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Properties of some C_{18} stationary phases for preparative liquid chromatography I[☆]. Equilibrium isotherms

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Abstract

Isotherm data were measured by frontal analysis and elution by characteristic points for 2,6-dimethylphenol, 3-phenyl-1-propanol and methyl benzoate on six columns packed with two commercial octadecyl silicas, using methanol–water solutions as the mobile phase. The retention factors and isotherm coefficients depend on the packing density. This density varies markedly from column to column, at least with some phases for which it is also a function of the column length. The determination of the thermodynamic data required for the modelling of chromatography must be done with an accuracy which seems to exceed what the current level of column to column reproducibility of these data permits at present, even when care is taken to control the experimental parameters, make precise measurements and report the data to the mass of silica.

1. Introduction

Reversed-phase liquid chromatography is by far the domineering technique in analytical chromatography [1]. Octadecyl-bonded silica adsorbents (ODS), used as the non-polar stationary phase of choice in this method, have been extensively studied for the last twenty years [2–16]. ODS adsorbents are also widely used in preparative chromatography, unless the feed components are too poorly soluble in the strongly polar solvents which must be used as the mobile phase to ensure significant retention. In spite of a most abundant literature discussing the use of these phases, their analytical applications and their chromatographic properties, some important questions remain unanswered, especially regarding their behavior under overloading conditions and the reproducibility of their performance in preparative chromatography.

Much fundamental work has been done on the comparison of the properties of columns packed with ODS and with other packing materials [6–8], of columns packed with ODS prepared with different reagents [9], and of columns packed with ODS produced by different manufacturers [10–12]. However, almost all these studies were undertaken using methods of linear chromatography, with the single aim to facilitate its ana-

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lytical applications. Accordingly, they are mainly focused on the retention characteristics of the columns being compared, on the dependence of the retention factor on various experimental parameters, and on the column efficiency. While this last parameter depends mostly on the packing method used and on the skill of the operator and, with proper care, can be improved to a certain extent, the other two have been found to depend essentially on the origin of the material used, to be most difficult to adjust, and uneasy to reproduce. Considerable attention has been paid to the column-to-column, batch-to-batch, and manufacturer-to-manufacturer reproducibilities of analytical performance [17–19]. The first has generally been found to be excellent, the second acceptable, the third poor, as if the ODS materials produced using different processes were almost entirely different products.

Except for a recent report discussing the performance of overloaded chromatographic columns [13], little attention has been paid to the column characteristics which are important in preparative chromatography. These are the kinetics of mass transfer and of adsorption–desorption in the column and the equilibrium isotherms of the components of interest between the stationary and the mobile phases. The column efficiency is a measure of the rate of the mass-transfer kinetics in the column but it must be used with caution. The initial slope of the isotherm is proportional to the retention factor, so many of the conclusions of analytical studies are applicable to this isotherm parameter. The nature of the isotherm, its initial curvature, and its saturation capacity are other parameters of importance for preparative chromatography. There is at present little comparative information on these parameters.

The goal of this work is an investigation of the feasibility of a comparison of the performance of stationary phases for preparative liquid chromatography. A number of issues must be clarified, such as (i) which compounds should be chosen for comparative studies; (ii) what is the reproducibility of column parameters when several similar columns are prepared at the same time; (iii) how close are the parameters of columns of different lengths, packed at the same time; (iv) are classical mass-transfer kinetics data, such as plots of the reduced column efficiency versus the reduced velocity, relevant in preparative chromatography and how could they be used. The amount of data required for a thorough comparison could be considerable. The scope of the work has to be limited to the essential if the approach is to be useful for the selection of the best stationary phase for practical applications. For example, it has been shown theoretically [14,15] that, in analytical chromatography, the use of 2-μm particles would be optimal for the separation of complex mixtures. It has also been proven that the practical difficulties experienced when trying to prepare and operate columns packed with such small particles are serious [16]. Preparative chromatography is best carried out with particles between 10 and 40 μm in diameter [20]. We have limited the present study to 10-μm particles.

In this paper, we discuss only the data of thermodynamics nature. Emphasis is placed on the column-to-column reproducibility of the data obtained with a given material. In order to be able to measure thermodynamic data on analytical columns and use these data to predict accurately band profiles on large-size preparative columns, relative differences between the values of the numerical coefficients of the isotherm not exceeding a few percents are desirable. Otherwise, a proper correction would be needed.

2. Theory

Adsorption isotherms were measured by frontal analysis (FA) and by the elution by characteristic point (ECP) method. The ECP method is based on the ideal model of chromatography [20], which assumes that the column efficiency is infinite. In this case, each concentration has a retention time which is simply related to the slope of the isotherm. A large sample is injected in the column. The peak recorded has a sharp

shock layer, usually at the front, and a diffuse boundary [20]. The equation which relates the profile of the diffuse boundary, $V(C)$, the amount of the compound adsorbed on the stationary phase at equilibrium, q, and the mobile phase concentration, C, can be written as follows

$$q = \frac{1}{V_a} \cdot \sum_{0}^{C} (V - V_0)\delta_i C \tag{1}$$

where V_a is the volume of adsorbent in the column, V_0 is the column hold-up volume, V is the retention volume of the characteristic point of the diffuse profile at concentration C, and $\delta_i C$ is the concentration increment (with $\sum \delta_i C = C$). Since all actual columns have a finite efficiency, the ECP method introduces a model error, as shown by Huber and Gerritse [21,22]. In a previous publication [23], we have made a numerical study of this error and shown that an efficiency of approximately 5000 theoretical plates is required to reduce below 1% the systematic error made on the determination of the isotherm coefficients.

When the column efficiency is not as high as required by ECP for accurate determinations, FA [20–22,24–27] is used. This method requires the measurement of the retention times of successive abrupt step changes of increasing (for peaks with self-sharpening fronts) or decreasing (for peaks with self-sharpening rear boundaries) sample concentration. The isotherm is derived by using the following equation for each concentration step:

$$q_{i+1} = q_i + \frac{(C_{i+1} - C_i)(V_{F,i+1} - V_0)}{V_a} \tag{2}$$

where q_i and q_{i-1} are the amounts of compound adsorbed by the column packing at equilibrium, after the end of the ith and $i+1$th breakthrough curves and $V_{F,i+1}$ is the retention volume of the breakthrough curve obtained when the concentration is raised from C_i to C_{i+1}.

The FA method has serious advantages over ECP. The retention volumes measured are independent of the column efficiency, no detector calibration is needed since the solutions used have known concentrations, and measurements can easily be carried out at mobile phase concentrations which are much higher than in ECP. The main drawbacks of the FA method are that it requires considerable amount of time and sample and it gives only a small number of data points. Although frontal analysis by characteristic point (FACP) [20,28,29], which uses the diffuse boundary of a negative step in the frontal analysis mode, permits the determination of isotherm data points at high concentrations, the need of calibrating the detector and the model error associated with the ECP method subsist while the requirement for a large amount of sample common to all FA methods remains. We have used both the FA and the ECP methods in the present work.

Statistical thermodynamics suggests the following general equation for liquid–solid isotherms [20]

$$q = q_S \cdot \frac{C(b_1 + 2b_2C + \cdots + nb_nC^{n-1})}{1 + b_1C + b_2C^2 + \cdots + b_nC^n} \tag{3}$$

where q_S is the saturation capacity of the adsorbent and the coefficients b_i are numerical coefficients related to the adsorbate–adsorbent and the adsorbate–adsorbate interaction energies. Eq. 3 must be used with few coefficients only. Otherwise, it is too flexible. The popular Langmuir isotherm is obtained for $n = 1$

$$q = \frac{q_S b_1 C}{1 + b_1 C} \tag{4}$$

The quadratic isotherm is obtained for $n = 2$

$$q = q_s \cdot \frac{C(b_1 + 2b_2C)}{1 + b_1C + b_2C^2} \tag{5}$$

It is the simplest isotherm with an inflection point. It has been used previously with success to account for the isotherm data of components which exhibit anti-Langmuir behavior [30].

The coefficients of the isotherm were obtained

by least-square fit of the data to minimize the square of the relative difference between the experimental data points and the corresponding points of the fitted isotherm [20,31,32].

3. Experimental

3.1. Equipment

The ECP determinations were performed using a Perkin-Elmer (Norwalk, CT, USA) Model 400 liquid chromatograph solvent-delivery system, a VICI (Valco, Houston, TX, USA) 6-port motor-driven sample injection valve with 0.25-, 0.50- and 1-ml sample loops, and a Spectra-Flow 757 UV absorbance detector (Kratos, Ramsey, NJ, USA). Data were acquired simultaneously with a Spectra-Physics (San Jose, CA, USA) SP 4270 integrator, for the real-time display of the chromatograms during the experiment, and an Autolab software (Spectra-Physics part No. A0099-207 10/86 A), for storing data files in an IBM personal computer for further analysis or for downloading to one of the VAX computers at the University of Tennessee Computer Center.

The frontal analysis measurements were performed using a Hewlett-Packard (Palo Alto, CA, USA) HP1090 liquid chromatograph, equipped with a multisolvent delivery system, a diode-array UV detector, and a computer data acquisition system.

3.2. Columns

Two samples of spherical ODS, both 10 μm average particle size, were obtained from YMC (Wilmington, NC, USA) with catalog No. BS-1010 S-10 120A ODS and lot No. EC03817, and Vydac (Hesperia, CA, USA) with catalog No. 201HSB10 and lot No. 910611-12-1, respectively. Six stainless columns (Alltech, Deerfield, IL, USA), all 0.46 cm I.D., were packed in our laboratory, using the slurry technique and a maximum pressure of 6000 p.s.i. (1 p.s.i. = 6894.76 Pa). Two 10 cm long and two 25 cm long columns were packed with the Vydac ODS material, one 10 cm long and one 25 cm long columns were packed with the YMC ODS material.

3.3. Chemicals

2,6-Dimethylphenol, 99% pure, solid, formula weight (FW) 122.17 [CAS Ref. No. 576-26-1], catalog No. D17,500-5 and methyl benzoate, 99%, liquid, FW 136.15 [93-58-3], catalog No. M2,990-8 were obtained from Aldrich (Milwaukee, WI, USA). 3-Phenyl-1-propanol, >98% (GC), liquid, FW 136.20, catalog No. 79 000 was obtained from Fluka (Buchs, Switzerland).

The mobile phase was a mixture of 40% methanol (Baker Analyzed HPLC reagent, catalog No. 9093-33; J.T. Baker, Phillipsburg, NJ, USA) and 60% water. Deionized water was freshly made in the laboratory using a Barnstead/Thermolyne (Dubuque, IA, USA) water-deionizing system consisting in two cartridges, one HN high-capacity DI cartridge (catalog No. D8901) and one HG organic-removal cartridge (catalog No. D8904).

3.4. Procedures

Sample preparation

For each compound, the most concentrated solution possible to prepare, based on its solubility in a methanol–water (40:60) mixture, was made first. Weighed amounts of the compounds studied were introduced into volumetric flasks with known volumes of mobile phase (methanol–water, 40:60) and thoroughly shaken in a sonic water bath, until all the solute dissolves or for 30 min. Failure for the solute to dissolve resulted in the addition of a small volume of fresh solvent and the procedure was repeated. This allowed the preparation of nearly saturated solutions. More dilute solutions were prepared by introducing weighted amounts of the solutes into known volumes of mobile phase. There was no secondary dilution of standard solutions to avoid errors due to sample loss during transfers. Solutions in the following concentration ranges were prepared: 2,6-dimethylphenol from 18.50 to

Table 1
Characteristics of the columns studied

	YMC		Vydac			
	YS (10 cm long)	YL (25 cm long)	VS1 (10 cm long)	VS2 (10 cm long)	VL1 (25 cm long)	VL2 (25 cm long)
ϵ_1	0.680	0.671	0.692	0.722	0.682	0.674
ϵ_2	0.754	0.753	0.755	0.749	0.686	0.724
ϵ_3	0.694	0.671	0.729	0.744	0.669	0.646
W_{Si}/V_c	0.64	0.67	0.61	0.58	0.71	0.70

ϵ_1 = Total porosity from uracil retention time; ϵ_2 = total porosity from the mass of dry silica (silica density = 2.21 g/ml) in the column; ϵ_3 = total porosity from the mass of methanol in the column; W_{Si}/V_c (g/ml) = ratio of the mass of stationary phase in the column and the column volume, as results from the average porosity.

4.609 mg/ml; 3-phenyl-1-propanol from 25.336 to 5.606 mg/ml; methyl benzoate from 11.76 to 2.52 mg/ml.

Detector calibration

The output of the detector is an electrical voltage. It is converted into the concentration (mg/ml) of the corresponding solute using a detector calibration curve obtained by flushing the detector cell with each solution prepared until a stable signal is obtained. For each of the three compounds studied, a third-order polyno-

Table 2
Column hold-up times (t_0), efficiency (N_0) and sample capacity factor (k') at infinite sample dilution

Compound	Parameter	Columns					
		YS	YL	VS1	VS2	VL1	VL2
	t_0 (min)	1.26	2.94	1.26	1.31	2.93	2.95
PP	k'	9.74	9.85	6.52	6.27	7.11	7.25
	Reproducibility[a]	0.01		0.06			
	$k'/(W_{Si}L)$			10.47			
	Reproducibility		−0.04				0.03
	N_0	1860	3050	1250	1640	3160	4070
DMP	k'	11.2	12.1	7.13	6.99	7.91	8.08
	Reproducibility[a]		0.07				0.06
	$k'/(W_{Si}L)$						11.61
	Reproducibility		0.03				0.03
	N_0	1890	3000	1290	1650	3210	3630
MB	k'	13.2	14.1	8.79	8.60	9.81	10.3
	Reproducibility[a]		0.06				0.07
	$k'/(W_{Si}L)$			14.43			
	Reproducibility		0.02				0.03
	N_0	1680	3130	1240	1360	3320	3220

For columns, see Table 1. PP = 3-Phenyl-1-propanol; DMP = 2,6-dimethylphenol; MB = methyl benzoate; L = column length.
[a] Reproducibility for YMC columns: $(k'_{YL} - k'_{YS})/k'_{YL}$; for Vydac columns: σ of the four results.

mial gave a close fit to the experimental data. The best coefficients of the fit were obtained by applying the polynomial regression in SigmaPlot (Jandel, San Rafael, CA, USA) to the experimental data points. The calibration data were used to convert the recorded chromatograms into the plots of concentration versus time shown below.

Column parameters

The column porosity was derived by three different methods, using the column holdup volume, the amount of silica contained in the column and the amount of methanol. The column hold-up time (t_0) was derived from the retention time of uracil. A solution of 0.15 mg uracil in 1.0 ml mobile phase was used for these measurements. Samples of 0.010 and 0.020 ml were injected into the 10 and 25 cm long columns, respectively. After completion of all the experiments reported here and in the companion paper [33], the columns were dried under a stream of nitrogen for 15 h, then filled with pure methanol and weighed again, giving the amount of methanol filling the pores. Finally, the columns were dried again, weighed and emptied. The mass of silica contained in the column permits the calculation of the porosity from the density of silica (2.21 g/ml). These results are summarized in Table 1.

The retention factor of each compound (k') and its limit efficiency for a zero-size sample (N_0) were measured by injecting the same volumes of the least concentrated solution used, at a flow-rate of 1.0 ml/min. The efficiency was derived from the conventional equation

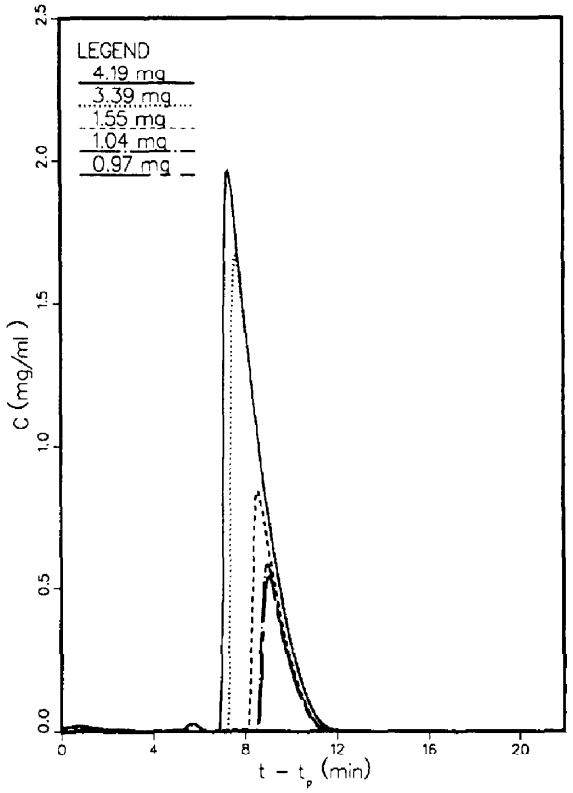

Fig. 1. Experimental chromatogram for sample 2,6-dimethylphenol on column VS2 (L = 10 cm, Vydac ODS). t_p = Duration of the injection.

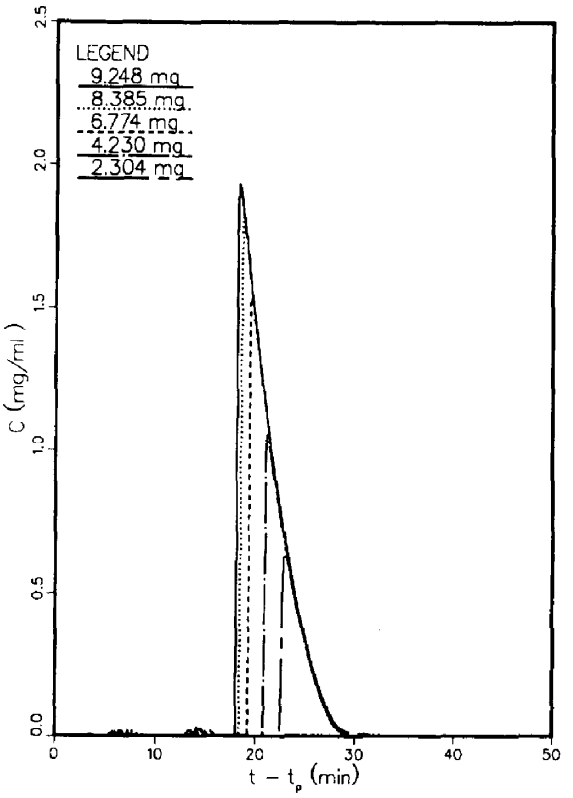

Fig. 2. Experimental chromatogram for sample 2,6-dimethylphenol on column VL2 (L = 25 cm, Vydac ODS).

$$N_0 = 5.54\left(\frac{t_R}{W_{0.5}}\right)^2 \quad (6)$$

The retention factor k' of each compound was calculated from

$$k' = \frac{t_{R,0} - t_0}{t_0} \quad (7)$$

The results obtained are summarized in Table 2. The retention factors of all three components are nearly the same on the two Vydac columns having the same length, but they are systematically lower on the shorter columns, by a few percents on the YMC column, by around 13% on the Vydac columns. By contrast, the height equivalent to a theoretical plate (HETP) at 1.0 ml/min (reduced velocities between 12 and 15) is nearly the same on the Vydac columns of different lengths (i.e., the plate number is proportional to the column length), but almost 50% higher on the longer YMC column than on the shorter one. While column-to-column fluctuations of efficiency are common and are usually attributed to fluctuations in packing homogeneity, variations in retention factors between columns packed with the same material can be easily explained only by variations in the packing density, which affect the phase ratio. Indeed, the amount of adsorbent introduced in the longer Vydac columns is approximately 2.75 times as large as the amount packed in the shorter columns, corresponding to an average packing density 10% larger.

ECP Measurements

The large samples required by this method

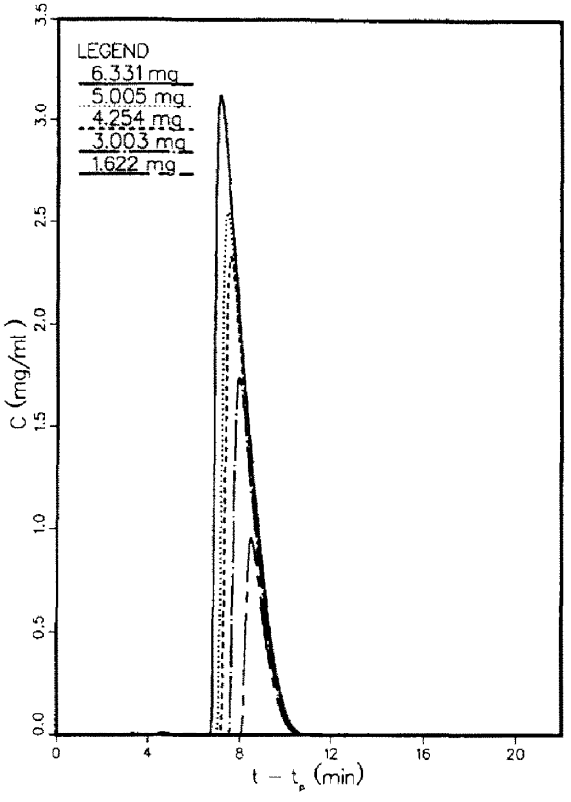

Fig. 3. Experimental chromatogram for sample 3-phenyl-1-propanol on column VS2 (L = 10 cm, Vydac ODS).

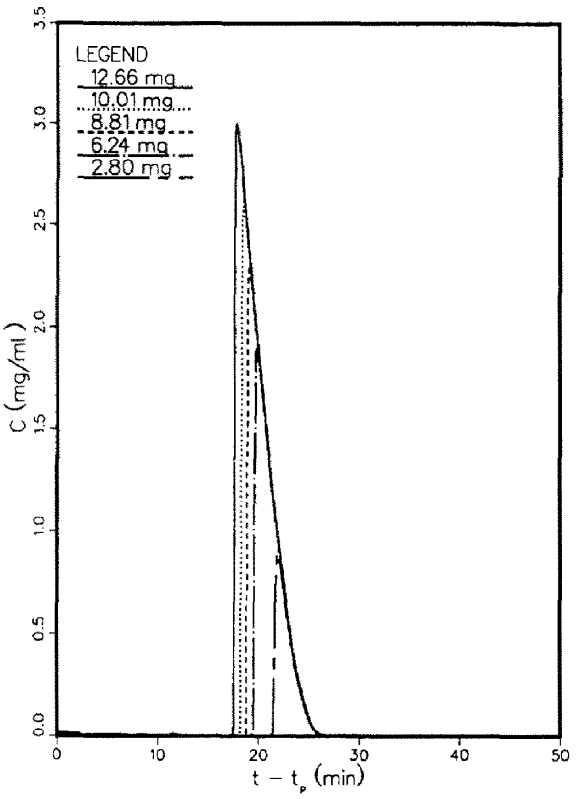

Fig. 4. Experimental chromatogram for sample 3-phenyl-1-propanol on column VL2 (L = 25 cm, Vydac ODS).

were obtained by injecting 0.25 ml of each solution on the 10 cm long columns, 0.50 ml of the concentrated 2,6-dimethylphenol and 3-phenyl-1-propanol solutions, and 1.0 ml of the concentrated methyl benzoate solution on the 25 cm long columns. In each of these experiments, the flow-rate was set at 1.0 ml/min. The use of large sample volumes in ECP is conventional. The proper correction was applied by taking the end of the injection as time origin.

4. Results and discussion

The experimental results in Table 1 lead to several unexpected conclusions. First, the packing density of columns is not very reproducible. Second, the three methods used to determine the column porosity do not agree closely. Third, the packing density varies with the length of the column, the longer columns having, rather surprisingly, a higher packing density. The trend is only marginal with the YMC material, with a packing density nearly 5% higher for the longer column. The difference is important for the columns packed with the Vydac ODS, a material which is known for being difficult to pack. Both 25 cm long columns and both 10 cm long ones have nearly the same packing density, but the former are nearly 15% denser than the latter. Since the columns were packed with the same material, using the same method, it is unlikely that the specific surface area of the adsorbent be different. Then the thermodynamic properties should be reported to the mass of packing in the column rather than to its volume.

The lack of close agreement between the three methods used to determine the column porosity

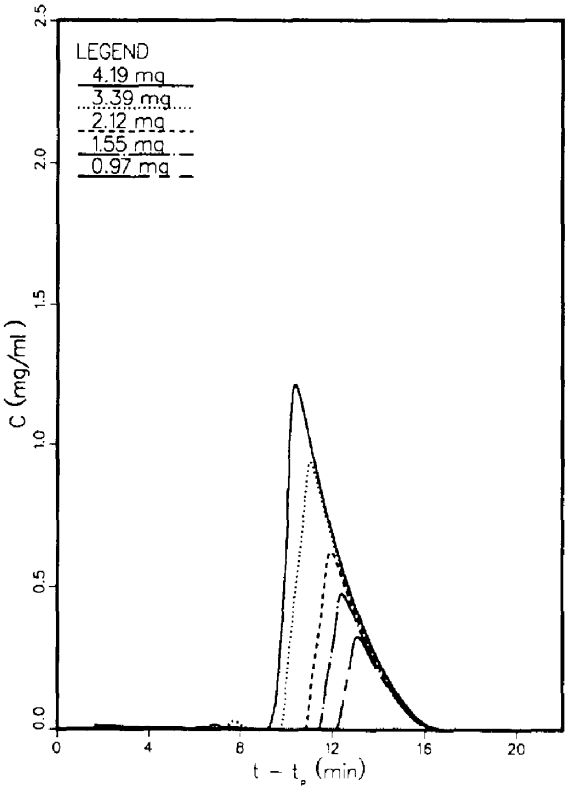

Fig. 5. Experimental chromatogram for sample 2,6-dimethylphenol on column YS (L = 10 cm, YMC ODS).

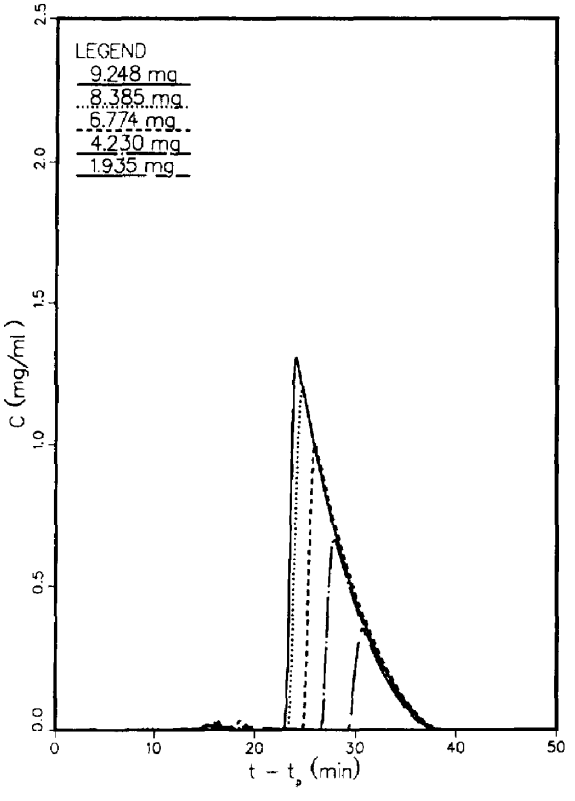

Fig. 6. Experimental chromatogram for sample 2,6-dimethylphenol on column YL (L = 25 cm, YMC ODS).

is not surprising. There is no guarantee that uracil is a non-retained, non-excluded tracer. The calculation of the volume of solid stationary phase using 2.21 g/ml for the density of silica is approximate, as this number is not universally accepted for amorphous silica and it neglects the contribution of the C_{18} bonded groups. Finally, the retention volume of uracil in methanol–water (40:60) may differ slightly from the volume of pure methanol contained in the column if the wettability of silica by the two liquids is different.

The retention factors of the three probes at infinite dilution[1] are reported in Table 2. The standard deviation of the measurement of k' on any single column is 0.5 to 1%. If the data for the four Vydac columns are not corrected for the variable packing density, the standard deviation for the four values of k' is 6 to 7% (Table 2). If the data are corrected, it is only 3%. A similar but less conclusive result is observed for the two YMC ODS columns, the relative difference of the two values being used instead. This result confirms the importance of taking the column packing density into account when comparing thermodynamic data acquired on different columns. This should be of major concern when trying to predict elution band profiles on preparative columns using data acquired with analytical size columns [34].

2,6-Dimethylphenol and 3-phenyl-1-propanol were found to have a near-Langmuirian equilibrium behavior on all six columns, while methyl benzoate has an anti-Langmuirian behavior. The

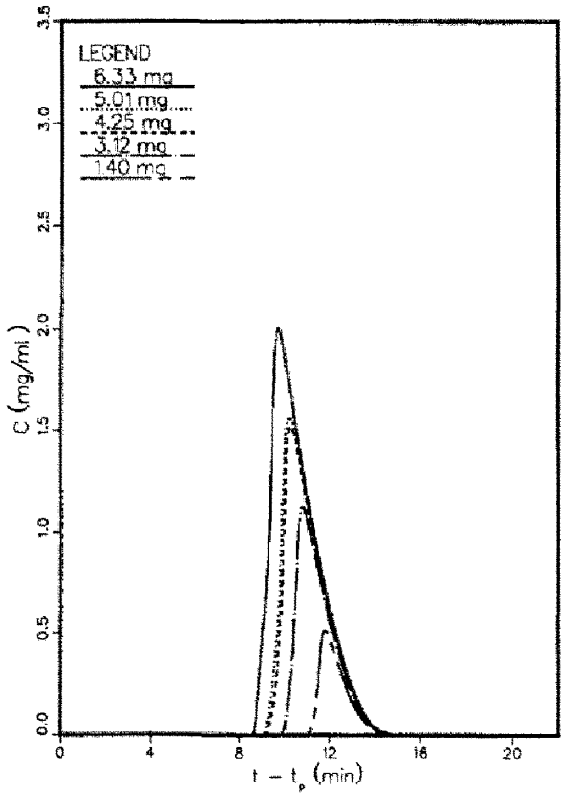

Fig. 7. Experimental chromatogram for sample 3-phenyl-1-propanol on column YS (L = 10 cm, YMC ODS).

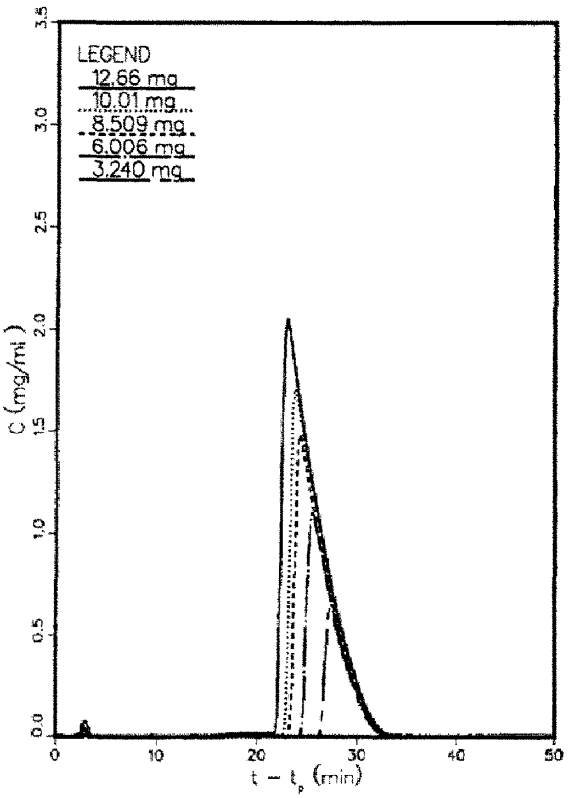

Fig. 8. Experimental chromatogram for sample 3-phenyl-1-propanol on column YL (L = 25 cm, YMC ODS).

[1] If we refer to Eq. 4, we see that we can consider the isotherm as linear as long as b_1C is negligible compared to 1. Values of C were such that b_1C was smaller than 0.001.

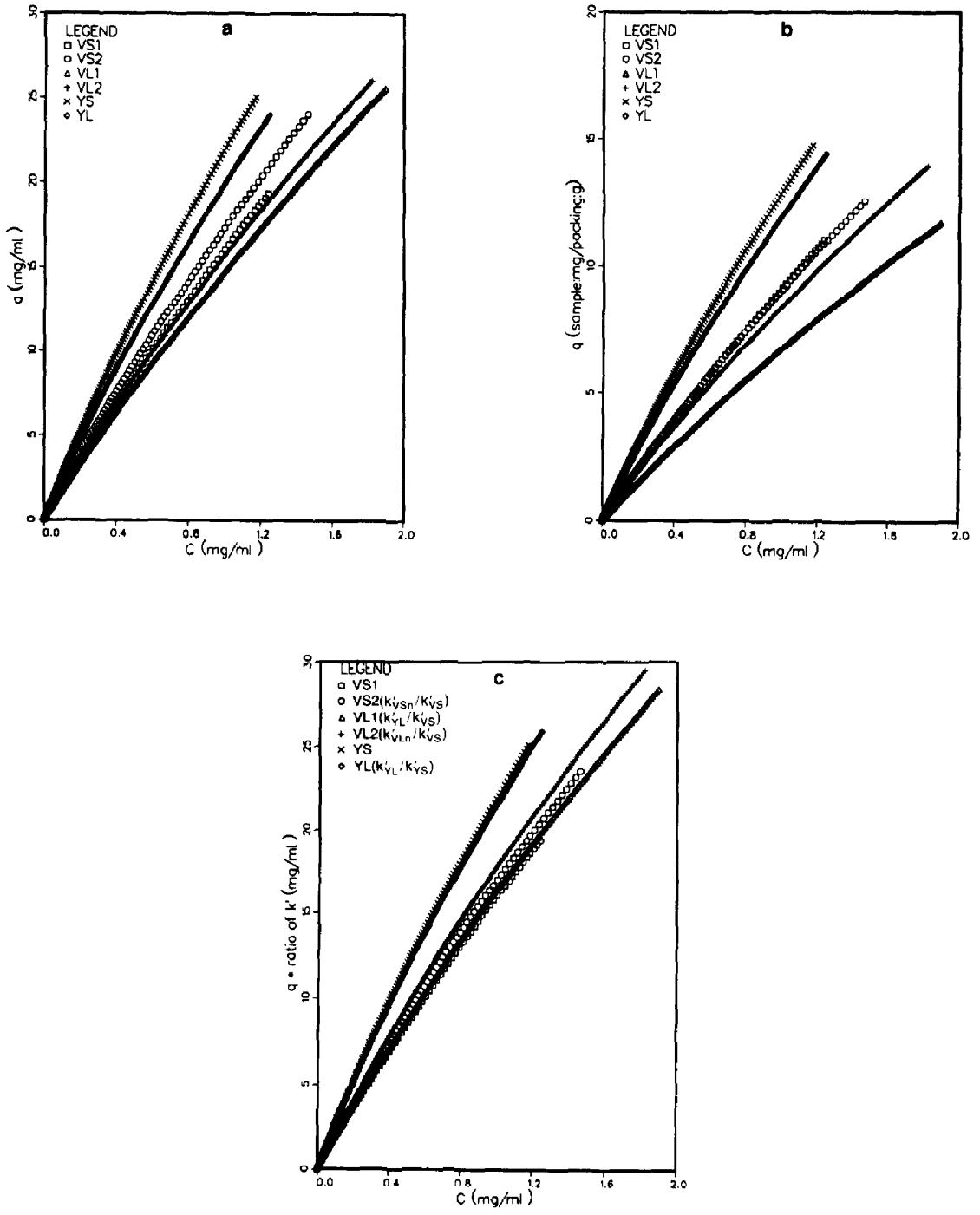

Fig. 9. Isotherms measured by ECP for 2,6-dimethylphenol on all six columns examined. (a) Isotherm data reported to the volume of the column. (b) Isotherm data reported to the mass of silica. (c) Isotherm data reported to the retention factor under analytical conditions.

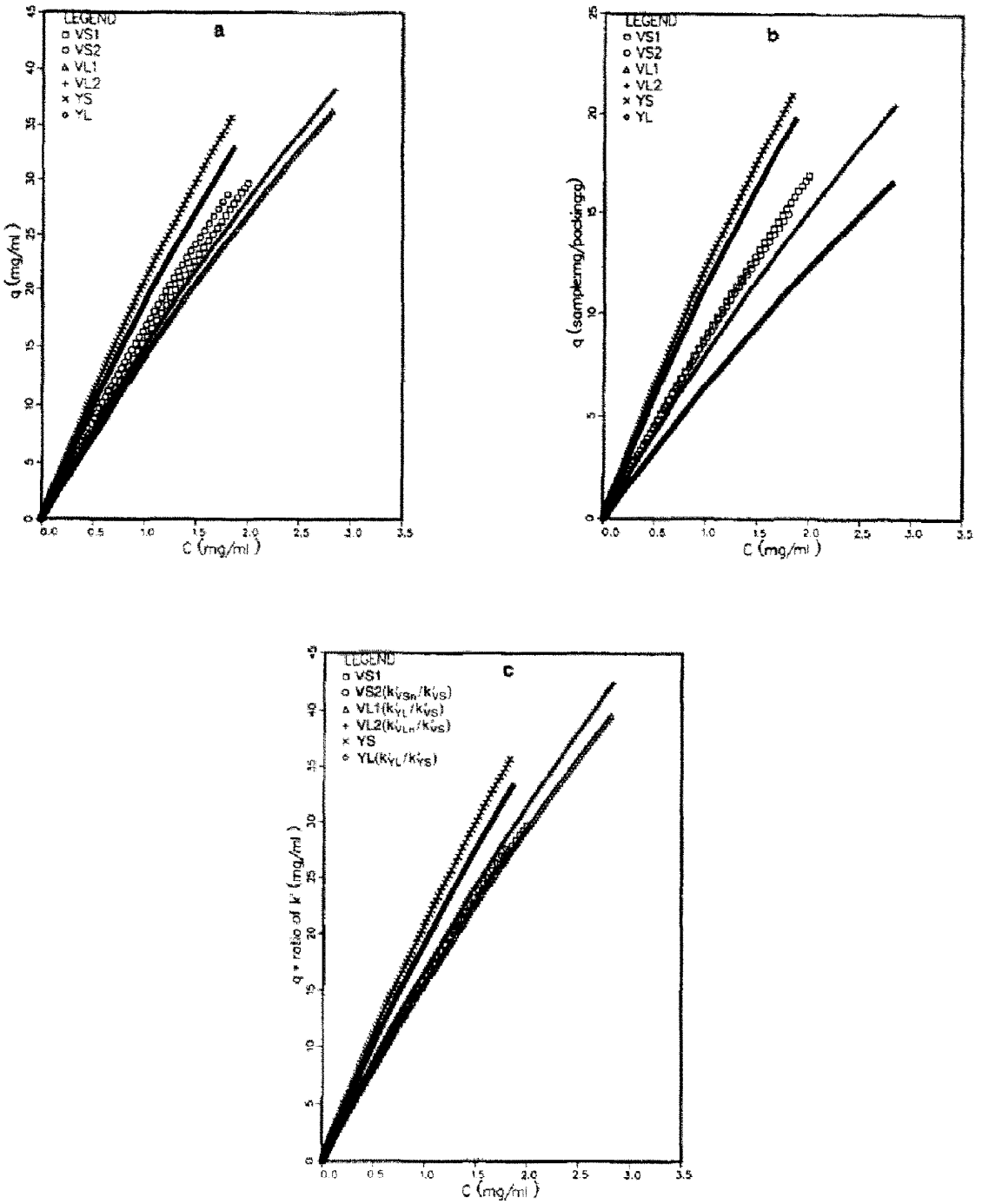

Fig. 10. Isotherms measured by ECP for 3-phenyl-1-propanol on all six columns examined. (a) Isotherm data reported to the volume of the column. (b) Isotherm data reported to the mass of silica. (c) Isotherm data reported to the retention factor under analytical conditions.

major differences in this behavior and in the procedures required for the modeling of the equilibrium isotherms and of the band profiles justify the separate discussion of these two sets of results.

4.1. Langmuirian compounds

The Langmuir isotherm is most popular because it is simple and it accounts quite well for the single-component equilibrium data obtained in a vast majority of the cases encountered in liquid chromatography and particularly in reversed-phase HPLC. The behavior of 2,6-dimethylphenol and 3-phenyl-1-propanol on the Vydac and YMC ODS are cases in point.

Both compounds exhibit Langmuirian band profiles on all six columns. Data are reported only for one set of Vydac columns (Figs. 1–4) and for the YMC columns (Figs. 5–8). In all cases, the front of the profiles obtained with the 25 cm long columns (Figs. 2, 4, 6 and 8) are steeper than those recorded with the 10 cm long columns (Figs. 1, 3, 5 and 7), in relation to the higher efficiency of the former, longer columns. The front of the band profiles tend to be steeper and the profiles to be narrower on the Vydac ODS columns.

The ECP method was applied to each of the profiles obtained and the experimental isotherms were calculated. The results are shown in Figs. 9 and 10. In Figs. 9a and 10a, the amounts adsorbed at equilibrium are reported to the volume of column, as classical in the chromatographic determination of isotherms. The curves obtained

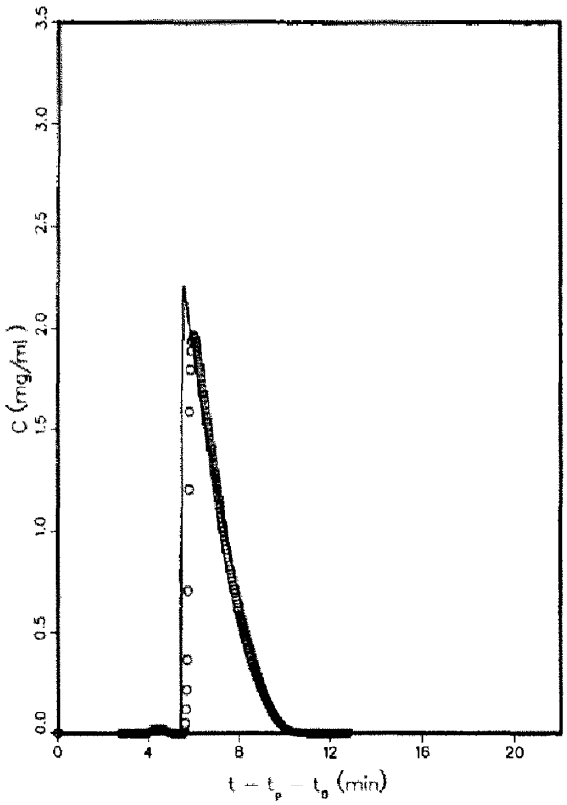

Fig. 11. Comparison between calculated (solid line) and experimental (symbols) band profiles on column VS2 ($L = 10$ cm, Vydac ODS). Sample: 2,6-dimethylphenol; amount: 4.2 mg. Mobile phase: methanol–water (40:60, v/v). Langmuir isotherm coefficients, $q_s = 144$ and $b = 0.14$.

are spread and no patterns emerge. In Figs. 9b and 10b, the amounts adsorbed have been reported to the mass of packing contained in the column. The curves corresponding to the six

Table 3
Isotherm coefficients for 2,6-dimethylphenol

2,6-Dimethylphenol	Columns					
	YS	YL	VS1	VS2	VL1	VL2
q_s from ECP	119	113	136	144	131	124
b_1 from ECP	0.226	0.213	0.134	0.144	0.128	0.145

For columns, see Table 1.

Table 4
Isotherm coefficients for 3-phenyl-1-propanol

3-Phenyl-1-propanol	Columns					
	YS	YL	VS1	VS2	VL1	VL2
q_S from ECP	208.96	199.79	264.33	264.84	228.88	225.68
b_1 from ECP	0.1117	0.1052	0.0632	0.0708	0.0668	0.0715
q_S from FA	203.83	196.36	246.11	265.81	232.48	220.03
b_1 from FA	0.1009	0.0960	0.0514	0.0626	0.0584	0.0643

For columns, see Table 1.

columns now form two better defined clusters, corresponding to the two different adsorbents. Inside each cluster, there is still a significant difference between the isotherms obtained for different columns packed with the same stationary phase. This difference is small, about 5%, in the case of the two YMC columns, YS and YL. It is negligible in the case of the two short columns packed with the Vydac material, VS1 and VS2 (less than 1%). Column VL2 gives an isotherm which is close to those given by columns VS1 and VS2 (the difference is 6%) but the isotherm derived from column VL1 is quite different, with an amount adsorbed at equilibrium with the same mobile phase concentration nearly 20% lower. The same conclusions apply to both compounds. It seems unlikely that such a large error could be made on the determination of the amount of stationary phase in the column. It is at least as unlikely that the specific surface area of the material contained in the two columns be that different. There is a possibility that the adsorbent surface in column VL1 has been modified but this is dubious, artifacts of that kind being common in normal-phase chromatography but rare in reversed-phase chromatography. The reason for the spread of the isotherm data still eludes us. In our opinion, the solution to this problem is an important prerequisite for the systematic use of isotherm data for the optimization of the experimental conditions in preparative chromatography.

In Figs. 9c and 10c, the amounts adsorbed have been reported to the retention factor measured under analytical conditions (very small sample amount, so the product b_1C (Eq. 4) is negligible compared to unity). The lines obtained for the columns of both series become much closer. In all cases, for the YMC and the Vydac columns, for PP and for DMP, the range of amounts adsorbed for a given mobile phase

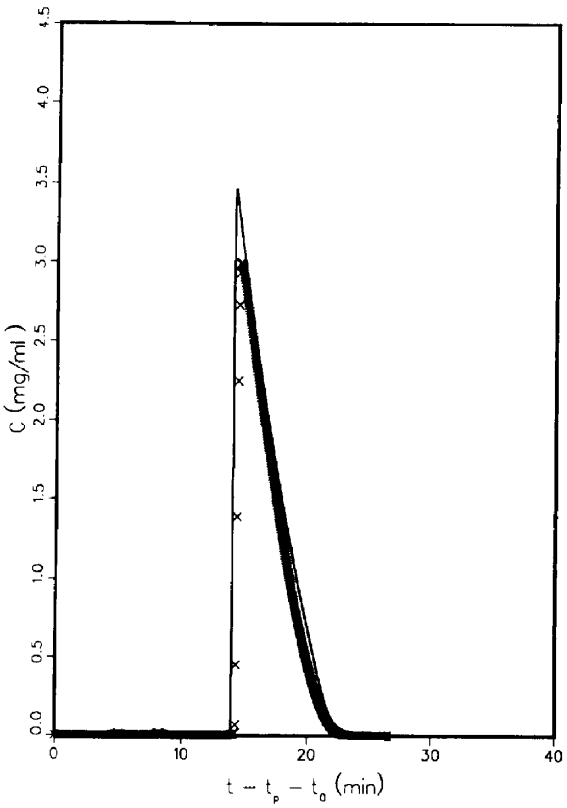

Fig. 12. Comparison between calculated (solid line) and experimental (symbols) band profiles on column VL2 ($L = 25$ cm, Vydac ODS). Sample: 3-phenyl-1-propanol; amount: 12.7 mg. Mobile phase: methanol–water (40:60, v/v). Langmuir isotherm coefficients: $q_S = 225.7$ and $b = 0.072$.

concentration is ± 4%, which is quite satisfactory. The rationale for this correction is that the retention factor at infinite dilution, k', is equal to $Fa = Fb_1q_s$ where F is the phase ratio $[(1-\varepsilon)/\varepsilon$, ε is the total porosity of the column the product of the phase ratio and the initial slope of the isotherm. So, if the isotherm is a characteristic of the stationary phase which depends only on the chemistry of the surface and the specific surface area, the differences between isotherms measured on different columns should be proportional to the differences between retention factors, because they are due to differences in packing density, hence in phase ratio. This assumption seems to prove approximately correct.

Since each of these isotherms appears to be Langmuirian, the experimental data were fitted to this model (Eq. 4). The coefficients q_S and b_1 were calculated for the isotherm derived from the elution band profile of the largest sample, as previously described [23]. The values obtained are reported in Tables 3 (2,6-dimethylphenol) and 4 (3-phenyl-1-propanol). The values corresponding to the two phases are significantly different, with the Vydac phase exhibiting a higher saturation capacity and a higher retention factor than the YMC phase. Inside each group, there is a spread of the numerical results, with a relative standard deviation of 6% for both q_S and b_1 on the Vydac material (the only one for which a meaningful relative standard deviation

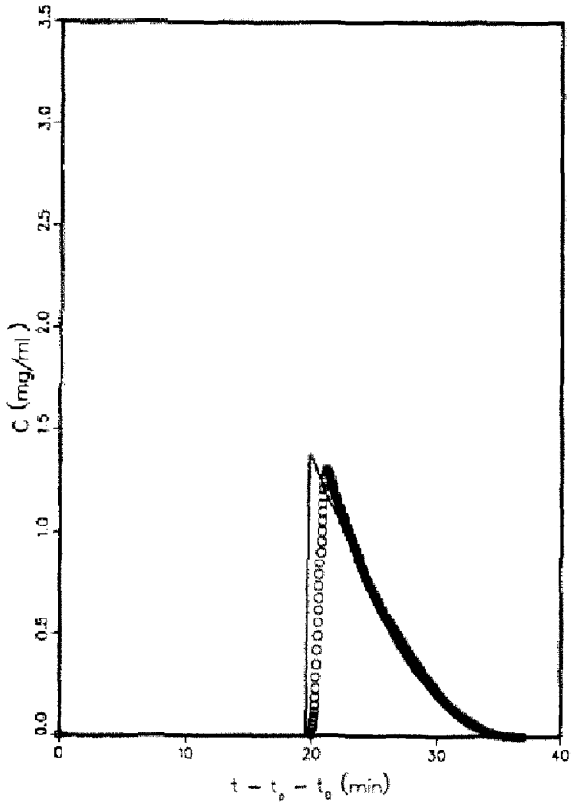

Fig. 13. Comparison between calculated (solid line) and experimental (symbols) band profiles on column YL (L = 25 cm, YMC ODS). Sample: 2,6-dimethylphenol; amount injected: 9.25 mg; amount used in the calculation: 8.40 mg. Mobile phase: methanol–water (40:60, v/v). Langmuir isotherm coefficients: q_S = 113.6 and b = 0.21.

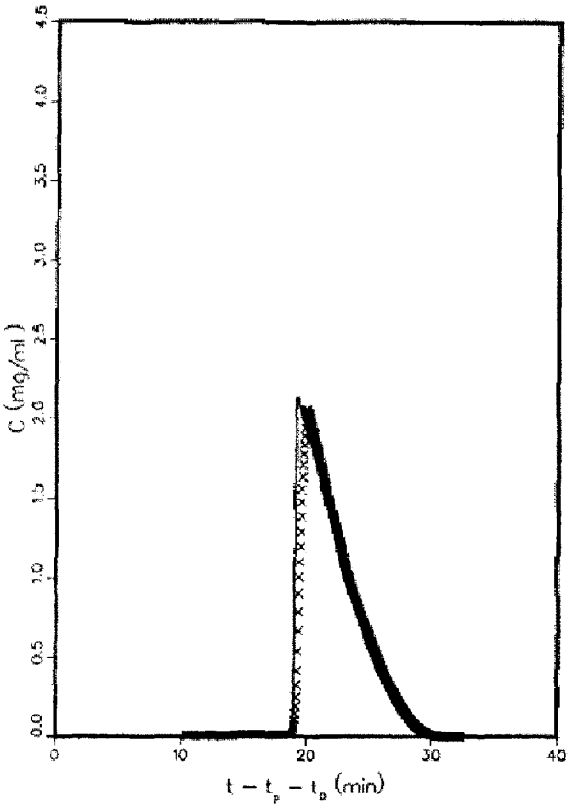

Fig. 14. Comparison between calculated (solid line) and experimental (symbols) band profiles on column YL2 (L = 25 cm, YMC ODS). Sample: 3-phenyl-1-propanol; amount injected: 12.7 mg; amount used in the calculation: 10.50 mg. Mobile phase: methanol–water (40:60, v/v). Langmuir isotherm coefficients: q_S = 199.8 and b = 0.11.

can be calculated). This error appears to be reasonable for experimental results involving complex determinations. Unfortunately, it is too large to permit accurate calculations of band profiles.

Isotherm data were also determined using the method of upward-staircase frontal analysis for 3-phenyl-1-propanol. The values obtained for the coefficients of the Langmuir equation are also given in Table 4. There is a very good general agreement with the results of the ECP method.

Knowing the isotherm and the column efficiency, it is possible to calculate band profiles using the equilibrium-dispersive model of chromatography [20,35,36]. Some of the results obtained are compared with the experimental band profiles in Figs. 11–14. In principle, this comparison is a circular argument. However, two model errors are made, one in each part of the circular argument. The first error is made in the ECP method which assumes the column efficiency to be infinite. Its consequences on the accuracy of the isotherm coefficients and on the selection of an isotherm model have been discussed previously [23]. In this earlier study based on the simulation of the measurement process using a Langmuir isotherm, we showed that there is a systematic difference between the values of the amount adsorbed at equilibrium given by the best-fit Langmuir isotherm and by the ECP isotherm at the same mobile phase concentration. In Figs. 15 and 16, these residuals are plotted versus the mobile phase concentration (C) for the two compounds studied. The shape of the curves obtained matches the result reported earlier.

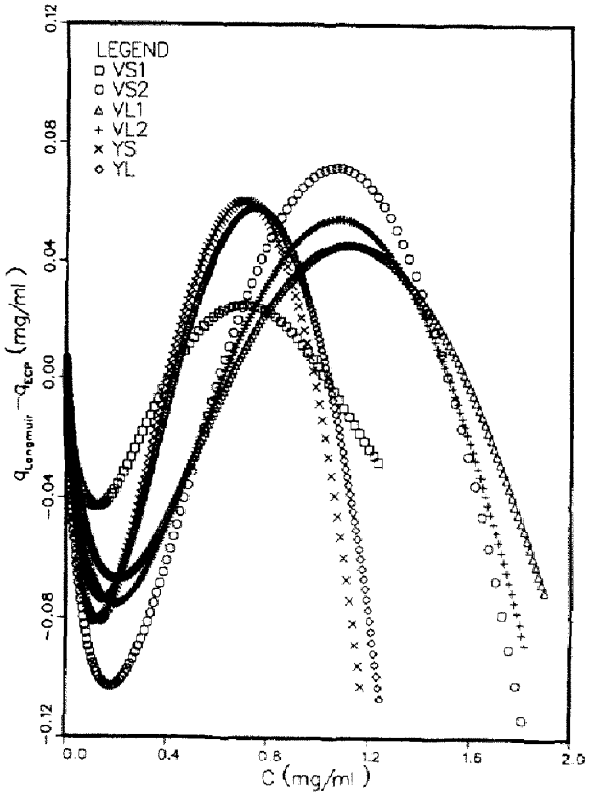

Fig. 15. Illustration of the model error caused by the use of ECP to derive the isotherm of 2,6-dimethylphenol on the six columns studied.

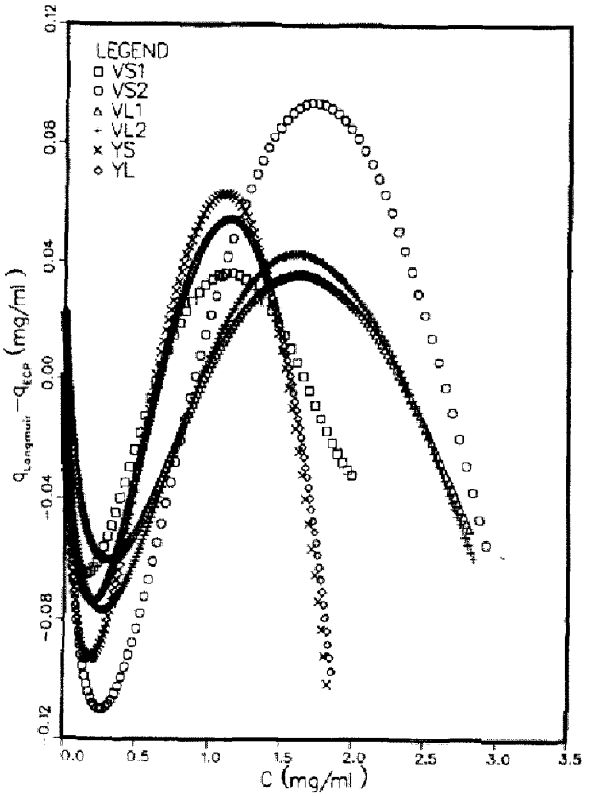

Fig. 16. Illustration of the model error caused by the use of ECP to derive the isotherm of 3-phenyl-1-propanol on the six columns studied.

A second model error is made in the equilibrium-dispersive model itself. This model assumes that the effect of a finite column efficiency on the band profile can be accounted for by a constant coefficient of apparent axial dispersion [20]. However, this coefficient varies with the solute concentration, causing a difference between the actual and the calculated band profiles. This difference is small if the rate constant of the mass-transfer kinetics is large, as is usually the case in reversed-phase chromatography of low-molecular-mass molecules [20]. Comparison between the calculated and experimental band profiles permits an assessment of the importance of these errors and of the possibility to correct them.

The agreement between calculated and experimental band profiles is very good for both compounds on the columns packed with both ODS phases (Figs. 11–14). However, the calculated peaks have a steeper front, are sharper at the peak maximum and less diffused at the rear of the tail. The difference in the steepness of the band front is slightly more important with the YMC than with the Vydac columns, suggesting a slower rate of mass transfer. Also, comparison of peak areas shows a slightly lower response on the former phase, suggesting a small sample loss on these columns.

4.2. Anti-Langmuirian compound

The behavior of the third compound studied, methyl benzoate, is opposite to that of the other two compounds. All overloaded bands of methyl benzoate exhibit a self-sharpening rear and a front diffuse boundary, a typical anti-Langmuirian behavior. The same type of profile was

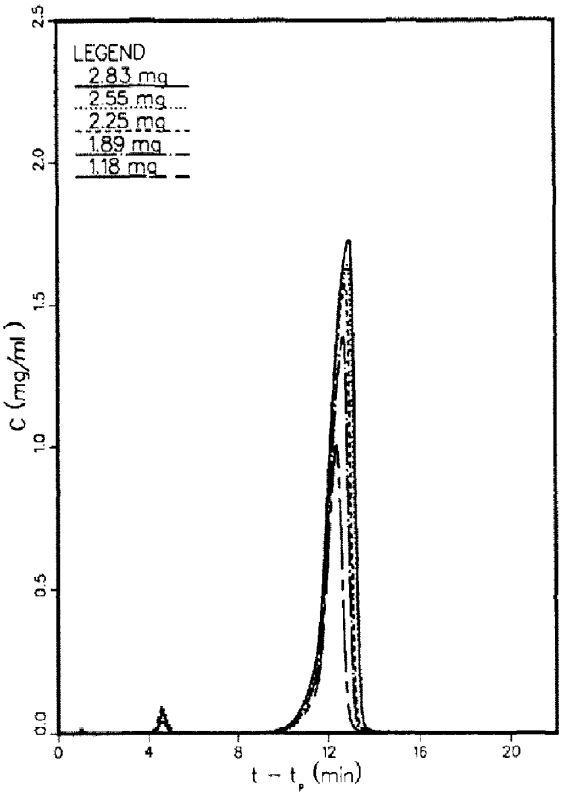

Fig. 17. Experimental chromatogram of methyl benzoate on column VS1 (L = 10 cm, Vydac ODS).

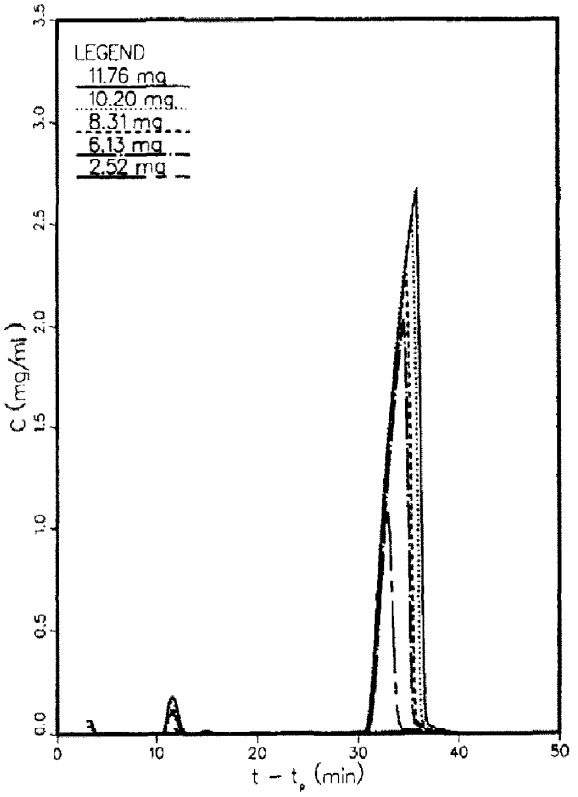

Fig. 18. Experimental chromatogram of methyl benzoate on column VL2 (L = 25 cm, Vydac ODS).

observed on all six columns, regardless of their length and of the nature of the stationary phase, as shown in Figs. 17–20. As a result of the shape of the isotherm, the elution time of the peak maximum increases and the rear shock becomes sharper with increasing sample size. Such an effect has rarely been reported in reversed-phase liquid chromatography and never studied in detail.

The isotherm of methyl benzoate was determined by applying the ECP method to the diffuse front of the band profiles obtained under overloading conditions. To verify this isotherm, determinations of the solute amount adsorbed at various mobile phase concentrations were carried out by FA, using negative concentration steps to generate self-sharpening rear shock layers. The isotherms obtained for all six columns are shown in Figs. 21 and 22. Difficulties

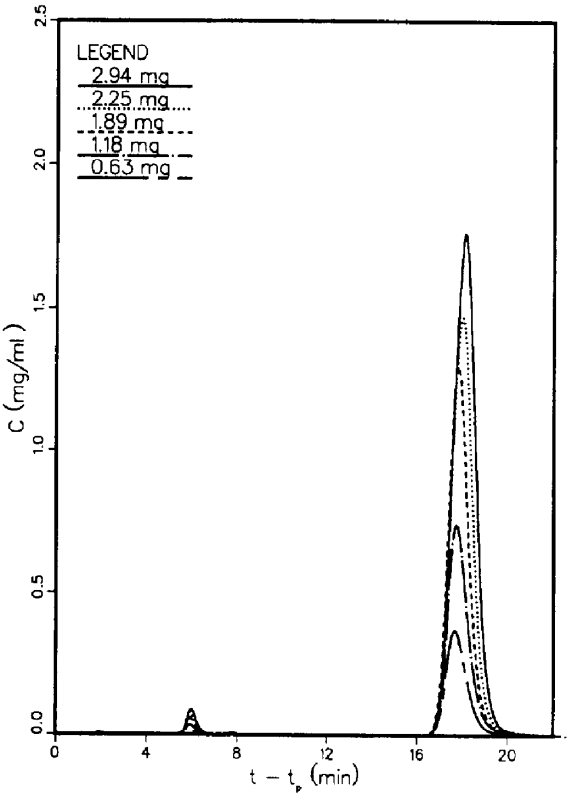

Fig. 19. Experimental chromatogram of methyl benzoate on column YS (L = 10 cm, YMC ODS).

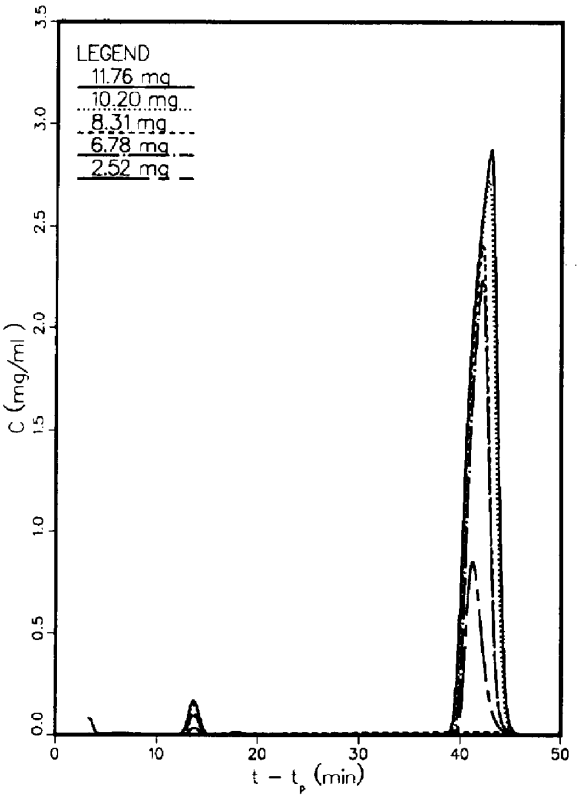

Fig. 20. Experimental chromatogram of methyl benzoate on column YL (L = 25 cm, YMC ODS).

arise for the determination of the isotherm at high concentrations because of the poor solubility of methyl benzoate in methanol–water solutions, which is probably related with the shape of the isotherm. In a methanol–water (40:60) mobile phase, the solubility is approximately 11.7 mg/ml. It was not possible to inject large enough samples and carry out the ECP determinations for concentrations exceeding 2.5 mg/ml (Fig. 21). By contrast, FA determinations could be done at concentrations up to 11.5 mg/ml (Fig. 22). This explains why the ECP isotherms appear to be almost linear while the curvature of the FA isotherms is apparent. Measurements were also made in a methanol–water (60:40) mixture in which the methyl benzoate solubility is much larger, ca 71.1 mg/ml. As seen in Fig. 23, the ECP and FA results are in excellent agreement in the low and medium

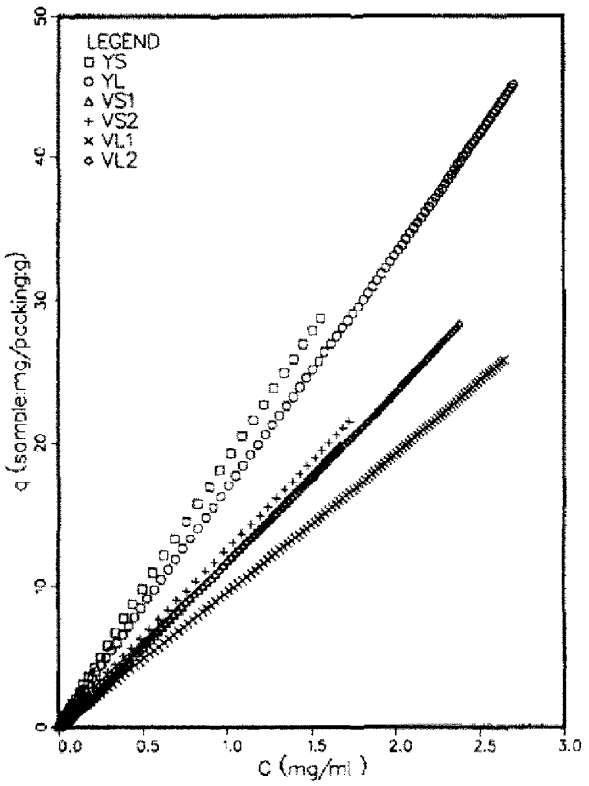

Fig. 21. Isotherms measured by ECP for methyl benzoate on the six columns studied. Mobile phase: methanol–water (40:60, v/v). Data reported to the mass of silica in the column.

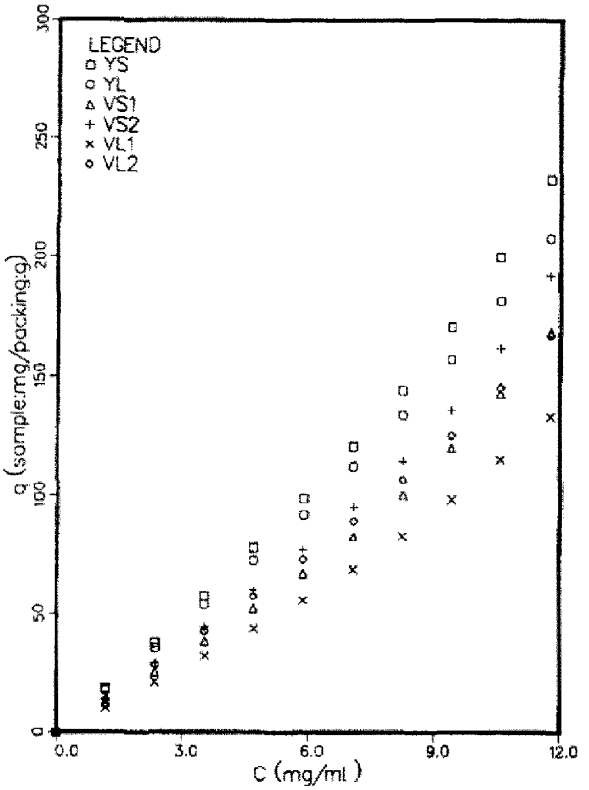

Fig. 22. Isotherms measured by FA for methyl benzoate on the six columns studied. Data reported to the mass of silica in the column. Mobile phase: methanol–water (40:60, v/v).

range of concentrations but diverge above approximately 30 mg/ml.

A convex-downward isotherm can be accounted for by accepting a negative value of b in Eq. 4 (Langmuir isotherm) or by using the second-order approximation of the general isotherm (Eq. 3). The former approach is purely empirical as it leads to an infinite value of the amount adsorbed for some finite mobile phase concentration, a conclusion which is physically meaningless. It also results in negative values of q_S (Table 5). Isotherms of type III in the Brunauer–Emmett–Teller classification are not encountered in liquid–solid equilibria. More probably, a convex-downward isotherm is an isotherm of type V whose inflection point is not accessible because it takes place at a concentration higher than the solubility. Accordingly, the true saturation capacity, q_S, is difficult to estimate.

The experimental adsorption data derived by ECP and FA were fitted to the two models listed above. The numerical coefficients obtained are reported in Table 5. There seems to be as much fluctuations between coefficients obtained for columns packed with the same phase as differences between the results obtained for columns packed with different phases.

Using these isotherm data, band profiles of methyl benzoate were calculated using a forward-backward algorithm [20,35]. The program aborted for instability reasons when the Langmuir equation with a negative b value was used. The results of two different calculations are reported here. The first used a four-term Taylor expansion of the Langmuir equation, the second used the three-term isotherm (Eq. 5). Com-

parison between experimental and calculated band profiles on two columns are illustrated in Figs. 24 and 25. The results obtained with the other four columns were similar. There are two major differences between these profiles. First, the calculated peak tends to be much higher than the experimental one. Second, the experimental band front is more diffuse than calculated, and its steep rear elutes slightly earlier. The differences in time and profile are less significant, however, when band profiles are calculated with the second isotherm (Eq. 5).

The simplest possible reason to explain the difference between experimental and calculated profiles around their maximum would be a decrease in the solubility of methyl benzoate in methanol–water solutions with increasing pressure. The injection of a saturated solution would result in the precipitation of the excess amount injected followed by its slow redissolution in the mobile phase. This would effectively widen the injection profile, decrease its maximum concentration and increase the retention time of the rear part of the profile. However, the rear shock layer originating from the end of the injection profile would be dispersed in the process, which is not the case. Furthermore, practically the same difference between calculated and mea-

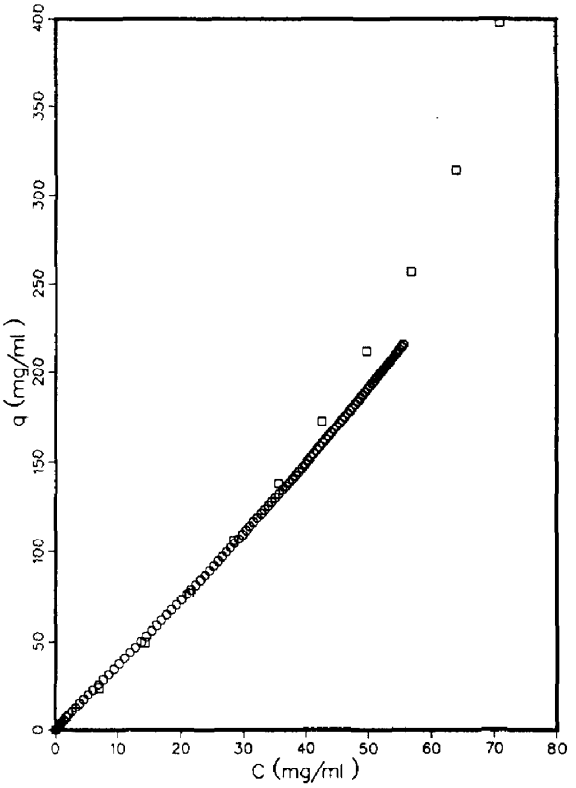

Fig. 23. Comparison of the isotherms derived from ECP (○) and FA (□) for methyl benzoate on column VL2 ($L = 25$ cm, Vydac ODS). Mobile phase: methanol–water (60:40, v/v).

Table 5
Isotherm coefficients for methyl benzoate

	Columns					
	YS	YL	VS1	VS2	VL1	VL2
ECP[a] q_S	na	na	−533	815	−1510	−1740
ECP[b] b_1	na	na	−0.037	0.032	−0.0136	−0.0123
FA[c] q_S	−1160	−1420	−601	−813	−794	−1160
FA[d] b_1	−0.021	−0.0165	−0.028	−0.027	−0.023	−0.018
FA[e] q_S	−600	−511	−285	−401	−368	−660
FA[f] b_1	−0.039	−0.041	−0.053	−0.054	−0.045	−0.031
FA[g] b_2	0.0003	0.0003	0.0005	0.0007	0.0004	0.0002

For columns, see Table 1. na = Not available.
[a] q_S obtained by ECP; first-order approximation isotherm.
[b] b_1 obtained by ECP; first-order approximation isotherm.
[c] q_S obtained by FA; first-order approximation isotherm.
[d] b_1 obtained by FA; first-order approximation isotherm.
[e] q_S obtained by FA; second-order approximation isotherm.
[f] b_1 obtained by FA; second-order approximation isotherm.
[g] b_2 obtained by FA; second-order approximation isotherm.

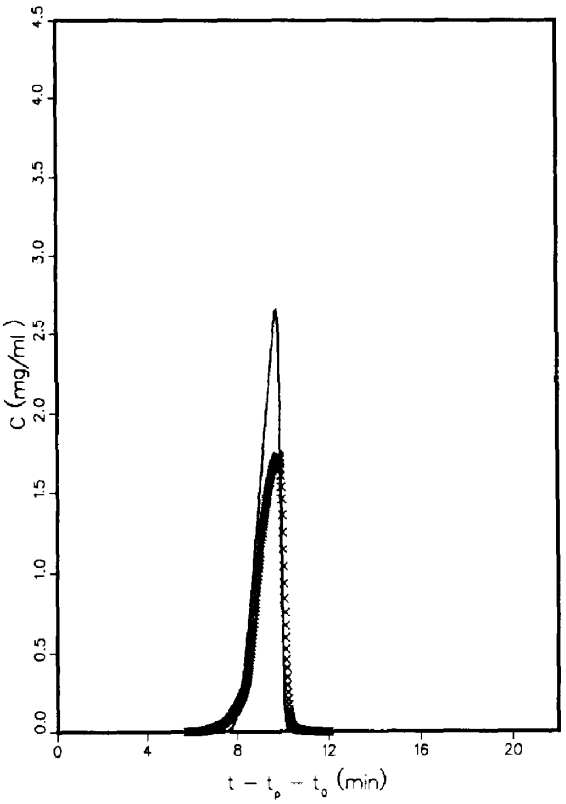

Fig. 24. Comparison between calculated (solid line) and experimental (symbols) band profiles for column VS1 (L = 10 cm, Vydac ODS). Sample: methyl benzoate; amount 2.83 mg. Mobile phase: methanol–water (40:60, v/v). Langmuir isotherm coefficients: $q_S = -640.5$ and $b_1 = -0.028$.

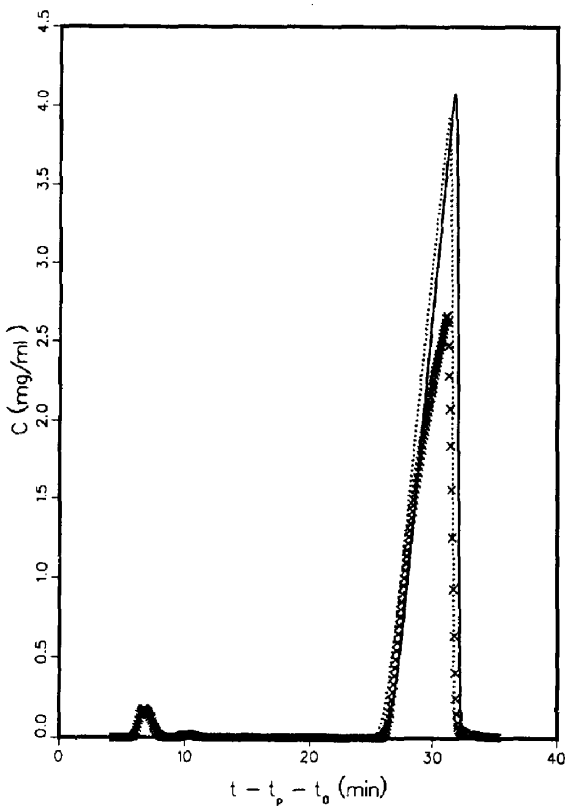

Fig. 25. Comparison between experimental (symbols) and calculated band profiles using isotherm data measured by FA and the Langmuir (solid line) or the quadratic (dotted line) isotherm models, for column VL2 (L = 25 cm, Vydac ODS). Sample: methyl benzoate; amount 11.76 mg. Mobile phase: methanol–water (40:60, v/v). Isotherm coefficients, Langmuir: $q_S = -1158.05$ and $b_1 = -0.018$; quadratic isotherm: $q_S = -660.66$, $b_1 = -0.031$ and $b_2 = 0.0002$.

sured profiles is observed with less concentrated solutions (Fig. 26).

5. Conclusions

The conclusions of a study based on the determination of isotherm data of three compounds on six chromatographic columns packed with ODS from two different companies can only be very limited. It is noteworthy, however, that the behavior of these two stationary phases is very similar. Both give Langmuir isotherms for 2,6-dimethylphenol and 3-phenyl-1-propanol and anti-Langmuir isotherms for methyl benzoate. The sharpness of the shock layers and the extent of dispersion of the diffuse boundary vary from column to column only in relation with their efficiency.

If allowance is made for the differences in the packing density of the different columns packed with the same stationary phase, the column-to-column reproducibility of isotherm data is markedly improved for the components giving a Langmuir isotherm. It is also better, although not as satisfactory, for methyl benzoate which gives an anti-Langmuir isotherm. The ratio of the retention factors of the compounds studied on the two phases investigated is close in spite of the important differences between these adsorbents.

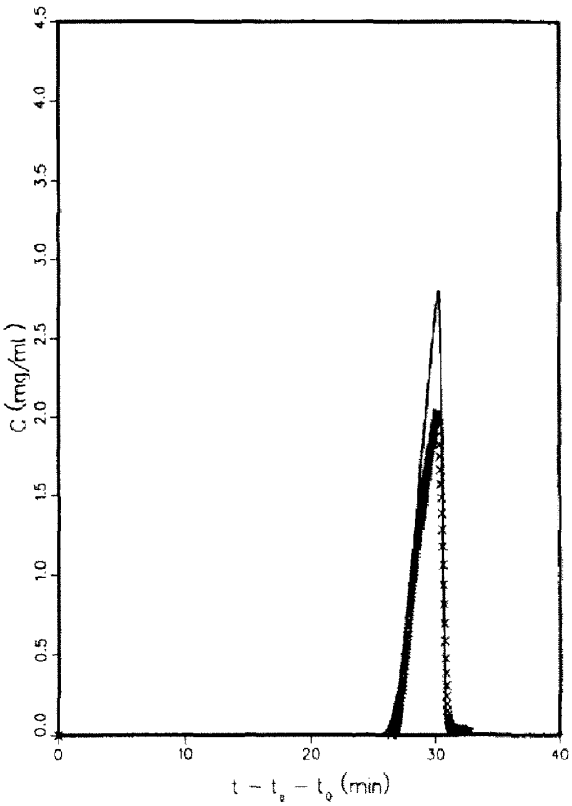

Fig. 26. Same comparison as in Fig. 25, except sample amount, 6.1 mg.

The isotherm data obtained by the ECP and FA methods are in excellent agreement for components giving Langmuir isotherms, as often reported previously [21,22]. However, this agreement is only fair for methyl benzoate at high concentrations. It is unclear whether the differences observed are due to experimental problems related to the anti-Langmuirian nature of the isotherm or to the low solubility of the compound.

Acknowledgements

We are grateful to YMC and Vydac companies for the gift of the ODS packing materials used throughout our studies. This work was supported in part by grant CHE-9201663 from the National Science Foundation and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory. We acknowledge support of our computational effort by the University of Tennessee Computing Center.

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