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Reproducibility, accuracy, and correction of isotherm data measured by chromatography

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Abstract

Using the methods of frontal analysis and elution by characteristic points, the isotherm of 3-phenyl-1-propanol was determined on four commercially available 10-μm ODS C_{18} packing materials: KROMASIL, VYDAC, YMC and ZORBAX. Each stationary phase was packed into five 10×0.46 cm I.D. columns. A methanol–water mixture (45:55, v/v) was used as the mobile phase and solvent. The column efficiencies and capacity factors were also determined for a series of compounds: *m*-cresol, benzyl alcohol, methyl benzoate, benzyl acetate, 2-isopropylphenol, and 2,6-dimethylphenol, all eluted under infinite dilution conditions. The average total column porosities were derived from the uracil retention volume, the internal and external porosities by reversed size-exclusion chromatography. Different columns packed with material from the same lot have significantly different thermodynamic properties. Corrections of isotherm data based on the difference between column retention factors and phase ratios do not compensate correctly for the deviations observed but corrections based on the difference of external porosities of the columns do.

Keywords: Stationary phases, LC; Isotherm data; Adsorption isotherms

1. Introduction

In spite of specific problems related to the relatively poor solubility of organic compounds in the aqueous mobile phases which have to be used in this mode, reversed-phase chromatography is widely used in preparative applications for the same convenience reasons which have caused it to become the most important mode of analytical liquid chromatography for over twenty years [1]. The most popular adsorbents used in this method is octadecyl-bonded silica (ODS). Much work has been done on the acquisition, compilation, comparison, correlation, and prediction of retention data on ODS [2–16]. Comparisons have been made of the properties of ODS-packed columns with those of columns made of other packing materials [8,10,15], of columns packed with ODS prepared with different reagents [9], and of columns packed with ODS produced by different manufacturers [12–14]. However, all these studies are mainly concerned with analytical applications.

In preparative chromatography, characteristics of the column or the packing material which are secondary in analytical applications acquire a great importance. The equilibrium isotherms of the feed

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components of interest between the stationary and the mobile phase and the parameters of the mass transfer kinetics become dominant factors that affect directly column performance. Of special concern is the possibility to scale-up design and operating conditions acquired on small columns or to predict such conditions on the basis of theoretical considerations and characteristics of the packings measured on analytical scale columns [17]. Because of the complexity of this issue whose importance has been recognized only recently, there is comparatively little information available in this area.

Previous studies [18,19] have shown a limited column-to-column reproducibility of the equilibrium isotherms, whether the columns had the same dimensions or not. This difference could be corrected for to a large extent by normalizing the equilibrium isotherm using the ratio of the retention factors. This correction allowed the use of the isotherm measured on one column for the prediction of the band profiles obtained with another one [18]. It is completely empirical, however, and provides no clues regarding the origin of the problem. Further investigations during which the actual packing density of the columns could be directly or indirectly determined appeared necessary.

The goal of this paper is a further investigation of the cause of the lack of reproducibility of isotherm data and an attempt at relating it to the lack of reproducibility of the apparent density of the column packing.

2. Theory

Under linear conditions, the sample concentrations in the stationary and the mobile phase at equilibrium are proportional and two parameters suffice to characterize the elution profile of a component, the retention factor k'

$$k' = \frac{t_R - t_0}{t_0} \tag{1}$$

where t_R is the retention time of the infinite diluted sample and t_0 is the column hold-up time, and the column efficiency for a zero sample size, N_0,

$$N_0 = 5.54\left(\frac{t_R}{\Delta t_w}\right)^2 \tag{2}$$

where Δt_w is the band width at half height.

When the column is overloaded, the equilibrium concentrations in the stationary and mobile phase are no longer proportional. The retention factor does not suffice to characterize equilibrium but the whole equilibrium isotherm is needed. It can be determined by elution by characteristic points (ECP) or frontal analysis (FA). The relative advantages and inconveniences of these methods are discussed elsewhere [17,18,20–22].

The ECP method relates the equilibrium isotherm and the profile of the diffuse boundary of a large concentration band by

$$q = \frac{1}{V_a}\sum_0^C (V - V_0)\delta_i C \tag{3}$$

where V_a is the stationary phase volume in the column, V_0 is the column hold-up volume, V is the retention volume of the point of the diffuse boundary at concentration C, and $\delta_i C$ is the increment ($\sum \delta_i C = C$). Since the ECP method assumes infinite column efficiency [17] and all columns have a finite efficiency, the isotherm data contains a model error [20–22] which is minimized by using columns having a minimum of 2000 theoretical plates [22].

The FA method is independent of column efficiency [19–21,23–26]. It measures the retention times of successive, abrupt concentration steps. The isotherm can then be determined by

$$q_{i+1} = q_i + \frac{(C_{i+1} - C_i)\,(V_{F,i+1} - V_0)}{V_a} \tag{4}$$

where q_i and q_{i+1} are the stationary phase concentrations in equilibrium with the ith and $i+1$th concentration steps, which have the mobile phase concentrations C_i and C_{i+1}, respectively.

The isotherm model used in this work is the Langmuir isotherm

$$q = \frac{aC}{1 + bC} \tag{5}$$

where q is the stationary phase concentration, C is the mobile phase concentration, a and b are numerical coefficients.

3. Experimental

3.1. Equipment

All experiments were made on a Hewlett-Packard (Palo Alto, CA, USA) HP1090M liquid chromatograph with a diode-array UV detector and a computerized data acquisition system. All measurements were done with a flow-rate of 1.000 ml/min and a detector wavelength of 250 nm, except FA which was run at 270 nm.

3.2. Columns

The empty stainless steel columns were purchased from Alltech (Deerfield, IL, USA). All columns are 10×0.46 cm I.D. Samples of four 10-μm spherical C_{18} ODS packing materials were obtained: KROMASIL (Eka-Nobel, Stratford, CT, USA), VYDAC (Hesperia, CA, USA), YMC (Wilmington, NC, USA), and ZORBAX (BTR, Wilmington, DE, USA). Each material was slurry-packed into five columns on the same day, starting with column #1 and ending with column #5. The lot numbers of each material and the column packing conditions are listed in Table 1. The methods used for packing the columns were those recommended by the respective manufacturers.

3.3. Chemicals

Uracil (Cat. No. 13078-8), *m*-cresol (Cat. No. C8572-7, 99%), benzyl alcohol (Cat. No. B1620-8, >99%), methyl benzoate (Cat. No. M2990-8, 99%) and 2,6-dimethyl phenol (Cat. No. D17500-5, 99%) were purchased from Aldrich (Milwaukee, WI, USA); acetone (Cat. No. AX0120-8, >99.5%) was purchased from EM Science (Gibbstown, NJ, USA); benzyl acetate (Cat. No. 45850, >99%), 2-isopropyl phenol (Cat. No. 59720, >98%) and 3-phenyl-1-propanol (Cat. No. 79000, >98%) were purchased from Fluka (Buchs, Switzerland).

Both the mobile phase and the solvent used to dissolve samples was a methanol–water mixture (45:55, v/v). Methanol (Cat. No. 9093-33) was purchased from J.T. Baker (Phillipsburg, NJ, USA). Water was freshly bidistilled/deionized in the laboratory, using a Thermolyne (Barnstead, Dubuque, IA, USA) water-deionizing system consisting of two cartridges, one HN high-capacity DI cartridge (Cat. No. D8901) and one HG organic-removal cartridge (Cat. No. D8904).

All samples and solvents were filtered on 0.45-μm pore size filters before use.

3.4. Procedures

Samples used for the measurements of k' and N_0 were 75.7 μg acetone and 25.0 μg each of *m*-cresol, benzyl alcohol, methyl benzoate, benzyl acetate, 2-isopropyl phenol, 2,6-dimethyl phenol and 3-phenyl-1-propanol. A 0.66-μg uracil sample was injected into each column for the determination of the system

Table 1
Column packing conditions (10-μm particles)

Brand	KROMASIL	VYDAC	YMC	ZORBAX
Lot. no.	DT0080	920714-28-1	EC16717	B32110
Pore size (Å)	100	90	120	150
Slurry solvent	30% CH_2Cl_2 and 70% isopr.	60% $CHCl_3$ and 40% acetone	CH_2Cl_2	30% CH_2Cl_2 and 70% isopr.
Pushing solvent	Same as above	33% Isopr. and 67% methanol	Methanol	Same as above
Pressure increase	Direct	Step	Direct	Direct
Maximum pressure (p.s.i.)	10 000	7000	4000	8000
Ca. packing time (min)	5	20	10	5
Ca. settle time (min)	5	30	5	5

Column dimensions, 10 cm×0.46 cm I.D.

hold-up time and the average total porosity of the column, data which were later used for the equilibrium isotherm determinations by ECP and FA. The phase ratios were determined from the column porosities derived from inverse size-exclusion chromatography [27]. Before using them for the calculation of k', ECP or FA data, retention volumes are corrected for the hold-up volume of the apparatus. The later is determined by replacing the column with a small connecting union, 2 mm long and 0.78 mm I.D. (volume ca. 1 μl). The results are summarized in Table 2. Each k' and N_0 measurement was made twice and the data listed in the Table is their average.

The ECP measurements were made by injecting 0.120 ml of 5.00 mg/ml, 20.00 mg/ml and 34.78 mg/ml 3-phenyl-1-propanol solutions into each column. The chromatograms obtained with the largest sample were used to calculate the ECP isotherm coefficients for each column [22]. Each measurement was made twice and the final result was taken as the average.

FA measurements were made by designing a program that has sixteen successive concentration step changes. Two pumps, one for the 45:55 (v/v) methanol–water mobile phase and the other one for a 34.00 mg/ml solution of 3-phenyl-1-propanol in the same solvent, were used for this purpose. The detector response was saturated at concentrations above $\approx$ 22 mg/ml, preventing the acquisition of data at higher concentrations.

3.5. Detector calibration

ECP requires detector calibration in order to translate the detector signal (mAu) into solute concentration (mg/ml). Calibration was performed by pumping solutions of known concentration directly into the detector cell until a stable signal was obtained. A third-order polynomial gave an excellent fit to the experimental data. The best coefficients of the fit were obtained by applying the polynomial

Table 2
Column characteristics

Columns		# 1	# 2	# 3	# 4	# 5
KROMASIL	V_a	0.684	0.698	0.690	0.697	0.696
	V_o	0.978	0.964	0.972	0.965	0.966
	ϵ	0.588	0.580	0.585	0.581	0.581
	ϵ_e	0.383	0.370	0.371	0.366	0.368
	F	0.645	0.675	0.672	0.681	0.681
VYDAC	V_a	0.608	0.611	0.601	0.609	0.597
	V_o	1.054	1.051	1.061	1.053	1.065
	ϵ	0.634	0.632	0.638	0.634	0.641
	ϵ_e	0.372	0.368	0.369	0.366	0.366
	F	0.570	0.580	0.575	0.577	0.575
YMC	V_a	0.502	0.497	0.504	0.503	0.499
	V_o	1.160	1.165	1.158	1.159	1.163
	ϵ	0.698	0.701	0.697	0.697	0.700
	ϵ_e	0.394	0.394	0.393	0.393	0.396
	F	0.414	0.416	0.422	0.418	0.416
ZORBAX	V_a	0.676	0.673	0.681	0.674	0.681
	V_o	0.986	0.989	0.981	0.988	0.981
	ϵ	0.593	0.595	0.590	0.594	0.590
	ϵ_e	0.399	0.401	0.395	0.398	0.398
	F	0.658	0.650	0.669	0.658	0.667

V_a: Column stationary phase volume. V_o: Column void volume. ϵ: Average column porosity measured by uracil. ϵ_e: Column external porosity determined by inverse size-exclusion chromatography [27]. F: Phase ratio $F=(1-\epsilon_T)/\epsilon_T$ ($\epsilon_T=\epsilon_i+\epsilon_e$, total porosity) determined by inverse size-exclusion chromatography [27].

regression in SigmaPlot (Jandel, San Rafael, CA, USA) to the experimental data points.

4. Results and discussion

4.1. Linear chromatography data (k' and N_0)

The values obtained for k' and N_0 are listed in Table 3 (KROMASIL), Table 4 (VYDAC), Table 5 (YMC), and Table 6 (ZORBAX). Although the columns were packed successively, with materials of the same lot, and had the same dimensions, there are usually minor column to column fluctuations of k' but very large such fluctuations of N_0. For the retention factor, the relative standard deviation (R.S.D.) is always less than 2%; it is less than 1% with VYDAC (except with acetone). For the column efficiency, the R.S.D. is 15 to 20% for KROMASIL, slightly less for VYDAC, and of the order of 5% for YMC and ZORBAX. This may be explained in part by the tough conditions of packing the KROMASIL columns. For KROMASIL and VYDAC, the efficiency of the first column packed was markedly poorer than the efficiency of the other four columns. The efficiency varies significantly from one compound to another, with the highest efficiency being recorded for benzyl acetate (only two minor exceptions).

As previously reported [12–14], columns packed with different stationary phases give different values of k' and N_0 for the same compounds, although these packing materials differ only in the particle pore size distribution. Retention tends to be highest on KROMASIL and lowest on ZORBAX. Although the packing pressure used for the YMC and ZORBAX

Table 3
KROMASIL: k' and N_0 measurements

Samples		Columns					Ave.[i]±Std.[j]
		# 1	# 2	# 3	# 4	# 5	
URA[a]	N_0	1552	1926	2278	2256	2177	2038±305
ACE[b]	k'	0.291	0.300	0.300	0.303	0.300	0.299±0.004
	N_0	1666	2247	2765	2848	2571	2419±480
MCR[c]	k'	4.516	4.645	4.701	4.707	4.680	4.650±0.079
	N_0	2133	3057	3562	3502	3484	3148±602
BAL[d]	k'	2.044	2.106	2.120	2.132	2.116	2.104±0.035
	N_0	2144	2888	3438	3377	3355	3040±547
MBE[e]	k'	10.143	10.446	10.496	10.543	10.471	10.420±0.159
	N_0	2672	3767	4329	4083	4264	3823±679
BAC[f]	k'	9.921	10.226	10.243	10.313	10.225	10.186±0.152
	N_0	3708	3865	4568	4054	4328	4105±347
DPH[g]	k'	9.181	9.479	9.463	9.571	9.432	9.425±0.146
	N_0	2284	3275	3859	3695	3910	3405±674
PPR[h]	k'	7.583	7.832	7.799	7.900	7.783	7.779±0.119
	N_0	2301	3254	3705	3710	3779	3350±622

[a]URA: 0.00066 mg uracil.
[b]ACE: 0.076 mg acetone.
[c]MCR: 0.025 mg *m*-cresol.
[d]BAL: 0.025 mg benzyl alcohol.
[e]MBE: 0.025 mg methyl benzoate.
[f]BAC: 0.025 mg benzyl acetate.
[g]DPH: 0.025 mg 2,6-dimethyl phenol.
[h]PPR: 0.025 mg 3-phenyl-1-propanol.
[i]Ave.: Average of all five columns.
[j]Std.: Standard deviation for the average of all five columns.

Table 4
VYDAC: k' and N_0 measurements

Samples		Columns					Ave.[i]±Std.[j]
		# 1	# 2	# 3	# 4	# 5	
URA[a]	N_0	1185	1353	1169	1284	1012	1200±129
ACE[b]	k'	0.287	0.291	0.289	0.290	0.280	0.287±0.004
	N_0	1277	1529	1385	1533	1617	1470±136
MCR[c]	k'	3.419	3.420	3.450	3.472	3.440	3.440±0.022
	N_0	1063	1469	1143	1158	959	1160±191
BAL[d]	k'	1.650	1.658	1.649	1.650	1.634	1.648±0.009
	N_0	1270	1565	1400	1525	1577	1470±131
MBE[e]	k'	8.496	8.493	8.427	8.371	8.355	8.428±0.066
	N_0	1793	2530	2237	2405	2572	2310±316
BAC[f]	k'	8.104	8.112	8.035	8.003	7.977	8.046±0.060
	N_0	1909	3676	3394	3677	3717	3275±774
DPH[g]	k'	6.511	6.519	6.517	6.588	6.536	6.534±0.031
	N_0	1162	1741	1266	1496	1497	1430±226
PPR[h]	k'	5.633	5.658	5.617	5.630	5.603	5.628±0.020
	N_0	1430	1749	1637	1784	1853	1690±165

Footnotes as in Table 3.

columns differ by 50% (Table 1), their efficiencies are close.

Acetone appears to be slightly retained on all the columns. This compound was used by some to measure the column hold-up volume in reversed-phase liquid chromatography. This is not a safe practice [28].

4.2. Chromatograms

The chromatograms obtained for the largest sample injected on each column are shown in Fig. 1, Fig. 2, Fig. 3, Fig. 4. They differ from column to column, the main differences being in the retention time of the band front. The differences are much more important between band profiles on columns packed

Table 5
YMC: k' and N_0 measurements

Samples		Columns					Ave.[i]±Std.[j]
		# 1	# 2	# 3	# 4	# 5	
URA[a]	N_0	2650	2878	2548	2745	2664	2700±120
ACE[b]	k'	0.232	0.228	0.230	0.230	0.226	0.229±0.002
	N_0	3066	3385	3144	3602	3366	3300±210
MCR[c]	k'	3.796	3.796	3.843	3.756	3.691	3.776±0.057
	N_0	3149	3397	3281	3197	3442	3300±130
BAL[d]	k'	1.760	1.756	1.770	1.745	1.715	1.749±0.021
	N_0	3207	3471	3315	3462	3460	3400±118
MBE[e]	k'	8.632	8.629	8.693	8.518	8.353	8.565±0.134
	N_0	3602	4035	3931	4015	4034	3900±185
BAC[f]	k'	8.421	8.421	8.473	8.318	8.152	8.357±0.128
	N_0	4254	5446	5154	6083	5572	5300±675
DPH[g]	k'	7.494	7.483	7.534	7.401	7.255	7.433±0.111
	N_0	3248	3636	3445	3676	3592	3500±175
PPR[h]	k'	6.329	6.322	6.355	6.246	6.118	6.274±0.096
	N_0	3437	3579	3449	3593	3573	3500±76

Footnotes as in Table 3.

Table 6
ZORBAX: k' and N_0 measurements

Samples		Columns					Ave.[i] ± Std.[j]
		# 1	# 2	# 3	# 4	# 5	
URA[a]	N_0	2546	2671	2630	2666	2745	2650±70
ACE[b]	k'	0.219	0.218	0.221	0.220	0.223	0.220±0.002
	N_0	3222	3237	3201	3242	3339	3250±53
MCR[c]	k'	2.994	3.061	3.041	3.033	3.102	3.046±0.040
	N_0	3200	3141	3206	3276	3193	3200±48
BAL[d]	k'	1.428	1.447	1.443	1.442	1.474	1.447±0.017
	N_0	3258	3266	3269	3311	3224	3270±31
MBE[e]	k'	7.752	7.877	7.845	7.891	8.098	7.893±0.127
	N_0	3980	3884	3840	3911	4524	4030±282
BAC[f]	k'	7.405	7.508	7.472	7.533	7.729	7.529±0.121
	N_0	4484	4018	4791	4322	4269	4400±286
DPH[g]	k'	5.959	6.054	6.018	6.093	6.272	6.079±0.118
	N_0	3473	3374	3402	3451	3414	3420±39
PPR[h]	k'	5.191	5.264	5.237	5.303	5.450	5.289±0.099
	N_0	3378	3347	3422	3408	3365	3380±31

Footnotes same as in Table 3.

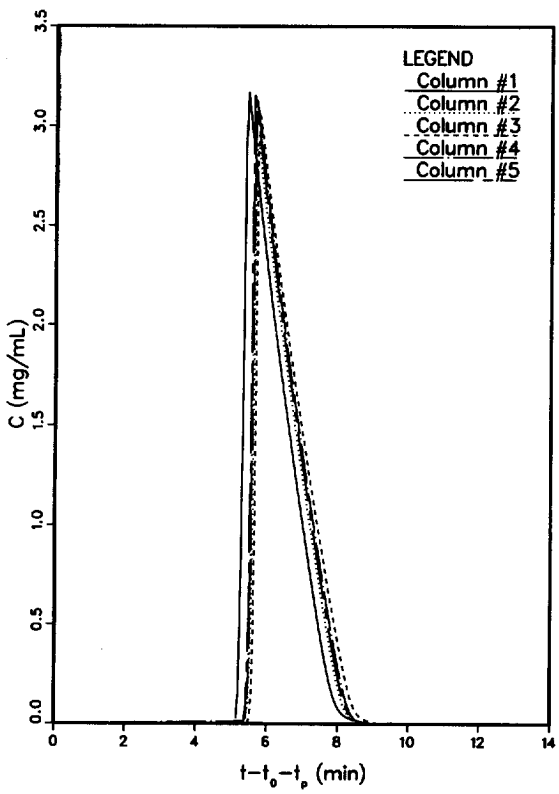

Fig. 1. Band profile used for the determination of the isotherm by ECP; KROMASIL columns.

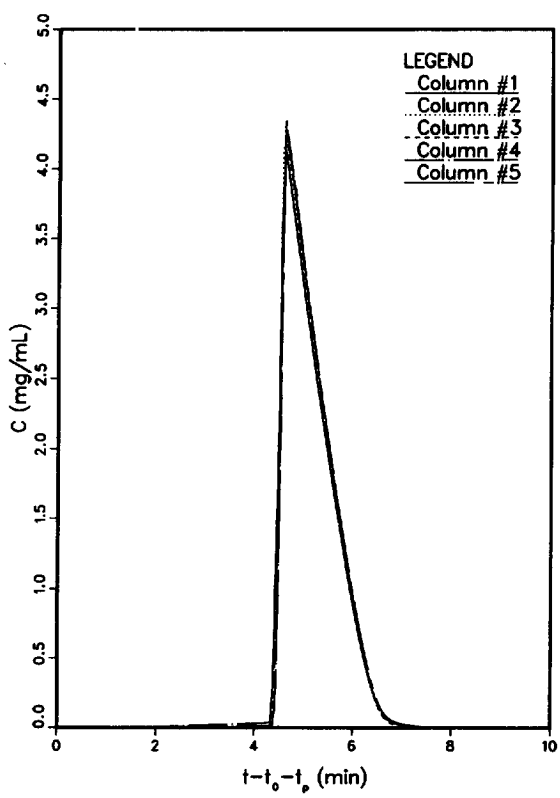

Fig. 2. Band profile used for the determination of the isotherm by ECP; VYDAC columns.

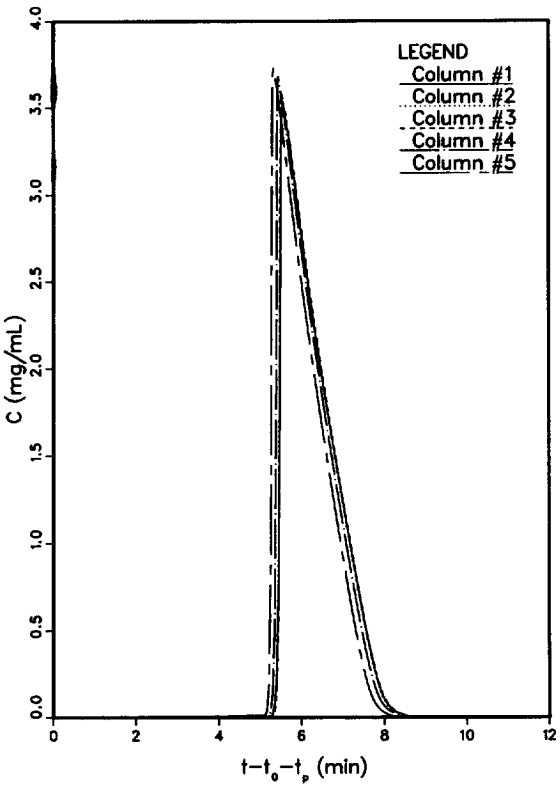

Fig. 3. Band profile used for the determination of the isotherm by ECP; YMC columns.

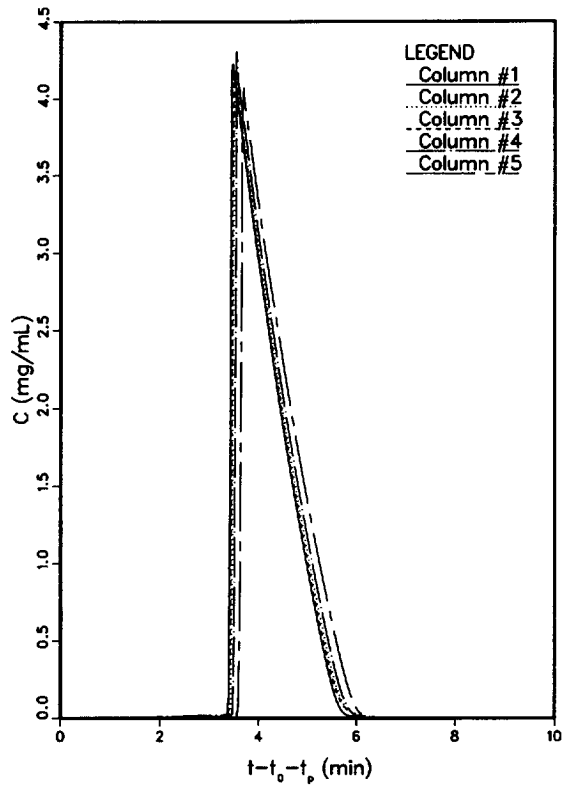

Fig. 4. Band profile used for the determination of the isotherm by ECP; ZORBAX columns.

with different materials, indicating markedly different equilibrium isotherms.

4.3. Equilibrium isotherms

The equilibrium isotherms were determined using both the ECP and FA methods. The FA isotherms are illustrated in Fig. 5, Fig. 6, Fig. 7, Fig. 8. As in previous work [18], the differences between equilibrium isotherms obtained for columns packed with the same ODS phase are significant. By contrast, isotherms obtained at several weeks intervals on the same column would not be distinguished from each other on the figures. The isotherm coefficients were derived by applying the SAS non-linear regression routine available at the University of Tennessee Computer Center (UTCC) to the isotherms obtained by both ECP and FA methods. The results are listed in Table 7 (KROMASIL), Table 8 (VYDAC), Table 9 (YMC), and Table 10 (ZORBAX). As we can see, the overall agreement between the isotherm coefficients derived by ECP and FA is quite good. Note also that the R.S.D. of the isotherm coefficients are generally between 1 and 2%, which can be considered as quite satisfactory.

In general, ECP tends to give a better estimate of coefficient a since it gives many data points in the low concentration range where the isotherm data is mainly controlled by the initial slope of the isotherm. Frontal analysis, on the other hand, gives a better estimate of the coefficient b since it is possible to reach much higher concentrations in FA than in ECP. An excellent fit of the experimental data and an accurate isotherm can be obtained by combining in the nonlinear regression the FA and ECP data and

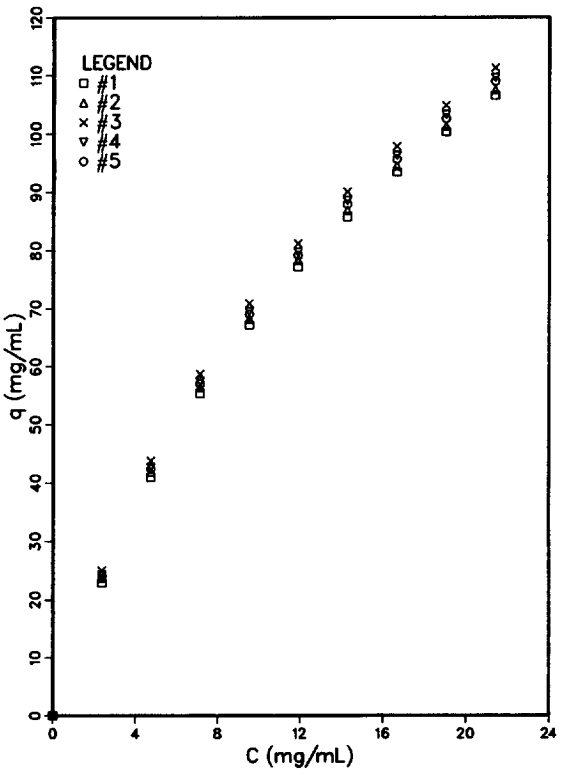

Fig. 5. FA isotherms of 3-phenyl-1-propanol on KROMASIL.

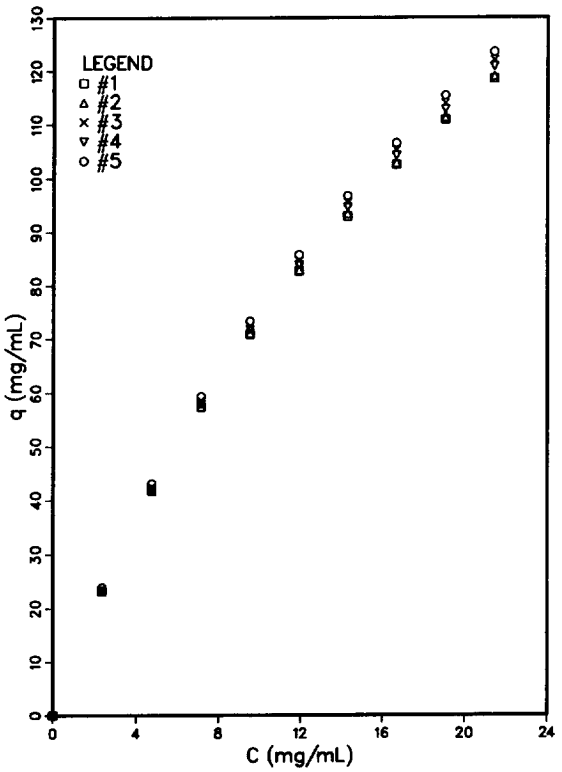

Fig. 6. FA isotherms of 3-phenyl-1-propanol on VYDAC.

giving the same total weight to each set of data points.

Based upon these isotherm coefficients, it is possible to calculate the band profiles and compare them with the experimental chromatograms. The agreement between the two sets of profiles was excellent for all the columns examined when the calculation is performed with the isotherm measured on the column on which the profiles are recorded. Data are shown only for the YMC phase (Fig. 9, solid line and symbols). A comparison of the experimental band profiles (symbols) and the profiles calculated using the isotherm determined on a different column is also shown in Fig. 9 (dotted line). The agreement is poor and illustrates the difficulties which may be encountered when trying to optimize the experimental conditions of a preparative separations using data measured with an analytical column.

4.4. Isotherms normalized by the ratios of k's

The most probable source of the differences observed between the equilibrium isotherms measured on columns packed with the same ODS phase is a difference in packing density [29]. In this case, it is possible to correct for these differences by using the ratio of the retention factors on two columns. This correction is justified by the relationship

$$k' = Fa = \frac{1 - \epsilon_T}{\epsilon_T} a \tag{6}$$

where F is the phase ratio and ϵ_T the total column porosity [17]. If the packing densities of two columns are different, their phase ratios must be different, while the coefficients of the isotherm should remain unaffected. This correction is practical because k' is easy to measure on a preparative column.

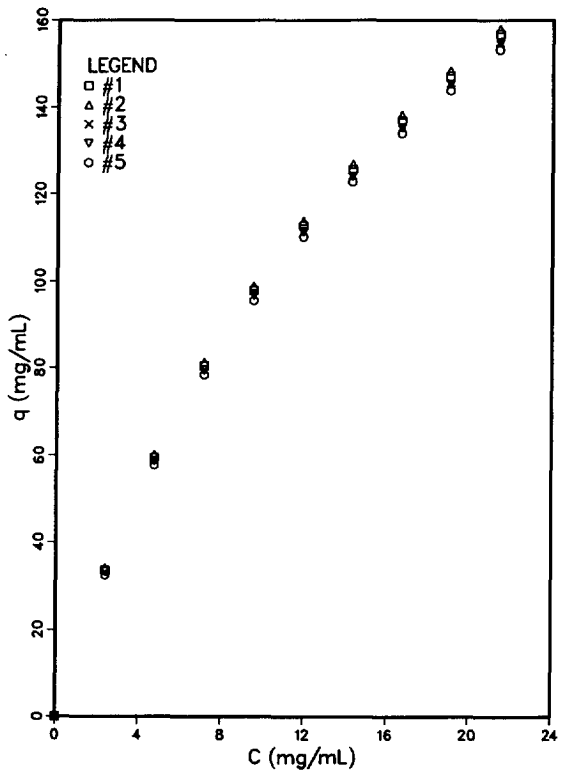

Fig. 7. FA isotherms of 3-phenyl-1-propanol on YMC.

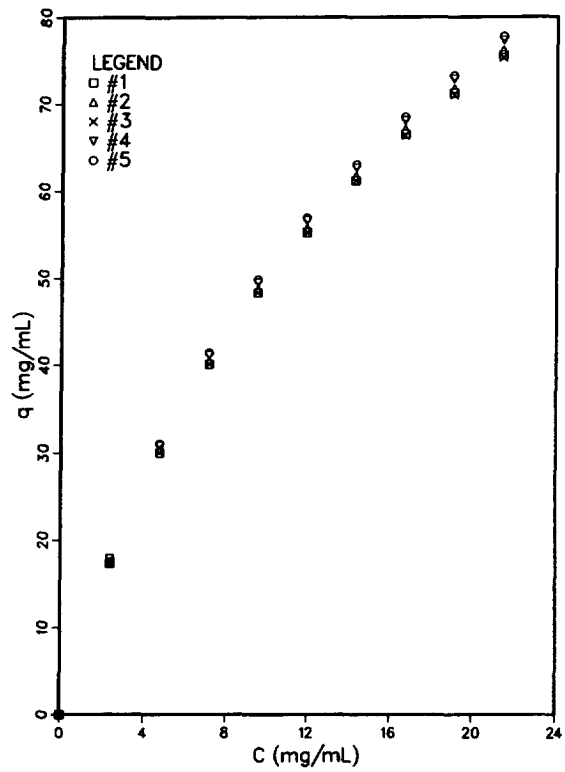

Fig. 8. FA isotherms of 3-phenyl-1-propanol on ZORBAX.

This procedure could be used to adjust the isotherm data measured on an analytical column for its use in calculating the band profiles on a new preparative column. The choice of the base k' to be used for the normalization is arbitrary. We used here the average value of the retention factors k'_{ave} obtained for each set of 5 columns (Tables 3–6, far right column, line PPR).

For each column, a new isotherm was obtained by multiplying the experimental values of q obtained originally by the ECP and FA methods by the ratio of the retention factor of the corresponding column (k'_i) to k'_{ave}. By applying the SAS nonlinear regression routine to this newly obtained set of isotherms we can calculate the new isotherm coefficients. The results in the case of the FA isotherms are given in Tables 7–10 (lines k'_i/k'_{ave} and k'-ECP). The same trend was observed for the FA isotherms. After normalization by the ratios of the retention factors, the R.S.D. of the new set of values of the coefficient a for the KROMASIL, YMC, and ZORBAX columns is larger than before, meaning that the correction is not successful (for the VYDAC columns, the R.S.D. is unchanged). If we eliminate the data for the worst of the five columns in the case, there is some improvement. On the other hand, no changes are observed for the coefficient b.

These results confirm a previous finding, that the isotherm coefficient b is much less affected by changes in the experimental parameters than the coefficient a [22]. Eq. 5 can be rewritten

$$q = \frac{bq_sC}{1 + bC} \tag{7}$$

where q_s is the column saturation capacity. Obviously, q_s is a function of the packing density while b is not. However, the previous result, that the normalization of the isotherms obtained from columns packed

Table 7
Isotherm studies on KROMASIL columns

		Columns				
		# 1	# 2	# 3	# 4	# 5
ECP	a	11.608 ±0.003	11.791 ±0.002	12.326 ±0.001	11.965 ±0.001	11.907 ±0.001
		Average of all 5 columns: 11.919±0.265				
	b (ml/mg)	0.0690 ±0.0001	0.0689 ±0.0001	0.0707 ±0.0001	0.0681 ±0.0000	0.0681 ±0.0000
		Average of all 5 columns: 0.0690±0.0011				
FA	a	10.763 ±0.075	11.099 ±0.089	11.652 ±0.114	11.302 ±0.098	11.211 ±0.102
		Average of all 5 columns: 11.205±0.322				
	b (ml/mg)	0.0548 ±0.0008	0.0572 ±0.0010	0.0587 ±0.0012	0.0570 ±0.0011	0.0569 ±0.0011
		Average of all 5 columns: 0.0569±0.0014				
k'_i/k'_{ave} ratio		0.975	1.007	1.003	1.015	1.000
k'-ECP	a	11.318 ±0.003	11.873 ±0.002	12.363 ±0.001	12.145 ±0.001	11.907 ±0.001
		Average of all 5 columns: 11.921±0.391				
	b (ml/mg)	0.0690 ±0.0001	0.0689 ±0.0001	0.0707 ±0.0001	0.0681 ±0.0000	0.0681 ±0.0000
$\epsilon_{e,i}/\epsilon_{e,ave.}$ ratio	1.030	0.995	0.997	0.984	0.989	
ϵ_e-ECP	a	11.957 ±0.003	11.732 ±0.001	12.289 ±0.001	11.774 ±0.001	11.776 ±0.001
		Average of all 5 columns: 11.906±0.231				
	b (ml/mg)	0.0690 ±0.0001	0.0689 ±0.0001	0.0707 ±0.0001	0.0681 ±0.0000	0.0681 ±0.0000
$a=k'/F$	a	11.757	11.603	11.606	11.601	11.429
		Average of all 5 columns: 11.599±0.116				

with a given ODS phase by the ratios of the retention factors could decrease moderately the extent of the deviations between these isotherms [17] is not confirmed.

The failure of a correction by the retention factor does not invalidate the assumption that column-to-column fluctuations of the packing density explain the lack of reproducibility of the isotherm data. This failure results probably from the serious difficulties presented by the precise determination of thermodynamic data, retention factors under linear conditions or isotherm data.

4.5. Alternate determination of the coefficient a

In principle, the retention factor is related to the first coefficient of the equilibrium isotherm by Eq. 6. Rather than determining a from the ECP of FA measurements, it is possible to derive it from retention data measured under linear conditions and the phase ratio. The corresponding values of a for all columns are also listed in Tables 7–10 (last row). It is observed that the relative standard deviation of these values is smaller than the R.S.D. of the values of a derived from either ECP or FA, two to three

Table 8
Isotherm studies on VYDAC columns

		Columns				
		# 1	# 2	# 3	# 4	# 5
ECP	*a*	10.827 ±0.004	10.739 ±0.003	10.917 ±0.003	10.790 ±0.003	11.040 ±0.003
		Average of all 5 columns: 10.863±0.119				
	b (ml/mg)	0.0483 ±0.0001	0.0462 ±0.0001	0.0457 ±0.0001	0.0454 ±0.0001	0.0457 ±0.0001
		Average of all 5 columns: 0.0463±0.0012				
FA	*a*	10.442 ±0.072	10.420 ±0.061	10.561 ±0.069	10.448 ±0.054	10.693 ±0.068
		Average of all 5 columns: 10.513±0.115				
	b (mL/mg)	0.0417 ±0.0007	0.0411 ±0.0006	0.0402 ±0.0007	0.0401 ±0.0005	0.0402 ±0.0007
		Average of all 5 columns: 0.0407±0.0007				
k_i'/k_{ave}' ratio		1.001	1.005	0.998	1.000	0.996
k'-ECP	*a*	10.837 ±0.004	10.792 ±0.003	10.895 ±0.003	10.790 ±0.003	10.996 ±0.003
		Average of all 5 columns: 10.862±0.086				
	b (ml/mg)	0.0483 ±0.0001	0.0462 ±0.0001	0.0457 ±0.0001	0.0454 ±0.0001	0.0457 ±0.0001
$\epsilon_{e,i}/\epsilon_{e,ave}$ ratio		1.011	1.000	1.003	0.995	0.995
ϵ_e-ECP	*a*	10.946 ±0.004	10.739 ±0.003	10.950 ±0.003	10.736 ±0.003	10.985 ±0.003
		Average of all 5 columns: 10.871±0.108				
	b (ml/mg)	0.0483 ±0.0001	0.0462 ±0.0001	0.0457 ±0.0001	0.0454 ±0.0001	0.0457 ±0.0001
$a=k'/F$	*a*	9.882	9.755	9.769	9.757	9.744
		Average of all 5 columns: 9.781±0.057				

times smaller in the cases of KROMASIL and VYDAC. However, the average value obtained by this method is significantly lower than the ECP value, by 2.7, 10, 6.3, and 6.4% for KROMASIL, VYDAC, YMC, and ZORBAX, respectively. This systematic deviation is much larger than the correction which would be required for the ECP model error, a reduction by approximately 1.5% for a 3000 plate column [22]. It is too large to be acceptable.

A comparison of the experimental band profiles (symbols) to those calculated using the average values of *a* (from k'/F) and *b* and the values of the phase ratio and efficiency of the column considered (dashed line) is shown in Fig. 9. The agreement between the two sets of profiles is too poor to be acceptable and this invalidates the procedure.

4.6. Isotherms normalized by the ratios of ϵ_e's

The column external porosity (ϵ_e) can be determined directly by inverse size-exclusion chromatography [27]. The results of these measurements are listed in Table 2. Using the same method as in Section 4.4 (normalization by k'), but using the ϵ_e ratios instead, we obtained a new set of isotherm coefficients, listed in Tables 7–10 under lines $\epsilon_{e,i}/$

Table 9
Isotherm studies on YMC columns

		Columns				
		# 1	# 2	# 3	# 4	# 5
ECP	a	16.026 ±0.002	16.153 ±0.003	15.981 ±0.003	15.750 ±0.003	15.539 ±0.002
		Average of all 5 columns: 15.890±0.244				
	b (ml/mg)	0.0573 ±0.0001	0.0569 ±0.0001	0.0569 ±0.0001	0.0558 ±0.0001	0.0546 ±0.0001
		Average of all 5 columns: 0.0563±0.0011				
FA	a	15.478 ±0.142	15.723 ±0.136	15.320 ±0.121	15.298 ±0.133	15.027 ±0.107
		Average of all 5 columns: 15.369±0.256				
	b (ml/mg)	0.0526 ±0.0011	0.0534 ±0.0010	0.0528 ±0.0009	0.0528 ±0.0010	0.0519 ±0.0008
		Average of all 5 columns: 0.0527±0.0005				
k'_i/k'_{ave} ratio		1.009	1.008	1.013	0.995	0.975
k'-ECP	a	16.170 ±0.003	16.282 ±0.003	16.189 ±0.003	15.671 ±0.003	15.151 ±0.002
		Average of all 5 columns: 15.893±0.478				
	b (ml/mg)	0.0573 ±0.0001	0.0569 ±0.0001	0.0569 ±0.0001	0.0558 ±0.0001	0.0546 ±0.0001
$\epsilon_{e,i}/\epsilon_{e,ave}$ ratio		1.000	1.000	0.997	0.997	1.005
ϵ_e-ECP	a	16.026 ±0.003	16.153 ±0.003	15.933 ±0.003	15.702 ±0.003	15.617 ±0.002
		Average of all 5 columns: 15.886±0.223				
	b (ml/mg)	0.0573 ±0.0001	0.0569 ±0.0001	0.0569 ±0.0001	0.0558 ±0.0001	0.0546 ±0.0001
$a=k'/F$	a	15.287	15.197	15.059	14.943	14.707
		Average of all 5 columns: 15.039±0.227				

$\epsilon_{e,ave}$ ratio and ϵ_e-ECP. As seen in these Tables, the rsd of the new set of values of the coefficient a for the five KROMASIL, VYDAC, YMC columns is significantly reduced compared to the R.S.D. of the experimental data. For the five ZORBAX columns it increases only slightly. The same trend is observed for the FA isotherms (results not listed in the Tables). No changes were observed for the coefficient b.

Using the average value of the new set of coefficients a the band profile on the YMC column # 2 was calculated as before. The result is shown in Fig. 9 (chain-dot line). The calculated band profile is now very close to the experimental chromatogram. This suggests that the correction procedure used here is successful.

5. Conclusion

Neither directly nor indirectly can the use of the retention factor acquired under linear chromatography conditions permit the derivation of a suitable correction of isotherm data allowing the calculation of band profiles on a column using isotherm data measured on another column, packed with the same stationary phase.

Table 10
Isotherm studies on ZORBAX columns

		Columns				
		# 1	# 2	# 3	# 4	# 5
ECP	*a*	8.381 ±0.001	8.562 ±0.001	8.404 ±0.002	8.632 ±0.001	8.812 ±0.001
		Average of all 5 columns: 8.558±0.177				
		Average of columns 1,2,4,5: 8.597±0.178				
	b (ml/mg)	0.0648 ±0.0000	0.0666 ±0.0001	0.0662 ±0.0001	0.0661 ±0.0000	0.0677 ±0.0000
		Average of all 5 columns: 0.0663±0.0010				
FA	*a*	8.025 ±0.097	8.128 ±0.102	8.076 ±0.092	8.280 ±0.104	8.326 ±0.101
		Average of all 5 columns: 8.167±0.130				
	b (ml/mg)	0.0602 ±0.0015	0.0608 ±0.0016	0.0613 ±0.0014	0.0612 ±0.0016	0.0612 ±0.0015
		Average of all 5 columns: 0.0609±0.0005				
k'_i/k'_{ave} ratio		0.981	0.995	0.990	1.003	1.030
k'-ECP	*a*	8.222 ±0.001	8.519 ±0.001	8.320 ±0.002	8.658 ±0.001	9.076 ±0.001
		Average of all 5 columns: 8.559±0.335				
	b (ml/mg)	0.0648 ±0.0000	0.0666 ±0.0001	0.0662 ±0.0001	0.0661 ±0.0000	0.0677 ±0.0000
$\epsilon_{e,i}/\epsilon_{e,ave}$ ratio		1.002	1.007	0.992	1.000	1.000
ϵ_e-ECP	*a*	8.398 ±0.001	8.622 ±0.001	8.336 ±0.002	8.632 ±0.001	8.812 ±0.001
		Average of all 5 columns: 8.560±0.193				
		Average of columns 1,2,4,5: 8.616±0.169				
	b (ml/mg)	0.0648 ±0.0000	0.0666 ±0.0001	0.0662 ±0.0001	0.0661 ±0.0000	0.0677 ±0.0000
$a=k'/F$	*a*	7.889	8.098	7.828	8.059	8.171
		Average of all 5 columns: 8.009±0.145				

Other than the model error introduced by ECP, we have no satisfactory explanation for a value of a lower when derived from k' than from ECP measurements. A small density of high energy adsorption sites on the surface of the stationary phase, nothing rare with silica-based stationary phases, might cause a difference between the retention factor at infinite dilution (determined by the Henry constant on these high energy sites) and the limit slope of the isotherm (determined by the bulk properties of the surface) but the deviation would be in the opposite direction. More probably, the estimates of the column hold-up volumes and of the phase ratio are inaccurate. The errors made propagate differently to the values of the retention factors and of the isotherm coefficients derived from experimental data.

Another approach available for a correction of isotherm data is the direct determination of the

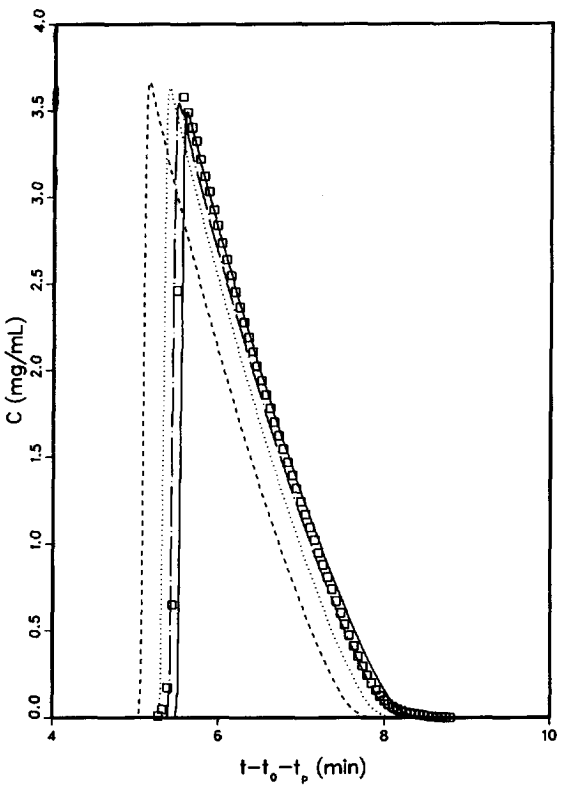

Fig. 9. Comparison between experimental (symbols) and calculated (lines) band profiles for column YMC # 2. Profiles calculated with the isotherm measured on the same column (solid line), with the isotherm measured on column # 5 (dotted line), with the isotherm obtained by taking the average values of a ($a=k'/F$, Table 9 last line) and b (Table 9, second row, third line) (dashed line), and with the isotherm obtained by taking the average values of a and b after correction by the porosity (Table 9, row ϵ_e-ECP) (chain-dotted line).

external porosity of the columns by inverse size-exclusion chromatography [27,30]. The procedures used for these determinations are discussed separately [30]. The correction provides satisfactory results and could be applied in actual practice without serious difficulties.

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