Isotherm Selection for Band Profile Simulations in Preparative Chromatography

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Equilibrium adsorption data for the N-benzoyl derivatives of D- and L-phenyl alanine in water/propanol solutions have been determined on a chiral-selective stationary phase, bovine serum albumin immobilized on silica. These data are well accounted for by an isotherm equation resulting from the sum of two Langmuir terms. The first term corresponds to the chiral-selective interactions, and the second to the nonselective molecular interactions between the enantiomers and the stationary phase. The individual band profiles of the two enantiomeric amino acids, either pure or in binary mixtures of various compositions, are predicted exactly by an equilibrium dispersive model using this bi-Langmuir isotherm. A classical Langmuir isotherm fails to predict these band profiles, illustrating the importance to collect accurate adsorption data and to use a correct isotherm model when calculating chromatographic band profiles.

Introduction

There is a considerable interest in the development of the applications of preparative chromatography as a separation and purification method in the pharmaceutical and biotechnological areas (Verzele and DeWaele, 1986). When a new separation technique such as chromatography moves from laboratory to production scale, new problems arise, especially in the design of scaled-up units and in the optimization of their operating conditions. A relatively easy solution to these problems can be achieved if we are able to simulate the chromatographic process and to calculate the individual elution profiles of the feed components. Performing such calculations under a wide enough range of experimental conditions permits a rapid selection of the best experiments to perform and a considerable reduction in the time spent in method development compared to an empirical approach.

Many authors have investigated the simulation of chromatographic bands and a number of different models have been recently suggested (Phillips et al., 1988; Howard et al., 1988; Golshan-Shirazi et al., 1989; Yu and Wang, 1989). These studies are focused on the investigation of the mass transfer phenomena in chromatographic columns and the influence of their kinetics on the elution band profiles. Although this is an important aspect of the problem, it is not critical for most applications because chromatographers have developed a variety of phase systems where mass transfer kinetics is fast (Unger, 1979). With these systems, the band profiles can be correctly accounted for using the equilibrium-dispersive model, in which the effects of axial dispersion and mass transfer kinetics are lumped into a single apparent dispersion coefficient, a function of the mobile-phase velocity. We have shown theoretically that the band profiles of single component (Guiochon et al., 1988) and binary mixtures (Golshan-Shirazi and Guiochon, 1990b) can be calculated by numerical integration of the equations of the equilibrium-dispersive model, using a fast finite difference algorithm (Rouchon et al., 1987; Czok and Guiochon, 1990a). Experimental demonstration of the validity of the band profiles calculated has been given by Golshan-Shirazi and Guiochon (1988) for single-component bands, and by Katti and Guiochon (1990) and Jacobson et al. (1990) for binary mixtures.

The aim of this article is to show that the main difficulty in the prediction of individual band profiles in chromatography resides at present in the proper choice of the model accounting for the competitive equilibrium isotherm. In many cases, a Langmuir (1916) model is used for theoretical calculations (Helfferich and Klein, 1970; Guiochon and Ghodbane, 1988). The agreement between the band profiles calculated with Lang-

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muir isotherms and those determined experimentally is often excellent for single-component bands (Golshan-Shirazi and Guiochon, 1988), but it is no better than good for binary mixtures (Katti and Guiochon, 1990). In simple cases, a better agreement could probably be obtained by using an ideal adsorbed solution (IAS) isotherm, such as the one described by Levan and Vermeulen (1981), which corrects for the thermodynamic inconsistency of the Langmuir competitive isotherm when the column saturation capacity is different for the two components of a binary mixture. In many cases, however, the Langmuir isotherm model is too simplistic. When some chromatographic systems, for example, extensive band profile tailing is observed. The experimental profile returns to the base line more slowly than predicted by the Langmuir model. By using a two-site bi-Langmuir model, a simple extension of the Langmuir isotherm, this tailing is more easily prognosticated. The bi-Langmuir isotherm assumes that two sites coexist which are both independent and noncooperative (Graham, 1953; Andrade, 1985; Laub, 1986).

As a case in point, we discuss here a simple separation problem that stems from the rapidly growing interest to see production-scale separations of enantiomeric pairs reduced to practice. Over the past decade, analytical-scale chiral separations by liquid chromatography have become commonplace (Armstrong and Han, 1988; Pirkle and Pochapsky, 1989). The advent of a variety of chiral stationary phases, including those where proteins [e.g., bovine serum albumin, BSA (Allenmark et al., 1982)] are covalently bound to the surface of a porous silica support, has widened the range of antipodes that can be analyzed. Under analytical conditions, the BSA stationary phase has been thoroughly examined, especially by the group of Allenmark (Allenmark, 1982; Allenmark and Bomgren, 1982; Allenmark et al., 1983, 1984; Allenmark and Andersson, 1986; Allenmark et al., 1988). Of the several families of molecules that can be separated by the BSA stationary phase, the *N*-benzoyl derivatives of amino acids are easily resolved and are readily available. At high concentrations, the band profiles of the enantiomers of *N*-benzoyl-*D*- and l-alanine have been simulated and compared to the experimental profiles of pure components and binary mixtures (Jacobson et al., 1990).

The irregularity of the adsorption surface caused by the presence of BSA bound to a solid support provides an excellent opportunity to demonstrate the existence of multiple bonding adsorption sites and to investigate their influence on chromatographic band profiles at high concentrations. The hypothesis that two distributions of sites—corresponding to a chiral-selective retention mechanism and an achiral, nonselective retention mechanism, respectively—exist seems plausible considering the environment in which the adsorption processes occur. This article provides evidence that the two-site bi-Langmuir model predicts the elution band profiles more precisely than the single-site Langmuir model.

Theory

The calculation of individual band profiles in chromatography requires the integration of the system of differential mass balance equations for the components involved. This integration is possible if a relationship between the local concentrations of each component in the stationary and the mobile phases is available.

Equilibrium dispersive model of chromatography

The differential mass balance equation for one compound in a slice of the chromatographic column at a given time is written:

$$u\frac{\partial C}{\partial z}+F\frac{\partial Q}{\partial t}+\frac{\partial C}{\partial t}=D\frac{\partial^2 C}{\partial z^2} \tag{1}$$

We need a relation between C and Q to calculate solutions of the partial differential equation (Eq. 1). Several authors have used the following kinetic equation (Whitham, 1974; Phillips et al., 1988; Golshan-Shirazi et al., 1989):

$$\frac{\partial Q}{\partial t}=K(q-Q) \tag{2}$$

Equation 2 assumes that the rate of variation of the concentration of the stationary with respect to time, t, is proportional to the difference between the actual (Q) and the equilibrium (q) solute concentrations in the stationary phase. A more general rate equation model has also been used to simulate the column dynamics in the case of multicomponent systems (Howard et al., 1988; Yu and Wang, 1989).

Assuming a semi-equilibrium model, Giddings (1965) and Haarhof and Van der Linde (1966) have shown that under this condition where the mass transfer kinetics in the column is fast, Eqs. 1 and 2 can be combined to form:

$$u\frac{\partial C}{\partial z}+F\frac{\partial q}{\partial t}+\frac{\partial C}{\partial t}=D_a\frac{\partial^2 C}{\partial z^2} \tag{3}$$

where D_a is an apparent dispersion coefficient that accounts for the combined effects of the axial dispersion and the finite rate of the mass transfer kinetics. Under linear conditions, a Gaussian peak is obtained. Its variance is given by $\sigma^2=HL=2D_at$, where H is the height equivalent to a theoretical plate of the column of length L (Giddings, 1965). The value of the apparent dispersion coefficient is given by $D_a=Hu/2$.

Equation 3 contains q, given by the equilibrium isotherm, but no more Q. When the apparent dispersion coefficient is set equal to zero, Eq. 3 represents the ideal model of chromatography (Wilson, 1940; DeVault, 1943; Glueckauf, 1946; Rhee et al., 1970). The ideal model can be solved in closed form for one of two situations: (1) one component using any isotherm (Glueckauf, 1947; Golshan-Shirazi and Guiochon, 1990a) or (2) two components using competitive Langmuir isotherms (Glueckauf, 1946; Helfferich and Klein, 1970; Golshan-Shirazi and Guiochon, 1989). For other cases, a numerical solution of Eq. 3 has to be calculated (Golshan-Shirazi and Guiochon, 1990b). Various numerical schemes can be used. For example, the finite difference equation corresponding to Eq. 3 with $D_a=0$ is (Golshan-Shirazi and Guiochon, 1990b):

$$\frac{C_{n+1}^j-C_n^j}{\Delta z}+\frac{1}{u}\frac{C_n^j-C_n^{j-1}+F(q_n^j-q_n^{j-1})}{\Delta t}=0 \tag{4}$$

Calculation of a solution using this equation introduces a numerical error equivalent to an apparent dispersion. Analysis

of this truncation error (Lin et al., 1989) shows that the numerical dispersion is given by:

$$D_n = \frac{\Delta z u}{2}(a_c - 1) \tag{5}$$

The Courant number of the numerical calculation, $a_c = (u\Delta t)/[(1+k_0')\Delta z]$ (Courant et al., 1928), must be greater than 1 to insure convergence of the calculations. In the work reported here, we have used Eq. 5, with a value of the Courant number set equal to 2 and a space increment Δz equal to the height equivalent to a theoretical plate of the column under linear conditions, so that $D_n = D_a$. This procedure is equivalent to the mixing cell in series model. It also assumes that the diffusion coefficients do not vary significantly with concentration, which is true in the concentration range used in chromatography (1% to 5%), at least for the enantiomers considered here, albeit probably not for proteins. Other methods, by which to calculate solutions of Eq. 3, are available; yet, they yield similar results (Czok and Guiochon, 1990b).

The missing link in obtaining a solution is the development of a relationship between the solute concentrations in the mobile phase, C, and in the stationary phase, q, at equilibrium.

Adsorption isotherm

The equilibrium isotherm defines the amount of the solute in the stationary phase, q, as a function of the amount of the solute in the mobile phase, C. The classical model for nonlinear chromatography is the Langmuir (1916) adsorption isotherm:

$$q_x = \frac{a_x C_x}{1 + b_x C_x} \tag{6}$$

As we show later, the single-site Langmuir isotherm fails to fit the experimental data accurately. A Scatchard plot of the data suggests the presence of two independent types of adsorption sites (Andrade, 1985). The corresponding adsorption model is the two-site bi-Langmuir model (Graham, 1953; Andrade, 1985; Laub, 1986)

$$q_x = \frac{a_{x,1} C_x}{1 + b_{x,1} C_x} + \frac{a_{x,2} C_x}{1 + b_{x,2} C_x} \tag{7}$$

Detailed investigations have shown that the first type of sites are the chiral recognition sites carried by the BSA that is bound to the surface of the silica support (Jacobson et al., 1990). The second type of sites account for the interactions of the enantiomers with the nonchiral-selective part of the surface: siloxane groups, unreacted silanol groups, amino-acid substituents, and peptide bonds.

To characterize the adsorption of binary mixtures where the two components vie for access to the adsorbent, the competitive versions of the respective adsorption models are required. The competitive Langmuir is accounted for as (Schwab, 1928):

$$q_x = \frac{a_x C_x}{1 + b_D C_D + b_L C_L} \tag{8}$$

and the competitive bi-Langmuir as (Jacobson et al., 1990):

$$q_x = \frac{a_{x,1} C_x}{1 + b_{D,1} C_D + b_{L,1} C_L} + \frac{a_{x,2} C_x}{1 + b_{D,2} C_D + b_{L,2} C_L} \tag{9}$$

Since in the following we use the models with which the column saturation capacities are the same for both enantiomers, the Langmuir isotherms are thermodynamically consistent (Levan and Vermeulen, 1981). Finally, at high mobile-phase concentration, a monolayer coverage of the solute on the stationary phase known as the saturation capacity can be attained, and this limiting value is calculated by the following expression:

$$q_{s,x,y} = \frac{a_{x,y}}{b_{x,y}} \tag{10}$$

Experimental Studies

Equipment

The chromatographic experiments were performed on an HP 1090 Liquid Chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a diode array UV detector, a computer data acquisition system, and a multisolvent delivery system. Also, a Gilson 203 Fraction Collector (Middleton, WI) was used to complement the HP system. A Haake A81 Circulating Water Bath (Karlsruhe, Germany) was used to control the temperature (25°C) of the column during the entire analysis.

Chemicals

Column. A Resolvosil-BSA-7 column (Alltech, Deerfield, IL) was used. The column dimensions were 150 mm in length × 4 mm ID. The average particle size of the packing material was 7 μm and the average pore size 10 nm.

Materials. *N*-benzoyl-*L*-phenylalanine (Sigma, St. Louis, MO) and 1-propanol were used without further purification. *N*-benzoyl-*D*-phenylalanine was prepared in the laboratory following the method described by Allenmark (1983).

Mobile Phase. For all chromatographic experiments, the mobile phase was 0.1 M phosphate buffer aqueous solution (pH = 6.8) with a constant concentration of organic modifier, 7% 1-propanol (v/v).

Procedures

The elution profiles of the pure enantiomers obtained were converted by direct calibration of the detector response to a concentration profile. Since the response of the UV detector is the same for both enantiomers, only one calibration was necessary. The wavelengths were chosen so that the detector response was linear in the concentration range used.

To determine the individual concentrations of each of the antipodes in the mixed band region of the elution profile, fractions of the eluent were collected and reinjected on the same column under analytical conditions. Fractions were collected at 0.1-min intervals from the start of the elution of the first component through the end of the mixed zone and at 0.2-min intervals from just beyond the mixed zone to the end of the tail of the more retained enantiomer. The concentrations of each enantiomer at each collection time are calculated, and the elution trace of each enantiomer is derived. Note that because fractions must be collected and analyzed to determine the experimental band profiles and we can collect only ten

fractions per minute, it is not possible to obtain one fraction having the concentration corresponding to the peak maximum. This explains some minor discrepancies between the heights of the calculated profiles and those of the experimental profiles reported.

The adsorption isotherms of the two isomers were obtained by frontal analysis (James and Phillips, 1954; Schay and Szekely, 1954). Because the HP 1090 is not equipped with a large enough sample loop to handle profile measurements by frontal analysis in the standard fashion, the multisolvent delivery system was modified for the procedure as described in a previous work (Golshan-Shirazi et al., 1988). Since a broad concentration range was undertaken for the analysis, the adsorption profile for each isomer at each temperature was carefully divided into three smaller concentration ranges, and wavelengths from the diode array detector of 235, 270 and 280 nm were employed, depending on the concentration range under consideration.

The best-fit Langmuir and bi-Langmuir isotherm parameters were calculated using a SAS algorithm available on the University of Tennessee VAX 8800 computer. The procedure fitted the q/C vs. q data, weighing each data point accordingly. Linear regressions were carried out using a standard method.

Several additional parameters are required for the calculations. The flow rate was 1 mL/min, the dead time was 94 s, the column length was 15 cm, and the column efficiency was 700 plates for the enantiomers studied. The efficiency for a nonretained compound was 1,700 plates.

Results and Discussion

Isotherm model

The separation of enantiomers in chromatography requires the use of a chiral stationary phase. As shown in Pirkle and Pochapsky (1989), chiral recognition by a simple chemically-bonded phase needs the formation of a transient complex involving three nonplanar interactions between atoms of the solute close to its chiral group and atoms of the stationary phase close to one of its chiral centers. At least two of these interactions must be strong, such as hydrogen bonding. If the free energy of formation of the complex is different for the two enantiomers, they will be separated to a degree (Armstrong and Han, 1988; Pirkle and Pochapsky, 1989). The number of sites on the stationary phase available for the formation of the chiral recognition complex cannot exceed the number of chiral centers in the bonded phase.

However, in the case of a protein-bonded silica, such as the Bovine Serum Albumin bonded silica used here for the separation of enantiomeric amino-acids, two chiral recognition mechanisms have been suggested (Allenmark et al., 1982, 1983, 1984). Either the chiral carbon of the *N*-benzoyl amino-acid would form, as described above, a complex with the chiral carbon of one of the amino-acid residues of the bonded protein, or the amino-acid derivative would fit snugly inside a cavity between several such residues of the BSA. Both mechanisms are possible because a natural protein contains only [*L*]-amino-acids, which provides a chiral-selective environment. Some of the amino-acid residues, inside the globular structure of the protein, may not be accessible to the solutes. However, albumin is known to have large inner cavities that are readily accessible to hydrophobic molecules, which explains its role in the transfer of these compounds in the blood. The two mechanisms are not exclusive, but can coexist. Obviously, the former mechanism, which is the one operative with chiral-selective chemically-bonded phases (Pirkle and Pochapsky, 1989), would involve a higher interaction energy and provide a higher column saturation capacity than the latter.

We must also recognize that, besides the molecular interactions involved in the mechanism of chiral recognition, the solute molecules may undergo a variety of nonchiral interactions with a number of sites on the stationary phase, e.g., the patches of silica not covered by the protein or the nonchiral substituent groups of the amino-acid residues. Interactions between the protein and the enantiomeric solutes may also involve the nonchiral *N*-benzoyl group of the solutes (most nonderivatized amino acids are not retained on the BSA-silica column). These nonchiral interactions have much less stringent requirements regarding the steric orientation of the molecules than the formation of the chiral recognition complex (Allenmark et al., 1983; Armstrong and Han, 1988; Pirkle and Pochapsky, 1989). They are responsible for the retention on BSA of achiral solutes (e.g., *N*-benzoyl glycine).

Given this picture of the separation mechanism of enantiomers on the BSA bonded silica column, it is reasonable to assume a two-site adsorption model (Jacobson et al., 1990). The first site would have a high energy and a low saturation capacity and would account mainly for the first mechanism of chiral separation (complex formation). The second site would have a low energy and a high saturation capacity and would account primarily for the nonselective interactions between the sample components and the stationary phase. To some extent,

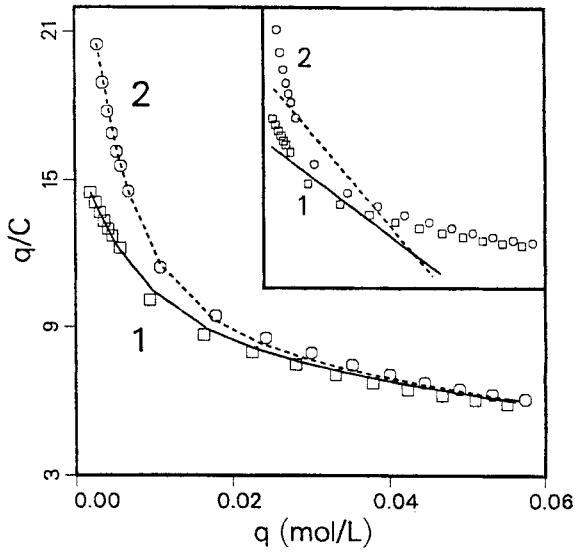

Figure 1. Scatchard plots of adsorption isotherms for (1) *N*-benzoyl-*D*-phenylalanine and (2) *N*-benzoyl-*L*-phenylalanine.

Experimental data (symbols) and best fit by each model.
For five parameter bi-Langmuir isotherm, see Eq. 7 for the isotherm and Table 2 for isotherm coefficients. On both types of sites, the column saturation capacities are the same for the two enantiomers.
For the inset of Langmuir isotherm, see Eq. 6 for the isotherm and Table 1 for isotherm coefficients.
Experimental conditions: stationary phase, immobilized BSA on silica; mobile phase, 0.10 M phosphate buffer with 7% *N*-propanol (v/v), pH 6.8; flow rate, 1 mL/min.

however, it could also account for the possible contribution of the second mechanism suggested in the chiral separation, the interaction between the enantiomers and several amino-acid residues of the BSA forming a suitable cavity. Since it is known that the BSA bonded columns have a low saturation capacity for chiral separations (Allenmark et al., 1988; Jacobson et al., 1990) and that the chiral recognition mechanism is saturated at rather low mobile-phase concentrations, we may assume a Langmuir model for each type of site (Eq. 7), although we know that in liquid-solid equilibria, this model is approximate in the best of cases.

We should further restrict the degree of freedom of the model by observing that the coefficients of the Langmuir term corresponding to the low-energy sites should be the same for the two enantiomers if there is no chiral selectivity involved and that the column saturation capacity for the high energy sites should be the same for the two enantiomers. This model has only five parameters. Because of the possibility of some residual interactions not accounted for in this first model, such as between the solutes and the chiral groups in the substituents of the amino acids of the proteins or with the cavities inside the BSA molecule, an additional degree of freedom could be provided by requiring only that each of the two sites has the same saturation capacity for the two enantiomers. This model would have six parameters. A nonrestricted bi-Langmuir model has eight parameters [and, different from the first two models, is not thermodynamically consistent (Levan and Vermeulen, 1981)]. We have used these three models and a single-site Langmuir model (Eq. 6) to account for the adsorption data and the chromatographic band profiles.

Determination of the isotherm

It was not possible to fit correctly the Langmuir model (Eq. 6) to the isotherm data in the whole concentration range. The reason why is clear from a Scatchard plot of these data (Figure 1, symbols). For a Langmuir isotherm, the plot of q/C vs. q should be linear (Andrade, 1985). The theory of chromatography shows that an elution profile depends only on the part of the isotherm in the concentration range between 0 and the largest concentration of the elution band, provided the isotherm has no inflection point (Aris and Amundson, 1973). Thus, the simple Langmuir isotherm was fit to the adsorption data in this narrower concentration range (Figure 1, inset and parameters in Table 1). The deviation between the experimental data in the selected range (symbols) and the calculated isotherms (straight lines 1 and 2) is significant, the deviation between the experimental data and the extrapolated isotherm is very important and the isotherms of the two enantiomers intersect, which is incorrect.

On the other hand, the flexibility of the five-parameter two-site model made it possible to calculate bi-Langmuir isotherm parameters using the entire concentration range in which adsorption data were measured, extending up to the injection concentration in the overloaded experiments (Figure 1, main). When fitting a model to experimental data, one can get different results depending on the relative weight given each data point, which in turn depends on the fitting procedure used (Andrade, 1985). Standard, nonlinear least-square regression of Eq. 7 proportionally weighs each point of the isotherm by its concentration in the mobile phase. This is inconvenient since in the present case we need the best possible accuracy at low concentrations. Because of the rigidity due to the lesser number of parameters, the quality of the five-parameter isotherm is much more sensitive to this effect than that of a six- or an eight-parameter isotherm. Thus, we have applied the standard SAS nonlinear least-square fit program to the regression of q/C vs. q, a procedure which gives a higher relative weight to the low concentration data points.

We have summarized in Table 2 the values obtained for the parameters of the five-, six- and eight-parameter bi-Langmuir isotherm models described above. Table 2 shows that there is physical continuity of the parameters of these three models. Figure 1 shows a plot of the five-parameter isotherm (lines), together with the experimental data (symbols). Excellent agreement is observed at low concentrations, while the agreement remains satisfactory in the high concentration range. The excellent agreement between experimental data and calculated isotherm extends to the whole concentration range with the six- and eight-parameter models.

As expected, the selective site parameters are quite different for the two enantiomers (Table 2). Both b coefficients are large and the saturation capacity is small, confirming that the sites capable of chiral recognition by complex formation are few and easily saturated. The low density of these selective sites is easily explained by the combined effect of the moderate concentration of chiral centers in the amino-acid residues of the protein and the strict steric requirements for the formation of the chiral recognition complex. Once the selective sites are saturated, adsorption takes place on the nonselective sites. This is confirmed by the substantial decrease in the slope of the isotherm, requiring the assistance of at least one additional parameter (five-parameter model) to match the changing conditions and achieve a good fit of the data in a wide concentration range. The nonselective sites have a saturation capacity 40 times greater than the selective sites, which explains why the column is overloaded with much smaller sample sizes for

Table 1. Langmuir Isotherm Parameters

Isomer	a	b L/mol	q_s mol/L
D	13.1	241	0.054
L	17.0	374	0.046

Table 2. Bi-Langmuir Isotherm Parameters

Parameters	Site	Isomer	a	b L/mol	q_s mol/L
5	Selective	*D*	9.13	1,640	0.00556
		L	20.1	3,610	
	Nonselective	*D*	7.09	32.7	0.217
		L			
6	Selective	*D*	9.47	1,900	0.00500
		L	20.0	4,010	
	Nonselective	*D*	7.15	36.6	0.195
		L	7.62	39.1	
8	Selective	*D*	9.71	1,640	0.00593
		L	20.1	4,220	0.00477
	Nonselective	*D*	6.67	27.7	0.241
		L	7.82	43.2	0.181

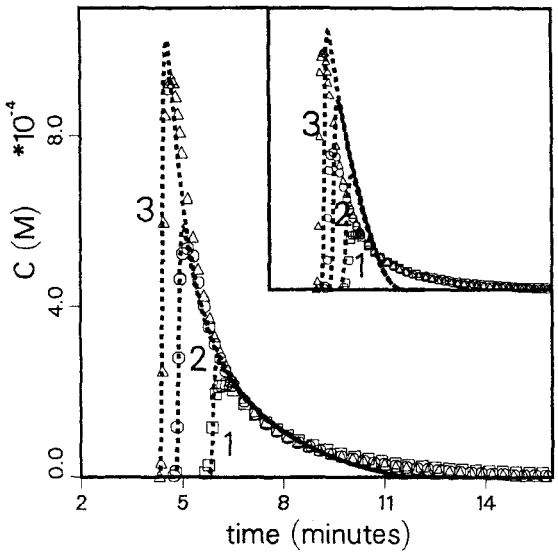

Figure 2. Experimental chromatograms (symbols) vs. calculated band profiles (lines) for *N*-benzoyl-*L*-phenylalanine.

The band profiles are calculated using the five-parameter bi-Langmuir isotherm (Figure 1). See Eq. 7 for the isotherm and Table 2 for the isotherm coefficients.
Sample size: (1) 0.494 μmol, (2) 0.989 μmol, and (3) 1.48 μmol. Same experimental conditions as for Figure 1.
Inset is the same as main figure, but the band profiles are calculated using the Langmuir isotherm. See Eq. 6 for the isotherm and Table 1 for the isotherm coefficients.

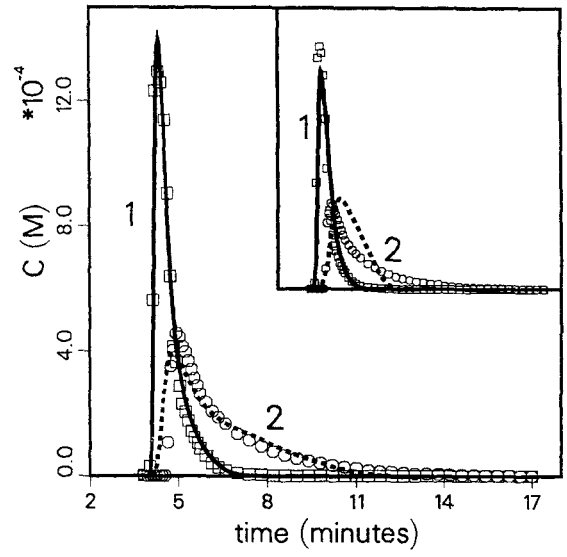

Figure 3. Experimental chromatograms (symbols) vs. calculated band profiles (lines) for a 1/1 binary mixture for *N*-benzoyl-*DL*-phenylalanine.

The band profiles are calculated using the five-parameter competitive bi-Langmuir isotherm. See Eq. 9 for the isotherm and Table 2 for isotherm coefficients.
Sample size: (1) 0.966 μmol-*D*-isomer and (2) 0.966-μmol *L*-isomer. Same experimental conditions as for Figure 2.
Inset is the same as main figure, but the band profiles are calculated using Langmuir isotherm (curves 1, 2, inset of Figure 1). See Eq. 8 for the isotherm and Table 1 for the isotherm coefficients.

chiral separations than for the separation of less closely related isomers.

The nonselective site parameters are expected to be identical for two enantiomers. Even with the eight-parameter model, with which all the parameters can float freely to optimize the fit, these parameters are close, although slightly greater for the more retained *L*-isomer, and the saturation capacities are not significantly different. The results obtained with the five-, six- and eight-parameter models yielded similar simulated chromatograms, suggesting that the difference between the parameters they provide is probably due to experimental errors in the adsorption isotherms.

When multiparameters models are used to fit data, results are poor if the number of parameters is insufficient. If these parameters are too many, the improvement is cosmetic at best, while, at worst, problems of indetermination arise. The eight-parameter model shows marginal improvements of the agreement with experimental isotherm and band profiles over the six-parameter model. The five-parameter model accounts well for the band profiles that are quite sensitive to the shape of the low concentration region of the isotherm. It performs much better than the competitive Langmuir model, demonstrating the sound physical basis of the two site model in the present case. Whether an additional parameter (i.e., in a six-parameter bi-Langmuir model) is needed to account for the isotherm at high concentrations and whether this parameter would account only for the concentration dependence of the activity coefficients of the enantiomers or also for chiral recognition by the second mechanism suggested by Allenmark et al. (1982, 1983, 1984) are yet unsolved questions under investigation. They are not directly relevant to the separation of the enantiomers by preparative chromatography, since multicomponent band profiles are correctly accounted for with a five-parameter model (see next section).

Study of band profiles

For all band profile calculations we have used only the competitive Langmuir isotherm and the five-parameter bi-Langmuir isotherm.

In Figure 2, we compare the experimental band profiles obtained for samples of three different sizes of the *L*-isomer with those calculated using both the bi-Langmuir and the Langmuir isotherms (Figure 2 inset). Similar results (not shown) were obtained with the lesser retained *D*-isomer. The agreement with experimental data is poor for the profiles calculated using the Langmuir isotherm. It is nearly perfect for the band profiles calculated using the bi-Langmuir model. The calculated band front elutes exactly on time, and the tails of the simulated profiles coincide with the experimental points. This result provides basic evidence that more than one mode of retention is present.

A more difficult test for the validity of band profile calculations is provided by the elution of binary mixtures of the two isomers under competitive conditions, i.e., at high concentrations. In the separation of enantiomers, the most important composition to study is the racemic or 1/1 (amount of *D*-isomer to amount of *L*-isomer) mixture (Figure 3). Two other mixtures were also studied, the 1/4 (Figure 4) and the 4/1 mixtures (Figure 5), to provide further evidence that the isotherm models as well as the finite difference procedure for the calculation of band profiles are valid. In each figure, we show the experimental data points obtained by fraction col-

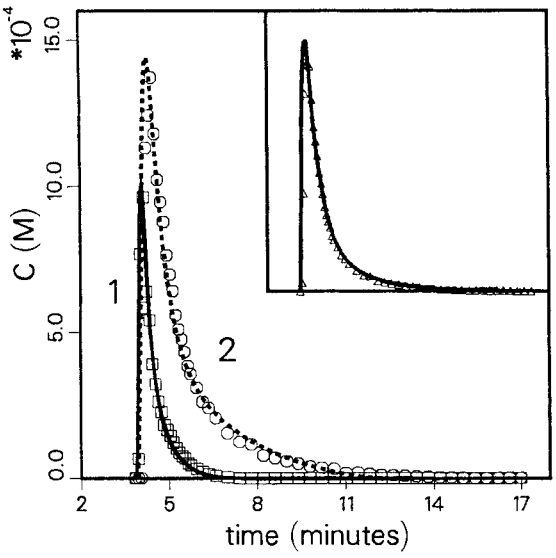

Figure 4. Experimental chromatograms (symbols) vs. calculated band profiles (lines) for a 1/4 binary mixture of *N*-benzoyl-*DL*-phenylalanine.

Band profiles calculated using the five-parameter competitive bi-Langmuir isotherm. See Eq. 9 for the isotherm and Table 2 for isotherm coefficients.
Sample size: (1) 0.532-μmol *D*-isomer and (2) 1.99-μmol *L*-isomer.
Same experimental conditions as for Figure 1.
Inset is the total chromatogram of the 1/4 binary mixture comparing the concentration profile derived from the detector response and the detector calibration curve (line) and the concentration profile obtained from the analysis of the collected fractions (symbols).

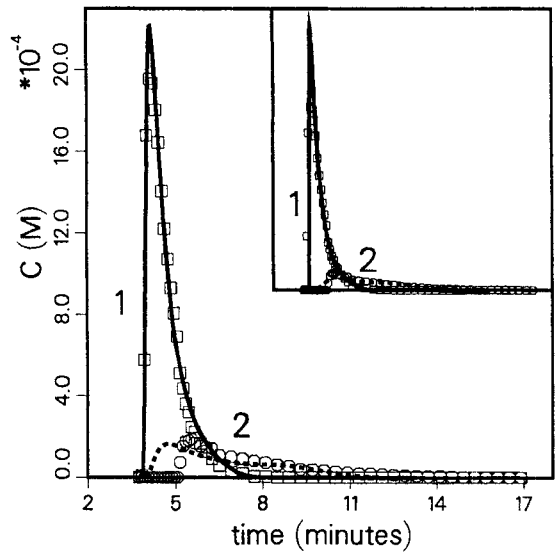

Figure 5. Experimental chromatograms (symbols) vs. calculated band profiles (lines) for a 4/1 binary mixture of *N*-benzoyl-*DL*-phenylalanine.

Band profiles calculated using the five-parameter competitive bi-Langmuir isotherm. See Eq. 9 for the isotherm and Table 2 for isotherm coefficients.
Sample size: (1) 2.03-μmol *D*-isomer and (2) 0.560-μmol *L*-isomer.
Same experimental conditions as for Figure 1.
Inset is the same as main figure, except that the calculation is performed using the Craig algorithm.

lection and analysis (symbols) and the profiles calculated using the bi-Langmuir isotherm (main figures). In the figure inset we show an additional chromatogram for comparison. The binary mixture simulations incorporate the competitive formulations of their respective models, Eq. 8 for the competitive Langmuir model, and Eq. 9 for the competitive bi-Langmuir model, allowing competition between solutes for the two types of adsorption sites.

In Figure 3 (racemic mixture), the band profiles calculated using the five parameter bi-Langmuir isotherm fits the experimental data very well, except that the calculated peak of the *L*-isomer is slightly too short. The band profiles calculated with the Langmuir isotherm (inset) fit the experimental profiles poorly, especially for the *L*-isomer (curve 2). The same poor agreement (not shown) was observed between the experimental band profiles and the profiles calculated with the Langmuir isotherm in the case of the 1/4 and the 4/1 mixtures.

In Figure 4 (1/4 mixture), there is again an excellent agreement between the experimental results and the band profiles calculated with the bi-Langmuir isotherm. The inset of Figure 4 compares the total chromatogram as seen with a nonselective detector (line) with the chromatogram reconstituted from the analysis of the collected fractions (symbols). The detector response is converted to concentration units with the aid of the calibration curve. The agreement between the two sets of experimental data validates the method used for determining the individual band profiles in the mixed band region (Experimental Studies section). The same degree of agreement was also observed in the cases of Figures 3 and 5 (not shown).

Finally, in Figure 5 (4/1 mixture), an excellent agreement is observed in the case of the first component (*D*-isomer) between the experimental profile and the solution calculated with the bi-Langmuir isotherm. For the second component, the strong tag-along effect is well accounted for by the calculated profile that approaches correctly the proneness of the ideal plateau. These simulations falter, however, in the band front of the *L*-isomer (curve 2 in Figure 5). It has been shown that this is an artifact of the calculation procedure (Czok and Guiochon, 1990b). The amount of numerical dispersion introduced by the finite difference technique on the front of the second component is greater than needed, and this aberration is seen only in a situation where the tag-along effect dominates. The inset shows a calculation performed using the Craig algorithm, which is slower than the scheme of Eq. 4 (Czok and Guiochon, 1990b). The agreement is now excellent for the front of the second peak, but somewhat less satisfactory for the first peak that becomes too high. This result illustrates the problems encountered in the calculation of numerical solutions of the system of Eq. 3 for two components when a finite difference method is used (Czok and Guiochon, 1990b).

Conclusion

There is an excellent agreement between experimental and calculated individual band profiles for binary mixtures of enantiomeric compounds. This result demonstrates the validity, in high-performance liquid chromatography, of the assumption made regarding the way to account for the finite rate of mass transfers in the column, lumping the various contributions to band broadening into an apparent dispersion coefficient. This result illustrates also the critical character of a proper choice of the isotherm model used in the calculations.

However, our results are in a large part explained by the choice of an enantiomeric system. Then, the column saturation capacity is the same for both isomers, and the Langmuir model is thermodynamically consistent (Levan and Vermeulen, 1981). Also, the column saturation capacity for the chiral recognition sites is small. Accordingly, nonlinear effects take place at low concentrations, the column is overloaded with small samples, and deviations from the competitive Langmuir model which assumes ideal behavior of the solution are less important at low concentrations. For all these reasons, the sum of two Langmuir competitive isotherms constitutes a suitable model of the competitive interaction between the two enantiomers.

Based on our present understanding of the retention mechanism of enantiomers on chiral-selective phases (Armstrong and Han, 1988; Pirkle and Pochapsky, 1989), the obvious choice for the adsorption isotherm of *N*-benzoyl-*DL*-phenylalanine is the bi-Langmuir model. The same conclusion applies to other amino-acid derivatives (Jacobson et al., 1990). None of the elution profiles, either for the pure compounds or for binary mixtures of the enantiomers, can be described properly by the single-site Langmuir model. Most probably, this result remains valid for many other chiral separations. Finally, we see that the study of equilibrium isotherms permits a more refined investigation of the retention mechanisms.

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Notation

a = Langmuir isotherm coefficient
a_c = Courant number, equal to $(u\Delta t)/(1+k'_0)\Delta z$
b = Langmuir isotherm coefficient
C = solute concentration in the mobile phase
D = coefficient of axial dispersion
D_a = apparent diffusion coefficient
D_n = numerical diffusion coefficient
F = phase ratio of the column packing with $F=(1-\epsilon)/\epsilon$
H = height equivalent to a theoretical plate
K = mass transfer coefficient
k'_0 = retention or column capacity factor at infinite dilution
q = solute concentration in the stationary phase at equilibrium
Q = solute concentration in the stationary phase
t = time
Δt = time increment
u = mobile phase velocity
z = column position
Δz = space increment
ϵ = column packing porosity

Subscripts

x = L, D corresponding to the L-isomer and the D-isomer, respectively
y = 1, 2
1 = selective site of adsorption
2 = nonselective site of adsorption

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