Modeling of the separation of two enantiomers using a microbore column

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Abstract

A microbore column packed with Chiralcel OB (cellulose tribenzoate coated silica) was used for the measurement of the single and competitive equilibrium-isotherm data of the 1-indanol enantiomers by frontal analysis. The amount of sample needed for the isotherm data acquisition was about 20 times less than that required with a conventional column. The data obtained were fitted to different single and competitive isotherm models. Both the single and the competitive data sets fitted best to the same Bilangmuir (BL) isotherm model with small differences in the numerical values of the parameters. The best fitted Bilangmuir single and competitive isotherm models were used to predict the overloaded experimental profiles of both pure enantiomers, of the racemic mixture, and of different enantiomeric mixtures. All the calculated profiles were in excellent agreement with the experimental ones. This agreement confirms that in many chiral separations, the competitive isotherms can be derived from data acquired from the mere racemic mixture with a sufficient accuracy for a correct prediction of the band profiles of all kinds of enantiomer mixtures, making possible the computer-assisted optimization of the experimental conditions.
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1. Introduction

The production of pure enantiomers has become important, due to the potential differences of the physiological activity and of the toxicity of the two enantiomers [1]. Chiral preparative chromatography, particularly as implemented in the simulated moving bed (SMB) process, has become the preferred method to carry out preparative enantiomeric separations or purifications [1–10]. Routine synthetic procedures produce racemic and/or diastereoisomeric mixtures. Enantioselective syntheses either are very expensive and/or often give a product of insufficient enantiomeric purity. The separations of racemic mixtures are difficult because, in most cases, the enantioselectivity of chiral stationary phases (CSPs) is relatively low. Furthermore, preparative separations should be

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performed under nonlinear conditions. It is then difficult and costly to optimize the process empirically, especially when the SMB implementation is used. This is why computer-assisted optimization is especially important for chiral preparative chromatography.

Frontal analysis [11] was the first developed LC method for the measurement of isotherm and it remains the most popular method because of its great accuracy. It is time-consuming, however, and it requires large amounts of pure compounds which are often expensive. To save the solvent and expensive enantiomeric sample and chiral stationary phase, a microbore column was used for the isotherm measurement in this work. Narrow-bore and microbore HPLC columns are increasingly used in analytical applications [12–16]. The use of small diameter columns affords large savings of expensive packing-materials. It reduces solvent consumption, it is more compatible to coupling with a mass spectrometer, and, in our investigations, it provides considerable savings by reducing the amounts of samples and consumables needed for the measurements.

Microbore columns have already been used for the determination of isotherms [17,18] because significant savings are made on the samples and the solvents needed for a measurement. Jandera et al. [18] 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 the same material. They compared also the isotherms of the enantiomers of mandelic acid on Teicoplanin, measured using commercial analytical and microbore columns packed with this CSP. The best parameters of the Langmuir (L) 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. Since the amount of sample necessary for the determination of the adsorption isotherms is reduced ten-fold when a 1 mm i.d. microbore column is substituted for a 4.6 mm i.d. conventional commercial analytical column, this approach is attractive for the determination of the isotherm data needed for the optimization of the preparative separations of expensive compounds such as pure enantiomers and biomolecules [18]. Cavazzini et al. [17]

investigated the adsorption equilibrium of the enantiomers of 1-phenyl-1-propanol on a microbore column packed with cellulose tribenzoate coated silica 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. The goal of this paper is to confirm that the competitive isotherm of two enantiomers derived from measurements made with the racemic mixture only is enough for the profile-prediction of all kinds of enantiomer mixtures and to show that the data required can easily be obtained using microbore columns.

2. Theory

2.1. Isotherm models

In many cases, different adsorption isotherm models can fit a set of adsorption data equally well. However, not all of them could also adequately predict overloaded band profiles. So, several isotherm models were investigated in this work [17,19–22].

2.1.1. Single compound isotherms

The isotherm data sets obtained by single-component frontal analysis of R-1-indanol and S-1-indanol were fitted to the three following isotherm models.

2.1.1.1. The Langmuir model (L).

$$q = \frac{q_s K C}{1 + K C} \tag{1}$$

where q_s is the specific saturation capacity of the adsorbent and K the equilibrium or binding constant of the compound considered in the phase system studied [11,20]. This model assumes a homogeneous surface with a uniform adsorption energy. However, when the adsorption energy is relatively low, it often fits the experimental data acquired on actual surfaces surprisingly well.

2.1.1.2. The Bilangmuir model (BL).

$$q = \frac{q_{s,1} K_1 C}{1 + K_1 C} + \frac{q_{s,2} K_2 C}{1 + K_2 C} \tag{2}$$

In this model, the two Langmuir terms refer to two different adsorption types of sites (e.g., enantioselective and nonselective sites) [21].

2.1.1.3. The Toth model (T).

$$q = \frac{q_s K C}{(1 + (KC)^v)^{1/v}} \tag{3}$$

where q_s is the saturation capacity, K the equilibrium constant, and v the heterogeneity parameter [11]. This model is a satisfactory empirical model for surfaces that are moderately heterogeneous. It is often suitable for polar compounds on conventional chromatographic stationary phases.

2.1.1.4. Loading factor.
The loading factor (L_f) is used to indicate the degree of overloading of the column. It is the ratio of the sample size to the amount of sample needed to make a complete monolayer on the stationary phase contained in the column. It is calculated from the following relationship [23]:

$$L_f = \frac{n}{(1 - \varepsilon_T) S L q_s} \tag{4}$$

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 the specific saturation capacity of the stationary phase.

2.1.2. Competitive isotherms
In multicomponent systems, the amount of any one of them adsorbed at equilibrium 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 isotherm models.

2.1.2.1. The competitive Langmuir model.

$$q_i = \frac{q_s K_i C_i}{1 + K_1 C_1 + K_2 C_2} \tag{5}$$

where q_s and K_i are the specific saturation capacity of the adsorbent and the equilibrium constant of component i, respectively.

2.1.2.2. The competitive Bilangmuir model (BL).

$$q_i = \frac{q_{ns} K_{ns} C_i}{1 + K_{ns}(C_1 + C_2)} + \frac{q_{es} K_{es,i} C_i}{1 + K_{es,1} C_1 + K_{es,2} C_2} \tag{6}$$

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 [11]. The subscript ns indicates the parameter of the first type of interactions, the subscript es those of the second one. Often, but not always, this model accounts well for the competitive adsorption data obtained with enantiomers.

2.1.2.3. The competitive Langmuir–Freundlich model (LF).

$$q_i = \frac{q_s (K_i C_i)^{v_i}}{1 + (K_1 C_1)^{v_1} + (K_2 C_2)^{v_2}} \tag{7}$$

where v_1 and v_2 ($v_i < 1$) represent the heterogeneity parameters for the two components on the surface considered [22]. The major drawback of this model is that the Henry constants are infinite since q_i/C_i tends toward infinity when C tends toward 0.

2.1.2.4. The competitive Toth model.

$$q_i = \frac{q_s K_i C_i}{(1 + (K_1 C_1 + K_2 C_2)^v)^{1/v}} \tag{8}$$

where v is the heterogeneity parameter for both components on the surface studied.

2.2. Model of chromatography

The simple equilibrium–dispersive (ED) model was used to describe the chromatographic process instead of the more rigorous GR and POR [11,24,25]. The ED model is the simplest realistic model and, for this reason, is also the most often used. The only parameter needed for this calculation is the plate number, easily derived from an analytical injection of the sample. When the mass transfer resistances are small, which is often the case with modern HPLC columns, particularly for small molecules, the ED model gives usually satisfactory predictions of chromatographic band profiles and the calculations are fast.

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

$$\frac{\partial C_i}{\partial t} + u \frac{\partial C_i}{\partial z} + F \frac{\partial q_i}{\partial t} = D_{a,i} \frac{\partial^2 C_i}{\partial z^2} \tag{9}$$

where t and z are the time elapsed from the beginning of the sample injection and the distance traveled by the molecules inside the column, respectively; u the interstitial mobile phase velocity; F the phase ratio obtained from 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 the solid-phase concentration. Since the ED model assumes instantaneous equilibrium between stationary and mobile phases, the solid phase concentration q_i is directly derived from the adsorption isotherm model, $q_i = f(C_1, C_2, \ldots, C_n)$. The contribution of the mass transfer resistances is included in the value of the apparent dispersion coefficient. This coefficient is related to the column efficiency by:

$$D_{a,i} = \frac{u_0 L}{2N_i} \tag{10}$$

where u_0 is the mobile phase linear velocity, L the column length and N_i the plate number for component i. In practice, it is assumed that all components have the same plate number. The use of one single kinetic parameter 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 calculation results.

The initial condition for Eq. (9) is:

$$C_i(t = 0, 0 < z < L) = 0 \tag{11}$$

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

$$\begin{aligned} C_i(t < t_p, z = 0) &= C_{i,f} \\ C_i(t > t_p, z = 0) &= 0 \end{aligned} \tag{12}$$

where t_p is the injection time 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:

$$\frac{\partial C_i}{\partial z} = 0 \tag{13}$$

The ED model was solved using a computer program based on an implementation of the method of orthogonal collocation on finite elements [11,26,27]. The set of discretized ordinary differential equations was solved with the Adams–Moulton method, implemented in the VODE procedure [28]. The relative and absolute errors of the numerical calculations were 1×10^{-6} and 1×10^{-8}, respectively.

3. Experimental

3.1. Equipment

A HP 1100 capillary chromatography system was used (Hewlett-Packard, now Agilent Technologies, Palo Alto, CA, USA). This system is equipped with a micro diode-array detector (cell volume, 500 nl), a solvent delivery system, a flow splitter with an electro-magnetic proportional valve connected to a flow sensor device, a temperature controlled oven, and a computer data station.

The method used for the measurement of FA data was previously developed by Cavazzini et al. [17]. Only one pump channel was used. It was connected to a six-way micro-valve (VICI, Cheminert CDX 0088, Valco Instrument, Houston, TX, USA) via 25 μm i.d. fused-silica capillaries (home made). Steel sample loops of different volumes (10, 20 and 150 μl) were used for FA and to generate overloaded elution profiles. The micro-valve was controlled by software (Ultra Plus II, Pump Controller Module-Interface Module; Micro-Tech Scientific, Sunnyvale, CA, USA). The system hold-up volume was 4.0 ± 0.1 μl.

3.2. Materials

The mobile phase was a solution of n-hexane and 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 tracer) 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 [19].

3.3. Column

The column used for the experiment was a 15 cm × 0.107 cm column packed with Chiralcel OB (cellulose tribenzoate coated on a silica support; Daicel, Tokyo, Japan). The column was packed by Micro-Tech Scientific. The amount of stationary phase necessary to pack this column was 160-fold smaller than that used to pack a 1 cm i.d. semi-preparative column [19]. The average particle diameter of the packing material is 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 0.694. The efficiencies of the column for R-1-indanol and S-1-indanol were approximately 700 and 600 theoretical plates, respectively.

The column inner diameter was carefully measured with an electronic caliper and found to be 0.107 ± 0.001 cm.

3.4. Measurements of the isotherm data

All the experimental data were measured at room temperature (25 °C), with 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, the selectivity factor was 1.74. The detector wavelength used was 283 nm.

An FA step is obtained by injecting a sufficiently large volume of a sample solution of suitable concentration into the column. This arrangement makes possible the measurement of isotherm data with the microsystem. It allows a dramatic reduction of the system dead volume and creates sufficiently high a back pressure for the flow-rate controller to work properly. The major disadvantages of this new experimental design compared to the conventional system are that (1) two dozen solutions having the appropriate concentrations must be prepared manually, in advance, and (2) the injection of the plug of sample solution causes pressure or flow-rate fluctuations, hence a loss in precision and accuracy in the measurements.

Since the micro-flow-rate was generated by splitting a higher flow from a HPLC pump, the actual pump flow was about 0.2–0.5 ml/min, and the volume of solvent needed to make measurements with this system is about 0.1–0.2 time that needed with the semi-preparative column. For the sake of accuracy, 1 ml of sample solutions at different concentrations were prepared manually using a volumetric flask though only about 0.2 ml needed. With this system, still about 17 times of sample were saved compared to what was needed for the semi-preparative column.

Single and competitive frontal analysis were performed at 25.0 ± 0.1 °C. The minimum sample volume necessary 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 and all the measurements were repeated two times. The average value was used for the determination of the isotherm parameters.

Finally, it must be emphasized that accurate knowledge of the column diameter or rather of the amount of packing material in the column is necessary in order to calculate accurate isotherm data. The considerable influence of an error made on the column diameter on the accuracy of these data has been shown elsewhere [30].

3.5. Modeling of the experimental isotherm data

The best numerical values of the Langmuir, Bilangmuir, Langmuir–Freundlich and Toth isotherm models were estimated by fitting the experimental adsorption data to the model equations, using the least-squares Marquardt method modified by Fletcher [29].

4. Results and discussion

4.1. Single compound frontal analysis

4.1.1. Single compound isotherms

The single-component isotherm data for R-1-indanol and S-1-indanol were measured by FA and the data set was fitted to different single-component isotherm models (see Eqs. (1)–(3)). Fig. 1 compares the experimental data (symbols) for R-1-indanol (Fig. 1a) and S-1-indanol (Fig. 1b), the best Langmuir isotherm (solid line), the best Bilangmuir isotherm (dashed line), and the best Toth isotherm (dotted line). The main figures show the data in the whole concentration range. The insets show only the data at low concentrations ($C_R < 1.5$ and $C_S < 2.5$ g/l). For R-1-indanol, the agreement between the experimental data on the one hand, the best Toth and the best Bilangmuir isotherm models on the other is excellent (the two corresponding lines cannot be distinguished in the main figure nor in the inset). The experimental data agree also fairly well with the Langmuir isotherm but exhibit systematic deviations at low and high concentrations. Such systematic differences are not seen with the other two models. For S-1-indanol, the agreement between the experimental data and the Bilangmuir model is slightly better than that with the Toth model, especially in the low concentration
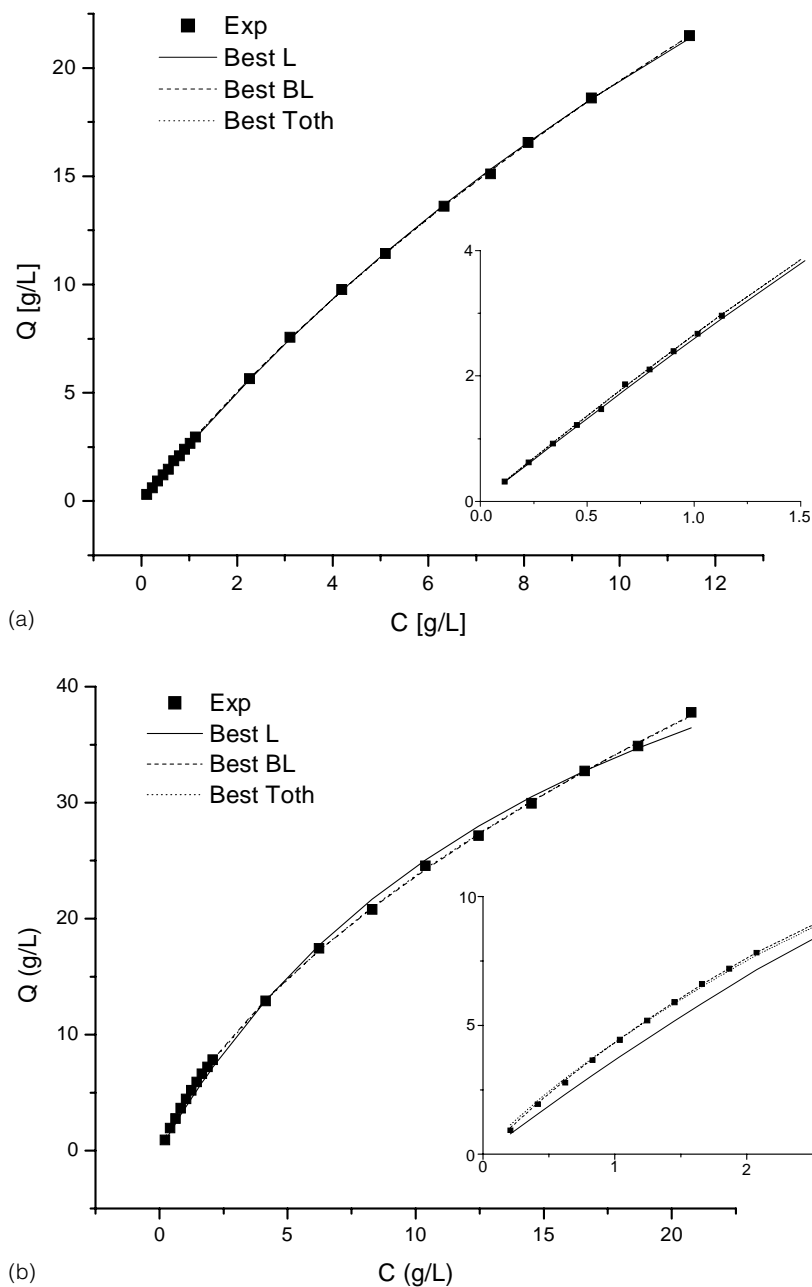
Fig. 1. Experimental isotherm data (symbols) obtained by single-component frontal analysis and the best Toth (dotted line), Langmuir (solid line), and Bilangmuir (dashed line) isotherm for R-1-indanol (a) and S-1-indanol (b).

range. The agreement between the experimental data and the Langmuir isotherm model is poor. These conclusions are confirmed by the values obtained for the Fisher test (see Table 1). These results are the same as

those previously obtained with several conventional columns [19,30].

The best values of the parameters obtained for the Langmuir, the Bilangmuir, and the Toth isotherm mod-

Table 1
Best estimates of the parameters of different isotherm models for single frontal analysis and Fisher's test values

Isotherm	Enantiomers	q_{ns} (g/l)	K_{ns} (l/g)	q_{es} (g/l)	K_{es} (l/g)	v	Fisher coefficient
Langmuir	R-1-indanol	69 ± 1	0.039 ± 0.001	NA	NA	NA	8.4×10^3
	S-1-indanol	67 ± 3	0.058 ± 0.004	NA	NA	NA	3.5×10^2
Bilangmuir	R-1-indanol	88 ± 7	0.022 ± 0.006	6.3 ± 4.9	0.14 ± 0.05	NA	1.8×10^4
	S-1-indanol	110 ± 36	0.016 ± 0.009	10 ± 5	0.35 ± 0.15	NA	7.0×10^3
Toth	R-1-indanol	114 ± 18	0.025 ± 0.003	NA	NA	0.75 ± 0.05	1.6×10^4
	S-1-indanol	489 ± 160	0.016 ± 0.004	NA	NA	0.35 ± 0.04	5.0×10^3

els and the corresponding Fisher coefficients for the two compounds are reported in Table 1. The value of the Fisher coefficient characterizes the quality of the fit of the data to the corresponding model; the larger the value, the better the fit. The values found for the Toth isotherm, 1.6×10^4 and 5.0×10^3 for R-1-indanol and S-1-indanol, respectively, are large, indicating an excellent fit, much better than the one afforded by the Langmuir isotherm (Table 1). For the Bilangmuir model, the Fisher coefficients are 1.8×10^4 and 7.0×10^3 for R-1-indanol and S-1-indanol, respectively, slightly higher than those for the Toth model but too close to allow a selection of the isotherm model on the basis of these statistical results. For the Langmuir model, the Fisher coefficients are markedly lower, particularly for S-1-indanol, but they would still be acceptable. Accordingly, all three models were used for the calculation of single-component band profiles for R-1-indanol and only the Toth and the Bilangmuir isotherm models were used for calculations of the S-1-indanol band profiles.

4.1.2. Validation of the single compound isotherms

Since at least two isotherm models fit the experimental adsorption equilibrium data equally well, as shown by the corresponding values of the Fisher test coefficients, these models were compared. All three models were used to calculate the overloaded elution band profiles of large samples of R-1-indanol, with a column efficiency of 700 theoretical plates. Only the Toth and Bilangmuir isotherm models were used to calculate the overloaded band profiles of S-1-indanol. Fig. 2 compares the experimental band profiles and the band profiles calculated with these different adsorption isotherm models, using

the equilibrium–dispersive model of chromatography (Eqs. (9)–(13)).

For R-1-indanol, all three isotherm models gave correct profiles, that agree well with the experimental ones (Fig. 2a). It is noteworthy that, in this case, in spite of the fact that the Langmuir model gave a much less good fit of the isotherm data than the other two models, the band profiles calculated from the Bilangmuir and the Toth models were only slightly better than those derived from the Langmuir model. For S-1-indanol, the profile derived from the Bilangmuir model was certainly better than the one afforded by the Toth model (Fig. 2b). Because of these results, the Bilangmuir model was chosen for further calculations of overloaded elution profiles. Note that, in this chiral separation, the simple Langmuir model allows the calculation of correct band profiles for the less retained R-1-indanol (Fig. 2a) while the band profiles of the more retained S-1-indanol must be calculated using a more complicated model such as the Bilangmuir or the Toth model.

Fig. 3 compares series of experimental profiles of pure R-1-(Fig. 3a) or S-1-indanol (Fig. 3b) with the corresponding profiles calculated using the Bilangmuir model. In Fig. 3a, the loading factor of R-1-indanol varies from 1.0 to 4.9%. In Fig. 3b, the loading factor of S-1-indanol varies from 0.48 to 4.2%. From Fig. 3, it can be concluded that the Bilangmuir model combined with the ED model of chromatography provides calculated band profiles of the pure compounds that are in excellent agreement with the experimental profiles at high loading factors. The experimental conditions and the loading factors of the chromatograms in Fig. 3 are summarized in Table 2.
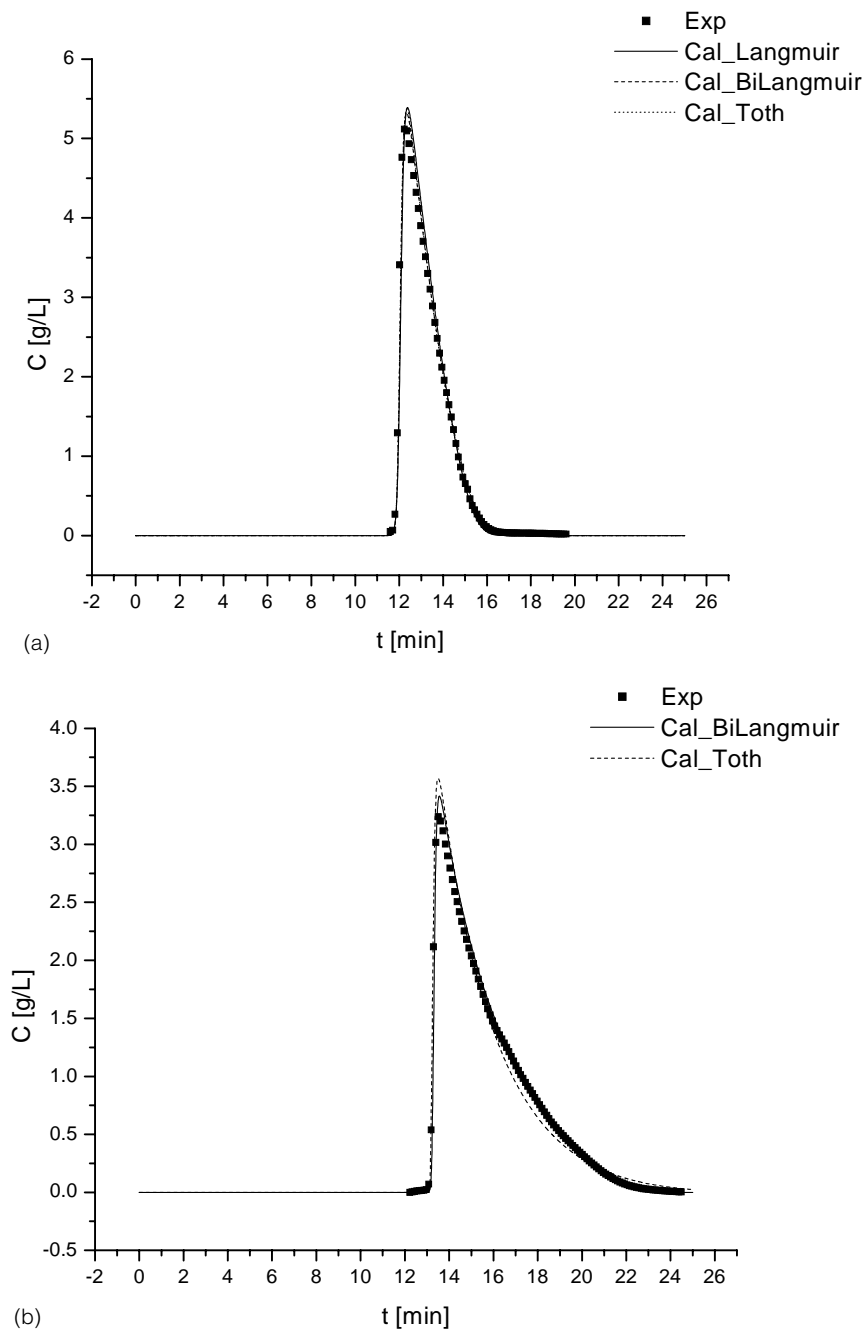
Fig. 2. Comparison of experimental profiles of *R*-1-indanol (a) and *S*-1-indanol (b) and the calculated profiles obtained with the Toth, the Bilangmuir, and the Langmuir isotherm models for *R*-1-indanol and the Toth and the Bilangmuir isotherm models for *S*-1-indanol.
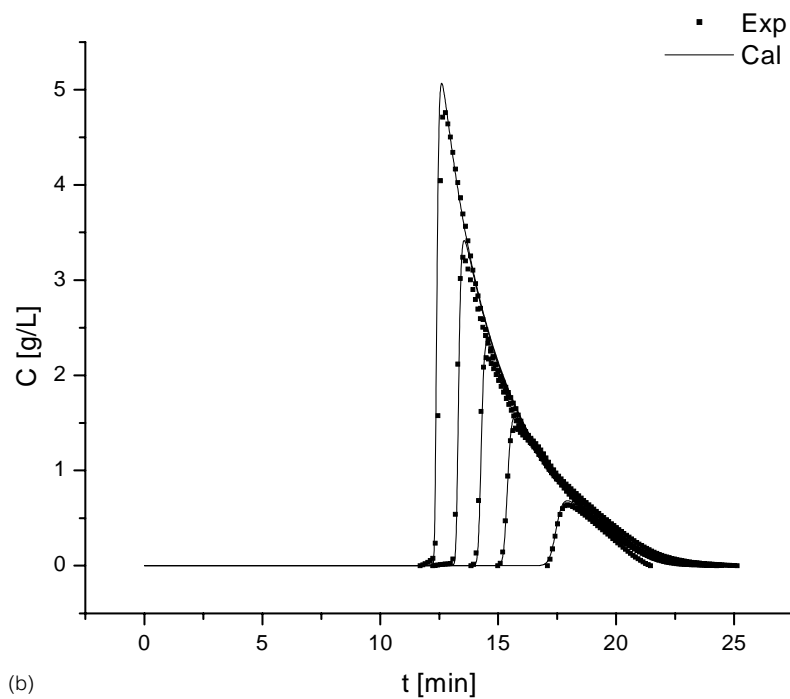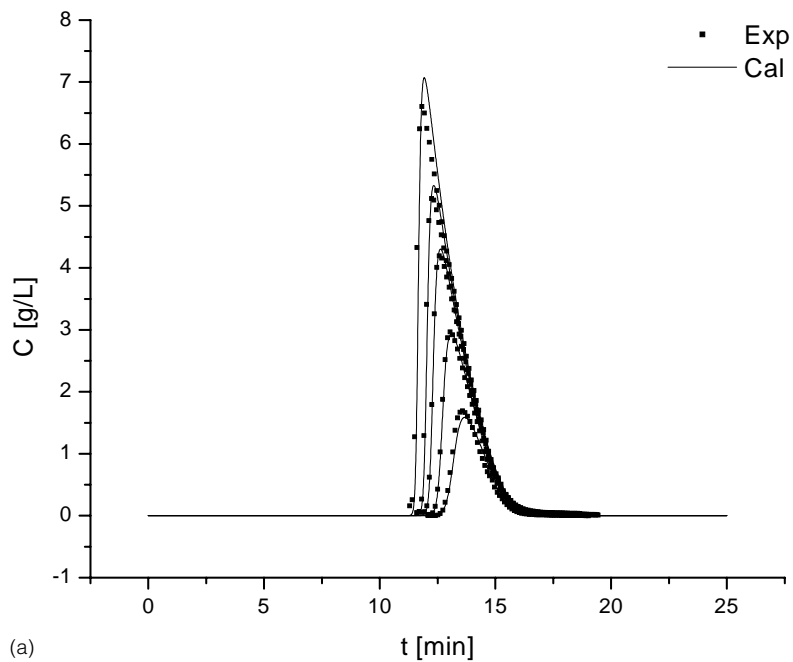
(a)

(b)

Fig. 3. Comparison of calculated (solid lines) and experimental (symbols) profiles for overloaded bands of R-1-indanol (a, $L_f = 1.0$, 1.9, 2.8, 3.6, and 4.9%), S-1-indanol (b, $L_f = 0.48$, 1.3, 2.0, 2.9. 4.2). Other experimental conditions are in Table 2.

Table 2
Loading factors, L_f, and other experimental conditions for Fig. 3a and b

	C_0 (g/l)	t_{inj} (min)	L_f (%)
R-1-indanol (Fig. 3a)	2.0	1.33	1.0
	3.7	1.33	1.9
	5.6	1.33	2.8
	7.1	1.33	3.6
	9.7	1.33	4.9
S-1-indanol (Fig. 3b)	2.5	0.67	0.48
	6.7	0.67	1.3
	10.5	0.67	2.0
	15.2	0.67	2.9
	21.9	0.67	4.2

4.2. Competitive frontal analysis

4.2.1. Competitive equilibrium isotherms

The competitive isotherm data were measured with the racemic mixture of 1-indanol. These data were fitted to different competitive isotherm models.

Fig. 4 compares the experimental adsorption data (symbols), the best Langmuir isotherm (solid line), the best Bilangmuir isotherm (dashed line), the best Langmuir–Freundlich (dotted line), and the best Toth isotherm (dash-dotted line). The lines show data calculated from the best isotherm corresponding to the competitive model, using Eqs. (5)–(8), respectively. While the main figure shows all the data, the inset shows only the data at low concentrations ($C < 1.5$ g/l). The agreement between the experimental data on the one hand and the best Langmuir–Freundlich and Bilangmuir isotherms on the other are excellent in the whole concentration range. The competitive Langmuir and Toth isotherms do not account as well for the experimental data. A similar conclusion can be derived from the values of the Fisher test (see Table 3). These results are quite similar to those obtained with the same stationary phase but with a conventional or a semi-preparative column [19].

The best values of the parameters obtained for the four isotherm models and the corresponding Fisher
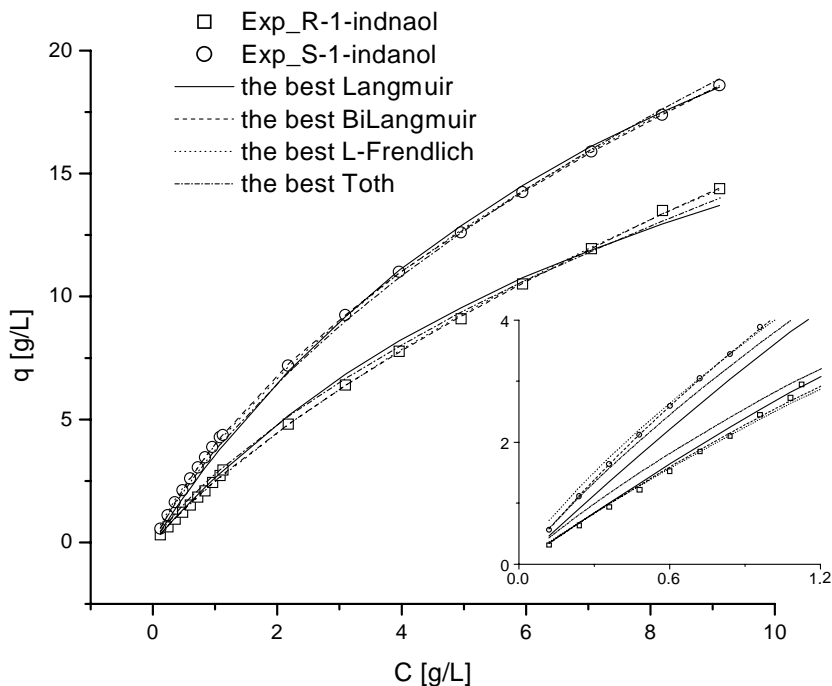

Fig. 4. Experimental isotherm data (symbols) obtained by competitive frontal analysis and best isotherms obtained by fitting these data to the Toth (dash-dotted line), the Bilangmuir (dashed line), the Langmuir–Freundlich (dotted line), and the Langmuir competitive isotherm models (solid line).

Table 3
Best estimates of the parameters of different competitive isotherm models and Fisher's test values

Isotherm type	Enantiomers	q_{ns} (g/l)	K_{ns} (l/g)	q_{es} (g/l)	K_{es} (l/g)	v	Fisher coefficient
Langmuir	*R*-1-indanol	68 ± 2	0.043 ± 0.003	NA	NA	NA	3.1×10^2
	S-1-indanol		0.058 ± 0.004	NA	NA	NA	
Bilangmuir	*R*-1-indanol	162 ± 25	0.091 ± 0.0019	13 ± 1	0.11 ± 0.00	NA	1.1×10^4
	S-1-indanol				0.27 ± 0.02	NA	
Toth	*R*-1-indanol	323 ± 220	0.014 ± 0.007	NA	NA	0.44	5.6×10^2
	S-1-indanol		0.018 ± 0.010	NA	NA		
Langmuir–Freudlich	*R*-1-indanol	114 ± 8	0.023 ± 0.002	NA	NA	0.93	3.8×10^3
	S-1-indanol		0.037 ± 0.003	NA	NA	0.82	

coefficients for the two compounds are reported in Table 3. The value found for the BL isotherm, 1.1×10^4, is very large, indicating an excellent fit, much better than those for the other three isotherms (Table 3). Accordingly, the BL model was used for band profile calculations for different mixtures of *R*-1-indanol and *S*-1-indanol. Note, however, a significant difference between the best numerical values of the coefficients of the isotherm obtained through the single-component fit and those obtained through the fit of the binary data, and an important confidence interval. These effects are related to the relatively narrow range of concentrations within which the experimental data could be acquired (100:1, see Figs. 1 and 4), itself limited by the low solubility of 1-indanol. In many earlier cases, data were acquired in a 1000:1 range of concentration. The set of data acquired here may be insufficient to warrant the use of multi-parameter models as complex as the Bilangmuir model and it might be more cautious to adopt a Langmuir model (for which the two effects observed, the significant differences between the numerical values of the best single-component and binary isotherms and fair precision of these values are not observed). The consideration of the Bilangmuir model is at the limit of overinterpretation of the data.

4.2.2. Validation of the competitive isotherm model

The best competitive Bilangmuir isotherm model was validated by using it to calculate the overloaded profiles of a series of samples of different sizes, some containing the racemic mixture, others made with different ratios of the two pure enantiomers, with relative compositions 1:3 and 3:1 of the *R*-1-indanol and

S-1-indanol. These calculated band profiles were compared to experimental band profiles obtained under the same conditions. The use of the ED model, rather than that of the more rigorous POR or GR models, is justified in this case by the relatively fast mass transfer kinetics observed in the system studied.

Fig. 5a–i compare to the experimental band profiles the profiles calculated for samples of different sizes of various mixtures of the two enantiomers of 1-indanol. These profiles were calculated with the best competitive Bilangmuir isotherm derived from FA data acquired with the racemic mixtures. The high concentration samples considered include the pure *R*-1-indanol or *S*-1-indanol (Fig. 5a and b), the racemic mixture (Fig. 5c–g) and two mixtures having different enantiomeric purities (approximate ratio 3:1 in Fig. 5h and 1:3 in Fig. 5i). In these calculations, we had to choose one plate number for the two compounds and used $N = 700$ theoretical plates as this unique plate number. The loading factors are between 1.0% (Fig. 5a) and 4.0% (Fig. 5g). The loading factors and the other experimental conditions are summarized in Table 4. The agreement between the experimental and the calculated profiles is always very good. The only significant discrepancies are in the profiles of the bottom of the valleys, which are generally predicted to be slightly less deep than they actually are, suggesting a slight underestimate of the mass transfer effect in the calculations. A slight model error is also introduced in the isotherm, as illustrated by the small difference between the isotherm parameters derived from the single-component data (Table 1) and the set of competitive data (Table 3). The consequence of this error is seen when com-
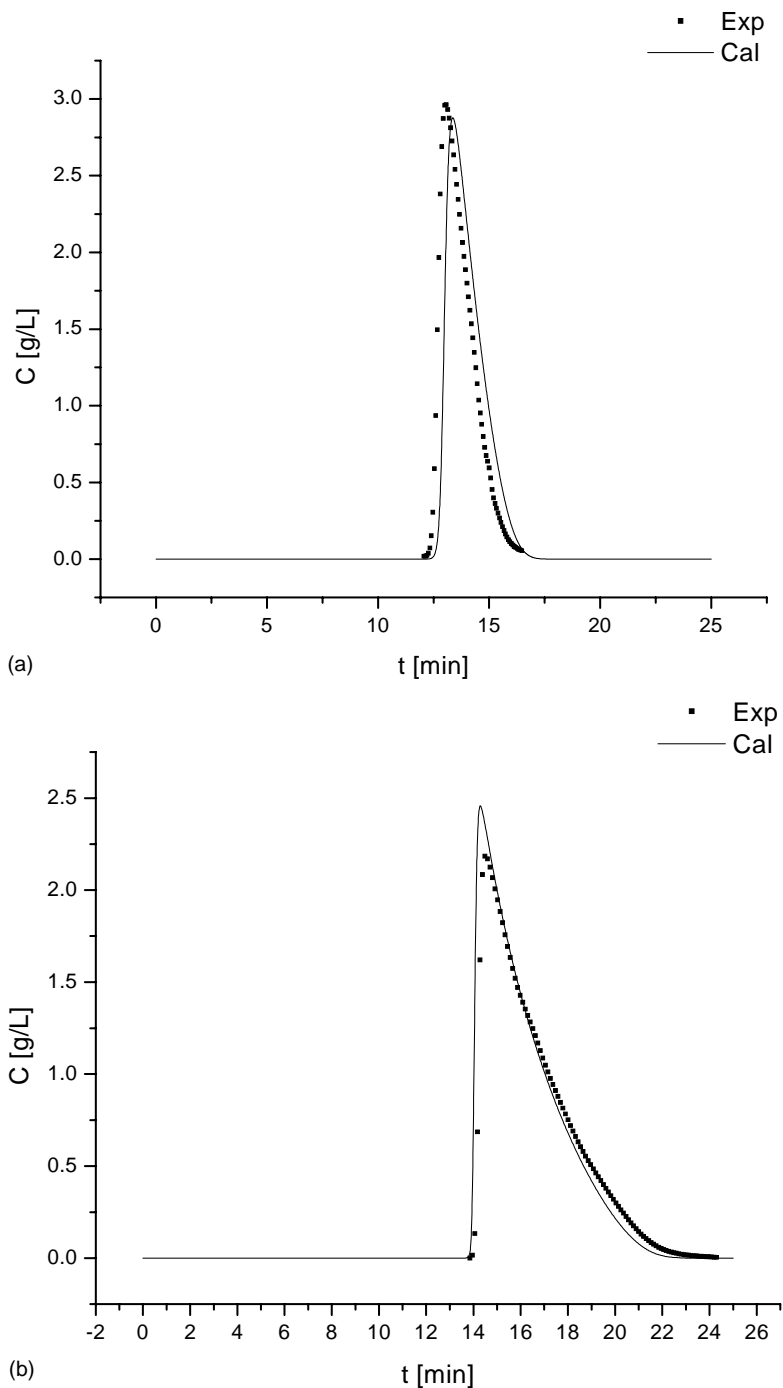
(a)

(b)

Fig. 5. Comparison of the experimental (symbols) and calculated (solid line) band profiles of various mixtures of the enantiomers of 1-indanol, including the pure *R*-1-indanol and *S*-1-indanol (a and b), the racemic mixture (c–g), and 1:3 and 3:1 mixtures (h and i). The loading factors are: 1.0, 1.4, 2.2, 2.5, 3.0, 3.7, 4.0, 2.6, 2.8%, respectively. The other experimental conditions are in Table 4.
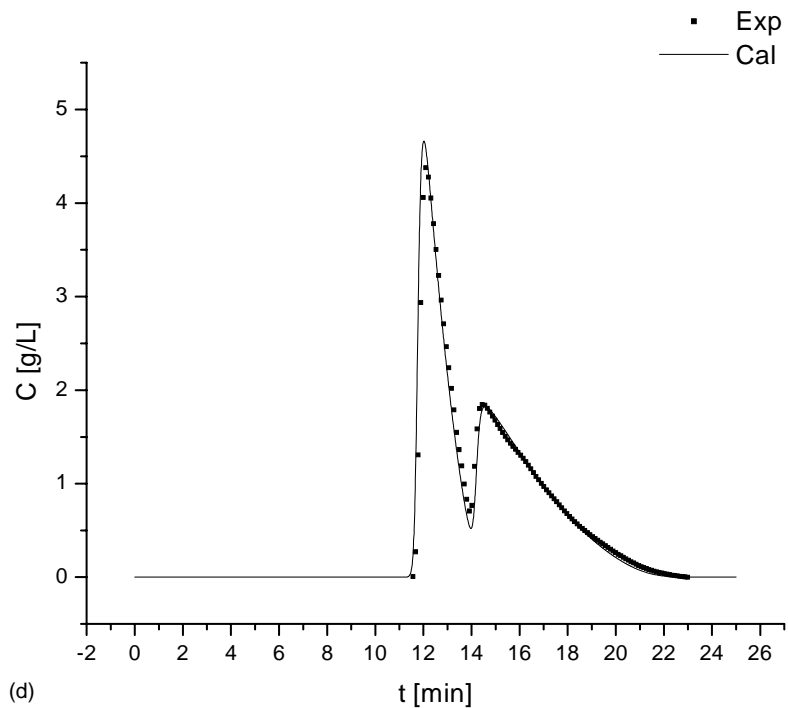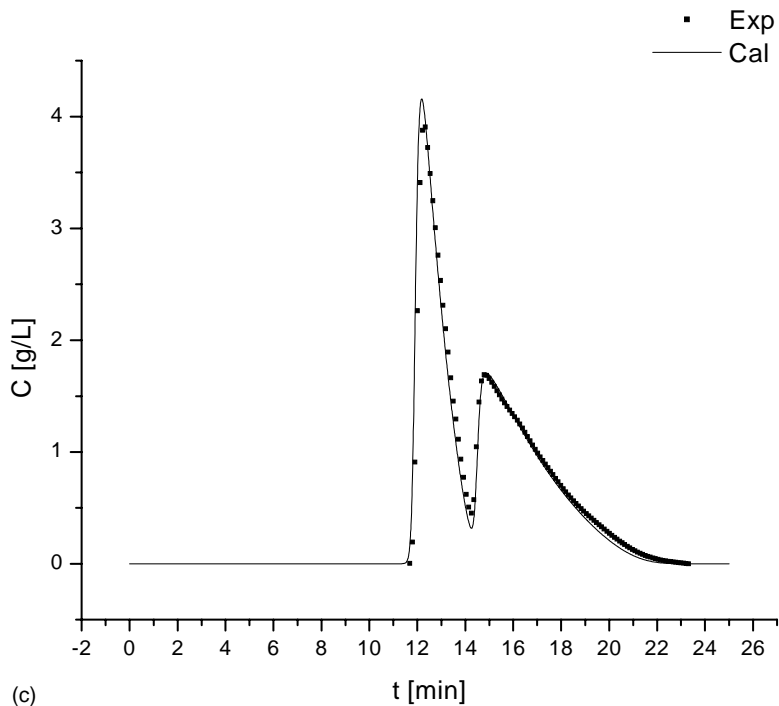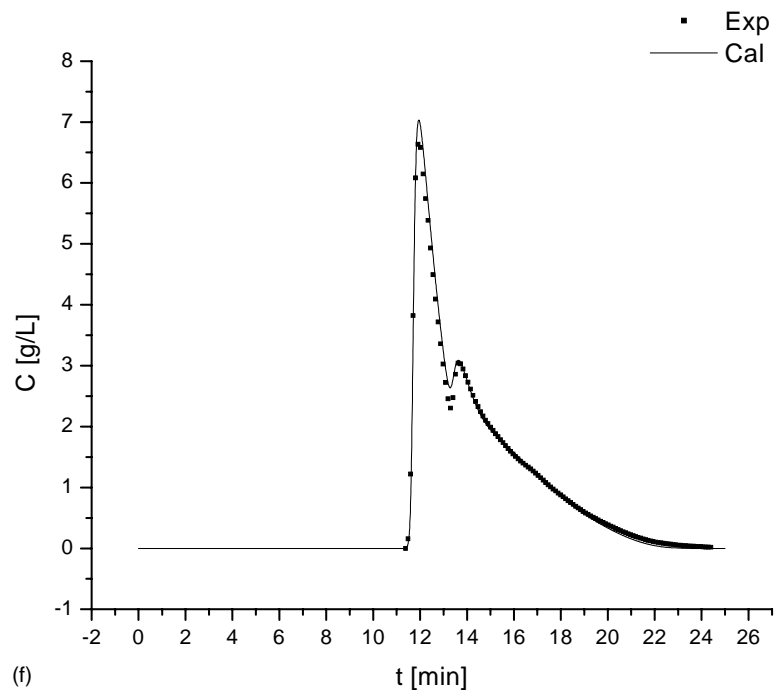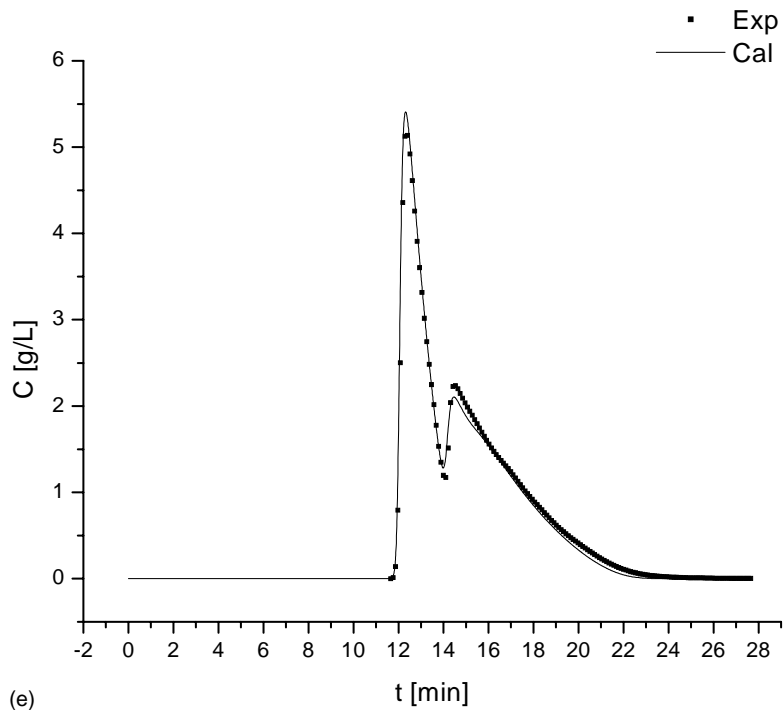
(c)

(d)

Fig. 5. (*Continued*)

(e)

(f)

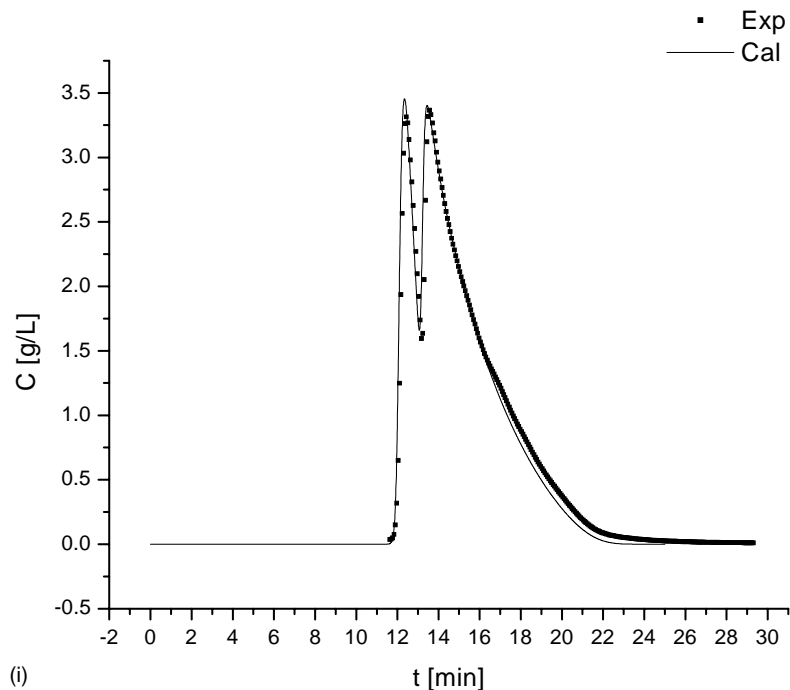Fig. 5. (*Continued*)

(g)

(h)

Fig. 5. (*Continued*)

Fig. 5. (*Continued*).

paring the single-component band profiles predicted with the best Bilangmuir models derived from the single-component data (Fig. 2a) and from the competitive data (Fig. 5a). This difference is small, however. The band profiles of the 3:1 and 1:3 mixtures are accurately predicted with the competitive isotherm and the differences observed in Fig. 5h and i are insignificant.

Table 4
Loading factors, L_f, and other experimental conditions for Fig. 5a–i

Fig. 5	C_r (g/l)	C_s (g/l)	t_{inj} (min)	L_f (%)
(a)	3.7	0	1.3	1.0
(b)	0	11	0.67	1.4
(c)	8.0	8.0	0.67	2.2
(d)	8.9	8.9	0.67	2.5
(e)	5.4	5.4	1.3	3.0
(f)	6.7	6.7	1.3	3.7
(g)	7.2	7.2	1.3	4.0
(h)	9.1	3.5	1.0	2.6
(i)	3.2	10.3	1.0	2.8

The importance of the consequences of the model error arising from the use of the competitive isotherm model to calculate single-component band profiles is further illustrated in Fig. 6 that compares the experimental profiles of large amounts of the two pure enantiomers and the profiles calculated using the competitive Bilangmuir isotherm model and the corresponding single-component Bilangmuir isotherm model, both combined with the ED model of chromatography. Both isotherm models predict the band profiles rather satisfactorily, although the competitive model underestimates slightly the retention time of the two enantiomers, by approximately 2 and 1% for the R- and the S-enantiomers, respectively. The Henry factors of R-1-indanol are 4.86 and 4.93, those of S-1-indanol 9.21 and 8.45 with the single-component and with the competitive Bilangmuir isotherms, respectively, with relative errors of 1.5 and 8.3% for R-1-indanol and S-1-indanol, respectively. These differences between the Henry factors are small for both enantiomers, specially for R-1-indanol. The difference is probably caused by a slight isotherm model error.
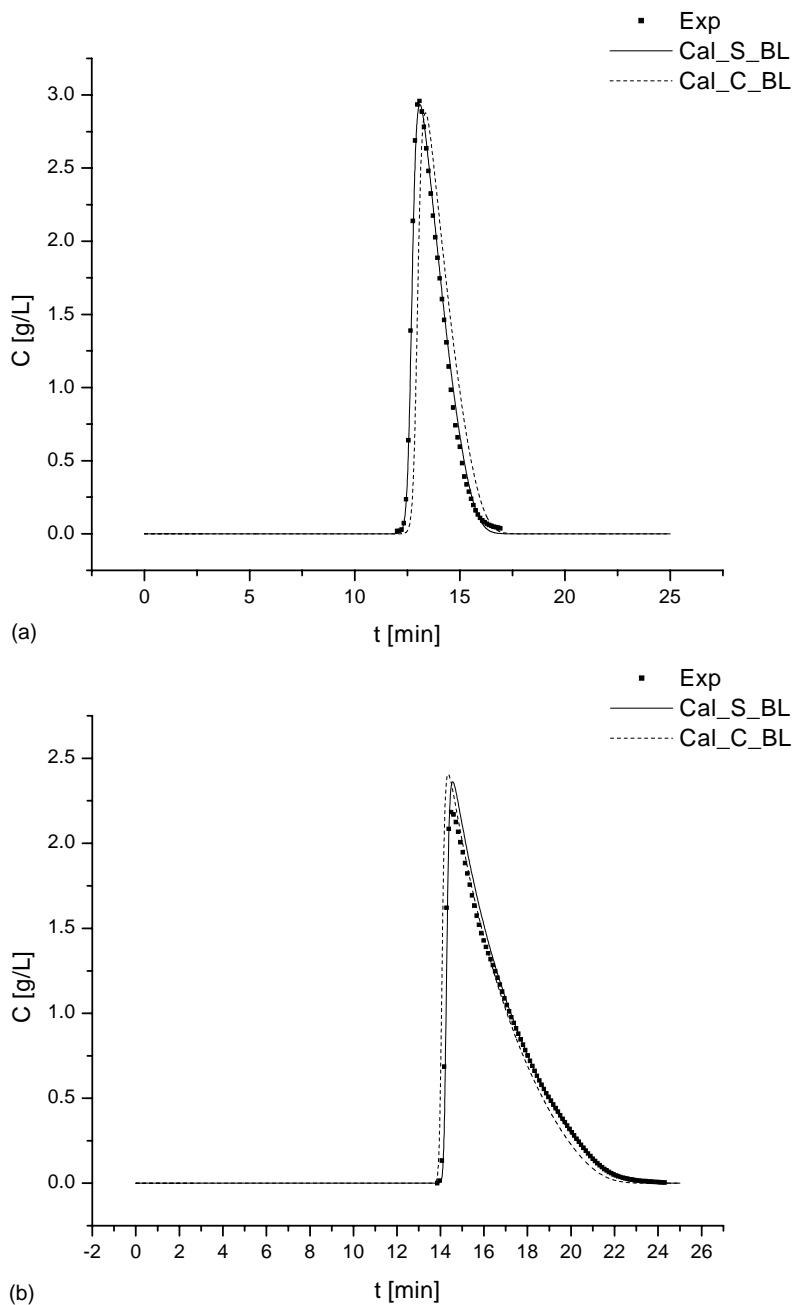
Fig. 6. Comparison of the experimental (symbols) profiles of samples of the pure enantiomers and of the profiles calculated using the competitive Bilangmuir isotherm model (dashed line) or the single-component Bilangmuir isotherm model (solid line). (a) *R*-1-indanol, (b) *S*-1-indanol.

5. Conclusions

The single-component and the competitive experimental isotherm data of *R*-1-indanol and *S*-1-indanol on Chiralcel OB are well accounted for by the Bilangmuir isotherm model, although the use of the Langmuir model gives also excellent results. The Bilangmuir model accounts often well for the data obtained in the study of enantiomeric separations [10,11, 17,19,30–33]. The single-component Bilangmuir isotherm model accounts well for the experimental isotherm data and the elution band profiles of the pure enantiomers. The competitive Bilangmuir isotherm model accounts well for the elution band profiles of a variety of enantiomeric mixtures, besides the racemic one, and accounts reasonably well for single-component band profiles. This general agreement between experimental and calculated profiles confirms again that the acquisition of competitive frontal analysis data only suffices often for the proper modeling of enantiomeric separations. Note, however, that accurate values of the numerical parameters of complex models such as the Bilangmuir model can be obtained only when the data are acquired in a wide concentration range. In many cases, the sample solubility limits this range. In such a case, the numerical values obtained have no physical sense.

Isotherm data can be obtained with a narrow bore column. This requires that more careful attention be paid to the control and to the accurate measurements of many experimental parameters. Most importantly, the column diameter has to be measured directly.

In the case in point, the simple ED model of chromatography gave calculated profiles that are in good agreement with those recorded experimentally. Although this model assumes instantaneous equilibrium of the sample between the mobile and the stationary phase and neglects the possible influence of the mass transfer resistances, lumping its effect into those of an axial dispersion, it accounts well in this case for results obtained with columns of only moderate efficiency (ca 700 theoretical plates).

Acknowledgements

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