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NON-LINEAR ELUTION PEAK MODEL

INFLUENCE OF THE MOBILE PHASE COMPOSITION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

The asymmetries of the peak profiles which are generally observed when a chromatographic column is overloaded are mainly caused by the non-linearity of the partition equilibrium isotherm. For moderate concentrations the isotherms can most often be replaced by a two-term expansion. Then the system of mass-balance equations can be solved and an analytical equation for the peak profiles is obtained. The changes in the peak shape and the retention time of the peak maximum with the amounts of benzyl alcohol injected have been studied for various eluent compositions in reversed-phase high-performance liquid chromatography. The experimental and theoretical elution profiles are in good agreement for the major part of the elution peak. The adsorption isotherm was shown to be of the Langmuir type. The maximum number of molecules adsorbed, as deduced from the values of the slope and curvature of the isotherm at the origin, does not change with the solvent composition. The peak asymmetry is independent of the mobile phase composition and is improved by increasing the column loading capacity per unit length.

INTRODUCTION

The study of the elution peaks obtained at finite concentrations in liquid chromatography[1] leads to the determination of the equilibrium isotherm, which gives information about the solute, solvent and stationary phase interactions[2]. Evidence of the importance of this approach in measuring physicochemical interactions is provided when the assumption of linear chromatography is not valid. When dealing with rare or expensive biological products, the use of small quantities of solutes is necessary. Therefore it is more appropriate to extract as much information as possible from the concentration profiles of the elution peak, than to use the frontal analysis method[3,4] which necessitates larger sample sizes.

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In previous work[1], we started with a simple mass-balance equation for liquid chromatography and derived an analytical solution of the peak-profile equation which is valid for concentrations leading to moderate overload. The model is based on a two-term expansion of the isotherm equation and is thus related to the first and second derivatives of the partition isotherm function at origin (for zero concentration). The retention behaviour of benzyl alcohol eluted at finite concentration in reversed-phase chromatography was investigated at various temperatures and its partition isotherm was shown to be of the Langmuir type.

The aim of the present work is to characterize the elution peak distortion observed at finite concentration and to test the validity of the model when changing the eluent composition.

THEORETICAL

The propagation of the concentration profile for a solute in a mobile phase is described by the mass-balance differential equation of liquid chromatography[1]

$$\frac{\partial[c + \mathrm{k}(c)]}{\partial t} + \frac{\partial(uc)}{\partial z} = D \cdot \frac{\partial^2 c}{\partial z^2} \tag{1}$$

where $\mathrm{k}(c)$ is the fictive concentration of adsorbed solute, c is the concentration of the solute in the mobile phase, t is the time elapsed from the injection time, z is the distance along the column, u is the velocity of the mobile phase and D is the apparent diffusion coefficient.

The equilibrium between the mobile and stationary phases is described by a relation involving n_{A}, the amount of solute in the stationary phase (in moles) and c, the solute concentration in the mobile phase

$$n_{\mathrm{A}}/V_{\mathrm{L}} = \mathrm{k}(c) \tag{2}$$

where V_{L} is the volume of the liquid phase in the column.

For a convex adsorption isotherm, the Langmuir equilibrium model (using two parameters α and β) is generally used

$$\frac{n_{\mathrm{A}}}{V_{\mathrm{L}}} = \frac{\alpha c}{1 + \beta c} \tag{3}$$

where the maximum solute amount adsorbed (in moles) is $N_{\mathrm{M}} = \alpha V_{\mathrm{L}}/\beta$.

An analytical expression for the elution peak profile can be deduced from eqn. 1 for a limited non-linearity of the partition isotherm, expanded to the second term

$$\frac{n_{\mathrm{A}}}{V_{\mathrm{L}}} = k'_0 c + \frac{k''_0 V_{\mathrm{L}} c^2}{2} \tag{4}$$

where the column capacity factor is defined as:

$$k' = \partial n_{\mathrm{A}}/\partial n_{\mathrm{L}} = k'_0 + k''_0 V_{\mathrm{L}} c \tag{5}$$

In the case of a Langmuir isotherm, k'_0 and k''_o are related to the parameters α and β of eqn. 3 according to:

$$k'_0 = \alpha \qquad k''_0 = -2\,\alpha\beta/V_L \tag{6}$$

Here n_L ($= V_L c$) is the number of moles of solute in the mobile phase.

The elution profile is then described by an equation which depends on four parameters:

(1) the peak size parameter, a, corresponding to the effective peak area, A_T, of the theoretical peak for small sample sizes

(2) the slope of the isotherm at the origin, k'_0, which is related to the retention time at infinite dilution, t_R:

$$t_R = (1 + k'_0)t_0 \tag{7}$$

(3) the global apparent dispersion coefficient, D', which is related to the standard deviation, σ_0, of a gaussian peak:

$$\sigma_0 = t_R \sqrt{2D'\,t_R/L} \tag{8}$$

(4) the curvature of the isotherm at the origin, k''_0, which is related to the peak slant coefficient, λ:

$$\lambda = k''_0 V_L/(1 + k'_0) \tag{9}$$

The elution profile expression in the case of a quasi-pulse injection is

$$c = \frac{2}{\lambda U}\left(\frac{D'}{\pi t}\right)^{1/2} \cdot \frac{\exp\left(-\dfrac{\xi^2}{4\,D't}\right)}{\coth\dfrac{\mu}{2} + \operatorname{erf}\,[\xi/2(D't)^{1/2}]} \tag{10}$$

where

$$U = L/t_R \quad \text{and} \quad \xi = L - Ut \tag{11}$$

$$D' = D/(1 + k'_0) \tag{12}$$

$$\mu = a\lambda U^2/2D' = a\lambda t_R/\sigma_0^2 \tag{13}$$

and L the column length. The peak-profile model (eqn. 10) is valid when $|\lambda|\, C_M \ll 1$. In this case $a = A_T$.

A simple equation exists between the peak slant coefficient and the coordinates, t_M and C_M, of the peak maximum:

$$C_M = (t_M - t_R)/\lambda t_M \tag{14}$$

This equation corresponds to an hyperbola, which may be reduced to a straight line for small values of C_{M}:

$$C_{\mathrm{M}} \approx (t_{\mathrm{M}} - t_R)/\lambda t_R \tag{15}$$

EXPERIMENTAL

The high-performance liquid chromatography (HPLC) system consisted of a Model 111 B pump (Beckman, Berkeley, CA, U.S.A.) and a Model 7125 sampling valve (Rheodyne, Berkeley, CA, U.S.A.) with a 40-μl loop. The detector used was a differential refractometer Model R401 (Waters, Milford, MA, U.S.A.). Its response to benzyl alcohol was linear over the whole concentration range studied.

A microcomputer CBM 4032 (Commodore, Santa Clara, CA, U.S.A.) interfaced with a 3497 digital voltmeter (Hewlett-Packard, Palo Alto, CA, U.S.A.) was used for the data-acquisition system with four-digit precision. The data were fitted to the theoretical model using a non-linear least squares program written in Fortran and the computer of the Centre Inter Régional de Calcul Electronique (Orsay, France).

The solvent used was a binary mixture of water and methanol (99% purity; Carlo Erba, Milan, Italy) at three different compositions, 70:30, 80:20 and 90:10 (w/w). The solute used was benzyl alcohol (99.5% purity, Carlo Erba).

The adsorbent, LiChrosorb C_{18}, particle size 10 μm (Merck, Darmstadt, F.R.G.), was packed into a 25 cm × 0.46 cm I.D. stainless-steel column, using the slurry technique. The column was placed in a water-bath at 20 ± 0.1°C.

The dead volume of the column, $V_{\mathrm{L}} = 2.7$ ml, was obtained by weighing it when filled either with carbon tetrachloride or methanol[5].

RESULTS

The characteristics of the partition isotherm at low surface coverage can be deduced either by plotting the positions of the peak maxima *versus* the sample sizes or by curve fitting of the model equation to the elution peak[1].

Plot of the retention time of the peak maximum with increasing sample size

When increasing size samples are injected, the determination of the coordinates of the peak maximum is easy: C_{M} is the maximum concentration of the experimental profile and t_{M} the corresponding elution time; C_{M} takes into account the detector calibration function.

The plot of the positions of the peak apices is well approximated by a hyperbola according to eqn. 14, for the whole concentration domain studied. From this plot (Fig. 1) we have derived the first and second derivatives of the equilibrium isotherm, the values of which are listed in Table I for various eluent compositions.

Adjustment of the model to the experimental elution peak

We have studied the modifications of the elution profiles obtained when increasing amounts of benzyl alcohol are injected into the column. The investigations were carried out at three different eluent compositions (Fig. 2). The least squares fit

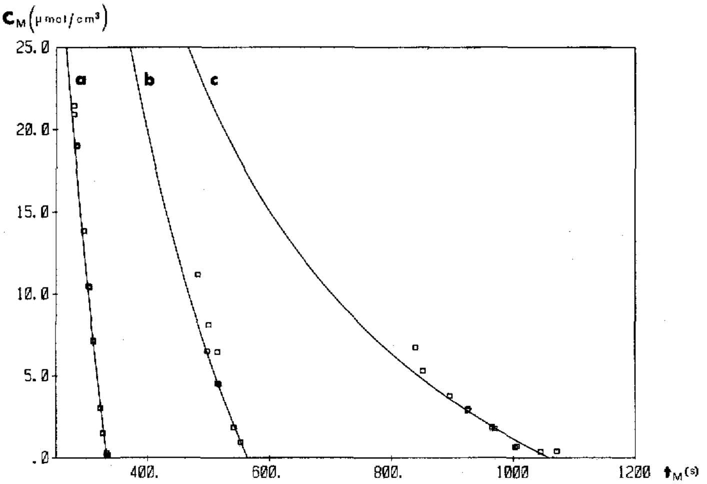

Fig. 1. Variation of the retention time of the peak maximum with the maximum concentration for benzyl alcohol. □, Experimental results; ——, theoretical curve (eqn. 14). Water–methanol mobile phases: (a) 70:30; (b) 80:20; (c) 90:10 (w/w).

of the theoretical model to the experimental data is good for the major part of the elution peak. The deviations which occur at both ends of the peak are caused by experimental distortions arising from inhomogeneities in the column packing.

The values of the four parameters necessary to define the elution profile are listed in Table II. The analytical expression of the peak profile is valid only for low concentrations, when the equilibrium isotherm can be expanded to its second term, and when $|\lambda| C_M \ll 1$. The first requirement is fulfilled when the plot of C_M *vs.* t_M can be approximated by an hyperbola. The validity of this hypothesis for the whole concentration range studied was demonstrated by the apex-locus method. The second one ($|\lambda| c_M \ll 1$) can be checked by comparing the peak-size parameter, *a*, and the effective peak area, A_T. The latter is always lower than *a* when the value of $|\lambda| C_M$ is large. For the maximum value of $|\lambda| C_M$ studied (0.30), a 10% deviation is observed between *a* and A_T. This figure may be used to estimate the degree of validity of the model at high concentrations. The values of k'_0 and k''_0, especially at high concentrations, are in good agreement with those obtained from the apex-locus method. The

TABLE I

COMPARISON OF THE CHARACTERISTICS OF ADSORPTION ISOTHERMS AT THREE DIFFERENT ELUENT COMPOSITIONS

Water–methanol eluent (w/w)	*Apex-locus method*				*Model-fitting method*			
	α	k''_0 (µmol^{-1})	$10^3 \cdot \beta$ (cm³/µmol)	N_M (µmol)	α	k''_0 (µmol^{-1})	$10^3 \cdot \beta$ (cm³/µmol)	N_M (µmol)
90:10	12.92	−0.240	25	1384	13.26	−0.25	26	1377
80:20	6.45	−0.058	12	1414	6.79	−0.057	11.6	1580
70:30	3.41	−0.016	6.4	1428	3.47	−0.016	6.3	1487

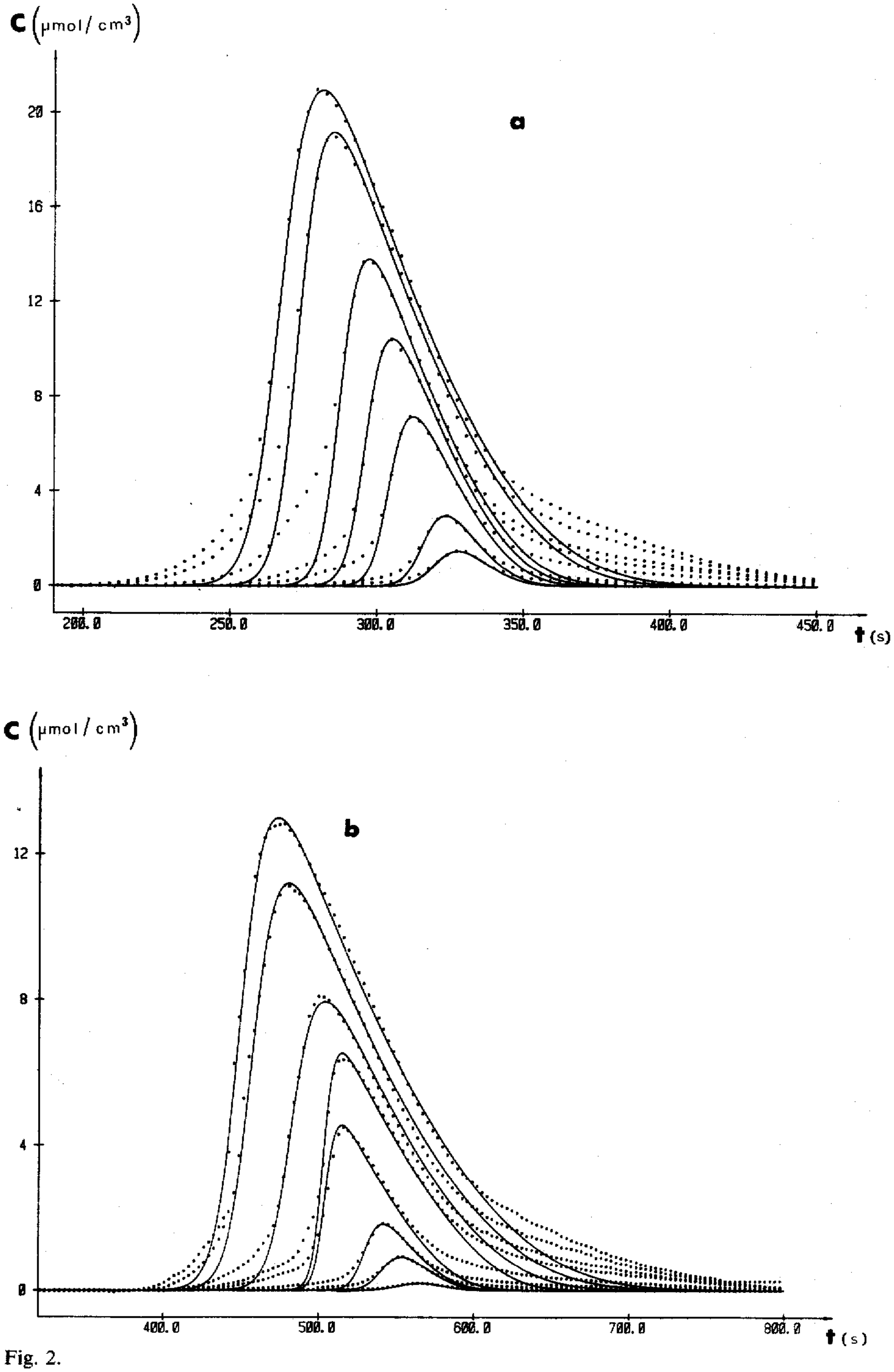
C (μmol/cm³)
a
20
16
12
8
4
0
200.0
250.0
300.0
350.0
400.0
450.0
t (s)
C (μmol/cm³)
b
12
8
4
0
400.0
500.0
600.0
700.0
800.0
t (s)

Fig. 2.

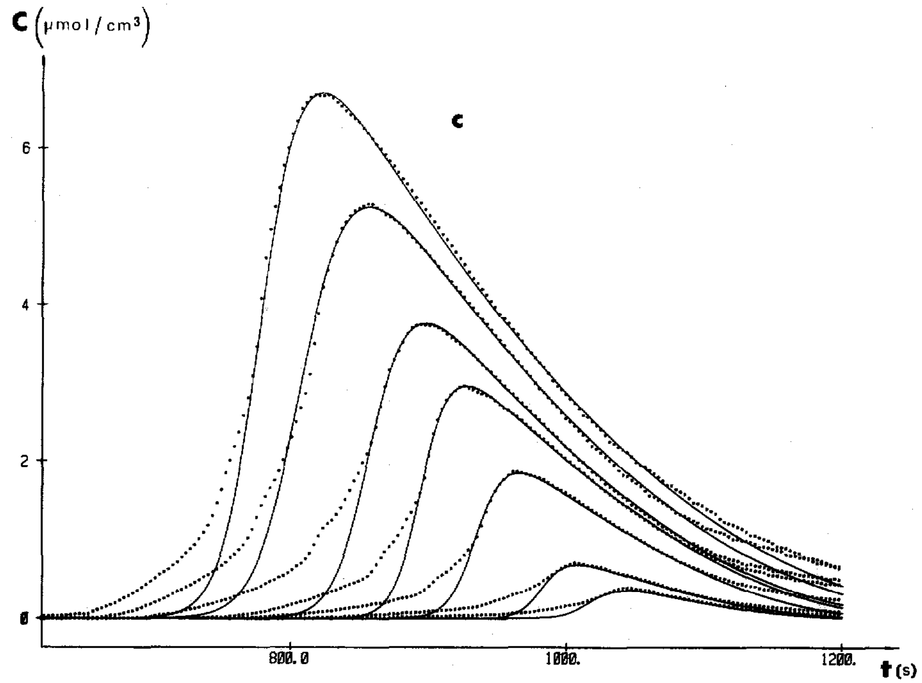

Fig. 2. Best fit of the moderate overload peak model. ——, Theoretical model; · · · ·, computer data-acquisition experimental points. Solute: benzyl alcohol. Adsorbent: LiChrosorb RP-18, 10 µm. Flow-rate: 2.14 ml/min. Mobile phases as in Fig. 1.

values of k_0'' are less significant at low concentrations, where peak distortions induced by isotherm non-linearity are less perceptible.

As observed previously for liquid chromatographic experiments, the dispersion coefficient increases markedly by a factor of 7 when the amount injected is increased from 2 to 60 mg. According to the theory, D' should not depend on the amount injected, in agreement with the behaviour of elution peaks at finite concentration in gas chromatography[6,7]. Since the theory does not take into account slow kinetic processes, the changes noticed in D' might be connected with a kinetic phenomenon.

DISCUSSION

The isotherm curvature values at the origin obtained either from the model-fitting method or from the apex-locus method are in good agreement (Table I).

We have shown previously[1] that, for benzyl alcohol in reversed-phase chromatography, with a negative isotherm curvature at the origin, the partition isotherm approximates to the Langmuir type. Here we have compared the Langmuir isotherm to its parabolic approximation for the various eluent compositions studied (Fig. 3). The validity of approximating a Langmuir isotherm by a parabola depends on the value of the product βC in eqn. 3. A 1% deviation from the parabolic approximation of the isotherm would be observed at the higher concentration studied.

TABLE II

INFLUENCE OF SAMPLE SIZES AND ELUENT COMPOSITIONS ON THE PARAMETERS OF PEAK PROFILES

Water–methanol eluent (w/w)	M (μmol)	t_M (s)	C_M ($\mu mol/cm^3$)	A_T ($\mu mol \cdot s/cm^3$)	a ($\mu mol \cdot s/cm^3$)	α	k''_0 (μmol^{-1})	$D' \cdot 10^4$ ($cm^2 \cdot s$)	$\lambda \cdot 10^3$ ($cm^3/\mu mol$)
90:10	1.84	1044.5	0.38	35.63	36.84	13.86	−1.25	3.1	−227.90
	3.67	1005.7	0.70	76.31	79.82	13.86	−0.96	3.6	−175.00
	13.32	964.4	1.86	255.68	271.41	13.75	−0.46	6.0	−85.49
	19.32	925.5	2.96	470.53	508.44	13.78	−0.38	7.9	−70.02
	30.88	895.0	3.77	674.37	731.96	13.46	−0.31	12.1	−58.62
	46.97	851.1	5.30	1108.30	1223.20	13.26	−0.25	19.6	−49.54
	63.34	839.2	6.71	1471.30	1657.96	13.46	−0.26	19.8	−49.20
80:20	1.8	552.4	0.93	35.49	35.86	6.51	−0.089	3.9	−32.16
	3.59	541.6	1.84	74.80	76.14	6.51	−0.077	4.2	−28.09
	12.04	516.6	4.49	231.85	240.71	6.56	−0.069	5.3	−24.83
	18.11	514.5	6.43	423.76	447.03	6.91	−0.073	7.0	−25.24
	30.93	500.4	8.09	693.31	731.96	6.75	−0.057	18.4	−20.76
	47.01	482.2	11.21	1155.00	1242.86	6.79	−0.057	26.0	−20.35
	63.46	473.5	12.96	1442.6	1567.08	6.88	−0.058	29.5	−20.00
70:30	1.75	327.6	1.47	33.73	34.0	3.41	−0.024	6.95	−15.00
	3.25	322.5	2.97	71.11	72.0	3.41	−0.019	7.50	−12.00
	11.75	311.5	7.15	209.59	220.0	3.43	−0.017	11.0	−10.60
	17.69	305.2	10.41	353.00	374.8	3.41	−0.015	14.96	−9.35
	30.93	295.9	13.79	532.27	570.0	3.41	−0.015	19.20	−9.20
	47.01	283.3	19.12	938.99	1045.0	3.47	−0.016	32.50	−9.80
	63.46	279.2	20.92	1214.5	1293.0	3.41	−0.014	47.00	−9.00

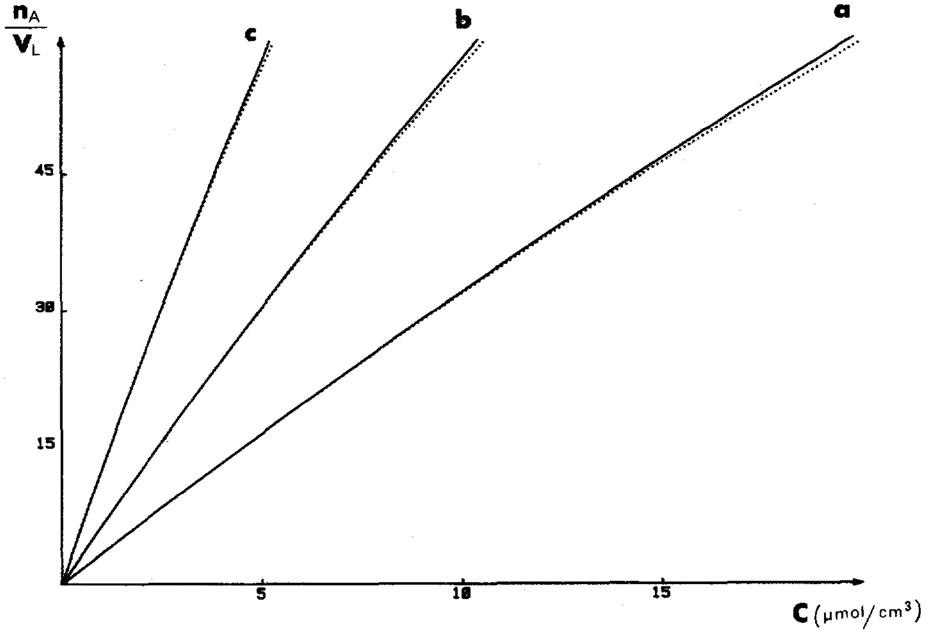

Fig. 3. Comparison of the Langmuir equilibrium isotherm (——) with its parabolic approximation (· · · ·). Mobile phases as in Fig. 1.

The values of the maximum number of moles adsorbed, N_M, deduced from the slope and curvature at the origin are similar for the various eluent compositions (Table I). This result demonstrates the validity of the Langmuir model assumed for the partition isotherm since N_M is related to the number of adsorption sites. Therefore, in agreement with studies of solvent effects in reversed-phase liquid chromatography[8], the retention mechanism as a function of eluent composition depends mainly on the interaction of the solute with the mobile phase.

Besides the physicochemical constants, which are deduced from the adjustment of the theoretical model to the experimental elution profiles obtained at finite concentration, one can derive a characterization of peak asymmetry. We define the experimental asymmetry factor of the elution peak by

$$S = w_2/w_1 \tag{16}$$

where w_1 and w_2 are the half-widths measured at 0.606 of the peak height before and after respectively the elution time at the peak maximum (ID = w_1 and IE = w_2 in Fig. 4). Such ratios are more commonly defined at 1/10 of the peak height, but we preferred to avoid the perturbations present in the low parts of the peak.

The asymmetry factor can be estimated from the parameters of the theoretical model. We shall assume that the elution peak can be approximated by a triangle ABC of height AH = C_M and of base BC. The location of point B is such that A, D, B lie on the same straight line (BH = w_B = $w_1/0.394$). The triangle base is equal to $\Delta t + 2\,w_B$, where $\Delta t = t_R - t_M$, t_R is the retention time at zero concentration and

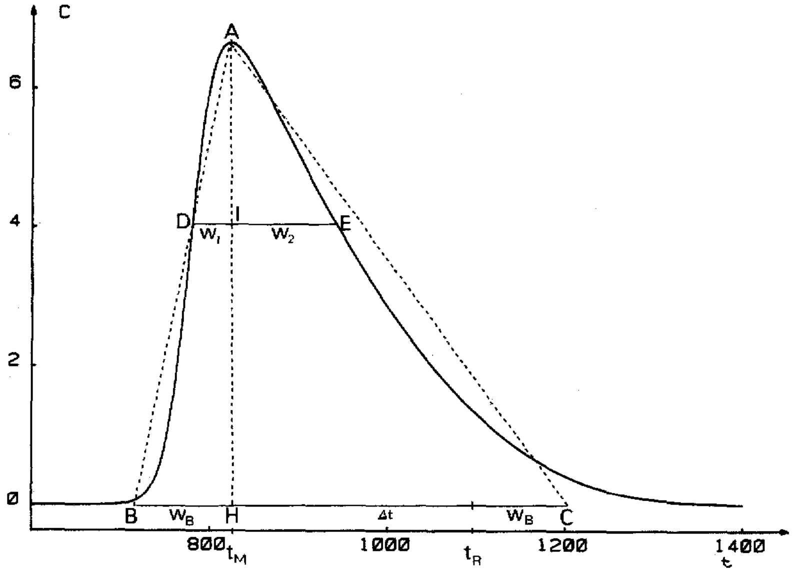

Fig. 4. Triangular diagram representing the elution peak.

t_M the retention time at the peak maximum. With such a choice for BC, the peak area, A, of the triangle is close to that of the elution peak, A_T.

Calculated at this basis, the asymmetry factor is:

$$S_0 = (\Delta t + w_B)/w_B \tag{17}$$

It is not rigorously equal to the asymmetry, S, measured at 0.606 of peak height since A, E, C are not on the same line.

A simple equation involving Δt and C_M can be deduced from the apex-locus expression (15) which relates the coordinates of the peak maximum to the peak slant coefficient:

$$C_M = \Delta t/(t_R|\lambda|) \tag{18}$$

Δt is also related to the area, A, of the triangle ABC representing the elution peak:

$$A = (\Delta t + 2w_B)C_M/2 \tag{19}$$

Combining the last two equations by elimination of C_M, a second degree equation is obtained

$$\Delta t^2 + 2w_B\Delta t - 2At_R|\lambda| = 0 \tag{20}$$

the solution of which is:

$$\Delta t = \sqrt{w_B^2 + 2At_R|\lambda|} - w_B \tag{21}$$

The calculated asymmetry factor is thence:

$$S_0 = \sqrt{1 + \frac{2At_R|\lambda|}{w_B^2}} \tag{22}$$

Assuming that the triangle area, A, is equal to the peak parameter a, a relationship involving the asymmetry factor and the peak parameter, μ, of the elution model (eqn. 13) is obtained:

$$S_0^2 = 1 + 2\left(\frac{\sigma_0}{w_B}\right)^2 |\mu| \tag{23}$$

We have reported (full points in Fig. 5) the values of S_0 calculated as a function of $|\mu|$, σ_0 and w_B, for all the experimental profiles studied in this work. The asymmetry factor is an increasing function of $|\mu|$, which is equal to 1 when $|\mu| = 0$. It is not a linear function because the ratio $(\sigma_0/w_B) = 0.394\ (\sigma_0/w_1)$ varies with μ.

In the domain of concentration studied, we have observed that the ratio σ_0/w_1 increases linearly with $|\mu|$ according to

$$\sigma_0/w_B = \gamma + \delta|\mu| \tag{24}$$

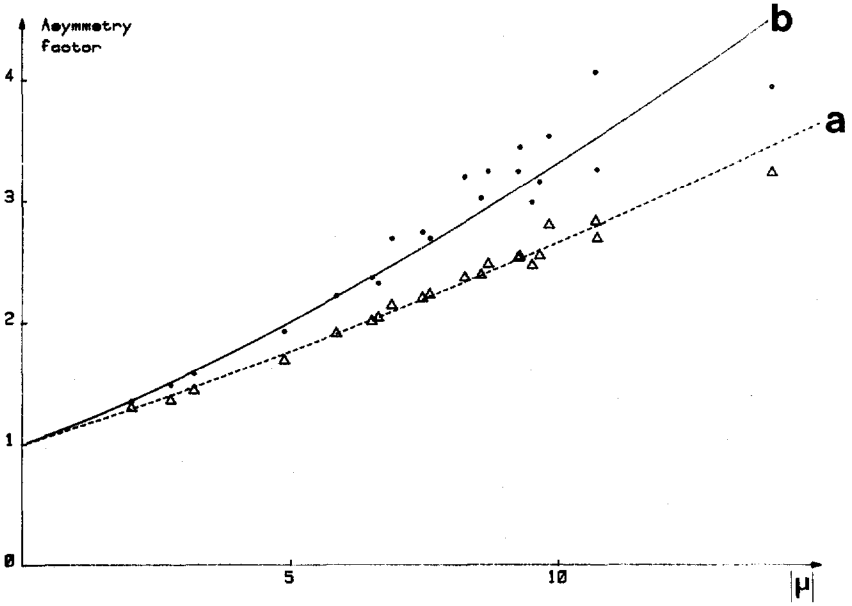

Fig. 5. Variation of the asymmetry of the peak with the parameter, μ, of the elution peak model. (a) Asymmetry measured at 0.606 of peak height: △, S; ----, $S = \sqrt{1 + 0.31|\mu|(0.93 + 0.047|\mu|)^2}$. (b) Asymmetry calculated at the base of the triangle: ●, S_0; ——, $S_0 = \sqrt{1 + 0.31|\mu|(1.04 + 0.079|\mu|)^2}$.

where the coefficients γ and δ were determined by linear regression to be 1.04 and 0.079, respectively. The variation of S_0 with $|\mu|$ is well represented (full line in Fig. 5) by the equation:

$$S_0^2 - 1 = 0.31|\mu|\,(\gamma + \delta|\mu|)^2 \tag{25}$$

The asymmetry factor, S_0, of the triangle symbolizing the peak is somewhat larger than S, that for the actual peak (Fig. 5), because of the approximations made in defining the trailing side of the peak: E is not located on the side AC of the triangle. Nevertheless the experimental factor S is well represented as a function of μ by an equation similar to eqn. 25, with $\gamma = 0.93$ and $\delta = 0.047$.

Therefore, an interesting relationship exists involving the asymmetry ratio coefficient, S, and the peak-profile parameter, μ, which is a useful parameter for estimating the degree of asymmetry of a peak in non-linear chromatography.

From the expression of $|\mu|$ in eqn. 13 we deduce

$$|\mu| = \frac{Q\,|k_0''|\,V_L u_0^2}{2FD(1 + k_0')^2} = \frac{Q\,|k_0''|\,V_L u_0}{2sD(1 + k_0')^2} \tag{26}$$

where Q is the mass injected, F the flow-rate, u_0 the mobile phase velocity and s the mobile phase cross-section. This expression may be used to fix the experimental conditions for reducing peak asymmetry. The parameter μ and thence the asymmetry are independent of the column length. The asymmetry increases with the mobile phase velocity and the amount injected, but decreases with increasing column cross-section.

In the special case of a Langmuir isotherm, the maximum number of molecules absorbed is $-2k_0'^2/k_0''$. For large values of k_0', μ is given by:

$$|\mu| = QLu_0/DN_M \tag{27}$$

For the same amount injected, the asymmetry remains roughly constant when the mobile phase composition is modified. It will be reduced by increasing the specific surface area per unit length.

CONCLUSIONS

The theory of the moderately overloaded peak model indicates that the change in the retention time with the concentration at the peak maximum is related to the first and second derivatives of the equilibrium isotherm function at the origin. In the case of the Langmuir isotherm, interesting information can be deduced about the maximum number of molecules adsorbed, which is independent of the mobile phase composition in reversed-phase HPLC.

In affinity chromatography where the equilibrium isotherms are often of the Langmuir type, an approach similar to the one presented could be useful to determine the adsorption capacity of the column which is difficult to measure by independent methods.

There is a relationship between the peak asymmetry factor, S, and one of the parameters of the theoretical profile (μ). It is shown that asymmetry is independent of the column length and increases with the mobile phase velocity, or with the amount injected, but decreases with increasing column cross-section. For a Langmuir isotherm, with the same amount injected, the asymmetry is independent of the mobile phase composition and is improved by increasing the loading capacity per unit length of the column. These results may be used to find the best experimental conditions in preparative liquid chromatography at moderate concentrations.

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