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PEAK BROADENING IN PAPER CHROMATOGRAPHY AND RELATED TECHNIQUES

VII. THE CONTRIBUTION OF THE MACROSCOPIC MOBILE PHASE VELOCITY PROFILE TO PEAK BROADENING IN PAPER AND THIN-LAYER CHROMATOGRAPHY

C. L. DE LIGNY AND W. VAN DE MEENT
Laboratory for Analytical Chemistry, State University, Croesestraat 77 A, Utrecht (The Netherlands)
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SUMMARY

The contribution of the macroscopic mobile phase velocity profile in a paper or thin-layer strip to longitudinal dispersion in chromatography was investigated. For this purpose the dependence of the plate height on the breadth of the paper or thin-layer strip, scanned by the densitometer, was determined using Whatman W2 paper and Camag D cellulose powder. As longitudinal dispersion depends on transversal (convective and diffusive) dispersion, the latter was determined for both chromatographic techniques.

INTRODUCTION

Longitudinal dispersion in chromatography can be described by the equation:

$$H = \frac{\sigma^2}{\mu} = B\frac{1}{\bar{u}} + C_M\bar{u} + C_S\bar{u} + C_F(u)\bar{u} + C_E(u)\bar{u} \qquad (1)$$

where

H = height equivalent to a theoretical plate,
σ = standard deviation of the solute distribution in the medium used for chromatography,
μ = distance travelled by the solute,
$\bar{u}$ = mean flow rate of the eluent.

The terms B, C_M, C_S, $C_F(u)$ and $C_E(u)$ are concerned with longitudinal diffusion, resistance to attainment of the partition equilibrium in the mobile and in the stationary phase, the macroscopic mobile phase velocity profile and eddy diffusion, respectively.

DE LIGNY AND BAX[1] showed that peak broadening in paper chromatography at low liquid velocity ($0.0005\ \mathrm{cm \cdot sec^{-1}}$) is exclusively caused by longitudinal diffusion, both in the mobile and the stationary phases. At higher liquid velocities (up to $0.002\ \mathrm{cm \cdot sec^{-1}}$) DE LIGNY AND REMIJNSE[2] demonstrated, in addition, the occurrence of slow attainment of the partition equilibrium. The slow mass transfer between the mobile and the stationary phase appeared to be caused mainly by slowness of diffusion in the mobile phase[3]. For thin-layer chromatography similar results were obtained[4]. A contribution of the mobile phase velocity profile (or, possibly, the $C_S\bar{u}$ and $C_E(u)\bar{u}$ terms) was found only in paper chromatography[3].

The various terms in the right-hand side of Eqn. 1 can be specified as follows for paper and thin-layer chromatography[3-5]:

$$B\,\frac{\bar{1}}{u} = \frac{\sigma^2_{\mathrm{diff}}}{\mu} = \frac{2\gamma_M D_M R_F t + 2\gamma_S D_S(1 - R_F)t}{\mu} \tag{2}$$

$$C_M\bar{u} = 0.01(1 - R_F)^2\,\frac{d_p{}^2}{D_M}\,\bar{u} \tag{3}$$

$$C_S\bar{u} = \frac{2}{3}\,R_F(1 - R_F)\,\frac{d_f{}^2}{D_S}\,\bar{u} \tag{4}$$

$$C_F(u)\bar{u} = \frac{2\kappa L^2}{\lambda_R d_p \bar{u} + \gamma_M D_M + \gamma_S D_S(1 - R_F)/R_F}\,\bar{u} \tag{5}$$

$$C_E(u)\bar{u} = \frac{2\kappa' d_p{}^2}{\lambda_R d_p \bar{u} + \gamma_M D_M + \gamma_S D_S(1 - R_F)/R_F}\,\bar{u} \tag{6}$$

where:

γ = tortuosity factor,
D = diffusion coefficient,
R_F = ratio of the distances travelled by the solute and by the eluent,
t = elution time,
d_p = diameter of the support particles,
d_f = thickness of the layer of stationary fluid on the support particles,
$\kappa, \kappa', \lambda_R$ = dimensionless constants, depending on the dynamics of flow,
L = dimension, characteristic for the flow profile (breadth of the paper or thin-layer strip scanned by the densitometer, *or* mean distance between maxima or minima in the flow profile, whichever is the smaller).

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

$$k = \frac{l_f{}^2 - l_0{}^2}{t}$$

l_f = distance from the surface of the eluent in the tank to the solvent front,
l_0 = distance from the surface of the eluent in the tank to the starting point.

Transversal dispersion can be described by[5]:

$$\sigma^2_{\mathrm{tr,\,chrom}} = \sigma^2_{\mathrm{tr,\,conv}} + \sigma^2_{\mathrm{tr,\,diff}} = 2(\lambda_R d_p \bar{u} + \gamma_M D_M + \gamma_S D_S(1 - R_F)/R_F)t \tag{7}$$

The first term between brackets represents convective dispersion, the other one diffusive dispersion. According to Eqn. 5, the contribution of the flow profile term can be assessed in a straightforward way by determining the plate height H as a function of the breadth L of the paper or thin-layer strip scanned by the densitometer. As the contributions of the $B \cdot \overline{1/u}$ and $C_M\bar{u}$ terms can be calculated from previous work[3,4], the contribution of the $C_S\bar{u}$ and $C_E(u)\bar{u}$ terms (if different from zero) can then be estimated from the extrapolated value of H at $L = 0$. When the transversal dispersion is also measured, the value of κ can be determined from Eqns. 5 and 7. Further, the value of λ_R can be obtained as estimates of d_p, γ, D and R_F have been made previously[1,3,4].

EXPERIMENTAL

Chemicals

L-Valine, Whatman W 2 paper, Camag D cellulose powder, were used.

Procedure

(*a*) *Determination of H as a function of L.* The chromatograms were obtained and stained as described earlier[6]. Then each band was divided in parts with a length equal to the slit length used in the densitometer. The travelled distance, μ, and the variance originating from the chromatographic transport, σ^2, were determined for each part*.

(*b*) *Determination of transversal dispersion.* The variance, originating from transversal convective and diffusive dispersion, $\sigma^2_{\mathrm{tr,chrom}}$, was determined by applying the bands parallel to the elution direction and eluting, staining and scanning them as described earlier (ref. 6)*. The variance, originating from transversal diffusive dispersion in paper chromatography, $\sigma^2_{\mathrm{tr,diff}}$, was determined by applying the bands parallel to the elution direction, leaving them to diffuse for a certain time, staining and scanning them as described earlier (ref. 1)*. The corresponding variance in thin-layer chromatography was calculated from the known values of γ, D and R_F**. The variance, originating from transversal convective dispersion, $\sigma^2_{\mathrm{tr,conv}}$, can then be calculated by difference.

Calculations

As described in the foregoing section under (a), each eluted and stained band 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 band. The variance, σ^2, and the position of the maximum, μ, of the solute distribution were determined for each part. On the score of the unevenness of flow over the distance b, each part of the band has its own particular value of σ^2 and of μ. So, the result of the measurements on one band is:

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

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

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* All variances were corrected for the variance originating from the application procedure and the equilibration period.

** This is not warranted in the case of paper chromatography, in view of the possibility that the tortuosity factors parallel and perpendicular to the machine direction are different.

TABLE I

TRANSVERSAL DISPERSION IN PAPER CHROMATOGRAPHY; CALCULATION OF λ_R

$\sigma^2_{tr,\,diff}$ (mm^2)	t (sec)	$\gamma_M D_M + \gamma_S D_S(1-R_F)/R_F$ ($mm^2 \cdot sec^{-1}$)	$\sigma^2_{tr,\,chrom}$ (mm^2)	t (sec)	$\lambda_R d_p \bar{u} + \gamma_M D_M + \gamma_S D_S(1-R_F)/R_F$ ($mm^2 \cdot sec^{-1}$)	$\lambda_R d_p \bar{u}$ ($mm^2 \cdot sec^{-1}$)	$\bar{u}$ ($mm \cdot sec^{-1}$)	$\lambda_R d_p$ (mm)	d_p (mm) (ref. 3)	λ_R
4.88	16 020	1.52×10^{-4}	8.39	13 720	3.06×10^{-4}	1.13×10^{-4}	75.3×10^{-4}	0.0150		
7.26	15 720	2.31	9.14	14 430	3.17	1.24	71.6	0.0173		
5.63	15 480	1.82	7.50	14 090	2.66	0.73	74.7	0.0098		
5.66	14 040	2.02	8.78	13 690	3.21	1.28	71.8	0.0178		
5.57	14 100	1.98								
Mean[a]		$(1.93 \pm 0.28) \times 10^{-4}$						0.0150 ± 0.0041	0.92 ± 0.36	0.016 ± 0.008

[a] Accuracy is given throughout this paper as the 90% probability interval.

TABLE II

TRANSVERSAL DISPERSION IN THIN-LAYER CHROMATOGRAPHY; CALCULATION OF λ_R

$\gamma_M D_M + \gamma_S D_S(1-R_F)/R_F$ ($mm^2 \cdot sec^{-1}$)	$\sigma^2_{tr,\,chrom}$ (mm^2)	t (sec)	$\lambda_R d_p \bar{u} + \gamma_M D_M + \gamma_S D_S(1-R_F)/R_F$ ($mm^2 \cdot sec^{-1}$)	$\lambda_R d_p \bar{u}$ ($mm^2 \cdot sec^{-1}$)	$\bar{u}$ ($mm \cdot sec^{-1}$)	$\lambda_R d_p$ (mm)	d_p (mm) (ref. 4)	λ_R
	3.00	7800	1.92×10^{-4}	0.62×10^{-4}	151×10^{-4}	0.0041		
	2.51	7800	1.61	0.31	151	0.0020		
	2.61	7800	1.67	0.37	149	0.0024		
	2.49	7800	1.60	0.30	147	0.0020		
	2.61	7800	1.67	0.37	146	0.0025		
$(1.30 \pm 0.13) \times 10^{-4}$						0.0026 ± 0.0008	0.32 ± 0.02	0.008 ± 0.003

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

$$\sigma_p^2 = \frac{1}{p} \sum_1^p \sigma_i^2 + \frac{1}{p} \sum_1^p (\mu_i - \mu_p)^2 \tag{8}$$

where:

$$\mu_p = \frac{1}{p} \sum_1^p \mu_i$$

Further:

$$H_p = \frac{\sigma_p^2}{\mu_p} = \frac{1}{p\mu_p} \sum_1^p \{\sigma_i^2 + (\mu_i - \mu_p)^2\} = c + dL^2 \tag{9}$$

where:

$$c = B\,\overline{\frac{1}{u}} + C_M\bar{u} + C_S\bar{u} + C_E(u)\bar{u} \tag{10}$$

and:

$$d = \frac{2\kappa}{\lambda_R d_p \bar{u} + \gamma_M D_M + \gamma_S D_S(1 - R_F)/R_F}\,\bar{u} \tag{11}$$

RESULTS

The values of μ_p at various values of p (or L) are shown in Fig. 1. The data on transversal dispersion are shown in Tables I and II.

DISCUSSION

We observe in Fig. 1 that H hardly increases further when L exceeds 6 mm. Therefore, to estimate c and d in Eqn. 9, straight lines were drawn through the points corresponding to the smallest two L values. Furthermore, lines parallel to the abscissa were drawn through the remaining points. The points of intersection with the sloping lines give approximately the distance between maxima or minima in the macroscopic flow velocity profile. The mean values of this distance are 9 and 8 mm in paper and thin-layer chromatography, respectively.

From the difference between the H values at $L = 0$ and $L > 9$, respectively 8 mm, the (maximum) value of the $C_F(u)\bar{u}$ term follows, that is observed in practice. For paper and thin-layer chromatography, this term amounts to 0.01 and 0.001 mm, respectively. Previously[4], it was found that in paper chromatography $C_F(u)\bar{u} + C_E(u)\bar{u} + C_S\bar{u} = 0.07 \pm 0.03$ mm (using a densitometer slit length L of 6 mm). It follows from these data that $C_E(u)\bar{u} + C_S\bar{u} = 0.06 \pm 0.03$ mm.

From the slopes of the graphs in Fig. 1 and $\bar{u}$, the values of

$$\frac{\kappa}{\lambda_R d_p \bar{u} + \gamma_M D_M + \gamma_S D_S(1 - R_F)/R_F}$$

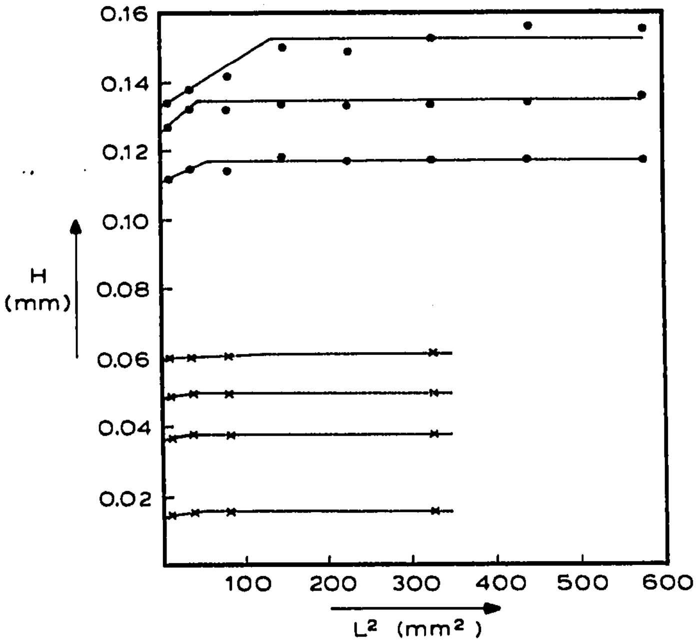

Fig. 1. Plate height H as a function of the breadth L of the paper or thin-layer strip scanned by the densitometer. ●, paper chromatography; ×, thin-layer chromatography.

TABLE III

CALCULATION OF κ IN PAPER CHROMATOGRAPHY

$\frac{\Delta H}{\Delta L^2}$ (mm^{-1})	$\bar{u}$ ($mm \cdot sec^{-1}$)	$\frac{\kappa}{\lambda_R d_p \bar{u} + \gamma_M D_M + \gamma_S D_S(1-R_F)/R_F}$ ($mm^{-2} \cdot sec$)	κ
1.89×10^{-4}	164×10^{-4}	57.6×10^{-4}	2.50×10^{-6}
0.96	169	28.5	1.26
1.59	190	41.9	2.08
Mean			$(1.9 \pm 0.8)\ 10^{-6}$

TABLE IV

CALCULATION OF κ IN THIN-LAYER CHROMATOGRAPHY

$\frac{\Delta H}{\Delta L^2}$ (mm^{-1})	$\bar{u}$ ($mm \cdot sec^{-1}$)	$\frac{\kappa}{\lambda_R d_p \bar{u} + \gamma_M D_M + \gamma_S D_S(1-R_F)/R_F}$ ($mm^{-2} \cdot sec$)	κ
0.259×10^{-4}	134×10^{-4}	9.6×10^{-4}	1.26×10^{-7}
0.074	134	2.8	0.36
0.222	135	8.2	1.07
0.259	135	9.6	1.25
Mean			$(1.0 \pm 0.5) \times 10^{-7}$

can be found. From the values of λ_R given in Tables I and II and the known values of D (ref. 1) d_p, γ, R_F (refs. 3, 4) and $\bar{u}$ the denominator can be calculated, so that κ is obtained (Tables III and IV). κ equals $2 \cdot 10^{-6}$ and $1 \cdot 10^{-7}$ for paper and thin-layer chromatography, respectively. These values are of the same magnitude as expected from the values found for packed columns*

The value of $\gamma_M D_M + \gamma_S D_S (1 - R_F)/R_F$ found for transversal diffusive dispersion in paper chromatography ($(1.93 \pm 0.19) \times 10^{-4}$ mm²·sec⁻¹) (Table I) is in good accord with the value that is calculated using the γ values for longitudinal diffusive dispersion[4] ($(2.05 \pm 0.40) \times 10^{-4}$ $\text{mm}^2 \cdot \text{sec}^{-1}$). So, the tortuosity factors parallel and perpendicular to the machine direction appear to be equal, *a posteriori*.

The values found for λ_R, 0.016 and 0.008 for paper and thin-layer chromatography, respectively, are about an order of magnitude smaller than the values holding for packed columns (ref. 5)**.

CONCLUSIONS

As a result of our investigations in the field of peak broadening in paper and thin-layer chromatography, the various parameters determining this phenomenon can be specified as given in Table V, for Whatman W2 paper and Camag D cellulose powder.

TABLE V

PARAMETERS DETERMINING PLATE HEIGHT IN PAPER AND THIN-LAYER CHROMATOGRAPHY

Parameter	*Dimension*	*Whatman W2 paper*	*Camag D cellulose powder*
γ_M		0.60 ± 0.12	0.42 ± 0.29
γ_S		0.03 ± 0.02	0.02 ± 0.08
d_p	mm	0.91 ± 0.36	0.32 ± 0.02
$C_S\bar{u} + C_E(u)\bar{u}$	mm	0.06 ± 0.03	negligible
λ_R		0.016 ± 0.008	0.008 ± 0.003
κ		$(1.9 \pm 0.8) \times 10^{-6}$	$(1.0 \pm 0.5) \times 10^{-7}$

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* If (see ref. 5, Eqn. 16 and Fig. 4) in the calculation of the expected value of $\kappa'/\rho^2 + \kappa \simeq \kappa, \rho$ is calculated from the distance between maxima or minima in the mobile phase flow profile (~ 8 mm) and the real fiber diameter of the paper or the real diameter of the cellulose powder particles (~ 0.01 mm).

** However, if the real paper fiber diameter or the real diameter of the cellulose powder particles as given by the manufacturer[3,4] are used in the calculation of λ_R, rather than the effective d_p values calculated from the $C_M\bar{u}$ term, values are found at least as large as the λ_R values holding for packed columns.