{ and } \frac{(C'_L)^2 d_p^2}{C_5 d_p \cdot |u| + D/C_4} |u|, \text{ respectively,} \tag{17}$$

or, as it is usually written:

$$\frac{\sigma^2}{|\mu|} = \frac{2K'' d_p^2}{\lambda''_R d_p \cdot |u| + \gamma D} |u| \text{ and } \frac{2K' d_p^2}{\lambda'_R d_p \cdot |u| + \gamma D} |u|, \text{ respectively.} \tag{18}$$

The coefficients for radial convective dispersion, $C_6 = \lambda_R''$ and $C_5 = \lambda_R'$, will be approximately equal to λ_R holding for chromatography.

Further, eqns. 16–18 give the dispersion due to microscopic inhomogeneities, on a scale of the order of the particle diameter d_p. This dispersion is proportional to d_p^2. It is decreased by radial convective ($\lambda_R d_p \cdot |u|$) and diffusive (γD) dispersion.

It is conceivable that the dispersion due to macroscopic inhomogeneities, on a scale of the order L, will be proportional to L^2. The analogon of eqn. 16 is then:

$$H = \frac{\sigma^2}{|l|} = \left(\frac{u' C'_L / C''_L + u''}{u} \right)^2 \frac{2KL^2}{\lambda_R d_p \cdot |u| + \gamma D} |u| = C_F(u',u'') L^2 \cdot |u| \tag{19}$$

or:

$$\frac{\sigma^2}{t_{eph}} = (u' C'_L / C''_L + u'')^2 \frac{2KL^2}{\lambda_R d_p \cdot |u| + \gamma D} = C'_F(u',u'') L^2 \tag{19a}$$

where K is proportional to $(C_L'')^2$.

From the values of $\Delta(\sigma^2/t_{eph})\Delta L^2 = C_F'(u',u'')$ in Table III, values of

$$(u'C'_L/C''_L + u'')^2 K \equiv y^2$$

can be calculated. As u'' is constant, C_L'/C_L'' and K can be calculated by the simple linear regression equation:

$$y = a + bx = u''\sqrt{K} + \frac{C'_L}{C''_L}\sqrt{K}\,u' \qquad (20)$$

It appears that y is given by this equation within the estimated experimental error. The value for K is found to be $5 \pm 1 \times 10^{-6}$ and for C_L'/C_L'' -0.04 ± 0.03. As C_L'/C_L'' cannot be negative (the inhomogeneities in the electric field and in the electroosmotic flow must be correlated) it follows that C_L'/C_L'' is equal to zero, which means that the inhomogeneities in the field strength are negligible compared to those in the electro-osmotic and sucking flow.

This would occur for a model of the cellulose thin layer consisting of a bundle of parallel capillaries, the diameters of the capillaries being uniform over their whole length, but varying from one capillary to another.

So, for a good approximation the dispersion due to macroscopic inhomogeneities is given by the following equation:

$$H = \frac{\sigma^2}{|l|} = \left(\frac{u''}{u}\right)^2 \frac{2KL^2}{\lambda_R d_p \cdot |u| + \gamma D}\,|u| \qquad (21)$$

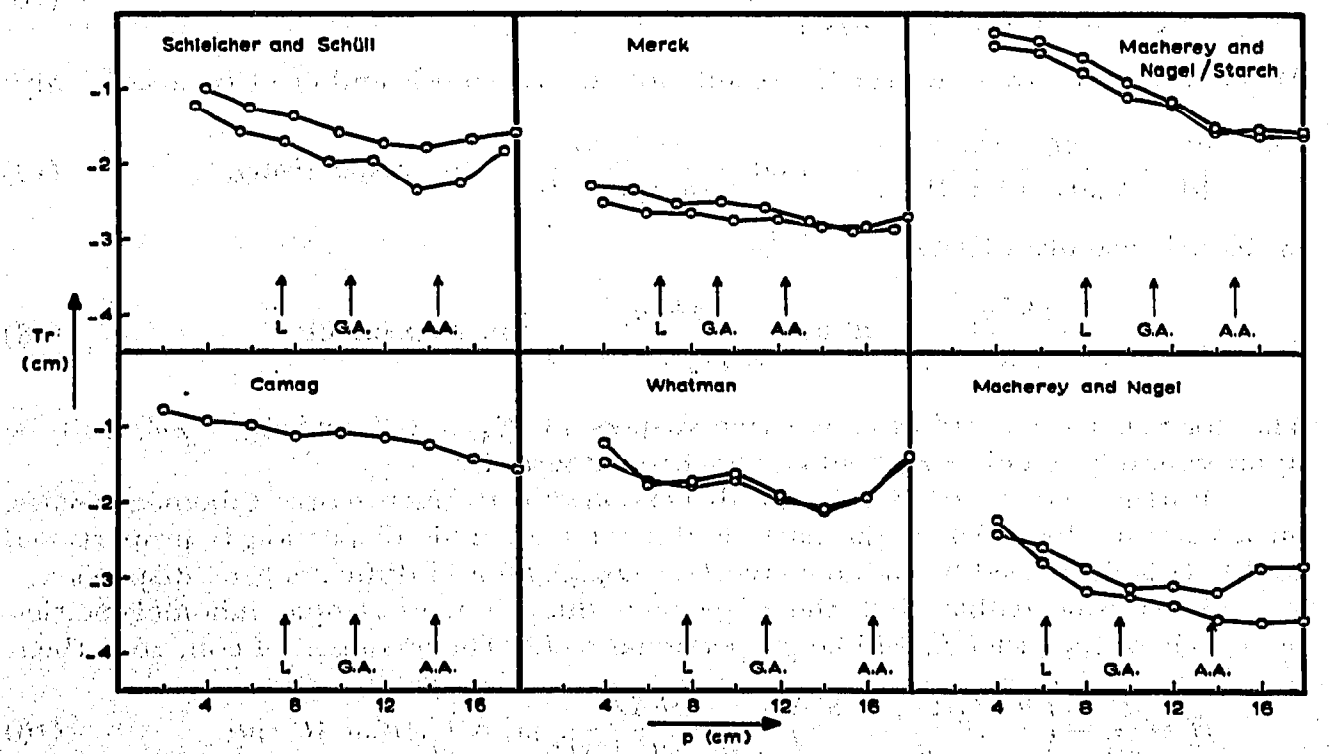

Fig. 2. Transport, Tr, of D(+)-xylose by the osmotic and sucking flow after exposure of the plates to a potential difference of 900 V for 1 h, as a function of its position p on the plate. The transport is directed toward the left (negative) side of the plate. The amino acid mixture is applied at p = 10 cm. The positions of the amino acids after electrophoresis are indicated by arrows (L = leucine, G. A. = glumatic acid, A.A. = aspartic acid). The graph for Camag DS-0 is the mean of determinations on four plates. For the other brands determinations on two plates were made which are shown separately.

A small value of u'', the electro-osmotic and sucking flow, is thus advantageous in high voltage thin-layer electrophoresis.

Fig. 2 shows that, of the "good" plates, Schleicher and Schüll 1440 pre-fabricated plates and Macherey and Nagel 300/starch home-made plates are superior to Merck pre-fabricated plates in this respect.

CONCLUSIONS

Schleicher and Schüll 1440 or Merck pre-fabricated cellulose thin-layer plates are superior to Camag DS-0 pre-fabricated and Macherey and Nagel 300 or Whatman CC 41 home-made plates, as regards peak dispersion by longitudinal diffusion. Addition of 2 wt % starch (to Macherey and Nagel 300 cellulose powder) results in plates that are also very good in this respect.

In the Shandon SAE 2525 electrophoresis apparatus application of the maximum voltage (1000 V) is advisable, not only to decrease separation time, but also to improve the resolution of mixtures. If cooling problems can be overcome, the best resolution in electrophoresis on cellulose thin-layer plates is obtained at a field strength of about 100 V/cm. Macroscopic inhomogeneities in the field strength are negligible compared to those in the electroosmotic and sucking flow. Schleicher and Schüll 1440 and Macherey and Nagel 300/starch plates are probably superior to Merck plates as regards peak dispersion by macroscopic inhomogeneities, and must be advocated therefore for high voltage thin-layer electrophoresis.

REFERENCES

1 C. L. De Ligny and D. Bax, *Z. Anal. Chem.*, 205 (1964) 333.
2 C. L. De Ligny and A. G. Remijnse, *J. Chromatogr.*, 35 (1968) 242.
3 C. L. De Ligny and A. G. Remijnse, *Rec. Trav. Chim. Pays-Bas*, 86 (1967) 410.
4 C. L. De Ligny and A. G. Remijnse, *J. Chromatogr.*, 35 (1968) 257.
5 C. L. De Ligny and W. Van De Meent, *J. Chromatogr.*, 53 (1970) 469.
6 C. L. De Ligny and A. G. Remijnse, *Rec. Trav. Chim. Pays-Bas*, 86 (1967) 421.
7 C. R. Wilke and Pin Chang, *Amer. Inst. Chem. Eng. J.*, 1 (1955) 264
8 D. E. Bidstrup and C. J. Geankoplis, *J. Chem. Eng. Data*, 8 (1963) 170.
9 C. L. De Ligny, *Chem. Eng. Sci.*, 25 (1970) 1177.
10 A. Klinkenberg and F. Sjenitzer, *Chem. Eng. Sci.*, 5 (1956) 258.