Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1015 (2003) 73–87
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Comparison of the binary equilibrium isotherms of the 1-indanol enantiomers on three high-performance liquid chromatography columns of different sizes

Dongmei Zhou [a,b], Krzysztof Kaczmarski [c], Georges Guiochon [a,b,*]

[a] *Department of Chemistry, University of Tennessee, 552 Buehler Hall, Knoxville, TN 37996-1600, USA*
[b] *Division of Chemical Sciences, Oak Ridge National Laboratory, Oak Ridge, TN, USA*
[c] *Faculty of Chemistry, Rzeszów University of Technology, 35-959 Rzeszów, Poland*

Received 17 February 2003; received in revised form 6 June 2003; accepted 4 July 2003

Abstract

The competitive isotherm data for the enantiomers of 1-indanol were measured on three columns, a microbore column (15 cm × 0.1 cm), a conventional analytical column (15 cm × 0.46 cm), and a semi-preparative column (20 cm × 1.0 cm), packed with Chiralcel OB. The sets of isotherm data measured on each one of these three columns could be fitted well by a bi-Langmuir isotherm model. The experimental elution band profiles of mixtures of the 1-indanol isomers were recorded on the three columns. The isotherm model, combined with the equilibrium dispersive model of chromatography, gave calculated profiles that are in excellent agreement with the experimental profiles in all cases investigated. It was found that the value of the inner diameter of the column is an important parameter in the calculation of the isotherm parameters from the measured isotherm data. In order to use isotherm data obtained on one column to account for the phase equilibrium on another one, the inner diameters of these columns must be measured accurately. The diameters of the three columns were all slightly off their nominal value. Without correction, an important systematic error was made on the isotherm data obtained with the microbore column while only negligible errors were made on the data obtained with the other two columns. After due correction for this effect, the relative difference between the isotherm data for the microbore and the semi-preparative column is still, on the average, about 10%, a difference that might be explained by the limited precision of the measurement of the microbore column diameter. The relative difference between the isotherm data for the analytical and the semi-preparative columns was about 1%, a reasonable value, since the two columns came from different batches of the same packing material.

Keywords: Adsorption isotherms; Enantiomer separation; Indanol; Cellulose tribenzoate

1. Introduction

Recent developments in the pharmaceutical industry make it increasingly important to prepare optically pure enantiomers for many of the modern pharmaceuticals, due to the potential differences of the

* Corresponding author. Tel.: +1-865-9740-733; fax: +1-865-9742-667.
E-mail address: guiochon@utk.edu (G. Guiochon).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0021-9673(03)01214-7

physiological activity and toxicity of the two enantiomers [1]. The preparation of optically pure enantiomers is an important and difficult task to which much attention has been devoted [2–6]. Chiral preparative chromatography is an effective and popular method of enantiomeric separation and/or purification [1,7–15]. Although this method is usually cheaper and easier to carry out than alternative ones, such as asymmetric synthesis, it remains difficult and costly. Chiral stationary phases (CSPs) are usually expensive, chiral selectivity is rarely high, and the saturation capacity of most CSPs is rather low, preventing from operating at high concentrations while still demanding that preparative separations be performed under nonlinear conditions to maximize the production rate and minimize solvent consumption and labor costs. This means that computer-assisted optimization is especially important for preparative chiral separations. To enable the computer-assisted optimization of a preparative chromatography process, it is necessary to have the competitive equilibrium isotherms of the two enantiomers in the phase system used. In previous publications [7–9,15–19], we measured the adsorption isotherm parameters of the components of a mixture and used them to predict the experimental band profiles obtained on the same column, under different sets of experimental conditions. Unfortunately, isotherm measurements are long and complex. Being long, they may require important volumes of solutions and large amounts of expensive compounds. They cannot be measured on the preparative column. The cost is reduced if narrow diameter columns can be used.

Single- and multi-component isotherms are now measured by dynamic methods [19]. The commonly used methods are frontal analysis (FA), elution by characteristic points (ECP), and the perturbation method (PT). The FA method is the most popular because of its accuracy. It is time-consuming, however, and it requires large amounts of pure compounds which are often expensive. ECP is another popular method. It is fast and needs smaller amounts of samples than FA but it requires an accurate calibration of the detector and it cannot be used for the determination of competitive isotherms. The PT method determines the isotherm by measuring the retention times of small-size perturbations (i.e. samples) injected onto the column equilibrated with sample solutions at different concentrations. Like FA, it requires large amounts of pure samples.

Narrow bore and microbore HPLC columns are increasingly used in analytical applications [20–24]. The use of small diameter columns affords large savings of expensive packing materials, especially when using CSPs, reduces solvent consumption, is more compatible to coupling with a mass spectrometer, and provides considerable savings by reducing the amounts of sample and consumable needed for the measurements. Several attempts have been made at using microbore columns for the determination of isotherms [25,26] because significant savings are made on the samples and the solvents needed for a measurement. Jandera et al. [26] compared the isotherm coefficients of benzophenone, phenol, and *o*-cresol measured under reversed-phase conditions on a packed HPLC capillary column and on a conventional analytical column packed with same material. They also compared the isotherms of the enantiomers of mandelic acid on Teicoplanin, using commercial analytical and microbore columns packed with this CSP [26]. The best parameters of the Langmuir isotherm model were in good agreement. Their results indicate that microbore or packed capillary columns can provide realistic values of the isotherm coefficients, comparable to the data which are obtained with conventional analytical HPLC columns, but great care should be paid to make accurate measurements. Since the amount of sample necessary for the determination of the adsorption isotherms is reduced 10-fold when a 1 mm i.d. microbore column is substituted for a 4.6 mm i.d. commercial analytical column, this approach is attractive for the determination of the isotherm data needed for the optimization of preparative separations of expensive compounds such as pure enantiomers and biomolecules [27]. Cavazzini et al. [25] investigated the adsorption equilibria of the enantiomers of 1-phenyl-1-propanol on the same microbore column used in this work by competitive frontal analysis. Accurate isotherm data were obtained. They were used to predict overloaded band profiles. The calculated profiles were in good agreement with the experimental profiles. The amounts of CSP, sample and mobile phase needed for the measurement were considerably decreased. However, an important disagreement was observed between the isotherms obtained for the two enantiomers of 1-phenyl-1-propanol on the same stationary phase

packed in a microbore and a conventional column [16,25].

In this paper, isotherm data were acquired using a microbore, an analytical and a semi-preparative column packed with the same stationary phase, and the bi-Langmuir isotherm model was used to account for the data and to calculate overloaded elution band profiles of single components, and of different binary mixtures of the two enantiomers on these columns. The sets of competitive isotherm data obtained on these three systems are compared. The main goal of this work was to find the source of the systematic errors that may explain the differences previously reported [25] between isotherm data obtained with columns of different sizes packed with the same stationary phase.

2. Theory

2.1. Isotherm models

In multi-component systems, the amount of one compound adsorbed at equilibrium with a solution of all of them depends on the concentration of all the other compounds present locally. The isotherm data obtained from competitive frontal analysis with rac-1-indanol were fitted to the following competitive bi-Langmuir isotherm model.

$$q_i = \frac{q_{ns} K_{ns} C_i}{1 + K_{ns}(C_1 + C_2)} + \frac{q_{es} K_{es,i} C_i}{1 + K_{es,1} C_1 + K_{es,2} C_2} \quad (1)$$

This isotherm model assumes that there are two types of sites on the surface, the nonselective sites (first term) that behave identically toward the two enantiomers and the enantioselective sites (second term) that are responsible for the chiral separation [19]. The subscript ns indicates the parameter of the first type of interactions, the subscript es those of the second one. The stricture of the model requires that the experimental data be fitted to Eq. (1) but without placing any restrictions on the numerical values of the coefficients (i.e. with eight degrees of freedom, not forcing and q_{ns}, K_{ns} and q_{es} to be equal for the two enantiomers). If the numerical values of the parameters are close and Eq. (1) is verified, the model is validated.

2.2. Model of chromatography

The chromatographic process is described by several models of increasing complexity [19]. The most important of these models are the general rate model (GR), the lumped pore diffusion model (POR), and the equilibrium-dispersive model (ED). In order to use the more rigorous GR and POR models for the calculation of band profiles, it is necessary to determine first the values of several kinetic parameters, which are often difficult to measure accurately or even to estimate reasonably. It is frequent that some of these parameters can be estimated only by using conventional correlations. For these reasons, the simple ED model is the most often used. This model assumes constant equilibrium between the stationary and the mobile phases and accounts for the mass transfer resistances through the use of an apparent axial dispersion coefficient. It gives most satisfactory results when the mass transfer resistances are small, which is often the case in the separation of low molecular weight compounds.

For each component i in the column, the mass balance equation of the ED model is:

$$\frac{\partial C_i}{\partial t} + u \cdot \frac{\partial C_i}{\partial z} + F \cdot \frac{\partial q_i}{\partial t} = D_{a,i} \cdot \frac{\partial^2 C_i}{\partial z^2} \quad (2)$$

where t and z are the time elapsed from the injection and the migration distance along the column, respectively; u the interstitial mobile-phase velocity; F the phase ratio related to the total porosity, ε_t, by $F = (1 - \varepsilon_t)/\varepsilon_t$; $D_{a,i}$ the apparent dispersion coefficient of component i; C_i the mobile-phase concentration; and q_i is the solid-phase concentration. Since the ED model assumes instantaneous equilibrium between stationary and mobile phases, the solid-phase concentration q_i is derived from the adsorption isotherm model, $q_i = f(C_1, C_2, \ldots, C_n)$. The contributions of the mass transfer resistances are included in the apparent dispersion coefficient. This coefficient is related to the column efficiency by:

$$D_{a,i} = \frac{u_0 L}{2N_i} \quad (3)$$

where u_0 is the mobile-phase linear velocity; L the column length; and N_i is the plate number for component i. In practice, it is assumed that all components have the same plate number. This is one of the reasons why

the calculations of overloaded band profiles are easier and much faster than those made with more complex models. However, this assumption may reduce the validity of the results.

The initial condition for Eq. (2) is:

$$C_i(t = 0,\ 0 < z < L) = 0 \tag{4}$$

The boundary conditions at the column inlet ($t > 0$ and $z = 0$) are:

$$C_i(t < t_p,\ z = 0) = C_{i,f} \qquad C_i(t > t_p,\ z = 0) = 0 \tag{5}$$

where t_p is the duration of the rectangular injection and the subscript f indicates a value at the column inlet. At the column outlet, the boundary condition for $t > 0$ and $z = L$ is:

$$\frac{\partial C_i}{\partial z} = 0 \tag{6}$$

2.3. Numerical solution of ED model

The ED model was solved using a computer program based on an implementation of the method of orthogonal collocation on finite elements [19,28,29]. The set of discretized ordinary differential equations was solved with the Adams–Moulton method implemented in the VODE procedure [30]. The relative and absolute errors of the numerical calculations were 1×10^{-6} and 1×10^{-8}, respectively.

3. Experimental

3.1. Equipment

3.1.1. Equipment for the analytical and semi-preparative columns

An HP 1090 instrument for liquid chromatography was used (Hewlett-Packard, now Agilent Technologies, Palo Alto, CA, USA). This system is equipped with a multi-solvent delivery system, an automatic injector with a 25 μl sample loop, a column oven, a diode-array detector, and a data acquisition system. The microcomputer of this system monitors the operations of the equipment and can be programmed, e.g. to perform a series of breakthrough curves (see Section 3.4).

3.1.2. Equipment for microbore column

An HP 1100 capillary chromatography system was used (Agilent Technologies). This system is equipped with a microdiode array detector (cell volume: 500 nl), a flow splitter with an electro-magnetic proportional valve connected to a flow sensor device and a computer workstation.

The same modifications to the instrument as were made by Cavazzini et al. [25] were used in this work. An FA step is obtained by injecting into the column a sufficiently large volume of a sample solution, at a suitable concentration. This connection dramatically reduces the system hold-up volume, helps in creating the back pressure that is needed for the flow-rate controller to work properly and allows the accurate measurement of isotherm data with this microsystem. The disadvantages of this new procedure compared to the conventional one are that samples of different concentrations have to be prepared separately, manually, in advance, and that the pressure or flow-rate perturbations due to the injection cause a certain loss of precision and accuracy. Steel sample loops of different volumes (10, 20 and 150 μl) were used for these FA measurements and to acquire the experimental overloaded band profiles. The total system holdup volume was measured at 4.0 ± 0.1 μl.

3.2. Materials

The mobile phase was a solution of *n*-hexane–2-propanol (92.5:7.5, v/v). Hexane and 2-propanol were HPLC grade solvents from Fisher Scientific (Fair Lawn, NJ, USA). 1,3,5-tri-*tert*-Butylbenzene (unretained solute) and 1-indanol were purchased from Aldrich (Milwaukee, WI, USA). Samples of pure *R*-1-indanol and *S*-1-indanol were also purchased from Aldrich and were purified in our laboratory [31].

3.3. Column

3.3.1. Column for semi-preparative system

This 20 cm × 1.0 cm column was packed in-house with Chiracel OB (cellulose tribenzoate coated on a silica support; Daicel, Tokyo, Japan). The column diameter was accurately measured using an electronic caliper and found to be 1.006 ± 0.001 cm. The average particle size of the packing material was 20 μm. The total column porosity, derived from

the retention volume of 1,3,5-tri-*tert*-butylbenzene, which was assumed to be an unretained tracer, was 0.697.

3.3.2. Column for microbore system

This 15 cm × 0.1 cm column was packed by Micro-Tech Scientific (Sunnyvale, CA, USA), with the same Chiracel OB as the semi-preparative column. The column diameter was measured with the same electronic caliper and found to be 0.107 ± 0.001 cm. The total column porosity, derived from the retention volume of 1,3,5-tri-*tert*-butylbenzene, was 0.694.

3.3.3. Column for analytical system

This 15 cm × 0.46 cm column was packed by Chiral Technologies (Exton, PA, USA) with Chiracel OB, but with a material coming from a different batch than the one used to pack the semi-preparative and the microbore column. The diameter was measured with the same electronic caliper and found to be 0.457 ± 0.001 cm. The total column porosity, derived from the retention volume of 1,3,5-tri-*tert*-butylbenzene, was 0.731.

3.4. Measurements of the isotherm data

3.4.1. Semi-preparative system

All experimental data were measured at room temperature (ca. 25 °C), at a 2.5 ml/min mobile-phase flow rate. The retention factors for *R*-1-indanol and *S*-1-indanol were 1.14 and 1.97, respectively; the selectivity factor was 1.73. The detector was used at a wavelength of 280 nm. The efficiencies of the column for both *R*-1-indanol and *S*-1-indanol were approximately 600 theoretical plates.

Competitive frontal analysis measurements were performed, following the conventional method [19], using the multi-channel solvent delivery system. One channel of this system was used to deliver the sample solution, the other to pump the pure mobile phase. The ratio of the flow rates of the two streams was adjusted periodically, by program, to increase the concentration of the sample solution by 10% increments from 0 to 100%.

This period was 7 min, corresponding to a volume of 17.5 ml, for the competitive frontal analysis measurements. The concentration range investigated was approximately 0.0–20 g/l. In this range, 19 data points were acquired.

3.4.2. Microbore system

All the experimental data were acquired at room temperature (25 °C), at a 15 μl/min mobile-phase flow rate. The retention factors for *R*-1-indanol and *S*-1-indanol were 1.18 and 2.06, respectively (i.e. 4% higher than on the semi-preparative column); the selectivity factor was 1.74. The detector wavelength used was 283 nm. The efficiency of the column for both enantiomers was approximately 600 theoretical plates.

Competitive frontal analysis measurements were also made at 25.0 ± 0.1 °C, at the flow rate of 15 μl/min. The minimum sample volume needed to reach the plateau concentration was 150 μl. The concentration range investigated was approximately 0–25 g/l. In this range, 19 data points were acquired, all the measurements being repeated twice. The average value was used for the determination of the isotherm parameters.

3.4.3. Analytical system

All the experimental data were measured at room temperature (ca. 25 °C), at a 0.4 ml/min mobile-phase flow rate. The retention factor for *R*-1-indanol and *S*-1-indanol were 1.07 and 1.88, respectively (i.e. approximately 7% lower than with the semi-preparative column); the selectivity factor was 1.76. The detector wavelength used was 280 nm. The efficiencies of the column for both *R*-1-indanol and *S*-1-indanol were approximately 600 theoretical plates.

The measurement of the competitive isotherm data and the overloaded band profiles were made as with the semi-preparative system. The concentration range investigated was approximately 0–20 g/l. In this range, 19 data points were acquired.

3.5. Modeling of the experimental isotherm data

The best numerical values of the parameters of the isotherm models were estimated by fitting the experimental adsorption data to the corresponding model equation, using the least-squares Marquardt method modified by Fletcher [32].

4. Results and discussion

4.1. Results of competitive frontal analysis

All three sets of competitive isotherm data were fitted to the bi-Langmuir isotherm model (Eq. (1)), first in the eight-parameter version (assuming that q_{ns}, k_{ns}, q_{es} and are different for the two enantiomers), second, since the numerical values obtained for the parameters of the first Langmuir term were not significantly different, to the five-parameter version that is given in Eq. (1) (q_{ns}, q_{es}, and K_{ns}, equal for the two enantiomers). The numerical values obtained for the best isotherm parameters of the three columns studied are reported in Table 1. For all three columns, the bi-Langmuir model accounts very well for the isotherm data. Fig. 1 compares the experimental isotherm data (■) and the curves corresponding to the best bi-Langmuir isotherm for each one of these three columns (—). The degree of scattering of the data around the best curves is small, in consistency with the regression coefficients being very close to unity. In all cases, the best bi-Langmuir isotherm is in excellent agreement with the experimental data, and the coefficient of regression (R^2) is always larger than 0.9998 (Table 1). The adsorption constants on the high-energy sites (enantioselective sites) for each compound are similar for all three columns. For *R*-1-indanol, the adsorption constants on the high-energy site is 0.11, 0.13, and 0.12 for the three columns; for *S*-1-indanol, they are 0.27, 0.28, and 0.33. For all three columns, the adsorption constants for *S*-1-indanol are two to three times larger than those for *R*-1-indanol. This confirms that only the high-energy sites are responsible for the chiral selectivity of the stationary phase.

4.2. Validation of bi-Langmuir isotherm model on three systems

The bi-Langmuir isotherm model was combined with the ED model to calculate the band profiles of samples of binary mixtures of *R*- and *S*-1-indanol. Fig. 2 compares the experimental (■) and the calculated (—) band profiles. The agreement between these profiles is excellent in all cases. This confirms the accuracy of the isotherm determined on each column. The experimental conditions under which the profiles shown in Fig. 2 were recorded are listed in Table 2.

The loading factor (L_f) was calculated from the following relationship [19]:

$$L_f = \frac{n}{(1-\varepsilon_t)SLq_s} \quad (7)$$

where n is the sample size; ε_t the total column porosity; S the column cross-section area; L the column length; and q_s is the saturation capacity of the stationary phase, estimated as the sum of the two saturation capacities of the model (Eq. (1)). The loading factors of the three profiles shown are 2.96, 5.04 and 3.40% for Fig. 2a–c, respectively.

4.3. Comparison of the competitive isotherm data on the three columns

In many previous publications [16–18], the adsorption isotherm parameters are used only to predict the experimental band profiles obtained with the same column, under different experimental conditions. Since the measurement of a complete set of equilibrium isotherm data requires long runs and usually consumes large amounts of samples and solvents,

Table 1
Best estimates of the parameters of the bi-Langmuir isotherm model derived from single frontal analysis data and regression coefficient

Isotherm	Enantiomers	Parameters				R^2
		q_{ns} (g/l)	K_{ns} (l/g)	q_{es} (g/l)	$K_{es,i}$ (l/g)	
Microbore column	*R*-1-indanol	162 ± 25	0.0091 ± 0.0019	13 ± 1	0.11 ± 0.00 ($i = 1$)	0.9999
	S-1-indanol				0.27 ± 0.02 ($i = 2$)	
Analytical column	*R*-1-indanol	224 ± 107	0.0046 ± 0.0025	15 ± 1	0.13 ± 0.00 ($i = 1$)	0.9998
	S-1-indanol				0.28 ± 0.02 ($i = 2$)	
Semi-preparative column	*R*-1-indanol	98 ± 6	0.015 ± 0.001	11 ± 0	0.12 ± 0.00 ($i = 1$)	1
	S-1-indanol				0.33 ± 0.00 ($i = 2$)	

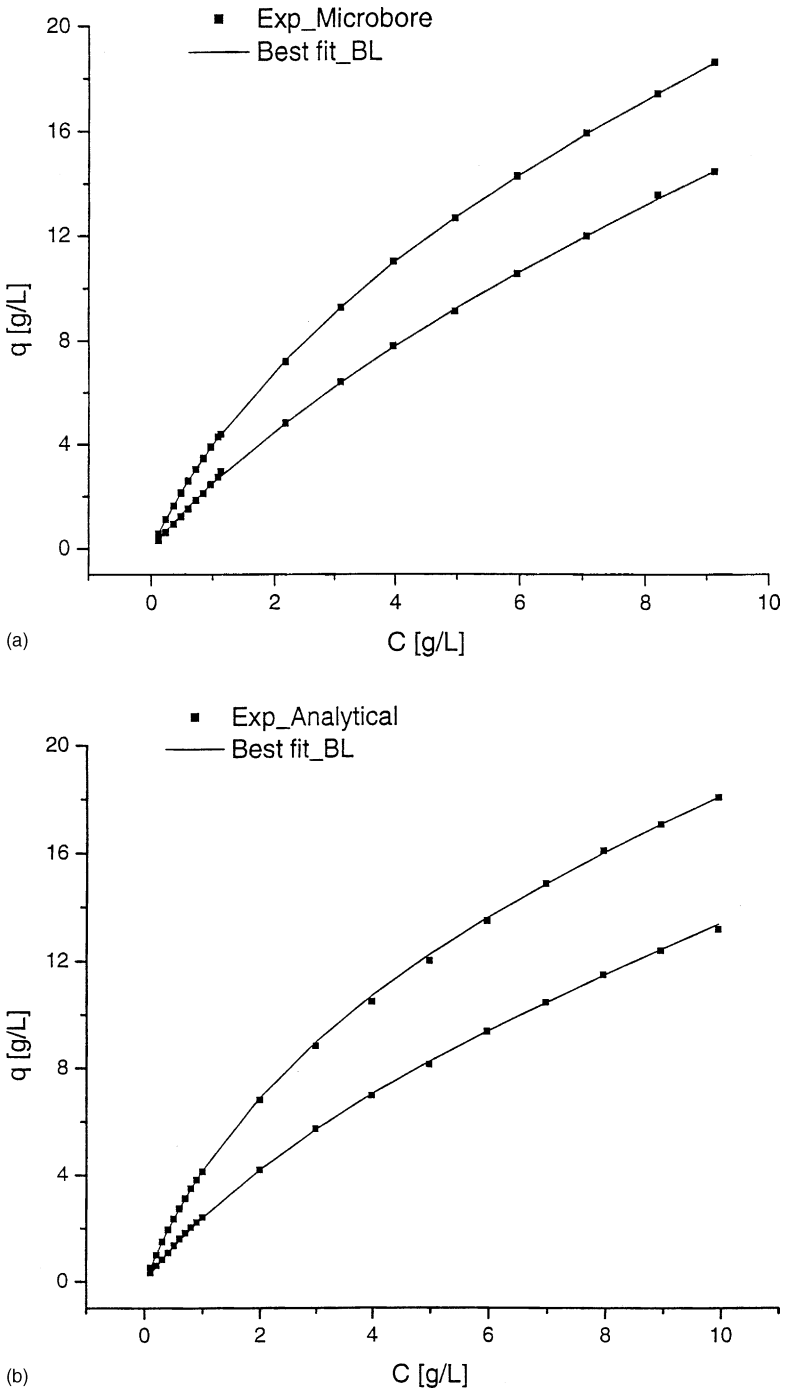

Fig. 1. Experimental isotherm data (■) and the best bi-Langmuir isotherm on three columns: (a) microbore column; (b) analytical column; (c) semi-preparative column.

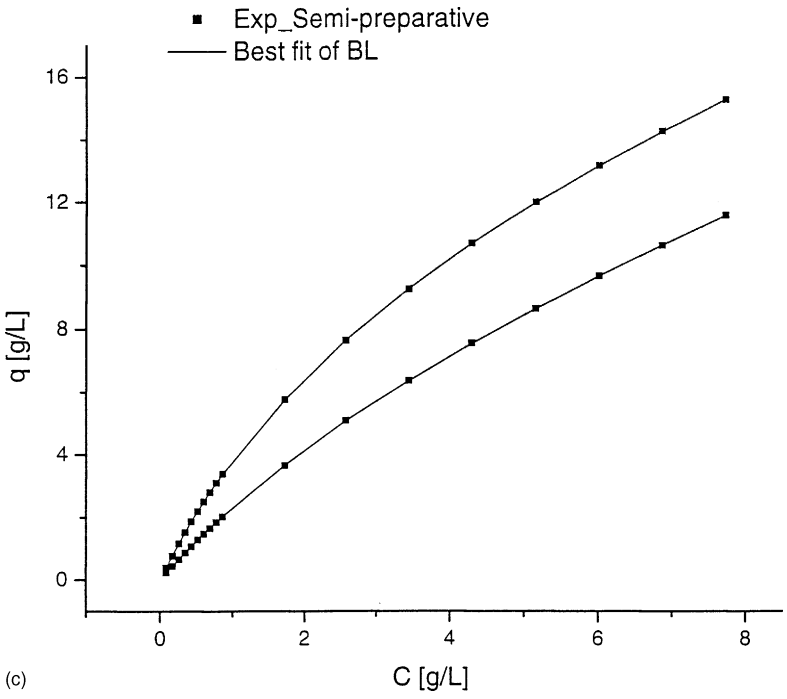

Fig. 1. (*Continued*).

using data acquired with a narrow bore column to measure the isotherm data and predict the behavior of large scale columns would bring large savings, especially when applied to expensive compounds. However, columns of different sizes may also have different packing density; it is, therefore, necessary to investigate under which conditions and with what corrections the isotherm data obtained with a microbore column can be used for the computer-assisted optimization of large preparative units.

The properties of the three columns and the experimental conditions under which they were used are listed in Table 3. The packing material are nearly the same, with minor differences at most (see later). The flow rate, the flow velocity, and the pressure drop are different; the column lengths are close. Finally, the retention factors, which are relative parameters, are the same for the three columns, within a few percent (see Section 3) and the separation factors on the three columns, 1.74, 1.76, and 1.73 for microbore, the analytical, and the semi-preparative column, respectively, are the same within experimental errors. This suggests that the stationary phases in the three columns have very similar properties.

Fig. 3 compares the three sets of isotherm data derived from the measurements. These isotherms are close, which was expected since the packing materials in the three columns are very similar if

Table 2
Experimental conditions of profiles in Figs. 2 and 4

Figure	Column	$C_{R\text{-1-indanol}}$ (g/l)	$C_{S\text{-1-indanol}}$ (g/l)	Injection time (min)	Loading factor (%)
Fig. 2a	Microbore	5.35	5.35	1.33	2.96
Fig. 2b	Analytical	3.80	3.80	1.05	5.04
Fig. 2c	Semi-preparative	3.57	3.57	1.00	3.40
Fig. 4	Semi-preparative	5.00	5.00	1.00	2.97

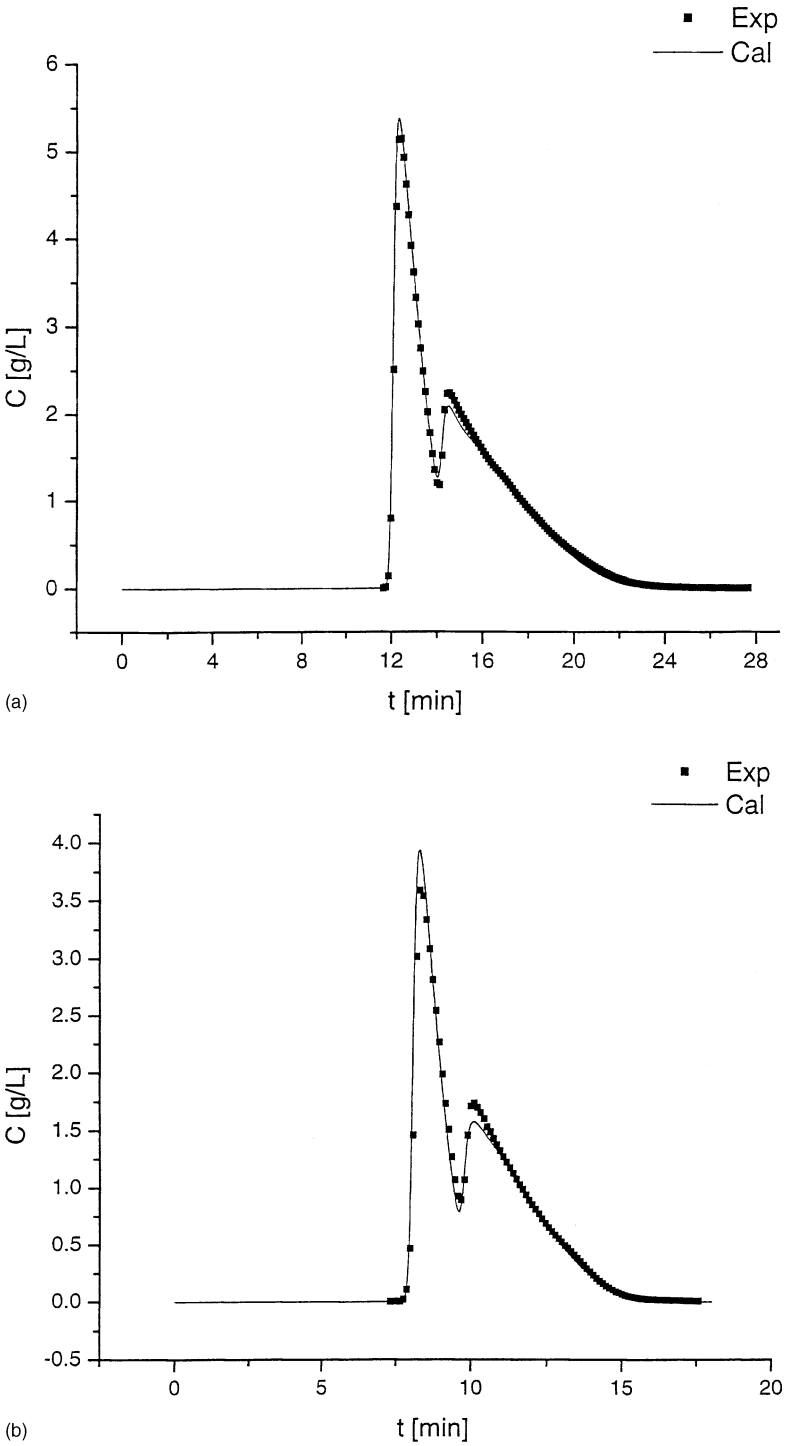

Fig. 2. Comparison of experimental and calculated profiles: (a) microbore column, $L_f = 2.96\%$; (b) analytical column, $L_f = 5.04\%$; (c) semi-preparative column, $L_f = 3.40\%$.

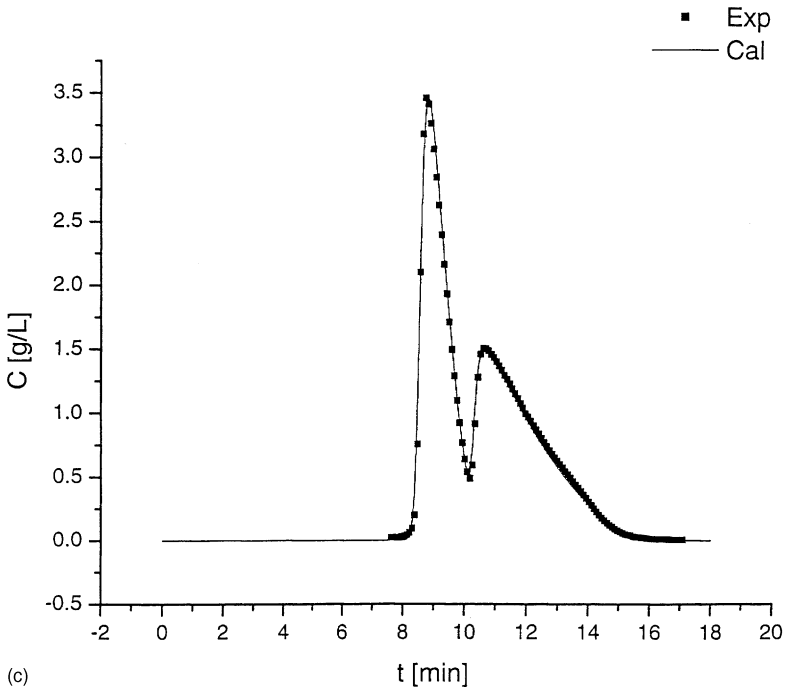

Fig. 2. (*Continued*).

not identical. The analytical column was new and have never been used before. The microbore column was used only to acquire the isotherm data of 1-phenyl-1-propanol [25] and the data reported in this work. The semi-preparative column had been used previously to acquire data for several investigations lasting a period of over 2 years, including continuous operations for several consecutive months at a time, as part of the column train of an SMB unit [19,33–36]. After 2 years of usage, the semi-preparative columns are still working well and satisfactorily [36]. Finally, the origins of the stationary phases are the same and the linear chromatography data obtained, k' and α, are nearly identical. So, the differences between the isotherms obtained with the three columns arise most probably from minor differences in the mechanical properties of these columns (e.g. fluctuations of the packing densities and/or errors made on their dimensions). For a convenient comparison, the isotherms in Fig. 3 were fitted to the following equation in

Table 3
Comparison of the experimental conditions and the isotherm models for the three columns

Parameter	Semi-preparative system	Microbore system	Analytical
Stationary phase	Chiracel OB, particle size: 20 μm		
Mobile phase	Hexane–isopropanol (92.5:7.5, v/v)		
Interstitial flow rate, u (cm/mm)	4.5	2.4	3.3
Pressure (bar)	12	35	11
Column length (cm)	20	15	15
Column diameter (cm)	1.0060 ± 0.001	0.107 ± 0.001	0.457 ± 0.001
Total porosity (ε_t)	0.697	0.694	0.731
Selectivity	1.73	1.74	1.76
Best competitive isotherm model	Bi-Langmuir		

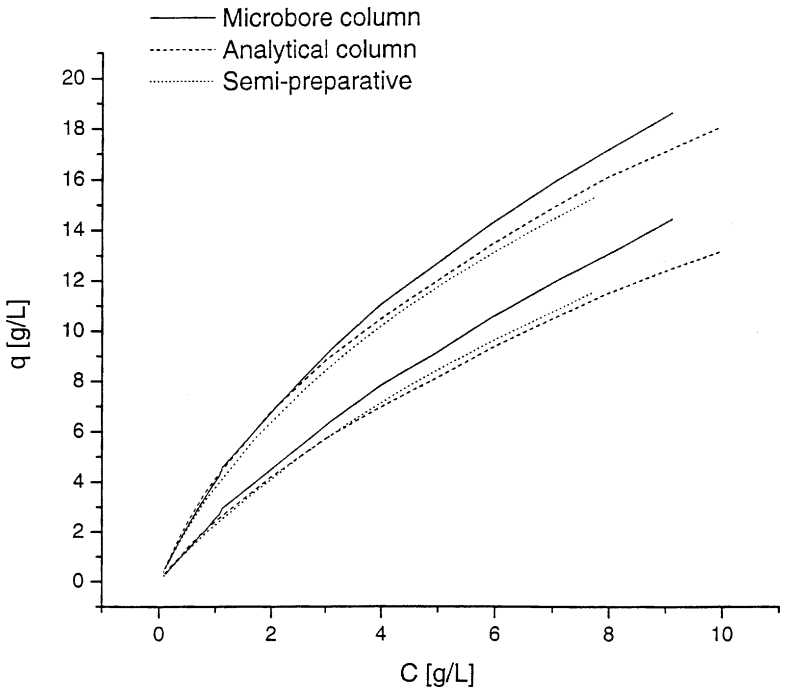

Fig. 3. Comparison of the isotherm data measured on the microbore column (—), the analytical column (- - -) and the semi-preparative column (· · ·).

which all the parameters are those determined for the semi-preparative column, so the correlation has only one degree of freedom, r,

$$q_i = \frac{(rq_{\mathrm{ns,s}})K_{\mathrm{ns,s}}C_i}{1 + K_{\mathrm{ns,s}}(C_1 + C_2)} + \frac{(rq_{\mathrm{es,s}})K_{\mathrm{es},i,\mathrm{s}}C_i}{1 + K_{\mathrm{es},1,\mathrm{s}}C_1 + K_{\mathrm{es},2,\mathrm{s}}C_2} \quad (8)$$

where the subscript s stands for the semi-preparative column. The best values of r obtained for the analytical and the microbore columns are: 1.01 ± 0.01 and 1.10 ± 0.00, respectively. So, the three sets of isotherm data are in close agreement with relative differences of +10% for the microbore column data and +1% for the analytical column, compared to the data obtained with the semi-preparative column.

We must note, however, that the initial isotherm derived from the microbore column deviated markedly from the one obtained for the other two columns. The reason was that, in the determination of the isotherm data, each FA measurement gives the amount of solute hold-up by the column when equilibrium has been reached after the stream of the mobile phase has been replaced by a stream of a solution of the solute in this liquid phase. The isotherm being a plot of the solid-phase concentration versus the liquid-phase concentration at equilibrium, the column diameter is needed to calculate the solid-phase concentration. Initially, we took for the column diameter, the value stated by the producer of the microbore column. Then, the value obtained for r in Eq. (8) was 1.79. In a similar earlier work, Cavazzini et al. [25] measured the isotherm of 1-phenyl-1-propanol on the same semi-preparative and microbore columns. They reported that the solid-phase concentration in equilibrium with a given solution concentration was 77% higher in the microbore than in the semi-preparative column (i.e. $r = 1.77$ for these two sets of data). These two values are almost identical. The difference is explained by the error made on the column diameter. That a relatively small error made on the column diameter could cause such a large systematic error in the isotherm data, requires ex-

planation. This is an important source of error to control.

In the derivation of the isotherm data from FA measurements, several parameters need to be determined: the amount of solute adsorbed at equilibrium (Q), the column length (L), the column diameter ($\varnothing$), the flow rate (F_v), and the hold-up time or retention time of an unretained compound (t_0). The solid-phase concentration is the amount adsorbed divided by the volume of adsorbent or:

$$q = \frac{Q}{V_a} = \frac{4Q}{\pi\varnothing^2 L - 4F_v t_0} \tag{9}$$

Among these parameters, Q, L, F_v and t_0 can be measured accurately, with a relative error that can easily be made smaller than 1%. Such small errors cannot cause the large difference observed on the isotherm data of our two columns. By contrast, the reproducibility of the diameter of the stainless steel tube used to manufacture columns is higher, the specifications of the tubing manufacturers are typically to ±0.02 in. (±0.05 mm) for most tubings used for the preparation of HPLC columns [37]. This error can be neglected for a 10 mm i.d. column, not for a 1 mm i.d. column. As we show below, the tube diameter has a large influence on the calculation of the isotherm data obtained, especially for microbore columns. Differentiating Eq. (9), we derive:

$$q\frac{d(1/q)}{d\varnothing} = \frac{2\pi L\varnothing}{\pi\varnothing^2 L - 4F_v t_0} \tag{10}$$

where the product $F_v t_0$ is equal to the hold-up volume of the column, which is measured directly and quite accurately. The total porosity is the ratio of the total pore volume to the geometrical volume:

$$\varepsilon_T = \frac{4F_v t_0}{\pi\varnothing^2 L} \tag{11}$$

and the phase ratio is

$$F = \frac{1-\varepsilon}{\varepsilon} = \frac{\pi\varnothing^2 L}{4F_v t_0} - 1 \tag{12}$$

hence

$$\frac{1}{F}\cdot\frac{dF}{d\varnothing} = \frac{2\pi L\varnothing}{\pi\varnothing^2 L - 4F_v t_0} = q\cdot\frac{d(1/q)}{d\varnothing} \tag{13}$$

If the total porosity is large, the difference between the geometrical and the hold-up volumes is relatively small and any error made on the geometrical volume will cause an important error on both the solid-phase concentration (Eq. (9)) and the phase ratio (Eq. (12)). If measurements are made with a column having a true internal diameter $\varnothing_t$ but the calculations are carried out assuming an erroneous diameter $\varnothing_e$, the ratio of the true and erroneous solid-phase concentrations is:

$$\frac{q_t}{q_e} = \frac{Q/V_{a,t}}{Q/V_{a,e}} = \frac{V_{a,e}}{V_{a,t}} = \frac{\pi\varnothing_e^2 L - 4V_0}{\pi\varnothing_t^2 L - 4V_0} \tag{14}$$

The true and calculated porosities are such that the hold-up volume remains constant:

$$4V_0 = \varepsilon_t\pi\varnothing_t^2 L = \varepsilon_e\pi\varnothing_e^2 L \tag{15}$$

Combination with Eq. (14) gives

$$\frac{q_t}{q_e} = \frac{\varnothing_e^2(1-\varepsilon_e)}{\varnothing_t^2(1-\varepsilon_t)} = \frac{1-\varepsilon_e}{(\varnothing_t/\varnothing_e)^2 - \varepsilon_e} \tag{16}$$

Since the total porosity is of the order of 0.70, a large systematic error on the isotherm data can take place. In both Tables 4 and 5, for the sake of illustration, the producer stated diameter value was used as the erroneous diameter value. Table 4 gives the relative error made on the isotherm data (i.e. on the value of q corresponding to a certain retention time of the breakthrough front) when the systematic error made in estimating the column diameter is 5%. This error is surprisingly large. It is larger for the microbore than for the semi-preparative column and it is not proportional to the relative error made on the column diameter. Table 5 shows the results of a similar calculation made by assuming, not a constant relative error, but a constant absolute error of ±0.05 mm for the three columns studied here. Obviously, the effect is more important for the microbore than for the analytical or the semi-preparative column. Since the measurement of the column diameter using the caliper still have an absolute error of ±0.01 mm, the probable error made on the isotherm data was calculated and is reported in Table 6. Although the actual diameters of the three columns had been accurately measured, with an error of 0.01 mm, the errors made on the isotherm data are still in the 6.5−6.8% range for the microbore column. This error may account for the residual discrepancy between the data obtained with the three columns. It suffices to explain completely the difference between the isotherms obtained with the analytical and the semi-preparative column on the one hand and the microbore column on the other (see Fig. 3).

Table 4
Influence of a given relative error on the column diameter on the values obtained for q and for the porosity of the three columns[a]

System	Diameter error (%)	Diameter (cm)	q_t/q_e	Total porosity	Phase ratio
Microbore column	−5	0.095	1.907	0.881	0.135
	0	0.100	1	0.795	0.258
	5	0.105	0.667	0.721	0.387
Analytical column	−5	0.437	1.538	0.799	0.252
	0	0.460	1	0.721	0.387
	5	0.483	0.731	0.654	0.529
Semi-preparative column	−5	0.95	1.493	0.781	0.280
	0	1	1	0.705	0.418
	5	1.05	0.742	0.639	0.565

[a] Relative difference between the measured diameter and the value given by the manufacturer.

Table 5
Influence of a given absolute error on the column diameter on the values obtained for q and for the porosity of the three columns[a]

System	Diameter error (cm)	Diameter (cm)	q_t/q_e	Total porosity	Phase ratio
Microbore column	−0.005	0.095	1.907	0.881	0.135
	0	0.100	1	0.795	0.258
	0.005	0.105	0.667	0.721	0.387
Analytical column	−0.005	0.455	1.084	0.737	0.356
	0	0.460	1	0.721	0.387
	0.005	0.465	0.829	0.706	0.417
Semi-preparative column	−0.005	0.995	1.035	0.712	0.404
	0	1	1	0.705	0.418
	0.005	1.005	0.967	0.698	0.433

[a] Relative difference between the measured diameter and the value given by the manufacturer.

The set of isotherm parameters for the microbore column given in Table 1 were used to predict the overloaded band profiles on the semi-preparative column. The corresponding experimental conditions are listed in Table 2. Fig. 4 compares the experimental overloaded profiles recorded on the semi-preparative column and the profiles calculated with the ED model, using the isotherm parameters obtained for the microbore column. The agreement between these two profiles is most satisfactory. The retention times of the

Table 6
Contribution to the error made on the isotherm parameters by an absolute error of ±0.01 mm on the column diameter

System	Diameter error (cm)	Diameter (cm)	q_{real}/q_{cal}	Total porosity	Phase ratio
Microbore column	−0.001	0.106	1.065	0.708	0.413
	0	0.107	1	0.694	0.440
	0.001	0.108	0.942	0.682	0.467
Analytical column	−0.001	0.456	1.017	0.734	0.362
	0	0.457	1	0.731	0.368
	0.001	0.458	0.984	0.728	0.374
Semi-preparative column	−0.001	1.005	1.007	0.698	0.432
	0	1.006	1	0.697	0.436
	0.001	1.007	0.993	0.695	0.439

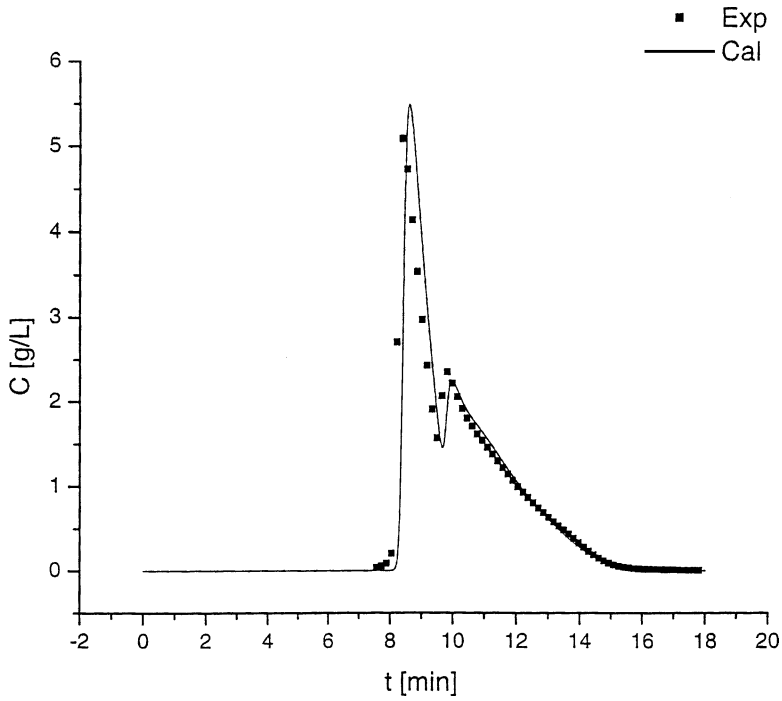

Fig. 4. Comparison of overloaded profiles on the semi-preparative column and the profiles calculated using the correct isotherm measured for the microbore column.

peaks in the calculated profile is slightly longer that those of the experimental profiles, which is explained by the small differences between the isotherm of the two columns. Yet, the agreement is sufficient to justify the use of the isotherm model obtained with the microbore column in the computer-assisted optimization of a separation carried out on a large preparative column.

5. Conclusions

The competitive isotherm data obtained for the enantiomers of 1-indanol on a microbore column, an analytical column, and a semi-preparative column fit equally well to the same isotherm model, the bi-Langmuir model. In all three cases, the best values of the parameters of the two enantiomers on the high-energy sites differ strongly, the adsorption constants for the more retained *S*-1-indanol being two to three times larger than those for the less retained *R*-1-indanol. The high-energy sites are thus responsible for the chiral separation studied. The low-energy sites behave identically toward both enantiomers and do not contribute to their separation.

Provided the correct column diameters are used to calculate the isotherm data and derive the values of the parameters of the isotherm model, there are no important differences between the values of these parameters whether they are derived from measurements carried out with the microbore, the analytical, or the semi-preparative column. However, there are significant differences between the precision of the isotherm parameters. A seemingly small error made on the diameter of a column may result in a considerable error on the isotherm data and hence on the isotherm parameters. The effect of a given relative error on the column diameter increases with decreasing column diameter. The systematic errors made on the solid-phase concentration at equilibrium are of the order of 6.5, 1.7, and 0.7% for the microbore, the analytical and the semi-preparative columns, respectively, while all diameters were measured with an error of 0.01 mm. So, particularly when narrow bore or microbore columns are used, it is most important accurately to measure

this diameter. The inner diameter of narrow bore or microbore columns should best be measured before they are packed. For example, weighing the column empty and filled with a dense liquid (e.g. mercury, EPA permitting) would be a useful complement to optical measures of the inlet and outlet tubing diameters.

After the correction of the microbore column diameter, the two sets of isotherm data on the microbore and semi-preparative column are similar, the residual difference being about 10%. Nevertheless, the isotherm measured on the microbore column could be used to achieve a reasonably accurate prediction of the profiles on the semi-preparative column. The agreement between the experimental and the calculated profiles was satisfactory. This work confirms that isotherms measured on a scaled-down column (e.g. narrow bore column) can be used for the calculation of the experimental band profiles obtained on wider columns (e.g. semi-preparative or preparative columns) provided the exact diameters of both columns is accurately known. Thus, the use of microbore columns can save large amounts of solute, solvent, time and labor.

Acknowledgements

This work was supported in part by Grant CHE-00-70548 of the National Science Foundation, and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory. The authors are grateful to Chiral Technologies (Exton, PA, USA) for the generous gift of Chiracel OB stationary phase.

References

[1] F.A. Maris, R.J.M. Vervoort, H. Hindriks, J. Chromatogr. 547 (1991) 45.
[2] M. Rouhi, Chem. Eng. News 80 (25) (2002) 13.
[3] D. Lee, S.A. Shamsi, Electrophoresis 23 (2002) 1314.
[4] J. Zukowski, V. De Biasi, A. Berthod, J. Chromatogr. A 948 (2002) 331.
[5] M.V. Russo, J. Pharm. Biomed. 29 (2002) 999.
[6] X.N. Lu, Y. Chen, J. Chromatogr. A 955 (2002) 133.
[7] F. Charton, S.C. Jacobson, G. Guiochon, J. Chromatogr. (1993) 630.
[8] A. Seidel-Morgenstem, G. Guiochon, AlChE J. 39 (1993) 809.
[9] F. Charton, M. Bailly, G. Guiochon, J. Chromatogr. A 687 (1994) 13.
[10] M. Krause, R. Galensa, J. Chromatogr. 441 (1988) 417.
[11] D. Armstrong, K. Gahm, L. Chang, Microchem. J. 57 (1997) 1779.
[12] A. Kunath, E. Hoft, H. Hamann, J. Chromatogr. 588 (1991) 352.
[13] A.M. Edge, D.M. Heaton, K.D. Bartle, Chromatographia 41 (1995) 161.
[14] A. Kunath, F. Theil, J. Wagner, J. Chromatogr. 588 (1991) 233.
[15] S. Jacobson, S.G. Shirazi, G. Guiochon, J. Am. Chem. Soc. 112 (1991) 6492.
[16] D.E. Cherrak, S. Khattabi, G. Guiochon, J. Chromatogr. A 877 (2000) 109.
[17] I. Quiñones, C.M. Grill, L. Miller, G. Guiochon, J. Chromatogr. A 867 (2000) 1.
[18] S. Khattabi, D.E. Cherrak, J. Fischer, P. Jandera, G. Guiochon, J. Chromatogr. A 877 (2000) 95.
[19] G. Guiochon, S.G. Shirazi, A.M. Katti, Fundamentals of Preparative and Nonilnear Chromatography, Academic Press, Boston, MA, 1994.
[20] H.E. Schwartz, B.L. Karger, P. Kucera, Anal. Chem. 55 (1983) 1752.
[21] S.R. Bakalyar, C. Phipps, B. Spruce, K. Olsen, J. Chromatogr. A (1997) 762.
[22] F. Houdiere, P.W.J. Fowler, N.M. Djordjevic, Anal. Chem. 69 (1997) 2589.
[23] K. Krause, M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 837 (1999) 51.
[24] D.B. Gomis, J.J. Mangas, A. Castaño, M.D. Gutiérrez, Anal. Chem. 68 (1996) 3867.
[25] A. Cavazzini, A. Felinger, K. Kaczmarski, P. Szabelski, G. Guiochon, J. Chromatogr. A 953 (2002) 55.
[26] P. Jandera, S. Bačkovská, K. Mihlbachler, G. Guiochon, V. Bunčeková, J. Planeta, J. Chromatogr. A 925 (2001) 19.
[27] R.O. Lawton, L.D. Alexander, W.N. Setzer, K.G. Byler, Biotropica 483 (1993) 25.
[28] K. Kaczmarski, G. Storti, M. Mazzotti, M. Morbidelli, Comput. Chem. Eng. 21 (1997) 641.
[29] J.A. Beminger, R.D. Whitley, X. Zhang, N.-H.L. Wang, Comput. Chem. Eng. 15 (1991) 641.
[30] P.N. Brown, A.C. Hindmarsh, G.D. Byrne, Variable Coefficient Ordinary Differential Equation Solver, procedure available on http//www.netlib.org.
[31] D. Zhou, D.E. Cherrak, K. Kaczmarski, A. Cavazzini, X. Liu, G. Guiochon, Chem. Eng. Sci. 58 (2003) 3257.
[32] R. Fletcher, A modified Marquardt sub-routine for nonlinear least squares, AERER6799-Harwell.
[33] S. Khattabi, D.E. Cherrak, K. Mihibachler, G. Guiochon, J. Chromatogr. A 893 (2000) 307.
[34] K. Mihlbachler, A. Jupke, A. Seidel-Morgenstern, H. Schmidt-Traub, G. Guiochon, J. Chromatogr. A 944 (2002) 3.
[35] K. Mihlbachler, K. Kaczmarski, A. Seidel-Morgenstern, G. Guiochon, J. Chromatogr. A 955 (2002) 35.
[36] K. Mihlbachler, PhD Dissertation, University of Magdeburg, Germany, 2002.
[37] A. Felinger, M. Kele, G. Guiochon, J. Chromatogr. A 913 (2001) 23.