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INFLUENCE OF THE ACCURACY OF THE EXTRA-COLUMN PEAK-WIDTH DETERMINATION ON THE VERIFICATION OF THEORETICAL PLATE-HEIGHT EQUATIONS*

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SUMMARY

The influence of extra-column peak-width contributions on the experimentally observed reduced theoretical plate height is discussed for high-performance liquid chromatographic systems as a function of the reduced flow velocity (Peclet number) and the capacity factor on the basis of model calculations. This allows an estimation of the minimum accuracy of the extra-column peak width, which is necessary for a detailed discussion of the in-column peak-broadening mechanism. The estimated minimum accuracy is compared with typical statistical and systematic errors, associated with the usual methods of measuring extra-column peak widths.

INTRODUCTION

Discussions of a comprehensive theory of chromatographic peak dispersion in column chromatography have been stimulated again by a number of recently published papers¹⁻⁵. The influence of the capacity factor on the contributions to peak variance caused by the mass transfer in the flowing fluid and in the fixed bed is still a subject of controversy^{1,3,6-10}. It seems that one reason for this is the inaccurate experimental determination of the peak width resulting exclusively from the chromatographic process in the column. This contribution is masked by other contributions to the peak width from outside the column. Each theoretical model is based on the assumption that this extra-column contribution to the peak width is known exactly, or that the error in determining it does not significantly affect the results. The validity of these assumptions and its implications for the accuracy of the model form the subject of the following discussion.

First, the systematic divergence between the measured peak width generated by the total chromatographic system and the peak width created by the column is investigated. Only the column contribution is the result of the kinetics of the chromatographic process and is therefore subject to theoretical interpretation^{1,6-12}. The

* Dedicated to Professor A. Neckel on the occasion of his 60th birthday.

superposition of different contributions to the peak variance is investigated by means of model calculations, using a set of reasonable and typical values for the peak-broadening parameters¹⁰. The results of these calculations permit an estimation of the maximum allowable error in the determination of the extra-column contribution to the peak width and specify those ranges of capacity factor and particle size values where the relative extra-column contribution to the total peak width becomes insignificant. Such experimental conditions should be used, if possible, for the experimental verification of theoretical models.

For small particle sizes and small capacity factors, the extra-column contribution to the peak width will not be negligible, and a highly accurate determination of this contribution is required in order to correct for it^{13,14}. Unfortunately, all methods described so far are subject to appreciable statistical or systematic errors. The systematic error involved in estimating the extra-column contribution by use of the linear extrapolation method¹⁵⁻¹⁸ is demonstrated by model calculations, and the approximate magnitude of the extra-column band broadening in typical chromatographic systems is reviewed.

THEORETICAL

The basic equation which relates the output variances of a system, σ^2 , with the variances of its N subsystems, σ_n^2 , is given by

$$\sigma^2 = \sum_n^N \sigma_n^2 \quad (1)$$

where n is the number of the subsystem. This can be used in chromatography¹⁹ to divide the total peak variance, σ_i^2 , into two contributions: one, $\sigma_{c,i}^2$, resulting from the chromatographic column and the second, σ_{ex}^2 , from all the other parts of the system, such as the injection device, connections, capillaries and detector cell:

$$\sigma_i^2 = \sigma_{c,i}^2 + \sigma_{ex}^2 \quad (2)$$

In this equation the subscript i denotes the analyte, indicating that the in-column peak broadening depends on data specific to a particular compound, *i.e.*, the diffusion coefficient and the capacity factor. The influence of the different diffusion coefficients of solutes on the extra-column contribution, σ_{ex}^2 , is neglected. The peak standard deviation, σ_i , can be measured as the half-peak-width at 0.607 of the peak height for pure Gaussian-type peaks, or, in the more general case and as required when measuring without a column, as the square root of the second moment of the peak.

The column variance, $\sigma_{c,i}^2$, given in volume units is related to the retention volume, V_{Ri} , of the compound, i , by the fundamental equation

$$\sigma_{c,i}^2 = \frac{H_i}{L} \cdot V_{Ri}^2 \quad (3)$$

where H_i is the theoretical plate height of the column for the compound i and L is the length of the column. Substitution of eqn. 3 into eqn. 2 leads to

$$\begin{aligned} \sigma_i^2 &= \frac{H_i}{L} \cdot V_{Ri}^2 + \sigma_{ex}^2 \\ &= \frac{H_i}{L} \cdot V_{Ro}^2 (1 + \kappa_i)^2 + \sigma_{ex}^2 \end{aligned} \tag{4}$$

where κ_i is the capacity factor of component i and V_{Ro} is the retention volume of an unretained substance. One of the two contributions to the total variance depends on the square of the retention volume, V_{Ri} , the other is independent of it. The retention-dependent contribution depends also on the theoretical plate height, H_i .

The theory of the chromatographic process^{1,6-12} shows that H_i is a function of the average flow velocity, v , the mean particle diameter, \bar{d}_p , of the fixed bed, the capacity factor, κ_i , and the diffusion coefficients, D_{mi} and D_{pi} , of the solute in the mobile phase, m , and the particles, p , of the fixed bed and of a number of geometry factors specific to the particles and the bed. Introducing dimensionless variables

$$h_i = H_i / \bar{d}_p \tag{5a}$$

$$v_i = v \bar{d}_p / D_{mi} \tag{5b}$$

where v_i is the reduced flow velocity, in chemical engineering the Peclet number (Pe). The theoretical plate-height equation of Huber^{7,10} is

$$h_i = \frac{a_1}{v_i} + \frac{a_2}{1 + a_3 v_i^{-\frac{1}{2}}} + a_4 v_i^{\frac{1}{2}} \left(\frac{\kappa_i^*}{1 + \kappa_i^*} \right)^2 + a_5 v_i \cdot \frac{\kappa_i^*}{(1 + \kappa_i^*)^2} \tag{6a}$$

or

$$h_i = h_{di} + h_{ci} + h_{fi} + h_{bi} \tag{6b}$$

The subscripts d, c, f and b indicate the contributions associated with various dispersion processes: mixing due to axial diffusion, convective mixing, mass exchange in the flowing fluid and mass exchange in the fixed bed (including the contributions from the stagnant mobile phase and the stationary phase). The factors, a_1 – a_5 , are constants for a given column, depending on the geometry of the particles and of the packing; κ_i^* is defined as the mass distribution coefficient between the fixed bed, b, and the flowing fluid, f

$$\kappa_i^* = \frac{\varepsilon_m}{\varepsilon_f} (\kappa_i + 1) - 1 \tag{7}$$

where κ_i is the mass distribution coefficient (capacity factor) between the stationary phase, s, and the mobile phase, m, ε_m is the fraction of the column volume occupied by the mobile phase and ε_f is the fraction occupied by the flowing part of the mobile phase.

If the geometry factors, a_1 – a_5 , in eqn. 6a are known, h_i can be calculated for various flow velocities, particle diameters, diffusion coefficients and mass distribution

coefficients. Huber *et al.*¹⁰ investigated silica-packed columns and determined the geometry factors by non-linear regression analysis of experimental σ_i^2 data, minimizing an appropriate least-squares function:

$$F = \sum_m \sum_i \left(\frac{h_{i,m}^{\text{exp}} - h_{i,m}^{\text{calc}}}{h_{i,m}^{\text{exp}}} \right)^2 \quad (8)$$

Here, the index i represents the solutes and m the experiments made for a solute i . In a given experiment, h_i^{exp} describes the experimentally found column band broadening, evaluated by means of eqn. 9 after measuring σ_i^{exp} , σ_{ex} and V_{Ri} :

$$h_i = \frac{(\sigma_i^{\text{exp}})^2 - \sigma_{\text{ex}}^2}{V_{Ri}^2} \cdot \frac{L}{\bar{d}_p} \quad (9)$$

h_i^{calc} is estimated by use of eqn. 6a.

Considering the influence of the capacity factor on the value of h_i , Huber *et al.*¹⁰ found for silica packings a significant dependence of h_i on κ_i , higher capacity factors resulting in higher values of h_i . Consideration of these results leads to the conclusion that eqn. 4 cannot give a linear dependence of σ_i^2 on V_{Ri}^2 , because both V_{Ri} and H_i are functions of κ_i . This means that a linear regression analysis, performed on σ_i^2 vs. V_{Ri}^2 data, will give an intercept which is not exactly identical to σ_{ex}^2 . Since H_i is also a function of D_{mi} , sometimes this effect may also mask the correlation of σ_i^2 with V_{Ri}^2 .

The geometry factors determined by Huber *et al.*¹⁰ show that the contribution h_{bi} is relatively small for the flow velocities and particle sizes used in practice. For a correct differentiation of the various h contributions in eqn. 6 and the estimation of their magnitude, especially of the two mass-exchange contributions, h_{fi} and h_{bi} , it is necessary to know the experimental value, h_i , with great accuracy. Eqn. 9 shows that the accuracy of h_i depends strongly on the accuracy of σ_{ex} . The error, Δh_i , in h_i which results purely from the error, $\Delta \sigma_{\text{ex}}$, in σ_{ex} , is given by eqn. 10a:

$$\Delta h_i = -2 \frac{\sigma_{\text{ex}} \Delta \sigma_{\text{ex}}}{V_{Ri}^2} \cdot \frac{L}{\bar{d}_p} \quad (10a)$$

$$\Delta \sigma_{\text{ex}} = -\frac{\bar{d}_p V_{Ri}^2}{2L} \cdot \frac{\Delta h_i}{\sigma_{\text{ex}}} \quad (10b)$$

Eqn. 10b gives the minimum accuracy in σ_{ex} which is necessary to keep the error in the reduced plate height below a desired level, Δh_i . If, for instance, the error, Δh_i , in the reduced theoretical plate height is required to be less than half of one of the four contributions to h_i , the maximum allowable error, $\Delta \sigma_{\text{ex}}^{\text{max}}$, in the determination of the extra-column peak width, at a given value of σ_{ex} , is obtained from eqn. 10b and given by the expression

$$\Delta \sigma_{\text{ex}}^{\text{max}} = \frac{1}{4} \cdot \frac{\sigma_{ki}^2}{\sigma_{\text{ex}}} \quad (11)$$

where k refers to the subscripts d, c, f, b in eqn. 6b and σ_{ki}^2 is correlated to h_{ki} by analogy with eqn. 3.

At a constant error $\Delta\sigma_{ex}$, one can calculate the maximum allowable extra-column peak width, σ_{ex}^{max} , which just gives the desired accuracy for a given peak-broadening contribution:

$$\sigma_{ex}^{max} = \frac{1}{4} \cdot \frac{\sigma_{ki}^2}{\Delta\sigma_{ex}} \tag{12}$$

RESULTS AND DISCUSSION

Effect of the extra-column peak-width contribution on the $h(v)$ curve

The strategy of model calculations was chosen to discuss the influence of the extra-column peak-width contribution on the accuracy of the theoretical plate-height determination. First, the distortion of a "true" $h_i(v_i)$ curve by an assumed extra-column contribution, σ_{ex} , is investigated for different capacity factors and for different particle diameters. In this approach a calculated $h_i(v_i)$ curve is assumed to be the "true" curve: the equation of Huber^{6,7} (eqn. 6a) is used to describe the dependence of h_i on the reduced flow velocity, v_i , and on the capacity factor, κ_i . For the geometry constants, a_1 - a_5 , the following values obtained for a well packed silica column¹⁰ were used: $a_1 = 2.90$, $a_2 = 1.30$, $a_3 = 7.60$, $a_4 = 0.92$ and $a_5 = 0.10$. The apparent values, h'_i , are now estimated by including also the assumed extra-column contributions to the peak width, σ_{ex}^2 . Three different assumptions, listed in Table I, have been made for σ_{ex} . These give σ_{ex} as a function of the flow-rate. Fig. 1 shows the influence of the different assumed σ_{ex} values on the $h'_i(v_i)$ curves for two different capacity factors. Fig. 1a deals with a small particle size ($\bar{d}_p = 5 \mu\text{m}$), Fig. 1b with a large particle size ($\bar{d}_p = 35 \mu\text{m}$). A marked influence of σ_{ex}^2 on h'_i is seen for small particles and for small capacity factors. The influence is much less pronounced for high capacity factors. In Fig. 1a the superposition of the extra-column variance produces a crossing of the h'_i curves corresponding to different capacity factors. In practice, this indicates appreciable extra-column contributions to the peak width. Such an effect is not observed with large particles.

From these calculations two important conclusions follow immediately: first,

TABLE I

ASSUMED VALUES FOR THE EXTRA-COLUMN PEAK WIDTH, σ_{ex} (μl), AS A FUNCTION OF THE FLOW-RATE

These data are used in the calculations shown in Fig. 1. A flow-rate of 1.0 ml/min corresponds to a reduced velocity of about 4.5 for a column of 4 mm I.D. and 5- μm particles. D_{mi} is assumed to be $3.7 \cdot 10^{-5} \text{ cm}^2/\text{s}$.

Assumption	Flow-rate (ml/min)					
	0.5	1.0	1.5	2.0	2.5	3.0
1	5	5	5	5	5	5
2	5	10	15	20	25	30
3	8	16	24	32	40	48

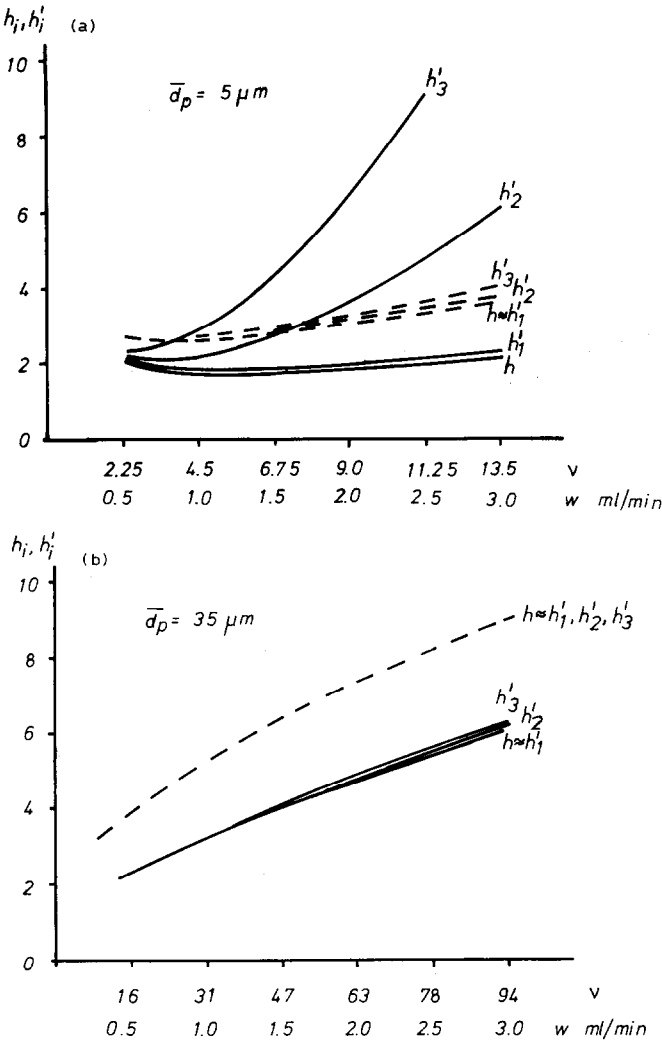


Fig. 1. Influences of the particle diameter and the solute capacity factor on the relative effect of the extra-column peak-width contribution to the h'_i vs. v_i curves. Particle size: (a) small ($\bar{d}_p = 5 \mu m$) and (b) large ($\bar{d}_p = 35 \mu m$). Capacity factors: small, $\kappa_i = 0.2$ (—); large, $\kappa_i = 5.0$ (-----). The h_i curves are calculated according to eqn. 6a by using the geometry factors $a_1 = 2.90$, $a_2 = 1.30$, $a_3 = 7.60$, $a_4 = 0.92$, $a_5 = 0.10^{10}$. The "apparent" curves, h'_1 , h'_2 , h'_3 , are obtained by superposition of the assumed extra-column band-broadening contributions of Table I on the corresponding h_i curves. The indices 1, 2, 3, refer to the various assumptions in Table I. Column data: 250 mm \times 4 mm I.D., ϵ_m 0.8, ϵ_r 0.4; D_{mi} $3.7 \cdot 10^{-5}$ cm²/s. w = flow-rate corresponding with v .

investigations on the influence of the capacity factor on the theoretical plate height should be performed with large particles, whenever possible, in order to avoid significant interference by the σ_{ex}^2 contribution and inadequate corrections. However, as a consequence, measurements near the minimum of the $h'_i(v_i)$ curve require very small flow velocities. Secondly, the influence of σ_{ex}^2 can be significantly reduced, even

for small particles, by using solutes with large capacity factors. However, it must be kept in mind that the functions $\left(\frac{\kappa_i^*}{1 + \kappa_i^*}\right)^2$ and $\frac{\kappa_i^*}{(1 + \kappa_i^*)^2}$ associated with the two mass-exchange terms vary slightly at high capacity factors. Hence the determination or verification of the different κ -dependence of these two terms should be done at small capacity factors.

Estimation of minimum accuracy of σ_{ex}^2

Based on calculations of h_i by means of the geometry factors a_1 - a_5 , given before¹⁰, the minimum accuracy of the extra-column variance, σ_{ex}^2 , is evaluated, which is needed to avoid a significant interference with the small contributions to h_i , e.g., the contribution h_{bi} in eqn. 6. An accurate quantitation of h_{bi} together with h_{fi} is important for estimating correctly the influence of the capacity factor, κ_i , on the $h_i(v_i)$ function. Eqn. 11 gives the maximum allowable error, $\Delta\sigma_{ex}^{max}$, in the extra-column peak width, producing an error in a particular contribution to h_i , which is just half of this contribution. It is seen from eqn. 10b that at small values of σ_{ex} a greater maximum allowable error, $\Delta\sigma_{ex}^{max}$, in its determination is allowed to achieve a constant error, Δh_i , in the theoretical plate height. This is of some importance, because the errors, $\Delta\sigma_{ex}$, (and difficulties) in the experimental determination of the extra-column peak width, σ_{ex} , increase at high flow-rates, as does the peak width itself.

Let us assume that the error, Δh_i , should be smaller than half of the mass-exchange term, h_{bi} , in the fixed bed, which has been recognized, at least for silica columns¹⁰, to be very small at moderate values of the reduced flow velocity, v_i . With this assumption, Fig. 2 gives the maximum allowable error, $\Delta\sigma_{ex}^{max}$, as a function of the magnitude of the extra-column band width, σ_{ex} , for various reduced flow velocities. The contribution, h_{bi} , is calculated from eqn. 6a using the value $a_5 = 0.10$ given before¹⁰.

Table II gives the maximum acceptable extra-column peak width, σ_{ex}^{max} , at various flow-rates, obtained by eqn. 12, assuming various constant errors, $\Delta\sigma_{ex}$, i.e., 3 and 10% relative error and 1 μl absolute error. In Table III these data are compared with typical values for σ_{ex} , measured in various chromatographic systems. It is seen that an accurate determination of the minor contributions to h_i requires that the extra-column peak width be smaller than the values usually obtained by using standard equipment.

Accuracy of the experimental determination of the extra-column peak width

In the discussion of the maximum acceptable error in the extra-column peak width, the possibilities of its accurate experimental determination must be considered critically. Several methods have been proposed for this purpose.

(i) Determination of the column contribution to the overall variance by varying the column length. Extrapolation to zero length gives the extra-column variance. This method requires columns of exactly the same dispersion per unit length, which cannot be assumed *a priori*. Variation of the column length by coupling of columns for which the peak dispersion characteristics have been determined previously may be an acceptable approach, providing no additional contributions are introduced by the connections between the columns. At the present time, no experimental values are available concerning the accuracy and precision of this method.

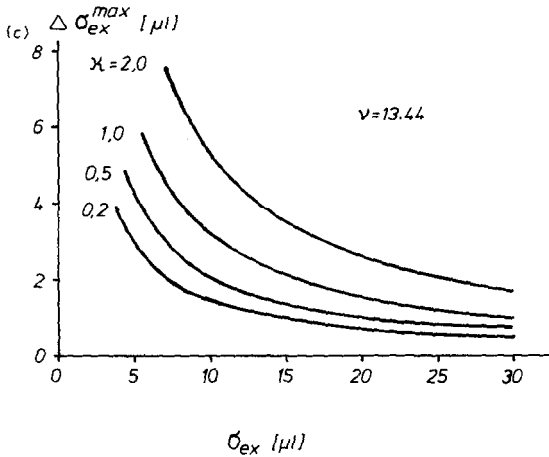
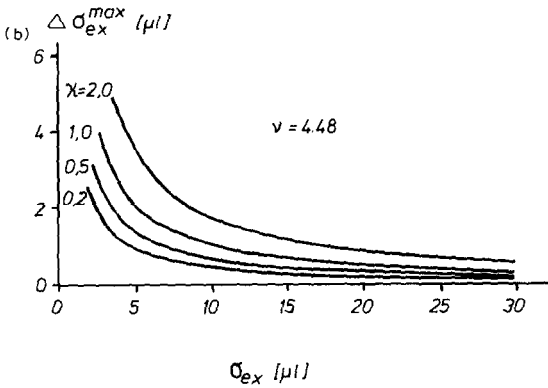
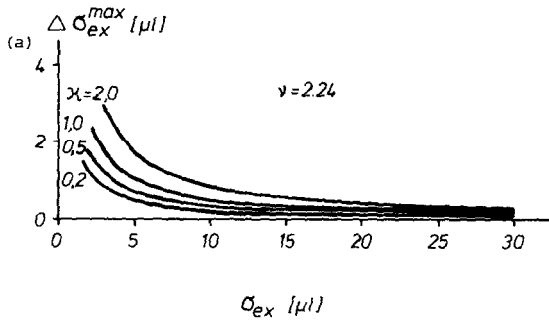


Fig. 2. Maximum allowable error, $\Delta \sigma_{ex}^{max}$ (μl), in the extra-column peak width as a function of the extra-column peak-width contribution, σ_{ex} , at various capacity factors, κ , and reduced flow velocities, v . The maximum allowable error is defined in this context as the error which allows the estimation of h_t with an accuracy and precision better than half of the contribution $h_{t,ex}$ in the fixed bed. Column data as in Fig. 1 except 5- μm particles and $v = 2.24$ (a), 4.48 (b) and 13.44 (c).

TABLE II

MAXIMUM ALLOWABLE EXTRA-COLUMN PEAK WIDTH, σ_{ex}^{max} (μl), FOR DIFFERENT VALUES OF THE ERROR, $\Delta\sigma_{ex}$, IN ITS DETERMINATION

The maximum allowable error is defined as the value which permits the estimation of h_i with an accuracy and precision equal to half of the contribution h_{bi} , where h_{bi} is calculated from eqn. 6a by use of the geometry factor $a_5 = 0.10$. Column data: 250 mm \times 4 mm I.D., 5- μm particles, ϵ_m 0.8, ϵ_r 0.4; D_{mi} $3.7 \cdot 10^{-5}$ cm²/s.

v_i	κ_i	$\Delta\sigma_{ex}$		
		+ 3%	+ 10%	+ 1 μl
2.25	0.2	9.0	5.1	2.4
	0.5	11.0	6.0	3.6
	1.0	13.3	7.3	5.3
4.50	0.2	13.0	7.1	5.1
	0.5	15.3	8.4	7.0
	1.0	18.8	10.3	10.6
6.75	0.2	15.9	8.7	7.6
	0.5	18.8	10.3	10.6
	1.0	23.1	12.7	16.0
13.5	0.2	22.3	12.2	14.8
	0.5	26.6	14.6	21.2
	1.0	32.7	17.9	31.9

TABLE III

APPROXIMATE EXPERIMENTAL EXTRA-COLUMN PEAK WIDTH, σ_{ex} (μl), REPORTED FOR TYPICAL CHROMATOGRAPHIC SYSTEMS EQUIPPED WITH SPECTROPHOTOMETRIC DETECTORS

Flow-rate (ml/min)	Reference				
	*	17	18	13	14
0.2			8-15		
0.5	12-17	7.5		7.5	2.5-15
1.0	17-22				5-22
1.2		9.7		9.0	
1.5	22-27				
2.4		13.3		9.9	
3.0	32-45			10.0	
Detector cell volume (μl)	8	4	8	4.5	1-4.5
Injection volume (μl)	10	16	1	1	0.5
Eluent constituents (volume ratio)	Heptane	Water- acetonitrile (3:2)	Water- methanol (1:1)	Water- acetonitrile (3:2)	

* Present measurements on a commercially available standard system without optimization.

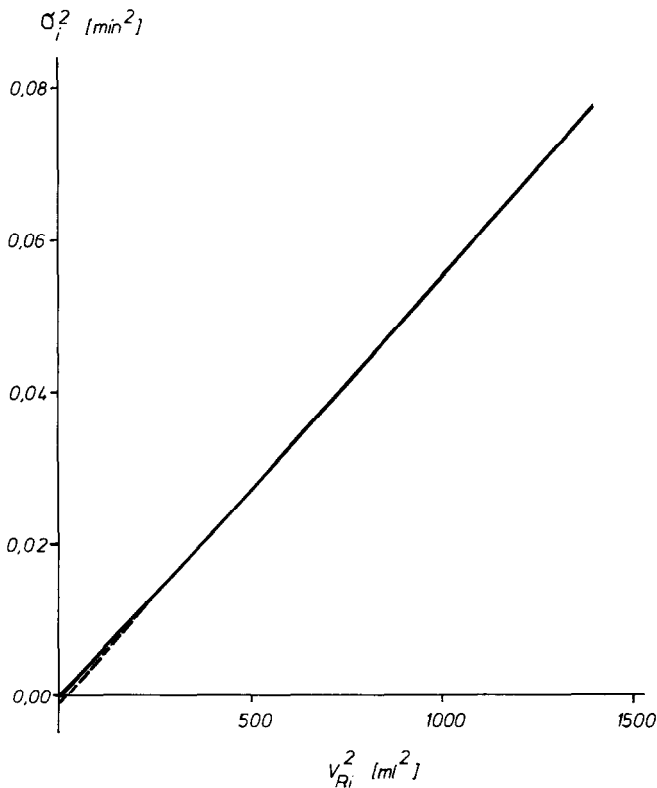


Fig. 3. Example of a σ_i^2 versus V_{Ri}^2 plot. The broken line represents the linear extrapolation from high V_{Ri}^2 values to zero retention volume. The data are calculated from eqn. 6a by using the geometry factors in Fig. 1. Column data as in Fig. 1 except 5- μ m particles and $v = 4.5$.

(ii) Measuring the band broadening in a system consisting of an injector, connectors and detector but without the column¹³. In this method the chromatographic system without the column must be simulated exactly, and this is quite difficult in practice. Due to the reduction of the flow resistance, the flow conditions and the streaming profiles are not exactly the same. An inaccuracy in σ_{ex} of at least 5% is expected although the measurements can be carried out with a precision of about 2%¹³.

(iii) A linear regression of the total peak variance, σ_i^2 , and the square of the retention volume, V_{Ri}^2 . The intercept of this straight line can be used as an approximate measure of the extra-column peak variance, σ_{ex}^2 (refs. 15–18). This approach neglects the fact that the theoretical plate height, H_i , depends on the capacity factor, κ_i , and on the diffusion coefficient, D_{mi} , of the solutes. The slope, H_i/L , of the $\sigma_i^2(V_{Ri}^2)$ function will therefore not be constant, unless accidentally in cases where the influences of the capacity factor and of diffusion coefficient compensate each other. Since h_i depends appreciably on κ_i (refs. 6, 7, 9 and 10), this strategy produces regression results (intercepts) that cannot be assumed to be a good approximation for σ_{ex}^2 . This is illustrated by the model calculations shown in Fig. 3: h_i values are cal-

TABLE IV

EXTRA-COLUMN PEAK-WIDTH CONTRIBUTION, σ_{ex} , OBTAINED FROM THE INTERCEPT OF THE $\sigma^2(V_{Ri}^2)$ REGRESSION LINE

The capacity factors ranged from 0.2 to 10.0.

Flow-rate (ml/min)	v	σ_{ex} (μ l)	
		Assumed*	Extrapolated**
0.5	2.25	0	-15.3
1.0	4.5	0	-17.2
2.0	9.0	0	-20.8
3.0	13.5	0	-22.6

* Value chosen for the calculation of the σ^2/V_{Ri}^2 function.** Estimated from eqn. 4, where H_i is calculated according to eqn. 6a using the geometry factors $a_1 = 2.90$, $a_2 = 1.30$, $a_3 = 7.60$, $a_4 = 0.92$, $a_5 = 0.10^{10}$. A constant diffusion coefficient was assumed for all solutes. Column data as in Table II.

culated for several solutes with equal diffusivity but different capacity factors, using the same geometry constants, a_1 - a_5 , as before¹⁰ and applying the Huber equation⁷. The extra-column peak width, σ_{ex} , is assumed to be zero. The σ_i^2 values corresponding to the calculated h_i data are plotted *versus* V_{Ri}^2 . A slightly concave curve is obtained in the usual working range. This implies that a linear regression of these data yields intercepts smaller than the true value of σ_{ex}^2 . Table IV summarizes the error in determining the extra-column peak width by this linear regression method. The negative values indicate that the true extra-column peak broadening is underestimated by this procedure. The errors obtained by this model calculation (capacity factor ranges from 0.1 to 10) are appreciable. Although the obtained error may be smaller when using a smaller capacity factor range, it is clear that this method cannot fulfil the requirements of an accurate determination.

CONCLUSIONS

Model calculations have been used to illustrate the importance of accuracy and precision in the measurement of the extra-column peak width when the peak dispersion process in the column is investigated. Since the "true" $h_i(v_i)$ curve has to be recalculated from the measured, "apparent" curve, $h'_i(v_i)$, a certain error is introduced in the model when the extra-column peak width is not negligible or cannot be determined accurately and precisely. This problem is not so important when large particles are used and for solutes with large capacity factors, since the column peak-width contribution is large under these circumstances. Microparticulate columns, however, are superior in separation performance and large particles imply very low flow velocities when working near the theoretical plate-height minimum. For the investigation of the capacity factor dependence of the theoretical plate height, solutes with low capacity factors have to be used in order to be able to separate the effects of the two mass-exchange terms, h_{fi} and h_{bi} . Therefore it is essential to minimize the extra-column peak width contribution and to reduce the error in its determination.

The maximum error in the extra-column peak width was calculated which still allows determination of the smallest contribution to the theoretical plate height, without this value being significantly falsified. This error is dependent on the extra-column variance (which itself depends on the chromatographic equipment and the flow-rate) on the one hand and the column volume and the capacity factor on the other hand. When assuming typical extra-column variances measured and reported in the literature, their errors should be less than a few microlitres at column volumes of a few millilitres. Unfortunately, the systematic error in the experimental determination of the extra-column peak width was found to be of the same order of magnitude in most cases.

Thus, the accuracy or the precision of the experimental determination of the extra-column peak width contribution in chromatographic systems must be carefully checked whenever a verification of theoretical models of chromatographic processes is attempted. The approximate evaluation of this value from the intercept of a linear regression in a $\sigma_i^2(V_{Ri}^2)$ diagram is unsatisfactory in this context.

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