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Effect of the packing pressure on the performance of C_{18} reversed-phase liquid chromatographic columns

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Abstract

Early evidence has shown that HPLC columns packed with a given ODS phase give different performance, related to differences in their packing densities and external porosities. This effect was systematically studied on two groups of ten analytical (10×0.46 cm) columns packed with Kromasil and Zorbax 10 μm spherical C_{18} , respectively, using chloroform as the slurry solvent and methanol as the pushing solvent. The packing pressure used for column j in each group was 1000j p.s.i. The mass of packing material inside each column increased with increasing packing pressure. The column external porosity, determined by inverse size-exclusion chromatography method, decreased as the packing pressure increased, while the internal porosity remained constant. With a methanol–water (45:55, v/v) solution as the mobile phase, the retention factors and column efficiencies were measured at infinite dilution for acetone, benzyl acetate, benzyl alcohol, *m*-cresol, 2,6-dimethyl phenol, methyl benzoate, 3-phenyl-1-propanol, and uracil (also used as non-retained compound to determine the hold-up times). The retention factor and the column efficiency of each compound increased linearly with increasing packing pressure. The isotherm of 3-phenyl-1-propanol was determined on each column by elution by characteristic points and frontal analysis methods. These data were normalized by the column geometrical volume (V_k : ml), by the stationary phase volume (V_p : ml), and by the mass of packing (W_p : g). The parameters obtained from the isotherm normalized by V_k cannot predict the band profile accurately. Better results were obtained with the parameters obtained from the isotherm normalized by V_p . Close agreement was obtained between the parameters obtained by mass normalization. This allowed a reasonable prediction of the band profiles recorded on one column using isotherm data measured on another column. Considering the difficulties encountered in volume determinations, normalization of isotherm data by the packing weight seems to be the most practical solution.

Keywords: Packing pressure; Stationary phases, LC; Preparative chromatography; Porosity; Band profiles; Isotherms; Phenylpropanol

1. Introduction

The optimization of the experimental conditions of

a preparative separation based on the use of the theory of nonlinear chromatography has become a distinct possibility [1] and an attractive proposition [2] because of the potential savings in wasted time and chemicals over the more traditional empirical approaches. However, the calculation of optimum experimental conditions requires the prior acquisition

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of relevant data regarding the equilibrium isotherms of the sample component and their mass transfer kinetics. The results of band profile calculations will be valid only as far as the thermodynamic and kinetic data used account accurately for the behavior of the column prepared for the actual separation or purification.

To achieve the potential savings in time and chemicals afforded by a fundamental approach to optimization, the experimental data must be acquired using analytical columns, not columns having a size of the same order of magnitude as the column actually needed for the industrial separation. Measurements on this column should be carried out only a few times, for the fine tuning eventually required. A major practical problem then arises, how to achieve a high degree of column-to-column reproducibility of chromatographic data. It has been repeatedly reported in the literature [3] that the reproducibility of the performance of columns packed with the same stationary phase is moderate at best, although such studies have been mainly carried out with analytical columns having practically all the same inner diameter [4–7]. The differences observed between equilibrium isotherms measured on chromatographic columns having different diameters can be important, precluding the use of a direct scale-up approach [8].

One of the important reasons explaining these differences is the fact that the procedures used to pack analytical- and preparative-scale columns are often quite different. The former are packed by dynamic compression of a slurry using the viscous shear of a high velocity stream as the source of the consolidation stress [3,9], while the latter are packed by applying directly to the packing a mechanical stress, under either radial [10] or axial compression [11]. Since stress does not convey homogeneously in a bed of pulverulent material [12], this latter process may not produce a column bed of constant density. Conversely, since the local flow velocity is proportional to the local column permeability, itself inversely related to the local packing density, the viscous shear is higher where the packing density is lower, which tends to result in a more homogeneous packing. We note, however, that these rationales are valid for the bulk packing but do not necessarily apply close to the column wall. Since first reported

by Kirkland [13] and Martin et al. [14], there is growing evidence that the wall smoothness has a positive influence on the quality of the column. This influence of the column wall cannot be the same for a 4.6 mm or for a 30 cm I.D. column. Thus, we should pay great attention to the possible difference in the packing densities of columns in comparing thermodynamic and kinetic data obtained with them. This is especially important when both analytical- and preparative-scale columns are involved.

In earlier studies [4–7], we have demonstrated that columns packed with the same stationary phase under the same conditions have different performance, related to different packing densities. These differences can be corrected, in part, by the ratio of the individual column external porosities, which, itself, can be determined by inverse size-exclusion chromatography (ISEC) [15–20] and which is found to vary significantly from one column to another. Several questions remain unsolved, however. For example, what is the direct link between the external porosity of the column and its packing density? The determination of the column external porosity by ISEC requires the accurate measurement of the retention volumes of polystyrene standards. These volumes are rather small and difficult to measure accurately. Therefore, it would be more practical, more precise and probably more accurate to correct the isotherm parameters otherwise than by using the individual column external porosity. The aim of this work is to show that the determination of the mass of packing material contained in the packed column affords a useful alternative approach.

2. Theory

2.1. Thermodynamics of phase equilibrium

Under linear conditions, i.e., for infinitely dilute samples (see a more accurate definition later in this section), the band profiles are usually nearly symmetrical. They can be modeled precisely enough by a Gaussian curve and two parameters are commonly used to characterize these elution profiles, the column efficiency, N_0 , and the retention factor, k'

$$k' = \frac{t_R - t_0}{t_0}; \quad N_0 = 5.54 \left(\frac{t_R}{\Delta w_{1/2}} \right)^2 \quad (1)$$

where t_R is the retention time of the band, t_0 is the column hold-up time, and $\Delta w_{1/2}$ the bandwidth at half-height.

In preparative chromatography, a large sample is used, the stationary phase concentration is no longer directly proportional to the mobile phase concentration, the column is said to be overloaded, the band profiles are no longer Gaussian nor even symmetrical, and the chromatographic separation is carried out under conditions which are no longer linear. The nonlinear behavior of equilibrium isotherms must be taken into account. The isotherm model used in this work is the Langmuir isotherm

$$q = \frac{q_s b C}{1 + b C} \quad (2)$$

where q is the stationary phase concentration, C is the mobile phase concentration, q_s is the saturation capacity, and b is a numerical coefficient. As seen in Eq. (2), linear conditions can be defined as those under which $bC \ll 1$, so the denominator in the RHS of Eq. (2) can be replaced by unity. The band profiles of large samples can be predicted accurately from the equilibrium isotherms [1,21].

The methods most commonly used for the experimental determination of isotherms are elution by characteristic points (ECP) [22–24] and frontal analysis (FA) [23–28]. In ECP, the amount adsorbed at equilibrium with concentration C is

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

where V_p is the volume of packing, V_0 is the column hold-up volume, V is the retention volume of the point at concentration C on the diffuse boundary, and $\delta_i C$ is the concentration increment ($\sum \delta_i C = C$). This equation is derived from the ideal model of chromatography, which assumes an infinite column efficiency. In order to limit the consequences of this model error, a column efficiency of no fewer than 2000 plates is required for the column [29].

In FA, the amount adsorbed at equilibrium with concentration C is calculated as

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

where $V_{F,i+1}$ is the retention volume of the i th

concentration step, q_i and q_{i+1} are the stationary phase concentrations in equilibrium with the i th and $i+1$ th steps which have mobile phase concentrations of C_i and C_{i+1} , respectively. The major advantage of FA is that the results obtained with this method are independent of the column efficiency [23–28]. However, recent studies have shown that this is not true when the retention volume of the breakthrough front is derived using the most conventional methods of measurement of this volume. A column efficiency exceeding approximately one hundred theoretical plates is required [30,31] in order to avoid a systematic error.

Note that the coefficient b in Eq. (2) has the dimension of the inverse of a mobile phase concentration. The product $b \cdot q_s$, however, may or may not be dimensionless, depending on what units are chosen for the denominator V_p in Eqs. (3,4). V_p is commonly taken as the volume of stationary phase in the column. The apparent column volume or its geometrical volume, V_k , are also used in some cases. Then, the product $b \cdot q_s$ is dimensionless. However, if we use the column packing mass W_p , instead, the product $b \cdot q_s$ has the units of *ml of solvent per mg of packing*.

2.2. Inverse size-exclusion chromatography (ISEC)

There is a correlation between the average molecular mass of the n th sample, Mr_n , and its molecular size, ϕ_n . For polystyrene dissolved in methylene chloride, we have [16]

$$\phi_n = 1.61 Mr_n^{0.59} \quad (5)$$

where ϕ_n is in angstrom (\AA) units. This correlation is the basis of the determination of molecular size and molecular mass by size-exclusion chromatography. In ISEC, the molecular masses of the polymeric samples are known and Eq. (5) is used to derive the integral pore volume distribution of the packing material from the retention volume distribution of the samples [7].

The total volume of a chromatographic column, V_k , can be written as the sum of three contributions, the inter-particle or external pore volume, V_e , the intra-particle or internal pore volume, V_i , and the stationary phase solid volume. These three terms can

be derived from the data given by ISEC, as explained in Section 3.

2.3. Determination of the different volume fractions

The volume fraction or porosity, ϵ_n , corresponding to a retention volume V_n , hence a certain pore size, ϕ_n , is defined by

$$\epsilon_n = \frac{V_n}{V_k} \quad (6)$$

According to this definition, the external porosity ϵ_e or total volume of the pores around the particles is

$$\epsilon_e = \frac{V_c}{V_k} \quad (7)$$

The internal porosity ϵ'_i is often defined in chromatography as the fractional column volume occupied by the internal pores

$$\epsilon'_i = \frac{V_i}{V_k} \quad (8)$$

The sum of these two porosities gives the total accessible porosity, ϵ_T , i.e.,

$$\epsilon_T = \epsilon_e + \epsilon'_i \quad (9)$$

This last definition leads to serious difficulties and may cause inconsistencies when the properties of beds of packing material consolidated to a different extent are compared. Then, the external porosity decreases with increasing value of the external stress applied to the column, while the true internal porosity of the particles decreases only slightly, the compressibility of the particle skeleton, made of solid silica, being limited [32]. Thus, it is more logical to define, as is commonly done in chemical engineering, the internal porosity as the fraction of the particle volume which is accessible to the mobile phase:

$$\epsilon_i = \frac{\epsilon'_i}{(1 - \epsilon_e)} \quad (10)$$

Then, the total column porosity becomes

$$\epsilon_T = \epsilon_e + \epsilon_i(1 - \epsilon_e) = \epsilon_e + \epsilon_i - \epsilon_i\epsilon_e \quad (11)$$

The accurate and precise determination of the inter-

nal and external porosities becomes a key to a better understanding of the packing behavior.

3. Experimental

3.1. Equipment

All experiments were made with a Hewlett–Packard (Palo Alto, CA, USA) HP1090M liquid chromatograph equipped with a diode-array UV detector and a computerized data acquisition system. Retention factors, column efficiencies and ECP measurements were carried out at a detector wavelength of 250 nm. Frontal analysis (FA) was run at 270 nm. Porosity measurements by ISEC were run at 254 nm.

3.2. Columns

The empty stainless steel columns were purchased from Alltech (Deerfield, IL, USA). All columns are 10 cm × 0.46 cm I.D. Two 10 μ m spherical C₁₈ ODS packing material, one from Kromasil (Eka-Nobel, Stratford, CT, USA) with Lot No. DT0132 and pore size 100 Å and the other from Zorbax (BTR, Wilmington, DE, USA) with Lot No. B32161 and pore size 150 Å, were obtained. Each material was slurry packed into 10 columns in our laboratory. The slurry solvent used was chloroform and the pushing solvent was methanol. The packing pressure used was 1000 p.s.i. (1 p.s.i. = 6894.76 Pa) for the 1st column of each group, 2000 p.s.i. for the 2nd column, ..., and 10 000 p.s.i. for the 10th column.

3.3. Chemicals

For thermodynamic studies, 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-dimethylphenol (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-isopropylphenol (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 the compounds listed above were a methanol–water (45:55, v/v) solution. 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.

For porosity measurements, polystyrene standards with molecular masses ranging from 2000 to 1 860 000 were purchased from Supelco (Bellefonte, PA, USA). Polystyrene standards with molecular masses of 456, 1050 and 3 840 000 were purchased from Tosoh (Tokyo, Japan). Benzene ($M_r=78.11$) was purchased from EM Science. Methylene chloride (J.T. Baker) was used both as the mobile phase and the sample solvent.

3.4. Procedures

The amounts of samples used for the measurements of k' and N_0 were 25 μg each of acetone, benzyl acetate, benzyl alcohol, *m*-cresol, 2,6-dimethyl phenol, methyl benzoate, 3-phenyl-1-propanol, and uracil. Uracil was also used for the determination of the system hold-up time, the stationary phase volume V_p , and the average total porosity of the column, data which were later used for the equilibrium isotherm determinations.

To determine the equilibrium isotherm, the ECP measurements were made by injecting 0.120 ml of 35 mg/ml 3-phenyl-1-propanol solutions into each column. The FA measurements were made by having the liquid chromatograph solvent delivery system run a dedicated gradient program requiring 20 successive concentration step changes. Two pumps, one for the methanol–water (45:55, v/v) mobile phase and the other for a 20 mg/ml solution of 3-phenyl-1-propanol in the same solvent, were used for this purpose. The isotherm data obtained were fitted to a Langmuir isotherm model (Eq. 2) and the best coefficients derived by applying the non-linear re-

gression routine available at the University of Tennessee Computing Center (UTCC). The band profiles were calculated by applying the isotherm coefficients to the equilibrium-dispersive model solved by the Rouchon algorithm [33].

To apply ISEC, a total of 15 polystyrene standards with molecular masses ranging from 456 to 3 840 000 were injected into each column. Benzene was used for calibration and for the determination of the total accessible porosity, ϵ_T , of the column, which is practically equal to that measured with uracil in reversed-phase liquid chromatography [7]. Each injection was repeated three times. The values reported in the tables are the average of these three results. Plots of the logarithm of the molecular masses of the probes versus their retention volumes showed a bimodal pore size distribution for each column. The external pore volume was derived from the intermediate point between the two modes of the pore size distributions [22]. The total column volume, V_k , was calculated from the geometric dimensions of the column tubing

$$V_k = \pi r^2 L \quad (12)$$

where r is the column radius (here $r=0.23$ cm) and L the column length (here $L=10.0$ cm). The external porosity, ϵ_e , was calculated from Eq. (7). Knowing ϵ_T and ϵ_e , the internal porosity, ϵ_i , is calculated from Eq. (11).

The empty mass of each column, W_i , was obtained prior to the packing procedure being applied. After the ISEC experiments, the column (filled with methylene chloride) was connected to a Hewlett–Packard 5840 A gas chromatography system and dried with a stream of ultra pure, dry nitrogen flown through the column at an inlet pressure of 30 p.s.i. The column was heated to 80°C at a rate of 5°C/min and kept at this temperature, under the nitrogen stream, for at least 20 h. After this step, the column was considered to be dried and its mass was measured as W_i^* . The packing weight, $W_{p,i}$, is then

$$W_{p,i} = W_i^* - W_i \quad (13)$$

An independent investigation has shown that under these experimental conditions, the column is dried to constant weight [34].

3.5. Detector calibration

ECP requires detector calibration in order to allow the translation of the detector signal (mAu) into the 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 regression in SigmaPlot (Jandel, San Rafael, CA, USA) to the experimental data points.

4. Results and discussion

4.1. Linear chromatography data

The chromatographic data obtained under linear conditions are presented in Fig. 1 and Fig. 2, in the format of the sample retention factors, k' (Fig. 1), or the column efficiency at infinite dilution, N_0 (Fig. 2), versus the packing pressure. For both packing materials, the values of both parameters, k' and N_0 , tend to increase with increasing packing pressure. The retention factor of each compound tends to be larger on the Kromasil columns (Fig. 1a) than on the Zorbax ones (Fig. 1b), in agreement with the lower average pore size and larger surface area of the former material. The relative standard deviation (R.S.D.) of the ten values of the retention factors of each of the compounds studied here is of the order of 3%, regardless of the column used. By contrast, the R.S.D. for columns packed under the same experimental conditions is between 1 and 2% [6]. Except for acetone (k' ca 0.27 on all columns), there is a slow, nearly linear, increase of the retention factors of the compounds studied with increasing packing pressure. The same result (not shown) was obtained when the retention factors were plotted versus the mass of packing material contained in the column (i.e., vs. its packing density). This last result is in agreement with those of an independent study on the reproducibility of slurry-packing of analytical and semi-preparative columns [34], although the range of packing densities experienced in this other study is quite narrower.

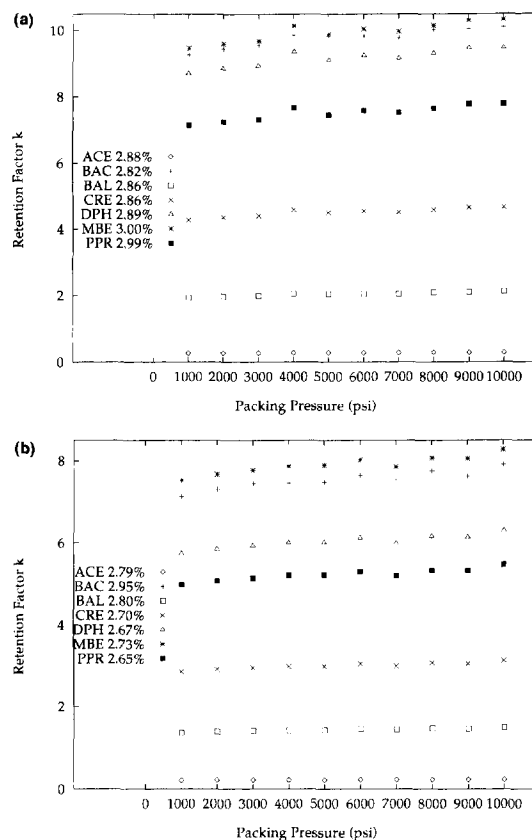


Fig. 1. Dependence of the retention factor on the packing pressure (p.s.i.). The percentage numbers in the figure legend represent the R.S.D. of the results obtained on all ten columns. ACE: acetone; BAC: benzyl acetate; BAL: benzyl alcohol; CRE: *m*-cresol; DPH: 2,6-dimethylphenol; MBE: methyl benzoate; PPR: 3-phenyl-1-propanol. (a) Kromasil. (b) Zorbax.

The R.S.D. of the column efficiencies is much larger. The column efficiency increases rapidly with increasing retention factor. Note that the efficiency was measured at a single flow-rate, which limits the validity of such comparison. For the Kromasil columns (Fig. 2a), this R.S.D. is on the order of 13%, comparable to the one observed for columns packed under the same pressure. On the other hand, for the Zorbax columns (Fig. 2b) this R.S.D. is around 11%, twice as much as for columns packed under the same pressure [6]. These results show that columns packed with the same stationary phase but under different packing pressures may give performance which are quite significantly different.

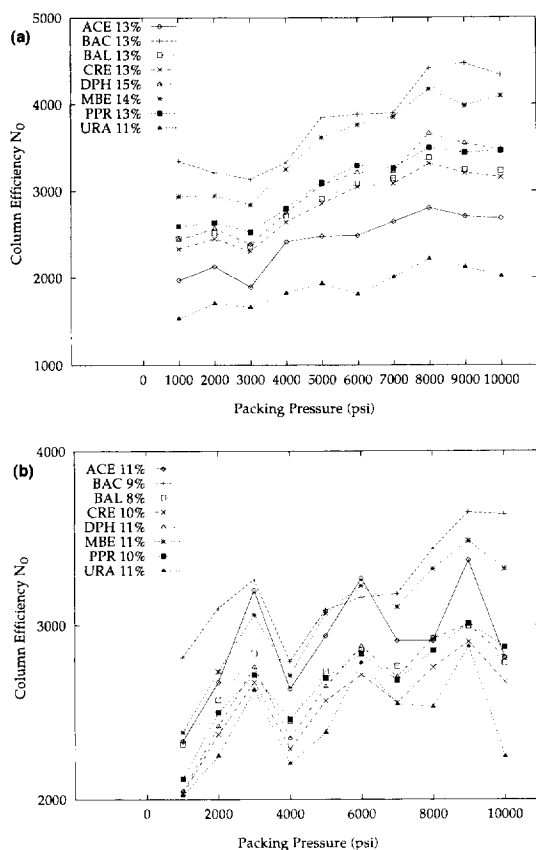


Fig. 2. Dependence of the column efficiency on the packing pressure (p.s.i.). The percentage numbers in the figure legend represent the R.S.D. of the results obtained on all ten columns. ACE, BAC, BAL, CRE, DPH, MBE, PPR: same as in Fig. 1. URA: uracil. (a) Kromasil. (b) Zorbax.

4.2. Chromatograms

Eight of the ten chromatograms obtained are shown in Fig. 3 (Kromasil) and Fig. 4 (Zorbax). Not shown for the sake of clarity of the figures are two chromatograms which are too close to some of those shown. These chromatograms are reported first as plots of the mobile phase concentration C versus the retention time (Fig. 3a, Fig. 4a), as commonly done. The relative difference between the retention times of the earliest and the last eluted chromatograms exceeds slightly 10%. The plots of C versus the dimensionless retention factor, k' , are illustrated in Fig. 3b and Fig. 4b. They are at least as different and

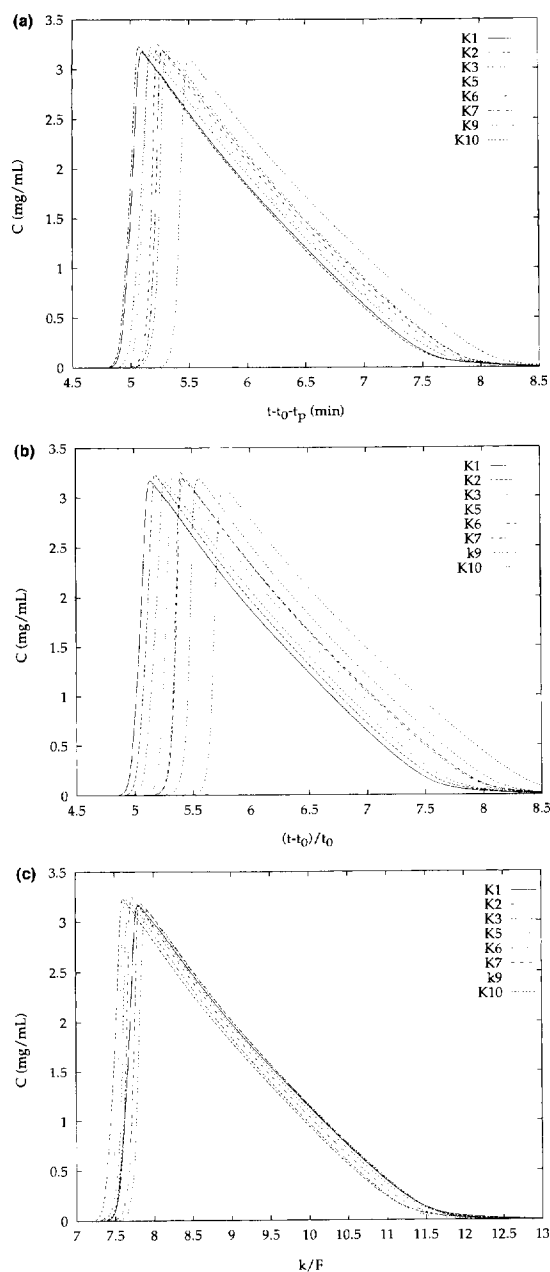


Fig. 3. Chromatograms obtained for 4.2 mg of 3-phenyl-1-propanol on eight of the ten columns. Mobile phase, methanol–water (45:55, v/v), 1 ml/min. Stationary phase, Kromasil. (a) Plots of C (mg/ml) versus the corrected time (min), with t_p , width of the rectangular injection pulse. (b) Plots of C (mg/ml) versus the retention factor. (c) Plots of C (mg/ml) versus the ratio of the retention factor and the phase ratio.

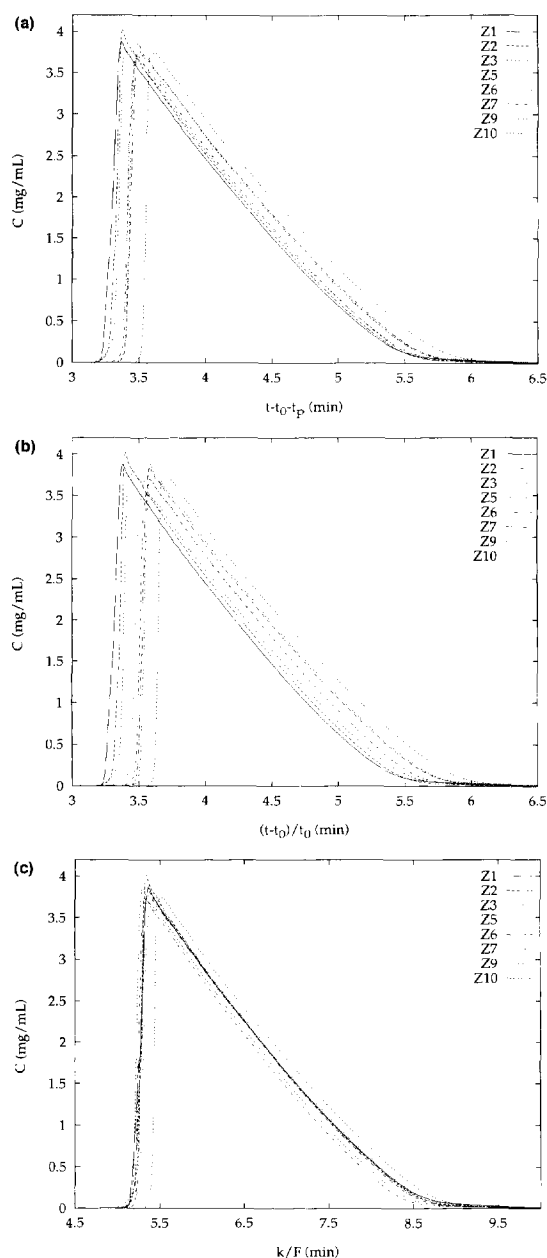


Fig. 4. Same as Fig. 3, but stationary phase, Zorbax. (a) Plots of C (mg/ml) versus time (min). (b) Plots of C (mg/ml) versus the retention factor. (c) Plots of C (mg/ml) versus the ratio of the retention factor and the phase ratio.

scattered as the previous ones. If we change the x -axis into the ratio of the sample retention factor k' and the column phase ratio F , i.e., if we plot C

versus the first parameter of the isotherm, the chromatograms become much closer for both packing materials, as shown in Fig. 3c (Kromasil) and Fig. 4c (Zorbax). This suggests that a normalization of the isotherm data by a proper function of the porosity could correct for column-to-column fluctuations of the packing density.

4.3. Equilibrium isotherms

The equilibrium isotherms of 3-phenyl-1-propanol on each column (not shown) were determined by both ECP and FA methods. The coefficients of the best Langmuir isotherm (Eq. 2) were derived using the SAS non-linear regression routine available at UTCC. The denominator, V_p , in both Eqs. (3,4) was first substituted by the column geometrical volume V_k (Eq. 12) then by the stationary phase volume, V_p . Table 1 (Kromasil) and Table 2 (Zorbax) list the FA results obtained from V_k (rows $q_{s,Vk}$ and b_{V_k}) and both the ECP and FA results obtained from V_p (rows $q_{s,Vp}$ and b_{V_p}). As shown by these tables, $q_{s,Vk}$ is much smaller than $q_{s,Vp}$ while the coefficient b remains the same. There is also an excellent agreement between the isotherm coefficients obtained by both ECP and FA.

In Fig. 5, four different calculated band profiles are compared with the experimental profile (symbols) recorded on the 10th column in each group. The solid line and the long-dashed line profiles were calculated with the isotherm coefficients obtained for this 10th column and derived by using either V_p or V_k in Eqs. (3,4), respectively. The short-dashed line and the dotted line profiles were calculated with the isotherm coefficients obtained for the first column, derived by using V_p . These coefficients were either not modified (short-dashed line) or modified (dotted) by the weight ratio of the packing materials in the two columns. The agreement between the experimental chromatogram and the two profiles calculated using the isotherm coefficients determined with V_p and the data acquired on the tenth column is excellent. By contrast, the band profiles calculated from the isotherm coefficients derived by using V_k are grossly incorrect. The excellent agreement between the experimental profile (symbols) and the band profile calculated with isotherm data acquired on a different column but corrected for the weight of

Table 1
Calculated equilibrium isotherm coefficients on Kromasil columns

Columns		K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
FA-V _k	$q_{s,vk}$	75.612±0.870	74.211±0.861	76.204±0.742	76.594±0.791	75.723±0.661	77.345±0.552	76.713±0.695	77.496±0.671	77.485±0.685	78.023±0.662
	b_{vk}	0.0582±0.0012	0.0602±0.0012	0.0587±0.0010	0.0623±0.0012	0.0593±0.0009	0.0579±0.0007	0.0593±0.0009	0.0586±0.0009	0.0597±0.0009	0.0602±0.0009
FA-V _p	$q_{s,vp}$	190.539±0.030	183.120±2.125	189.161±1.842	188.160±1.944	184.117±1.607	189.169±1.349	186.251±1.686	185.445±1.605	185.151±1.636	185.105±1.572
	b_{vp}	0.0582±0.0008	0.0602±0.0012	0.0587±0.0010	0.0623±0.0012	0.0593±0.0009	0.0579±0.0007	0.0593±0.0009	0.0586±0.0009	0.0597±0.0009	0.0602±0.0009
ECP-V _p	$q_{s,vp}$	160.049±0.267	157.077±0.259	161.011±0.249	164.247±0.181	161.012±0.217	164.609±0.192	163.072±0.209	160.293±0.289	163.042±0.166	164.235±0.148
	b_{vp}	0.0731±0.0001	0.0726±0.0001	0.0722±0.0001	0.0717±0.0001	0.0708±0.0001	0.0709±0.0001	0.0705±0.0001	0.0711±0.0001	0.0700±0.0001	0.0712±0.0001
FA-W	$q_{s,w}$	122.071±1.469	120.031±1.393	120.947±1.168	120.770±1.248	119.362±1.040	119.661±0.854	117.729±1.074	119.362±1.034	117.978±1.041	119.157±1.011
	b_w	0.0582±0.0008	0.0602±0.0012	0.0587±0.0010	0.0623±0.0012	0.0593±0.0009	0.0579±0.0007	0.0593±0.0009	0.0586±0.0009	0.0597±0.0009	0.0602±0.0009

$q_{s,vk}$ (mg; sample/ml; column) = 76.541 ± 1.143 (R.S.D. = 1.49%).

FA- $q_{s,vp}$ (mg; sample/ml; packing) = 186.622 ± 2.476 (R.S.D. = 1.33%).

$q_{s,w}$ (mg; sample/mg; packing) = 119.707 ± 1.325 (R.S.D. = 1.11%).

$b_{vk} = b_{vp} = b_w = 0.0594 ± 0.0013$ (R.S.D. = 2.14%).

Table 2
Calculated equilibrium isotherm coefficients on Zorbax columns

Columns		Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9	Z10
FA-V _k	$q_{s,vk}$	52.215±0.582	52.843±0.513	52.855±0.524	53.573±0.539	53.956±0.482	55.117±0.535	54.554±0.490	54.994±0.582	55.044±0.471	54.948±0.526
	b_{vk}	0.0611±0.0012	0.0605±0.0010	0.0610±0.0011	0.0619±0.0011	0.0607±0.0010	0.0592±0.0010	0.0589±0.0009	0.0590±0.0011	0.0616±0.0009	0.0599±0.0010
FA-V _p	$q_{s,vp}$	135.061±1.505	135.839±1.318	135.035±1.340	135.618±1.366	136.378±1.217	137.640±1.337	136.029±1.221	136.511±1.445	136.228±1.167	136.806±1.310
	b_{vp}	0.0611±0.0012	0.0605±0.0010	0.0610±0.0011	0.0619±0.0011	0.0607±0.0010	0.0592±0.0010	0.0589±0.0009	0.0590±0.0011	0.0616±0.0009	0.0599±0.0010
ECP-V _p	$q_{s,vp}$	115.674±0.228	121.622±0.128	118.979±0.950		127.946±0.660	120.391±0.101	121.146±0.193		120.058±0.108	122.585±0.115
	b_{vp}	0.0753±0.0002	0.0705±0.0001	0.0723±0.0007		0.0664±0.0004	0.0713±0.0001	0.0689±0.0001		0.0705±0.0001	0.0716±0.0001
FA-W	$q_{s,w}$	76.719±0.855	76.813±0.745	75.901±0.753	76.693±0.772	76.191±0.680	76.742±0.745	76.207±0.684	76.391±0.809	76.435±0.655	75.915±0.727
	b_w	0.0611±0.0012	0.0605±0.0010	0.0610±0.0011	0.0619±0.0011	0.0607±0.0010	0.0592±0.0010	0.0589±0.0009	0.0590±0.0011	0.0616±0.0009	0.0599±0.0010

$q_{s,vk}$ (mg; sample/ml; column) = 54.010 ± 1.083 (R.S.D. = 2.00%).

FA- $q_{s,vp}$ (mg; sample/ml; packing) = 136.114 ± 0.792 (R.S.D. = 0.58%).

$q_{s,w}$ (mg; sample/mg; packing) = 76.401 ± 0.340 (R.S.D. = 0.44%).

$b_{vk} = b_{vp} = b_w = 0.0604 ± 0.0011$ (R.S.D. = 1.79%).

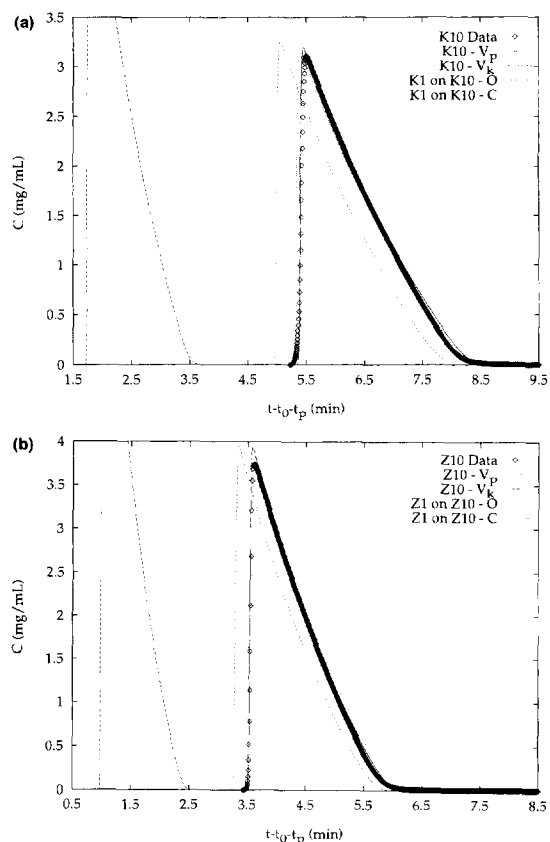


Fig. 5. Comparison between the experimental chromatogram (symbols) obtained on column 10 and band profiles calculated from the isotherm data obtained on columns 1 and 10 and derived using either V_p or V_k in Eqs. (3,4). Solid line: isotherm coefficients obtained on column 10 using the stationary phase volume V_p . Long chain line: isotherm coefficients obtained on column 10, using the column geometric volume V_k . Short chain line: isotherm coefficients obtained on column 1, without modification. Dotted line: calculations made with the isotherm coefficients obtained on column 1, modified by the mass ratio of packing material. (a) Kromasil. (b) Zorbax.

the packing (dotted line) demonstrates the validity of the correction.

4.4. Column volume fractions

The column total accessible volume was derived from the retention volumes of uracil in a methanol–water (45:55, v/v) solution ($V_{T,u}$) and of benzene in methylene chloride ($V_{T,b}$). The results are listed in Table 3 (Kromasil) and Table 4 (Zorbax). The agreement between these two sets of results is excellent, despite the systematic smaller value of $\epsilon_{T,u}$. This difference (ca 1.5%) could be explained by the partial or total collapse of the C_{18} chains onto the silica surface in the methanol–water solution while these chains are dissolved and extended in methylene chloride. Solvated chains leave between them a larger volume accessible to the mobile phase and to an unretained tracer than collapsed chains between which there are unaccessible cavities. Indeed, $V_{T,u}$ is smaller than $V_{T,b}$ (Tables 3,4) which shows that the unaccessible volume is larger in methanol–water solution than in methylene chloride solution. In Fig. 6, the column total porosity derived from the retention volume of benzene in methylene chloride, $\epsilon_{T,b}$, calculated from Eq. (7) is plotted versus the column packing pressure. A linear relationship is observed.

The column external porosity was determined by ISEC, using the procedure previously described [7]. The values of the column external porosity, ϵ_e , are listed in Tables 3 and 4 and plotted in Fig. 6 versus the packing pressure. As the column total porosity, the external porosity decreases linearly with increasing packing pressure, showing that the space between the packing particles becomes smaller as the

Table 3
Porosities of Kromasil columns

	Columns									
	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
$V_{T,u}$	1.002	0.988	0.992	0.985	0.978	0.982	0.977	0.967	0.966	0.961
$V_{T,b}$	1.014	1.006	1.010	0.999	0.997	0.993	0.991	0.982	0.978	0.973
$\epsilon_{T,b}$	0.610	0.605	0.608	0.601	0.600	0.597	0.596	0.591	0.588	0.585
ϵ_c	0.402	0.400	0.404	0.390	0.389	0.389	0.384	0.386	0.372	0.374
ϵ_i	0.347	0.342	0.341	0.346	0.346	0.342	0.345	0.334	0.345	0.338

Table 4
Porosities of Zorbax columns

	Columns									
	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9	Z10
$V_{T,u}$	1.019	1.015	1.011	1.005	1.004	0.996	0.995	0.992	0.990	0.994
$V_{T,b}$	1.035	1.029	1.023	1.020	1.021	1.012	1.011	1.008	1.004	1.007
$\epsilon_{T,b}$	0.623	0.619	0.615	0.614	0.614	0.609	0.608	0.606	0.604	0.606
ϵ_e	0.434	0.428	0.428	0.424	0.418	0.408	0.409	0.404	0.405	0.399
ϵ_i	0.334	0.334	0.328	0.329	0.337	0.338	0.337	0.339	0.335	0.344

external stress resulting from viscous shear becomes larger. The external porosity of column Z1 (Table 4) appears to be unusually large, at 0.434. The long-

term stability of the column was not studied. It is worth noting the columns were packed at UTK and studied at ORNL, a 50 km distance, nearly a month later. In the meantime, they were conserved, filled with methanol. To acquire the data reported here, the columns were operated under mobile phase stream for approximately 8 h. During that time the column beds were stable, the efficiency nearly constant, and the chromatographic properties measured were consistent. All this indicates that the packing beds were stable. This is consistent with the behavior of Zorbax under dynamic axial compression [34,35].

The column internal porosity, ϵ_i , is derived by difference between the column total and external porosities, from Eq. (11). The results are also listed in Tables 3 and 4 and plotted in Fig. 6. As expected, the packing pressure has little effect on the column internal porosity. The slight upward trend (ca. +2% over the 1000 to 10 000 p.s.i. pressure range studied) is marginally significant at best.

4.5. Packing material density

The mass of the packing material inside each column, $W_{p,i}$, the stationary phase volume, V_p , determined by injecting uracil in a methanol–water (45:55, v/v) solution, the ratio of $W_{p,i}$ and V_p , i.e., the packing material actual density, d_p , and its apparent density, $W_{p,i}/V_k$, are listed in Table 5. As expected, both $W_{p,i}$ and V_p increase in general as the packing pressure increases, while the ratio of these two quantities remains nearly constant. As a result, the higher the packing pressure, the more adsorbent particles are packed into the column, the heavier the packing material inside the column, the higher the apparent packing density, and the smaller the space between the particles, i.e., the external porosity. This

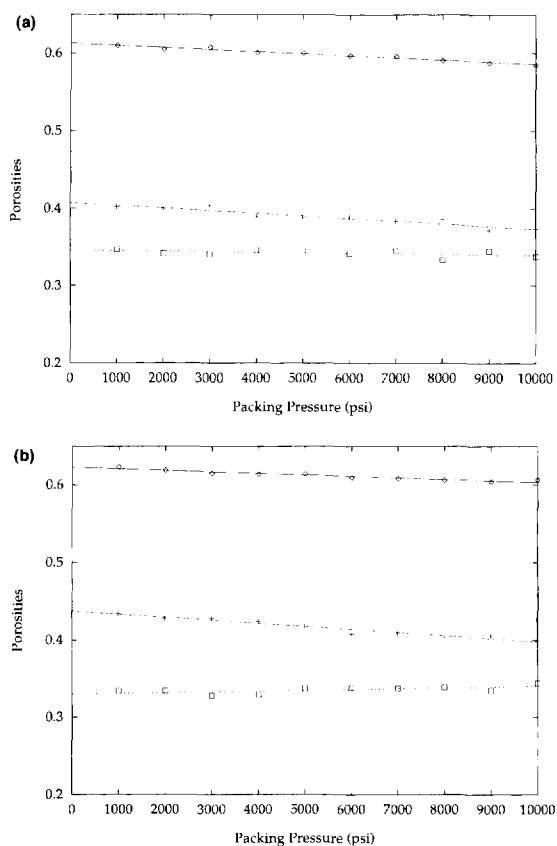


Fig. 6. Plot of the column total porosity, ϵ_T (\diamond), external porosity, ϵ_e (+), and internal porosity, ϵ_i (\square), versus the packing pressure P (p.s.i.). (a) Kromasil. Solid line: $\epsilon_T = -2.7 \cdot 10^{-6} \cdot P + 0.6$; Long chain line: $\epsilon_e = -3.4 \cdot 10^{-6} \cdot P + 0.4$; Short chain line: $\epsilon_i = -6.2 \cdot 10^{-7} \cdot P + 0.3$. (b) Zorbax. Solid line: $\epsilon_T = -2.0 \cdot 10^{-6} \cdot P + 0.6$; Long chain line: $\epsilon_e = -3.9 \cdot 10^{-6} \cdot P + 0.4$; Short chain line: $\epsilon_i = 1.1 \cdot 10^{-6} \cdot P + 0.3$.

Table 5
Packing density data for Kromasil and Zorbax columns

		Column no. ($V_k = 1.662$ ml)									
		1	2	3	4	5	6	7	8	9	10
Kromasil	V_p (ml)	0.659	0.673	0.669	0.676	0.683	0.679	0.684	0.694	0.695	0.700
	W_p (g)	1.029	1.027	1.047	1.054	1.054	1.074	1.083	1.079	1.091	1.088
	W_p/V_k (g/ml)	0.619	0.618	0.630	0.634	0.634	0.646	0.652	0.649	0.656	0.655
	W_p/V_p (g/ml)	1.562	1.527	1.565	1.559	1.544	1.582	1.583	1.555	1.570	1.554
Zorbax	V_p (ml)	0.642	0.646	0.650	0.656	0.657	0.665	0.666	0.669	0.671	0.667
	W_p (g)	1.131	1.143	1.157	1.161	1.177	1.194	1.190	1.196	1.197	1.203
	W_p/V_k (g/ml)	0.680	0.688	0.696	0.698	0.708	0.718	0.716	0.720	0.720	0.724
	W_p/V_p (g/ml)	1.762	1.770	1.780	1.770	1.791	1.795	1.786	1.788	1.784	1.803

agrees with the above external porosity data. The constancy of the actual density of the packing material is in agreement with that of the internal porosity.

4.6. Isotherm correction by packing mass

As discussed earlier, the denominator in Eqs. (3,4) can be substituted by other stationary phase parameters than the W_p/V_k volume. We have found out that the isotherm coefficients derived by use of V_p give much more accurate result than that of V_k (Fig. 5). However, in practice, it is more difficult to determine accurately a volume than a mass. Therefore, we now use the packing mass, W_p , obtained above to calculate the isotherm coefficients. The results are also listed in Tables 1 and 2 (rows $q_{s,w}$ and b_w). As expected, the coefficient b remains constant in all cases while q_s depends on which stationary phase parameter is used in the isotherm calculation.

Given the dispersion of the chromatograms obtained with the different columns of the batch studied (Figs. 3,4), it is necessary to find out an easy way to unify the isotherm coefficients so that, after simple modification, the data measured on one column can be easily applied to the prediction of the chromatograms observed on another one. Considering the ease of measuring the mass of packing in a column, we considered normalizing the isotherm coefficients ($q_{s,w}$ and b_w) by the packing mass in the column. If we want to apply the results obtained from column i to column j , we write

$$q_{s,w,j} = q_{s,w,i} \frac{W_{p,j}}{V_{p,j}} \quad (14)$$

By applying this procedure, we used the modified isotherm parameters obtained from column 1 onto 10 in both the Kromasil and the Zorbax column groups and recalculated the band profiles. The results are shown in Fig. 5 (dotted line). As we can see, the difference between the simulated band profile and the experimental chromatogram is minimal in this case.

5. Conclusion

Accurate and dependable measurements of the porosity of liquid-chromatography columns can be made with ISEC. The data derived from these measurements demonstrate that the column packing density is directly affected by the packing procedure. The higher the packing pressure, the more particles of packing material can be packed inside the column, the larger the column packing density, the smaller the column external porosity, and the lower its permeability [32].

The isotherm coefficients measured on a column and reported to its geometrical volume cannot be used to predict accurately the band profiles obtained on another column because the apparent density of the packing varies from column to column. However, satisfactory results are obtained when the isotherm data are reported to the actual volume of stationary phase held in the column or to the mass of packing used. Considering the difficulties encountered in accurate volume determinations, the normalization procedure that uses the column packing mass offers the most practical solution.

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References

- [1] G. Guiochon, S. Golshan-Shirazi and A.M. Katti, *Fundamentals of Preparative and Nonlinear Chromatography*, Academic Press, Boston, MA, 1994, Ch. XVI.
- [2] A. Felinger and G. Guiochon, *AIChE J.*, 40 (1994) 594.
- [3] C.F. Poole and S.K. Poole, *Chromatography Today*, Elsevier, Amsterdam, 2nd ed., 1993.
- [4] H. Guan and G. Guiochon, *J. Chromatogr. A*, 687 (1994) 179.
- [5] H. Guan and G. Guiochon, *J. Chromatogr. A*, 687 (1994) 201.
- [6] H. Guan and G. Guiochon, *J. Chromatogr. A*, 724 (1996) 39.
- [7] H. Guan and G. Guiochon, *J. Chromatogr. A*, 731 (1996) 27.
- [8] M. Sarker and G. Guiochon, *LC·GC*, 12 (1994) 300.
- [9] J.G. Kennedy and J.H. Knox, *J. Chromatogr. Sci.*, 10 (1972) 549.
- [10] J.N. Little, R.L. Cotter, J.A. Prendergast and P.D. McDonald, *J. Chromatogr.*, 126 (1976) 439.
- [11] E. Godbille and P. Devaux, *J. Chromatogr.*, 122 (1976) 317.
- [12] D. Train, *Trans. Instn. Chem. Engrs.*, 35 (1957) 258.
- [13] J.J. Kirkland, *J. Chromatogr.*, 83 (1973) 149.
- [14] M. Martin, F. Verillon, C. Eon and G. Guiochon, *J. Chromatogr.*, 125 (1976) 17.
- [15] I. Halász, *Ber. Bunsenges Phys. Chem.*, 79 (1975) 731.
- [16] I. Halász and K. Martin, *Angew. Chem., Int. Ed. Engl.*, 17 (1978) 901.
- [17] R. Nikolov, W. Werner and I. Halász, *J. Chromatogr. Sci.*, 18 (1980) 207.
- [18] W. Werner and I. Halász, *J. Chromatogr. Sci.*, 18 (1980) 277.
- [19] R. Groh and I. Halász, *Anal. Chem.*, 53 (1981) 1325.
- [20] T. Crispin and I. Halász, *J. Chromatogr.*, 239 (1982) 351.
- [21] G. Guiochon, A.M. Katti, M. Diack, M.Z. El Fallah, S. Golshan-Shirazi, S.C. Jacobson, and A. Seidel-Morgenstern, *Acc. Chem. Res.*, 25 (1992) 366.
- [22] G. Guiochon, S. Golshan-Shirazi and A.M. Katti, *Fundamentals of Preparative and Nonlinear Chromatography*, Academic Press, Boston, MA, 1994, Chapter III.
- [23] J.F.K. Huber and R.E. Gerritse, *J. Chromatogr.*, 58 (1971) 138.
- [24] R.E. Gerritse and J.F.K. Huber, *J. Chromatogr.*, 71 (1972) 173.
- [25] G. Schay and G. Szekely, *Acta Chim. Hung.*, 5 (1954) 167.
- [26] D.H. James and C.S.G. Phillips, *J. Chem. Soc.*, (1954) 1066.
- [27] Y.A. Eltekov, Y.V. Kazakevitch, A.V. Kiselev and A.A. Zhuchkov, *Chromatographia*, 20 (1985) 525.
- [28] Y.A. Eltekov and Y.V. Kazakevitch, *J. Chromatogr.*, 365 (1986) 213.
- [29] H. Guan, B.J. Stanley and G. Guiochon, *J. Chromatogr. A*, 659 (1994) 27.
- [30] P. Sajonz, G.M. Zhong and G. Guiochon, *J. Chromatogr. A*, 728 (1996) 15.
- [31] P. Sajonz, G.M. Zhong and G. Guiochon, *J. Chromatogr. A*, 731 (1996) 1.
- [32] G. Guiochon and M. Sarker, *J. Chromatogr.*, 704 (1994) 247.
- [33] G. Guiochon, S. Golshan-Shirazi and A.M. Katti, *Fundamentals of Preparative and Nonlinear Chromatography*, Academic Press, Boston, MA, 1994, Chapter X.
- [34] B. Stanley, C. Foster and G. Guiochon, in preparation.
- [35] M. Sarker, A.M. Katti, and G. Guiochon, *J. Chromatogr. A*, 719 (1996) 275.