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PEAK BROADENING IN PAPER CHROMATOGRAPHY AND RELATED TECHNIQUES

V. CONDITIONS FOR MINIMUM SEPARATION TIME

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SUMMARY

In paper and thin-layer chromatography peak broadening is a function of the mean flow rate of the eluent, which in turn is a function of the distances of the starting point and solvent front from the eluent in the tank.

Starting from the relationship between peak broadening and the positions of starting point and solvent front, formulae are derived for the elution time for a given separation problem as a function of both these distances. It appears that the elution time is at a minimum for certain positions of starting point and solvent front.

A method is outlined for calculating this minimum value for the elution time and the corresponding positions of starting point and solvent front.

INTRODUCTION

Peak broadening in GLC can be described by the VAN DEEMTER equation, as modified by SIE AND RIJNDERS[1]:

$$H \equiv \frac{\sigma^2}{l} = B\,1/u + C_M u + C_S u + C_F(u)u \qquad (1)$$

where

H = height equivalent to a theoretical plate
σ = standard deviation of the solute distribution in the chromatography column
l = distance travelled by the solute
u = flow rate of the eluent

The four terms in this equation account for peak broadening by molecular diffusion, resistance to mass transfer in the mobile and the stationary phase and unevenness of flow, respectively.

This formula is also applicable to paper and thin-layer chromatography, when we introduce the following modifications:

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(a) The flow rate of the eluent, u, is not constant, but decreases with time. We observe the mean flow rate $\bar{u}$ and the mean plate height $\bar{H}$.

(b) In thin-layer chromatography peak broadening is exclusively caused by longitudinal diffusion and by resistance to mass transfer in the mobile phase[2].

(c) In paper chromatography the mass transfer term does not stem exclusively from the $C_M u$ term, in contrast to the situation in thin-layer chromatography. It is most likely that there is also a contribution from the $C_F(u)u$ term, whereas the $C_S u$ term is negligible, as in thin-layer chromatography[3].

Accounting for these facts, we have the following equations:

Thin-layer chromatography

$$\bar{H} = B\,\overline{1/u} + C_M\,\bar{u} \tag{2}$$

Paper chromatography

$$\bar{H} = B\,\overline{1/u} + C_M\bar{u} + \overline{C_F(u)u} \tag{3}$$

The various terms in these equations are equal to[3]:

$$B = 2\,\gamma_M D_M + 2\,\gamma_S D_S\,\frac{1 - R_F}{R_F} \tag{4}$$

$$C_M = 0.01\,\frac{k^2}{(1+k)^2}\,\frac{{d_p}^2}{D_M} = 0.01\,(1 - R_F)^2\,\frac{{d_p}^2}{D_M} \tag{5}$$

$$C_F(u) = \frac{2K\,L^2}{\lambda d_p u + \gamma_M D_M} \tag{6}$$

where

γ = tortuosity factor
D = diffusion coefficient
R_F = ratio of the distances, covered by the solute and by the eluent
k = ratio of the amounts of solute in the stationary and the mobile phase, at equilibrium
d_p = diameter of the support particles
d_f = thickness of the layer of stationary fluid
K = dimensionless constant depending on the flow profile (10^{-3}–10^{-5})
L = dimension characteristic for the flow profile
λ = dimensionless constant depending on the packing geometry (≈ 0.03)

In paper and thin-layer chromatography flow velocity depends on the distance of the solvent front from the surface of the eluent in the tank. Therefore the mean flow rate $\bar{u}$ and, consequently, the mean plate height $\bar{H}$ too, depend on the distances of starting point and solvent front from the surface of the eluent in the tank. Optimum values for these distances in respect of separation time for a specified separation problem can be derived.

In doing this, it is appropriate to deal with paper and thin-layer chromatography separately because of the different C terms.

SEPARATION TIME IN THIN-LAYER CHROMATOGRAPHY

Since[4]:

$$u_f = \frac{k}{2l_f} \tag{7}$$

where

u_f = flow rate of the eluent at the solvent front
k = constant factor
l_f = distance from the surface of the eluent in the tank to the solvent front

and as the flow rate behind the front, u, is about 20% lower than u_f[4], it can be derived that[2]:

$$\bar{H} = B\,\frac{l_f + l_0}{0.8\,k} + C_M\,\frac{0.4\,k}{l_f - l_0}\ln\frac{l_f}{l_0} \tag{8}$$

where

l_0 = distance from the surface of the eluent in the tank to the starting point

$$\frac{l_f + l_0}{0.8\,k} = \overline{1/u} \tag{9}$$

$$\frac{0.4\,k}{l_f - l_0}\ln\frac{l_f}{l_0} = \bar{u} \tag{10}$$

In separating two or more solutes we are interested in those conditions, at which a prescribed separation is obtained in the shortest elution time.

For the total elution time, *i.e.*, the time needed for the eluent to cover the distance from 0 to l_f the following equation holds:

$$t_{\text{elution}} = \int_0^{l_f} \frac{dl_f}{u_f} = \frac{l_f^2}{k} \tag{11}$$

The separation of two solutes is described by the following equation[5]:

$$\frac{R^2}{l_A} = \frac{1}{4}\left(1 - \frac{R_{F(B)}}{R_{F(A)}}\right)^2 \frac{l_A}{\sigma_A^2} \tag{12}$$

wherein A and B designate the faster and the slower moving solute, respectively, and the *peak resolution* R is equal to:

$$R = \frac{l_A - l_B}{\sigma_A + \sigma_B} \tag{13}$$

As $\sigma_A^2/l_A = \bar{H}_A$ and $l_A = (l_f - l_0)\,R_{F(A)}$ eqn. (12) can be written as follows:

$$\frac{R^2}{l_f - l_0} = R_{F(A)}\left(1 - \frac{R_{F(B)}}{R_{F(A)}}\right)^2 \frac{1}{4\,\bar{H}} \tag{14}$$

or

$$l_f - l_0 = \frac{4\,R^2}{R_{F(A)}\left(1 - \frac{R_{F(B)}}{R_{F(A)}}\right)^2}\,\bar{H} \equiv p\,\bar{H} \tag{15}$$

By substitution of eqn. (8) into eqn. (15) and introduction of the parameter $x = (l_f - l_0)/(l_f + l_0)$, $0 < x < 1$, we can derive:

$$\frac{l_f^2}{b} = \frac{(x+1)^2 \ln (1+x)/(1-x)}{4x(x-a)} \qquad (16)$$

wherein $a = pB/0.8\,k$ and $b = 0.4\,pkC_M$, and l_f^2/b is equal to $t_{elution}\,k/b$.

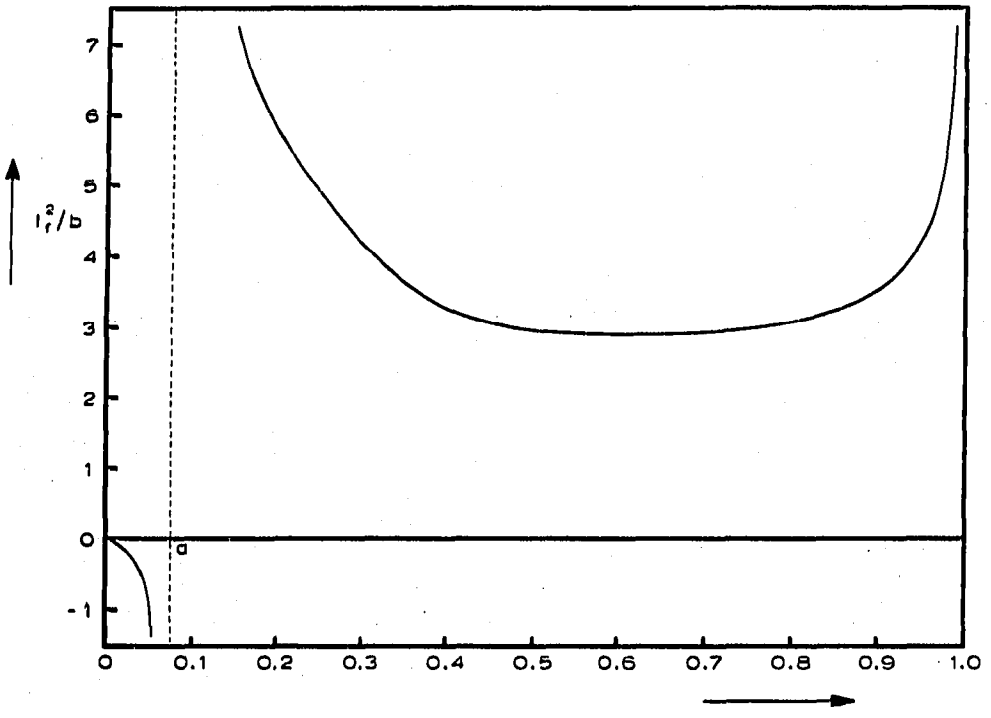

Fig. 1. Graph of eqn. (16). $a = 0.075$.

From Fig. 1 the following conclusions can be drawn:

(a) When $x < a$ it turns out that l_f^2/b is negative and it is impossible to obtain a separation. In this region the flow rate of the eluent, when reaching the starting point, is already so slow, that the peak maxima separate even more slowly, than the peaks broaden as a result of diffusion.

(b) When $a < x < 1$ the specified separation problem can be solved. The separation time involved depends on x and therefore on l_f and l_0. Optimum values of l_f and l_0, yielding the minimum separation time, can be calculated by determining the coordinates of the minimum in Fig. 1.

Before doing this, however, we shall turn to paper chromatography.

SEPARATION TIME IN PAPER CHROMATOGRAPHY

In paper chromatography the mass transfer term does not stem exclusively from the $C_M\bar{u}$ term, in contrast to the situation in thin-layer chromatography. There is a contribution from the $\overline{C_F(u)u}$ term too[3].

However, from eqn. (6) it follows that if $\bar{u}$ is so high that $\lambda\, d_p\bar{u} \gg \gamma_M D_M$, $\overline{C_F(u)u}$

is constant (C_F). It has been shown experimentally[3] that this situation occurs, when $\bar{u} > 0.0005$ cm · sec^{-1}. In this case it holds that:

$$\bar{H} = B \frac{l_f + l_0}{0.8\,k} + C_M \frac{0.4\,k}{l_f - l_0} \ln \frac{l_f}{l_0} + C_F \qquad (17)$$

By substitution of eqn. (17) into eqn. (15) and introduction of the parameter x we can derive (keeping in mind that l_f should be positive):

$$l_f = \frac{1 + x}{4\,(x - a)} \left\{ pC_F + \sqrt{p^2 C_F^2 + \frac{4\,(x - a)\,b}{x} \ln \frac{1 + x}{1 - x}} \right\} \qquad (18)$$

As l_f is at a minimum for the same value of x as the elution time l_f^2/k we can use eqn. (18) for determining the optimum values of l_f and l_0 corresponding with minimum elution time (see Fig. 2).

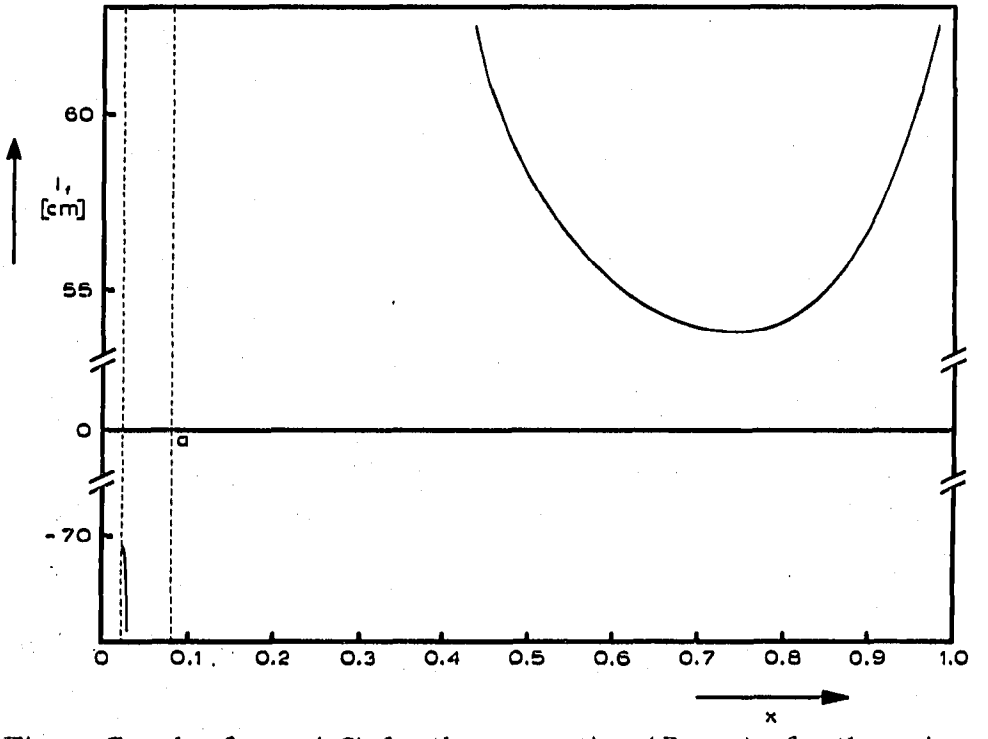

Fig. 2. Graph of eqn. (18) for the separation ($R = 4$) of L-threonine and L-α-aminobutyric acid on Whatman W 31 ET paper by means of a 4:1:5 butanol–acetic acid–water eluent at 21.5°. $a = 0.079$; $b = 597$ cm^2; $p = 2270$; $C_F = 0.007$ cm.

PROCEDURE FOR DETERMINING OPTIMUM VALUES OF l_f AND l_0

For determining optimum values of l_f, l_0 and $t_{elution}$ from eqns. (16) and (18) we must know the values of k, a, b and C_F.

These quantities can be calculated as follows:

k can be determined in a separate experiment, using eqn. (11).

$a = pB/0.8\,k$

p can be calculated by eqn. (15) from the values of R_F and the desired degree of separation R.

B can be calculated from eqn. (4). For doing this γ_M and γ_S must be known, while D_M and D_S can be calculated according to WILKE AND PIN CHANG[6].

$$b = 0.4\, pkC_M$$

C_M can be calculated from eqn. (5), if the value of d_p is known.

C_F can be taken constant and equal to 0.007 cm. (We should control afterwards if indeed $\bar{u} > 0.0005$ cm $\cdot$ sec^{-1})[3].

Values of k, γ_M, γ_S, d_p*, and C_F for some cellulose powders for thin-layer chromatography and some chromatography papers are given in Table I. These values were determined for elution of amino acids by a 4:1:5 butanol–acetic acid–water mixture[2,3]. It is probable, however, that they are not very dependent on experimental conditions, except the k values which are dependent on the properties of the eluent.

TABLE I

VALUES OF k, γ_M, γ_S, d_p AND C_F IN PAPER AND THIN-LAYER CHROMATOGRAPHY

	Material	k (cm^2 · sec^{-1})	γ_M	γ_S	d_p (cm)	C_F (cm)
Cellulose powder:	M & N 300	0.017	0.39	0.03	0.028	—
	Camag D	0.018	0 39	0.03	0.032	—
	Whatman CC41	0.029	0.39	0.03	0.047	—
	S & S 144	0.037	0.39	0.03	0.046	—
	S & S 142 dg	0.039	0.39	0.03	0.034	—
	S & S 140 dg	0.069	0.39	0.03	0.052	—
Paper:	W 1	0.032	0.62	0.03	0.080	0.007
	W 2	0.028	0.60	0.03	0.080	0.007
	W 3 MM	0.040	0.44	0.03	0.080	0.007
	W4	0.060	0.37	0.03	0.080	0.007
	W17	0.069	0.46	0.03	0.080	0.007
	W20	0.016	0.47	0.03	0.080	0.007
	W 31 ET	0.106	0.42	0.03	0.080	0.007
	W 54	0.071	0.36	0.03	0.080	0.007

Optimum values of l_f and l_0 follow from the coordinates of the minima of eqns. (16) and (18).

Eqn. (16) can be differentiated easily. In the minimum $\frac{\mathrm{d}\, l_f^2/b}{\mathrm{d}x} = 0$, from which it follows that:

$$a = \frac{2\,(1 - x)\ln(1 + x)/(1 - x) - 2x^2}{(1 - x)^2 \ln(1 + x)/(1 - x) - 2x} \qquad (19)$$

From this equation a can be calculated for any value of x. By substituting the appropriate values of a and x in eqn. (16) the minimum values of l_f^2/b and l_f can be calculated, and from l_f and x we obtain l_0.

This procedure can be simplified by constructing graphs of the optimum values of l_f^2/b and x both as a function of a. Then the minimum value of l_f^2/b and the corresponding value of x can be read directly from the graph for any value of a. This graph is shown in Fig. 3.

For paper chromatography the procedure is somewhat more complicated as

* The effective values of d_p are tabulated. These are about one order of magnitude larger than the values given by the manufacturer[2,3].

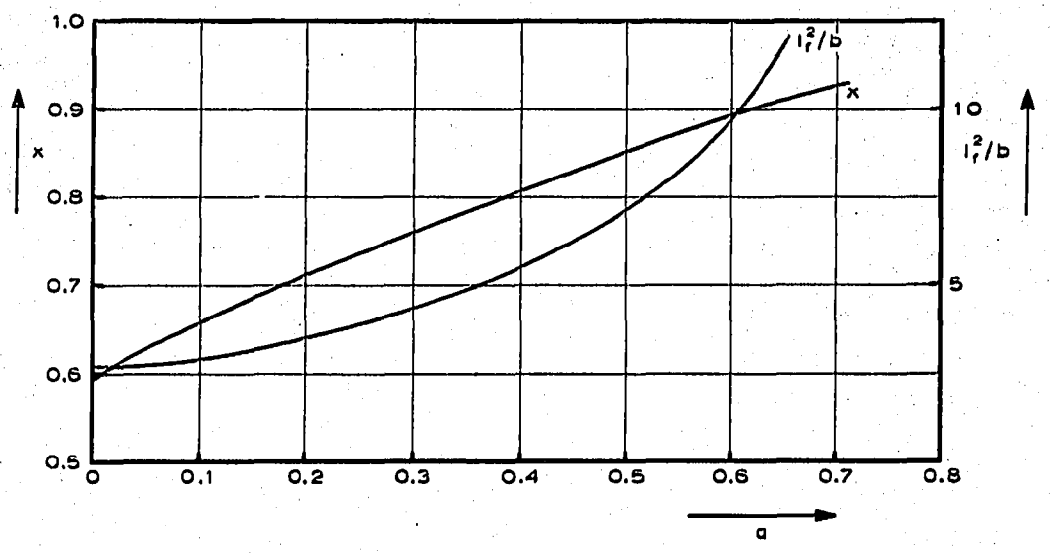

Fig. 3. Graph of the optimum values of l_f^2/b and x as a function of a.

differentiation of eqn. (18) results in an implicit function of x containing the three parameters a, b and pC_F.

In this case the easiest way is to determine the coordinates of the minimum graphically from eqn. (18) (see Fig. 2).

EXAMPLE

We want to separate a mixture of the two amino acids L-threonine and L-α-aminobutyric acid on M & N 300 cellulose powder and W 31 ET paper by means of a 4:1:5 butanol–acetic acid–water eluent. These substances have R_F values of 0.36 and 0.48, respectively, on the cellulose powder and of 0.39 and 0.51, respectively, on the paper[2,3].

Cellulose powder

We must know the values of k, a and b.

From Table I it follows that $k = 0.017$ cm$^2 \cdot$sec^{-1}

$a = pB/0.8\,k$

If we take R to be 4, p can be calculated from eqn. (15) to be 2130. Substitution into eqn. (4) of the γ values of Table I and the values of D_M and D_S, calculated[7] according to WILKE AND PIN CHANG[6] gives $B = 2.81 \cdot 10^{-6}$ cm$^2 \cdot$sec^{-1}. So we have: $a = 0.446$.

$b = 0.4\,pkC_M$

From eqn. (5) and the d_p value in Table I it follows that $C_M = 0.85$ sec. So, $b = 12.2$ cm^2. From Fig. 3 follows:

a	b	l_f^2/b	l_f^2	l_f	x	l_0	$t_{elution}$
0.446	12.2 cm^2	6.2	75.6 cm^2	8.7 cm	0.83	0.82 cm	4500 sec

Paper

$k = 0.106$ cm$^2 \cdot$sec^{-1}

Taking $R = 4$ again we calculate $p = 2270$ and $B = 2.94 \cdot 10^{-6}$ cm$^2 \cdot$sec^{-1}, so:
$a = 0.079$.
$C_M = 6.2$ sec, so $b = 597$ cm^2.

Using these values, we find from the graph of eqn. (18) (see Fig. (2)):

a	b	l_f	x	l_0	$t_{elution}$
0.079	597 cm^2	53.8 cm	0.75	5.8 cm	27 300 sec

According to eqn. (10), $\bar{u} = 0.00196$ cm$\cdot$sec^{-1}, so the assumption that $\overline{C_F(u)u}$ is constant was correct.

CONCLUSION

It appears, that for obtaining the same degree of separation, elution time for paper chromatography is much longer than for thin-layer chromatography .

REFERENCES

1 S. T. Sie and G. W. A. Rijnders, *Anal. Chim. Acta*, 38 (1967) 3.
2 C. L. de Ligny and A. G. Remijnse, *J. Chromatog.*, 33 (1968) 242.
3 C. L. de Ligny and A. G. Remijnse, *J. Chromatog.*, 35 (1968) 257.
4 J. C. Giddings, G. H. Stewart and A. L. Ruoff, *J. Chromatog.*, 3 (1960) 239.
5 C. L. de Ligny, H. M. Schmidt and Miss W. de Vries, *Rec. Trav. Chim.*, 82 (1963) 1051.
6 C. R. Wilke and Pin Chang, *Am. Inst. Chem. Engr. J.*, 1 (1955) 264.
7 C. L. de Ligny and D. Bax, *Z. Anal. Chem.*, 205 (1964) 333.