CHROM. 3409

PEAK BROADENING IN PAPER CHROMATOGRAPHY AND RELATED TECHNIQUES

IV. THE MECHANISM OF THE MASS TRANSFER TERM IN PAPER CHROMATOGRAPHY

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SUMMARY

The mechanism of peak broadening in paper chromatography is investigated by comparing the peak widths obtained in chromatography with those caused only by diffusion, for a set of amino acids of widely differing R_F values and with eight kinds of Whatman paper.

The results show that longitudinal diffusion occurs both in the mobile and the stationary phase in paper chromatography.

The mass transfer term is composed of a contribution of unevenness of flow throughout the paper and a contribution of the slowness of attainment of the partition equilibrium between the mobile and the stationary phase. The resistance to attainment of equilibrium resides predominantly in the mobile phase.

INTRODUCTION

The main features of peak broadening in chromatography, especially gas chromatography, can be described adequately by the VAN DEEMTER equation. However, in the course of years some modifications proved necessary, in particular concerning the eddy diffusion and mass transfer terms. Important improvements on the Van Deemter equation have been given by GIDDINGS, PRETORIUS *et al.* and, especially, by SIE AND RIJNDERS[1]. The equation, proposed by the last mentioned authors, is:

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

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

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u = flow rate of the eluent

The four terms in this equation account for peak broadening by longitudinal molecular diffusion, resistance to attainment of the partition equilibrium (this resistance residing both in the mobile and the stationary phase) and unevenness of flow throughout the column cross-section, respectively.

It has been shown[2,3] that in paper chromatography longitudinal molecular diffusion occurs both in the stationary and the mobile phase and that, accordingly:

$$\sigma^2_{\text{diff}} = Bl\frac{1}{u} = BR_F t = \{2\gamma_M D_M R_F + 2\gamma_S D_S(1 - R_F)\}\, t \tag{2}$$

where

γ = tortuosity factor
D = diffusion coefficient
R_F = ratio of the distances covered by the solute and by the eluent
t = diffusion time

The three mass transfer terms are equal to:

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

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

$$C_F(u)u = \frac{2KL^2}{\lambda d_p u + \gamma_M D_M}\,u \tag{5}$$

where

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} in packed columns)
L = dimension characteristic for the flow profile
λ = dimensionless constant depending on the packing geometry (≈ 0.03 in packed columns)

Only a limited number of investigations into peak broadening in paper chromatography in general and into the mechanism of the mass transfer term in particular have appeared. The last mentioned studies are reviewed briefly below.

By determining H as a function of u, the present authors found[3] the combined C terms to be equal to 4–10 sec (Whatman No. 1 paper, butanol–acetic acid–water eluent, amino acids, 21.5°). They did not attempt to estimate the relative importance of the three contributing terms.

By the same method, MALLIK AND GIDDINGS[4] found 1–4 sec for the mass transfer term (Whatman No. 31 ET, butanol–acetic acid–water eluent, amino acids, 28°). Arbitrarily, they equated this term to C_S.

STEWART[5] repeated these experiments, found 8 sec for the mass transfer term and, again arbitrarily, equated this term to C_M.

In later work he demonstrated[6] the occurrence of a large $C_F(u)$ term and expressed as his opinion that this term governs the mass transfer term*.

In view of these conflicting statements we thought it worthwhile to try to unravel the mechanism of the mass transfer term in paper chromatography, by the same method as proved to be successful in thin-layer chromatography[8].

In this method, the contribution of longitudinal molecular diffusion to peak broadening is determined in separate experiments and subtracted from the peak broadening obtained in chromatography, leaving:

$$(\sigma^2_{\text{chrom}} - \sigma^2_{\text{diff}})/l = C_M u + C_S u + C_F(u)\, u \qquad (6)$$

The relative importance of the three mass transfer terms can be estimated by investigating the dependence of the left hand side of eqn. (6) on R_F (compare eqns. (3), (4) and (5)).

EXPERIMENTAL

Chemicals

The following chemicals were used:

(1) L-α,γ-diaminobutyric acid; (2) L-ornithine; (3) L-aspartic acid; (4) L-glutamic acid; (5) L-threonine; (6) L-α-aminobutyric acid; (7) L-methionine; (8) L-valine; (9) L-norvaline.

All amino acids were purchased from Fluka and were "chromatographically pure".

Chromatography papers: Whatman No. W 1, W 2, W 3 MM, W 4, W 17, W 20, W 31 ET and W 54.

Procedure

Values of l, R_F and σ^2_{chrom} were taken from previous work[9]. Values of σ^2_{diff} were determined as described earlier[2].

RESULTS

The mean values of σ^2_{diff} and their 90 % probability intervals are shown as a function of R_F in Figs. 1 and 2. The mean values of l, R_F, σ^2_{chrom}, σ^2_{diff} (recalculated for a diffusion time equal to the elution time in the chromatography experiments) and $(\sigma^2_{\text{chrom}} - \sigma^2_{\text{diff}})/l$ are given in Table I.

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* The work of MALLIK AND GIDDINGS and of STEWART suffers from some minor imperfections: (1) The use of racemic mixtures of amino acids which introduces the possibility of excessive peak broadening by partial separation of the antipodes[7]. (2) The visual and therefore subjective method of determining peak widths. (3) In one case[6], the short presaturation period (1 h), which may lead to changes in the composition of the eluent during chromatography.

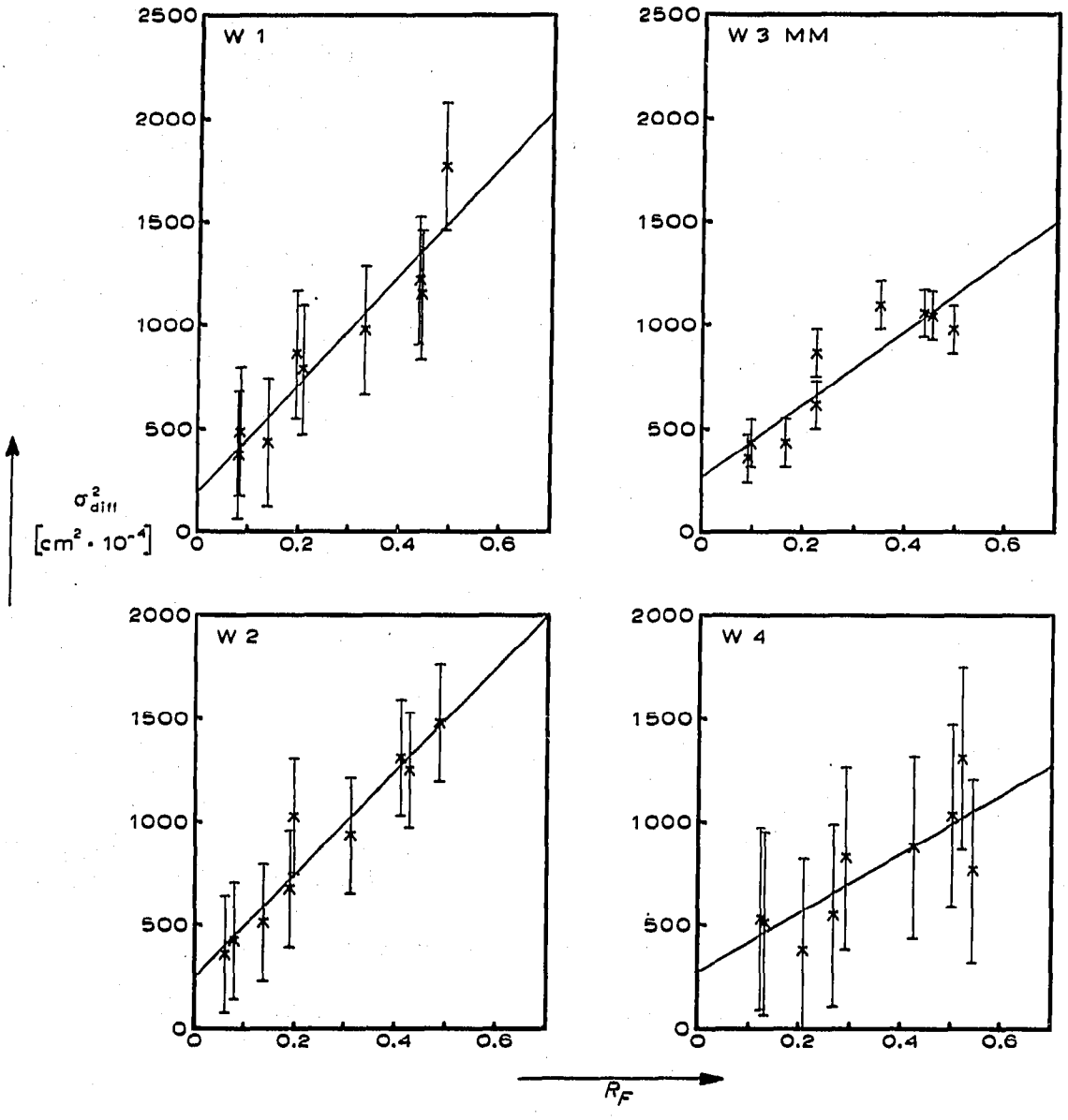

Fig. 1. Mean values of σ^2_{diff} and their 90% probability intervals as a function of R_F. Diffusion times are: Whatman No. W1, 74040 sec; W2, 74700 sec; W3 MM, 75000 sec; W4, 75600 sec.

DISCUSSION

The values of σ^2_{diff} and σ^2_{chrom} are mean values of two and three determinations, respectively. They have therefore an approximately normal distribution, so that the usual statistical methods can be applied.

(1) σ^2_{diff}

In the first place it was ascertained, by means of BARTLETT's tests, that the variances of σ^2_{diff} for a particular chromatography paper are homogeneous.

According to eqn. (2) a linear relationship exists between σ^2_{diff} and R_F, as the diffusion coefficients of the amino acids are almost equal[8]. This relationship was calculated by the method of least squares, allocating equal statistical weights to the

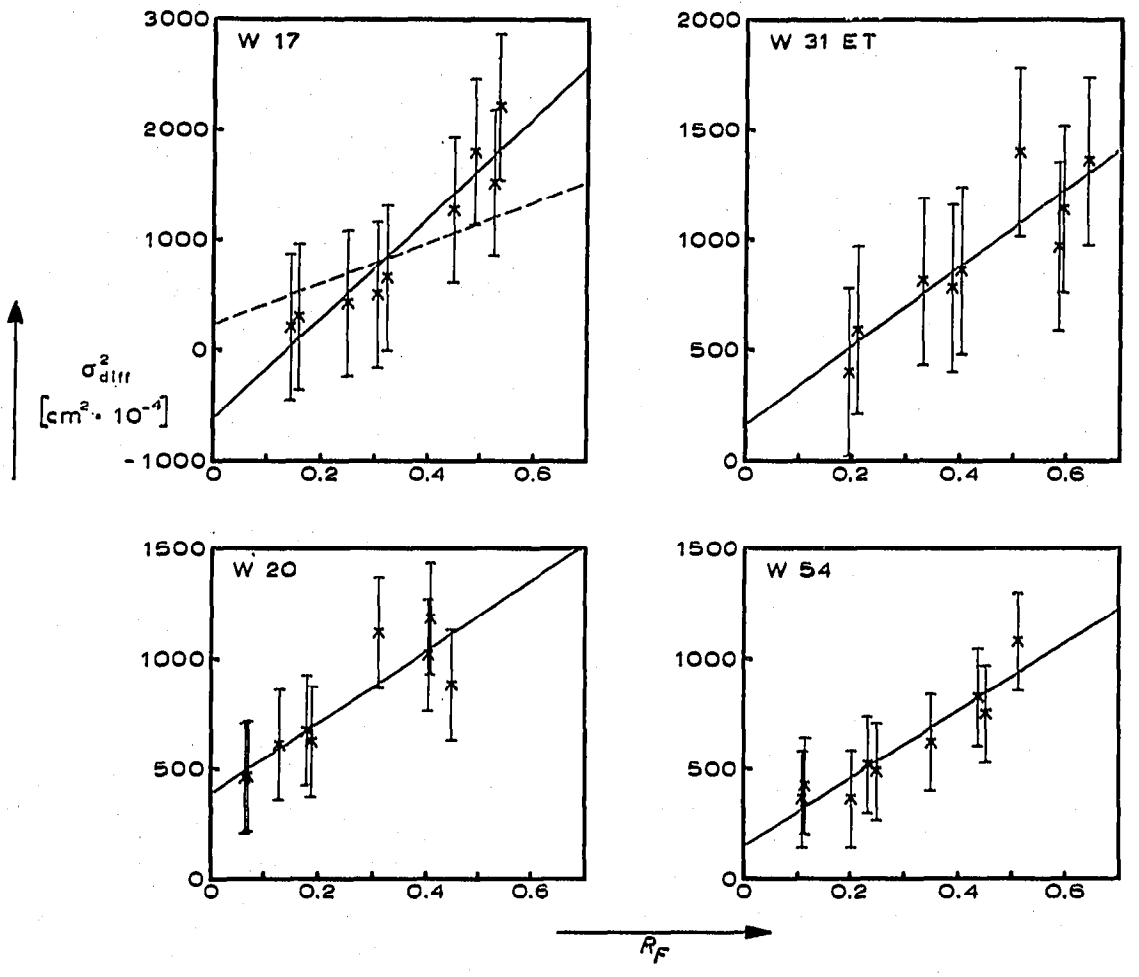

Fig. 2. Mean values of σ^2_{diff} and their 90% probability intervals as a function of R_F. Diffusion times are: Whatman No. W 17, 73500 sec; W 20, 69300 sec; W 31 ET, 76500 sec; W 54, 75600 sec.

values of σ^2_{diff}. The corresponding straight lines are shown in Figs. 1 and 2. From their slopes and intercepts, together with the calculated[2] values of $\bar{D}_M$ and $\bar{D}_S$ and the known diffusion times, γ_M and γ_S can be calculated. These values are given in Table II.

We note the following points:

(a) The calculated lines represent the measurements very well, passing through 67 out of the 72 90% probability intervals.

(b) Seven out of the eight γ_S values are positive. The possibility of obtaining this result if, in reality, γ_S is zero is only 1.5%[10]. This proves again that in paper chromatography longitudinal diffusion in the stationary phase contributes to peak broadening.

(c) Apart from the negative γ_S value found for W 17, the γ_S values are not significantly different. The mean value is 0.03 ± 0.01. Assuming that, in reality, γ_S for W 17 is equal to $\bar{\gamma}_S$, we obtain $\gamma_M = 0.46$ for this paper*.

In contrast to the values of γ_S, those of γ_M do show significant differences. They

* The corresponding straight line is dotted in Fig. 2. It still passes through eight out of the nine probability intervals.

TABLE I

PEAK BROADENING IN PAPER CHROMATOGRAPHY

Amino acid No.	W_1 ($t_{elution}$ = 27840 sec)					W_2 ($t_{elution}$ = 26280 sec)				
	l (cm)	R_F	σ^2_{chrom} (cm^2)	σ^2_{diff} (cm^2)	$(\sigma^2_{chrom}-\sigma^2_{diff})/l$ (cm)	l (cm)	R_F	σ^2_{chrom} (cm^2)	σ^2_{diff} (cm^2)	$(\sigma^2_{chrom}-\sigma^2_{diff})/l$ (cm)
1	2.02	0.085	0.062	0.018	0.0219	1.75	0.078	0.052	0.015	0.0214
2	2.15	0.084	0.097	0.014	0.0388	1.36	0.063	0.061	0.012	0.0357
3	3.28	0.138	0.087	0.016	0.0215	3.01	0.135	0.075	0.018	0.0189
4	5.32	0.209	0.179	0.029	0.0282	4.01	0.188	0.122	0.024	0.0245
5	4.67	0.197	0.114	0.032	0.0174	4.36	0.196	0.111	0.036	0.0172
6	8.43	0.330	0.187	0.037	0.0179	6.54	0.310	0.153	0.033	0.0183
7	10.40	0.438	0.225	0.046	0.0172	9.58	0.429	0.134	0.044	0.0094
8	11.28	0.442	0.245	0.043	0.0179	8.64	0.410	0.239	0.046	0.0223
9	11.61	0.489	0.248	0.066	0.0156	10.89	0.488	0.216	0.052	0.0150

Amino acid No.	W_{17} ($t_{elution}$ = 15420 sec)					W_{20} ($t_{elution}$ = 25500 sec)				
	l (cm)	R_F	σ^2_{chrom} (cm^2)	σ^2_{diff} (cm^2)	$(\sigma^2_{chrom}-\sigma^2_{diff})/l$ (cm)	l (cm)	R_F	σ^2_{chrom} (cm^2)	σ^2_{diff} (cm^2)	$(\sigma^2_{chrom}-\sigma^2_{diff})/l$ (cm)
1	3.91	0.147	0.247	0.004	0.0620	0.94	0.065	0.028	0.017	0.0123
2	4.41	0.160	0.182	0.006	0.0398	1.08	0.070	0.043	0.017	0.0240
3	6.66	0.250	0.249	0.009	0.0360	1.84	0.128	0.041	0.022	0.0099
4	8.98	0.323	0.298	0.014	0.0317	2.87	0.187	0.085	0.023	0.0217
5	8.09	0.305	0.311	0.010	0.0372	2.58	0.178	0.064	0.025	0.0154
6	12.56	0.451	0.500	0.027	0.0376	4.78	0.310	0.104	0.041	0.0131
7	12.91	0.486	0.300	0.038	0.0203	5.86	0.405	0.108	0.038	0.0121
8	14.64	0.526	0.682	0.032	0.0444	6.24	0.406	0.130	0.043	0.0138
9	14.21	0.535	0.704	0.046	0.0463	6.52	0.452	0.091	0.032	0.0089

range from one to two times the value found for cellulose powders for thin-layer chromatography[8].

(2) $(\sigma^2_{chrom}-\sigma^2_{diff})/l$

In the first place it was investigated, by means of BARTLETT's tests, whether the variances of σ^2_{chrom} for a particular chromatography paper are homogeneous. Four of the eight tests gave significant results on the 10% probability level. This strongly suggests that the variances are not homogeneous (the chance of obtaining the above mentioned results if the variances are, in reality, homogeneous is only $5 \cdot 10^{-5}$ %).

Reasoning that the variance of σ^2_{chrom} probably depends mainly on the magnitude of σ^2_{chrom}, log var (σ^2_{chrom}) was plotted *vs.* log σ^2_{chrom} and the best straight line through the points was determined assuming that the accuracies of log var (σ^2_{chrom}) and log σ^2_{chrom} are unknown[11]. It appeared that var (σ^2_{chrom}) is proportional to $(\sigma^2_{chrom})^{2.1}$. From this graph var (σ^2_{chrom}) was read and combined with var (σ^2_{diff}) to yield var $(\sigma^2_{chrom}-\sigma^2_{diff})/l$.

It can be seen from Table I that the values of $(\sigma^2_{chrom}-\sigma^2_{diff})/l$ decrease when R_F increases. This suggests that a large part of the mass transfer term stems from the C_{Mu} term, which is, according to eqn. (3) proportional to $(1-R_F)^2$.

W3MM ($t_{elution}$ = 24360 sec)					*W4* ($t_{elution}$ = 18240 sec)				
l (cm)	R_F	σ^2_{chrom} (cm^2)	σ^2_{diff} (cm^2)	$(\sigma^2_{chrom}-\sigma^2_{diff})/l$ (cm)	l (cm)	R_F	σ^2_{chrom} (cm^2)	σ^2_{diff} (cm^2)	$(\sigma^2_{chrom}-\sigma^2_{diff})/l$ (cm)
2.49	0.096	0.079	0.014	0.0261	3.58	0.132	0.142	0.012	0.0362
2.39	0.092	0.092	0.012	0.0335	3.48	0.124	0.329	0.013	0.0911
4.34	0.167	0.092	0.014	0.0181	5.68	0.209	0.116	0.009	0.0188
5.79	0.224	0.158	0.020	0.0239	8.21	0.291	0.314	0.020	0.0359
5.85	0.225	0.145	0.028	0.0200	7.24	0.267	0.227	0.013	0.0295
9.05	0.350	0.192	0.036	0.0173	11.95	0.425	0.341	0.021	0.0268
11.37	0.438	0.150	0.034	0.0102	13.53	0.501	0.248	0.025	0.0165
11.70	0.452	0.269	0.034	0.0201	14.66	0.522	0.287	0.032	0.0174
12.87	0.496	0.266	0.032	0.0183	14.68	0.544	0.275	0.018	0.0175
W31 ET ($t_{elution}$ = 9960 sec)					*W54* ($t_{elution}$ = 14160 sec)				
l (cm)	R_F	σ^2_{chrom} (cm^2)	σ^2_{diff} (cm^2)	$(\sigma^2_{chrom}-\sigma^2_{diff})/l$ (cm)	l (cm)	R_F	σ^2_{chrom} (cm^2)	σ^2_{diff} (cm^2)	$(\sigma^2_{chrom}-\sigma^2_{diff})/l$ (cm)
5.17	0.196	0.303	0.005	0.0575	2.94	0.111	0.135	0.007	0.0435
5.79	0.211	0.430	0.008	0.0730	3.05	0.115	0.135	0.008	0.0417
8.76	0.332	0.260	0.010	0.0285	5.35	0.204	0.105	0.007	0.0184
11.07	0.402	0.251	0.011	0.0217	6.14	0.233	0.142	0.010	0.0215
10.20	0.387	0.262	0.010	0.0247	6.54	0.249	0.156	0.009	0.0225
14.06	0.510	0.326	0.018	0.0219	9.18	0.349	0.149	0.012	0.0150
15.63	0.593	0.228	0.015	0.0137	11.88	0.452	0.135	0.014	0.0102
16.12	0.584	0.305	0.013	0.0182	11.50	0.438	0.175	0.015	0.0139
16.82	0.638	0.295	0.018	0.0165	13.42	0.511	0.257	0.020	0.0176

Therefore, the best linear relationship between $(\sigma^2_{\text{chrom}}-\sigma^2_{\text{diff}})/l$ and $(1-R_F)^2$ was calculated by the method of least squares, allocating to the values of $(\sigma^2_{\text{chrom}}-\sigma^2_{\text{diff}})/l$ statistical weights, inversely related to their variances (see Figs. 3 and 4).

We note the following points:

(a) All intercepts are positive, four of them even significantly larger than zero. This shows that 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.

Our data do not permit us to decide whether the $C_S u$ or the $C_F(u)u$ term is mainly responsible for the remainder of the mass transfer term. However, as STEWART has shown[6] that the flow profile term is very important in paper chromatography, we tentatively equate the mass transfer term to $C_M u + C_F(u)u$. As will be shown below this assumption leads to reasonable values of λ and K in eqn. (5).

(b) In the calculation of $C_M/(1-R_F)^2$ from the slopes it must be realized that in paper chromatography the flow rate of the eluent is not constant throughout the elution time, but is gradually decreasing. The mean flow rate is equal to[8]:

$$\bar{u} = \frac{0.4k}{l_f - l_0} \ln \frac{l_f}{l_0} \qquad (7)$$

TABLE II

VALUES OF γ_M AND γ_S IN PAPER CHROMATOGRAPHY

Chromatography paper	γ_M	γ_S
W 1	0.62 ± 0.16	0.03 ± 0.03
W 2	0.60 ± 0.12	0.03 ± 0.02
W 3 MM	0.44 ± 0.14	0.04 ± 0.03
W 4	0.37 ± 0.17	0.04 ± 0.04
W 17	0.87 ± 0.27	−0.09 ± 0.06
W 20	0.47 ± 0.15	0.06 ± 0.02
W 31 ET	0.42 ± 0.16	0.02 ± 0.04
W 54	0.36 ± 0.09	0.02 ± 0.02

where

k = $(l_f^2 - l_0^2)/t_{\text{elution}}$

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.

From the known values of l_f, l_0 and t_{elution}, the values of k and $\bar{u}$ can be calculated. From the values of $\bar{u}$ and the slopes of the lines in Figs. 3 and 4 $C_M/(1-R_F)^2$ is obtained. The intercepts are equal to $\overline{C_F(u)u}$. Finally, from the values of $C_M/(1-R_F)^2$ and of $\bar{D}_M$*, and eqn. (3), d_p is found. These calculations are summarized in Table III.

The values of d_p in Table III are not significantly different. Their mean value is 0.080 ± 0.010 cm (about twice the value found for thin-layer chromatography). However, just as was found for thin-layer chromatography, they are surprisingly large when compared with the estimated fiber diameter, 0.001 cm[4]. The reason for this discrepancy is probably the same as was postulated for thin-layer chromatography[8]: the fibers are clogging together, thus forming large aggregates.

According to eqn. (5), at the low-velocity limit:

$$\lambda d_p \bar{u} \ll \gamma_M D_M \text{ and } \overline{C_F(u)u} = \frac{2KL^2}{\gamma_M D_M}\bar{u} \qquad (8a,b)$$

whereas at the high-velocity limit

$$\lambda d_p \bar{u} \gg \gamma_M D_M \text{ and } \overline{C_F(u)u} = \frac{2KL^2}{\lambda d_p} \qquad (9a,b)$$

The values of $\overline{C_F(u)u}$ in Table III are not significantly different. Their mean value is 0.007 ± 0.003 cm. Accordingly, they do not depend on $\bar{u}$ so that conditions (9a,b) hold. Substituting into (9a) 0.08 cm for d_p, average values of 10^{-3} cm·sec^{-1} and 0.5 for $\bar{u}$ and γ_M, respectively and 3.10^{-6} cm^2·sec^{-1} for D_M, we get $\lambda \gg 0.015$. Thus, the order of magnitude of λ is the same as in packed columns. Substituting into (9b) 0.007 cm for $\overline{C_F(u)u}$, 0.6 cm for L (the average lateral distance on the paper between maxima or minima in the flow rate[6]), 0.1 for λ and 0.08 cm for d_p, we get $K = 8 \cdot 10^{-5}$, which is, again, of the right order of magnitude.

(c) The effect of unevenness of flow is not nearly as important as in gas chromatography where the $C_F(u)$ term is about 100 times larger than the C_M term.

* $\bar{D}_M = 3.07 \times 10^{-6}$ (cm^2·sec^{-1})^2.

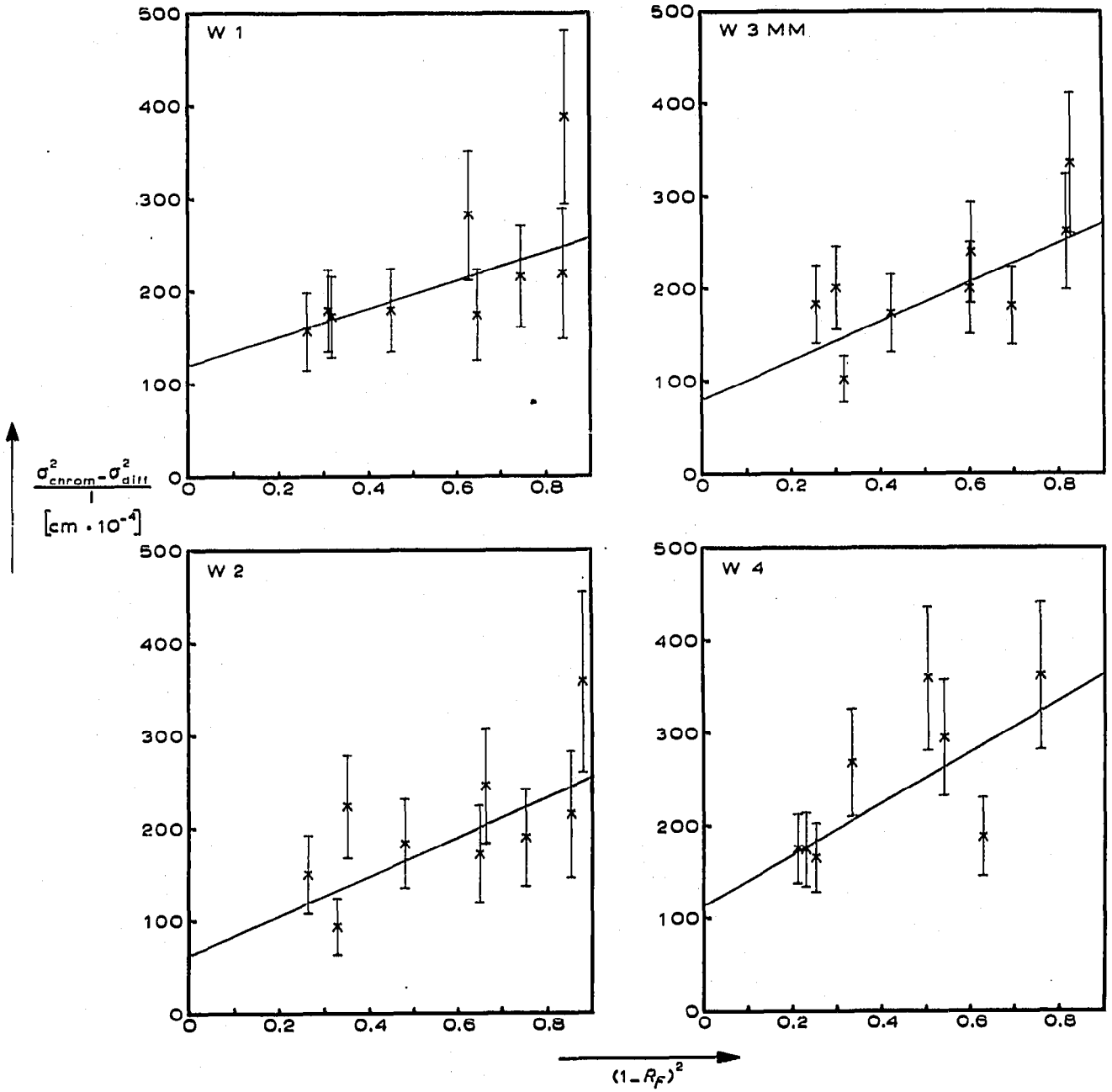

Fig. 3. Mean values of $(\sigma^2_{chrom} - \sigma^2_{diff})/l$ and their 90% probability intervals, as a function of $(1 - R_F)^2$.

In paper chromatography these terms are about equal for medium R_F values; for small R_F values $C_M\bar{u}$ is even larger than $\overline{C_F(u)u}$.

CONCLUSIONS

As found previously, the contribution of longitudinal diffusion in the stationary phase to peak broadening in paper chromatography cannot be neglected.

The mass transfer term is composed of a contribution due to the slowness of

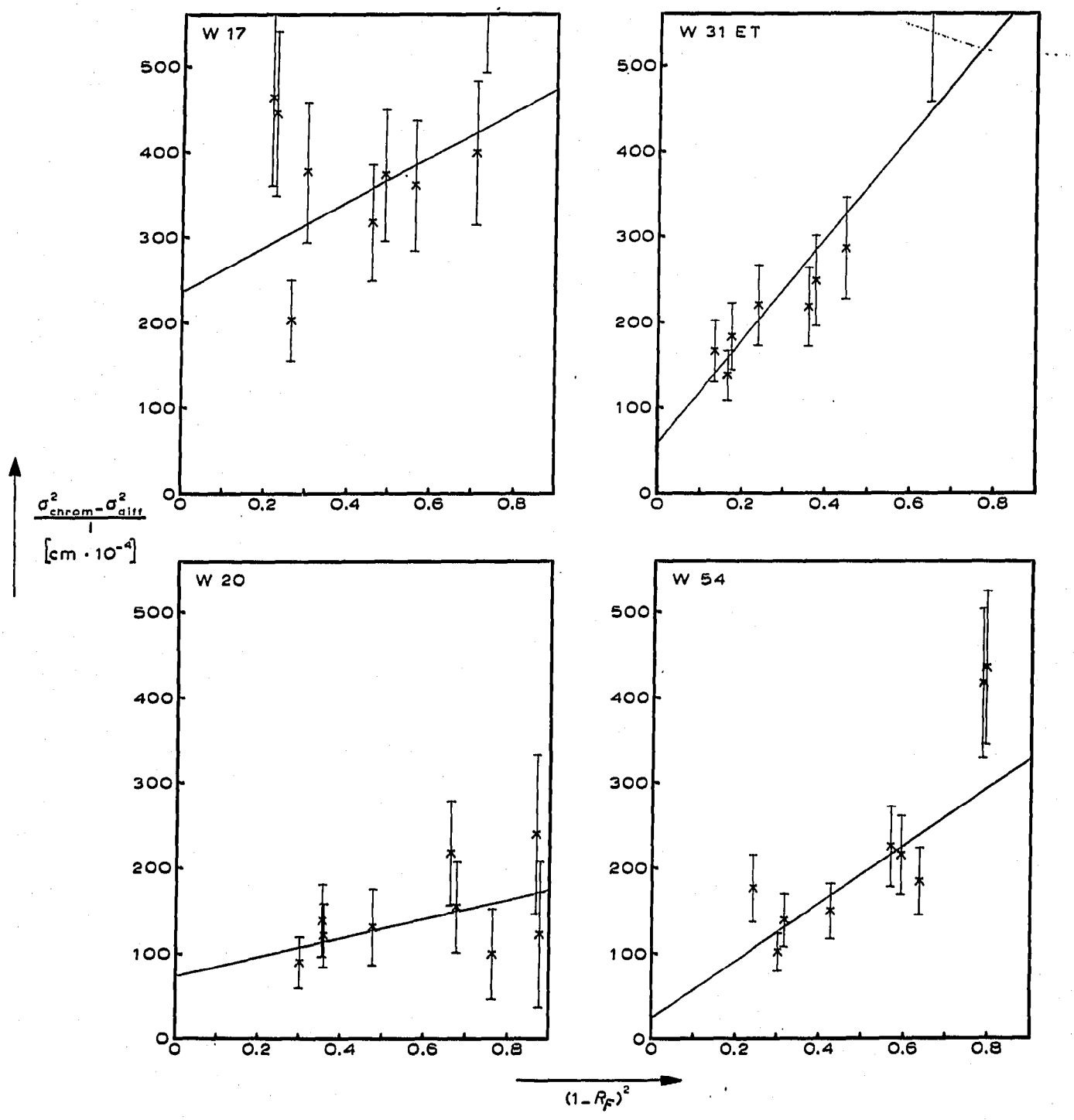

Fig. 4. Mean values of $(\sigma^2_{chrom} - \sigma^2_{diff})/l$ and their 90% probability intervals, as a function of $(1 - R_F)^2$.

attainment of the partition equilibrium between the mobile and the stationary phase (caused mainly by the slowness of diffusion in the mobile phase) and a contribution due to the unevenness of flow throughout the paper.

The values of the effective particle diameter d_p calculated from the values of the

TABLE III

RESISTANCE TO MASS TRANSFER IN PAPER CHROMATOGRAPHY

Chromatography paper	l_0 (cm)	l_f (cm)	k ($cm^2 \cdot sec^{-1}$)	$\bar{u}$ ($cm \cdot sec^{-1}$)	$C_M\bar{u}/(1-R_F)^2$ (cm)	$C_M/(1-R_F)^2 = 0.01 d_p^2/D_M$ (sec)	d_p (cm)	$\overline{C_F(u)u}$ (cm)
W1	6	30.6	0.032	0.00086	0.0151	18	0.074 ± 0.032	0.012 ± 0.007
W2	6	27.8	0.028	0.00079	0.0213	27	0.091 ± 0.036	0.006 ± 0.008
W3 MM	6	31.9	0.040	0.00098	0.0211	22	0.081 ± 0.034	0.008 ± 0.008
W 4	6	33.5	0.060	0.00149	0.0276	19	0.075 ± 0.041	0.011 ± 0.012
W 17	6	33.1	0.069	0.00173	0.0263	15	0.068 ± 0.056	0.024 ± 0.018
W 20	6	20.9	0.016	0.00053	0.0111	21	0.080 ± 0.045	0.007 ± 0.006
W 31 ET	6	33.0	0.106	0.00267	0.0606	23	0.084 ± 0.020	0.006 ± 0.004
W 54	6	32.2	0.071	0.00181	0.0339	19	0.076 ± 0.023	0.002 ± 0.008

former contribution are much larger than the diameter of the cellulose fibers. This is probably caused by the fibers clogging together, thus forming larger aggregates.

The values of the flow profile parameter K and the packing geometry parameter λ, calculated from the latter contribution are of the same order of magnitude as the values given by SIE AND RIJNDERS for packed columns.

Plate heights in paper chromatography are larger than in thin-layer chromatography on cellulose powder for the following reasons:

(1) the values of γ_M are one to two times as large as in thin-layer chromatography;

(2) the effective values of d_p are about twice the values found in thin-layer chromatography;

(3) in paper chromatography, unevenness of flow causes a large contribution to peak broadening. This effect does not occur in thin-layer chromatography.

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