ELSEVIER

Journal of Chromatography A, 905 (2001) 3–17

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Influence of the solute hydrophobicity on the enantioselective adsorption of β-blockers on a cellulase protein used as the chiral selector

Gustaf Götmar[a], Torgny Fornstedt[a], Marie Andersson[a], Georges Guiochon[b,*]

[a]*Department of Pharmacy, BMC, Box 580, S-751 23 Uppsala, Sweden*

[b]*Department of Chemistry, The University of Tennessee, Knoxville, TN, 37996-1600 and Division of Chemical and Analytical Science, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA*

Received 7 June 2000; received in revised form 13 September 2000; accepted 15 September 2000

Abstract

Adsorption isotherm data were acquired at different eluent pH values for the enantiomers of several β-blockers on cellobiohydrolase I on silica gel. They fit well to the biLangmuir model, allowing the determination of the equilibrium constants and the monolayer capacities for chiral and nonselective adsorption. The adsorption of the *S*-enantiomers (eluted second) is exothermic at low pH, endothermic at high pH, and athermal in a narrow pH range depending on the β-blocker. This transition pH range is lower for *S*-alprenolol than for the more hydrophobic *S*-propranolol, although their endothermic adsorption originates from hydrophobic interactions. This surprising observation is explained by the relative values of the isotherm coefficients. *S*-Alprenolol seems to have a more pronounced endothermic behavior than *S*-propranolol because the nonselective interactions of both compounds with the stationary phase are exothermic but their contribution to retention, relative to that of the endothermic chiral interactions, is less important for alprenolol. The order of increasing energy of the chiral interactions is the same as that of hydrophobicity, propranolol>alprenolol>metoprolol.

Keywords: Hydrophobicity; Enantiomer separation; Adsorption isotherms; Chiral stationary phases, LC; Immobilized proteins; β-Blockers; Alprenolol; Propranolol; Metoprolol

1. Introduction

A vast number of new chiral stationary phases (CSPs) for liquid chromatography were developed and studied during the last decade [1–9]. Unfortunately, although this principle has been validated beyond doubts in both HPLC [10–18] and GC [19], most of these investigations persist to ignore that chiral separations are generally carried out under such conditions that two retention mechanisms, one enantioselective, the other nonselective, coexist. Retention factors and even isotherms are often treated as if a selective retention mechanism only was involved [20–26]. More rigorous fundamental studies of the thermodynamics and kinetics of chiral separations by chromatography are needed for a better understanding of the chiral recognition mechanisms.

*Corresponding author. Tel.: +1-865-9740-733; fax: +1-865-9742-667.

E-mail address: guiochon@utk.edu (G. Guiochon).

0021-9673/01/$ – see front matter
PII: S0021-9673(00)00971-7

Most often, a CSP consists of an achiral matrix, e.g. porous silica, with bonded chiral ligands. These ligands can be small groups, e.g. Pirkle phases [1,2], or macromolecules such as cellulose derivatives [3] or proteins [5,6]. Protein ligands (e.g., α_1-acid glycoprotein [7–9], bovine serum albumin [5,10], ovomucoid [27], and α-chymotrypsin [28]) are most popular for bioanalytical applications since they give CSPs that are compatible with aqueous mobile phases. Cellobiohydrolase I (CBH I) immobilized on porous silica is the best CSP for resolving chiral drugs containing one or more basic nitrogen atoms and one or more hydrogen-acceptor or hydrogen-donor groups [29,30]. It gives the largest separation factors for almost all enantiomeric pairs of β-receptor antagonists (i.e. β-blockers), a group of amino alcohols. This last separation is particularly important because the two enantiomers of β-blockers often exhibit different pharmacological and metabolic behavior [31,32]. For example, L-propranolol is an efficient β-blocker while D-propranolol shows no such effects [31].

To better understand the mechanism of enantioseparations, thermodynamic data are needed. We showed previously that the adsorption isotherms of the enantiomers of propranolol on CBH I immobilized to silica are well described by a biLangmuir model [14]. One Langmuir term, the same for both enantiomers, accounts for nonselective interactions and the corresponding mass transfer kinetics is fast. The other term corresponds to enantioselective interactions and the kinetics is slow [14]. This heterogenous mass transfer kinetics explains the broad tailing peaks observed for the more retained enantiomer, even under analytical, linear, conditions [14]. The adsorption of *S*-propranolol on the chiral sites is endothermic and has an unusually high entropy. The adsorption of both enantiomers on the nonchiral sites is exothermic as is the adsorption of *R*-propranolol on the chiral sites [15]. This explains the exceptional temperature effect previously reported [25]. Finally, we also showed that the chiral sites are strongly ionic while the nonchiral ones are only weakly so [18] and that the number of nonchiral sites, the monolayer capacity of the chiral sites for *R*-propranolol, and the binding strength of *S*-propranolol to these chiral sites increase rapidly with increasing pH, between 4.7 and 6.0 [18].

There are some indications that hydrophobicity also plays a role in the chiral separations studied. Vandenbosch et al. found a correlation between the retention factors of the first eluted enantiomer of several compounds on a CBH I column and their hydrophobicity [33]. However, the use of retention factors does not allow accounting for the contribution of nonselective interactions, so the conclusion is misleading [14–18]. It was suggested by Henriksson et al. that at least one tryptophan group (Trp376) is involved in the chiral site [34,35]. The large positive entropy term obtained for the enantioselective interactions of *S*-propranolol strongly suggests that hydrophobic interaction is important for chiral recognition [15]. This result seems to contradict other experimental results. The separation factors are 5.95 and 3.24 at pH 5.0 and 9.74 and 5.83 at pH 7.0 for the enantiomers of alprenolol and propranolol, respectively [34]. The former (with a single aromatic ring) is less hydrophobic than the latter (with a naphthyl group). The aim of this work is to study the effect of hydrophobicity on the nonselective and the enantioselective interactions, to identify their origin, and to compare the relative importance of hydrophobic and ionic interactions in the retention.

2. Theory

Most adsorbent–adsorbate interactions are nonchiral and cannot distinguish the two enantiomers. We call the corresponding sites type-I sites, for the sake of brevity. The chiral selective sites, called type-II sites, are the parts of the protein molecules on which take place the interactions responsible for chiral recognition. The relationship between the equilibrium concentrations of a component in the stationary and the mobile phases at constant temperature is the adsorption isotherm equation. The contributions of type-I and type-II sites to this isotherm are additive, so the adsorption isotherms of the two enantiomers are the sum of two terms, accounting respectively for the contributions of the two types of sites. In most cases, this equation is:

$$q_1 = q_{1,\mathrm{I}} + q_{1,\mathrm{II}} = \frac{q_{1,\mathrm{I,s}} b_{1,\mathrm{I}} C_1}{1 + b_{1,\mathrm{I}} C_1} + \frac{q_{1,\mathrm{II,s}} b_{1,\mathrm{II}} C_1}{1 + b_{1,\mathrm{II}} C_1} \qquad (1)$$

$$q_2 = q_{2,\mathrm{I}} + q_{2,\mathrm{II}} = \frac{q_{2,\mathrm{I,s}} b_{2,\mathrm{I}} C_2}{1 + b_{2,\mathrm{I}} C_2} + \frac{q_{2,\mathrm{II,s}} b_{2,\mathrm{II}} C_2}{1 + b_{2,\mathrm{II}} C_2} \quad (2)$$

This isotherm model is the biLangmuir isotherm. C and q are the mobile and stationary phase concentrations, respectively, and q_s is the stationary phase concentration corresponding to a monolayer (surface completely covered), or specific saturation capacity of the stationary phase. The coefficient b (dimension of the reverse of a concentration) depends on the adsorption energy. The retention factor is related to the numerical coefficients of the biLangmuir isotherm by the following equation valid under linear conditions, i.e. at infinite dilution:

$$k' = F \frac{\partial q}{\partial C} = F \sum q_{\mathrm{i,s}} b_{\mathrm{i}} = F \sum a_{\mathrm{i}} \quad (3)$$

where a_i (i.e. $a_i = q_{i,s} b_i$) is the equilibrium or Henry constant; Σa_i is the initial slope of the adsorption isotherm, F is the phase ratio, with $F = (1 - \epsilon)/\epsilon$, where ϵ is the total porosity of the column. A general expression of the retention factors of the two enantiomers under linear conditions, i.e. at infinite dilution, can be derived from Eqs. (1–3), as follows:

$$k'_1 = k'_{1,\mathrm{I}} + k'_{1,\mathrm{II}} = F(q_{1,\mathrm{I,s}} b_{1,\mathrm{I}} + q_{1,\mathrm{II,s}} b_{1,\mathrm{II}}) = F(a_{1,\mathrm{I}} + a_{1,\mathrm{II}}) \quad (4)$$

$$k'_2 = k'_{2,\mathrm{I}} + k'_{2,\mathrm{II}} = F(q_{2,\mathrm{I,s}} b_{2,\mathrm{I}} + q_{2,\mathrm{II,s}} b_{2,\mathrm{II}}) = F(a_{2,\mathrm{I}} + a_{2,\mathrm{II}}) \quad (5)$$

When no isotherm data are available, the enantioselectivity must be characterized empirically, as the apparent separation factor of the two enantiomers, α_{app} (equal to α, the classical separation or selectivity factor). Using the initial slopes of the isotherms [see Eqs. (4) and (5)] this ratio is given by:

$$\alpha_{\mathrm{app}} = \frac{a_{2,\mathrm{I}} + a_{2,\mathrm{II}}}{a_{1,\mathrm{I}} + a_{1,\mathrm{II}}} \quad (6)$$

This factor characterizes the analytical separations given by a CSP. The contributions of type-II sites to the adsorption of the two enantiomers can be derived from the isotherms and the true chiral separation factor determined as $\alpha_{true} = a_{2,II}/a_{1,II}$. This latter value only is meaningful in a discussion of the mechanism of chiral recognition.

3. Experimental

3.1. Apparatus

The equipment was the same Shimadzu LC-10 system (Kyoto, Japan) as used in a previous study [18]. The column was kept at constant temperature with a circulating waterbath. The column hold-up volume, V_0, was determined to be 1.26 ml, derived from the elution time of the first buffer/water disturbance peak. The hold-up volume did not change with the mobile phase pH. All frontal analysis data were corrected for the dead volume contribution of the instrument and for the column hold-up volume. The total correction volume, V_T, was 1.58 ml (including V_0).

3.2. Chemicals

Astra Hässle (Mölndal, Sweden) kindly supplied 99% pure *R*-(+)- and *S*-(−)-metoprolol hydrochlorides, D-(+)- and L-(−)-alprenolol hydrogentartarate monohydrate. *R*-(+)- and *S*-(−)-propranolol hydrochloride (99% purity) were from Sigma (St. Louis, MO, USA). Acetic acid (>99.8%) and anhydrous sodium acetate (>99%) from Riedel-de-Haën (Seelze, Germany) were used as buffer salts. The water was from Millipore, Milli-Q grade. The stock solutions were filtered on 0.45-μm filters (Kebo, Spånga, Sweden) after dissolving the buffer salts.

3.3. Column and immobilization of the stationary phase

The protein CBH I, obtained from the filamentous fungus *Trichoderma reesei*, was immobilized on silica particles as described previously [14,30]. The material was then packed in a stainless steel column (100 mm×4.6 mm I.D.). The concentration of CBH I immobilized on the silica was determined by measuring the UV absorbance of the solution used at 280 nm, before and after reaction with aldehyde silica. A 50.7-mg amount of protein was bonded per gram of diol silica. The amount of protein in the column, 45.6 mg, was derived from the bonded protein/diol silica concentration and from the dry mass of packing material in the column.

3.4. Mobile phase

Three acetic buffers, prepared with pH values of 5.0, 5.5 and 6.0, were used as the mobile phase. Their ionic strength was kept the same, at $I = 0.10$, by using a constant concentration of sodium acetate (100 m*M*). The concentration of acetic acid needed to achieve the desired pH was calculated using the Henderson–Hasselbalch equation. A calibrated Metrohm 632 pH meter (Metrohm, Herisau, Switzerland) was used to measure the exact pH. In Tables 1–4 the exact values of the pH of the solutions used are reported. The volumetric flow-rate was 1.00 ml/min.

3.5. Procedures

The staircase frontal analysis and the calculation procedures used to acquire and model adsorption data was previously described [13,18]. All linear measurements were made twice successively, once with the racemic mixture, once with a pure enantiomer, allowing proper identification of the two peaks. Isotherm data were determined at each pH, in a concentration range extending between 0.25 μ*M* and 1.7 m*M* (29 data points), except for metoprolol for which the amount adsorbed was too low at the lowest pH.

Table 2
BiLangmuir isotherm parameters for *R*- and *S*-metoprolol at different eluent pH ($I = 0.10$)

Type of site	pH	a	RSD (%)	b (mM^{-1})	RSD* (%)	q_s (m*M*)
R, I	5.51	1.41	1.9	0.109	9.8	12.9
	6.02	1.75	2.4	0.157	8.4	11.1
S, I	5.51	1.38	4.3	0.114	16	12.1
	6.02	1.99	3.2	0.231	8.2	8.6
R, II	5.51	0.75	3.5	8.82	17	0.08
	6.02	1.10	3.0	7.24	15	0.15
S, II	5.51	1.30	3.6	3.85	11	0.34
	6.02	2.07	2.4	7.71	11	0.27

4. Results and discussion

4.1. CBH I protein as immobilized chiral selector

CBH I immobilized on silica gives a CSP that can separate many β-blockers, often with a high separation factor [26,29,30]. The most useful mobile phase is an aqueous buffer with a small concentration of an organic solvent, e.g. 2-propanol or acetonitrile. The retention time of the more retained *S*-enantiomer of a given β-blocker increases more rapidly with increasing mobile phase pH than that of the corresponding *R*-enantiomer, resulting in an enhanced enantioselectivity. The retention times of

Table 1
Analytical retention and separation factors of the *R*- and *S*-enantiomers of the β-blockers at different eluent pH ($I = 0.10$) and column temperatures

pH	T (°C)	Metoprolol			Alprenolol			Propranolol		
		k' (*R*)	k' (*S*)	α	k' (*R*)	k' (*S*)	α	k' (*R*)	k' (*S*)	α
5.03	10	0.77	0.83	1.08	1.44	2.29	1.59	5.20	6.01	1.16
	20	0.63	0.71	1.13	1.22	2.38	1.95	3.98	5.20	1.31
	30	0.54	0.63	1.17	1.05	2.42	2.30	3.16	4.70	1.49
	40	0.47	0.56	1.19	0.91	2.42	2.66	2.53	4.27	1.69
5.50	10	0.98	1.09	1.11	1.96	4.00	2.04	6.75	8.81	1.31
	20	0.83	1.01	1.22	1.68	4.55	2.71	5.26	8.36	1.59
	30	0.72	0.94	1.31	1.47	4.98	3.39	4.26	8.21	1.93
	40	0.63	0.90	1.43	1.30	5.26	4.05	3.50	8.08	2.31
6.00[a]	10	1.22	1.44	1.18	2.52	6.29	2.50	8.25	12.13	1.47
	20	1.04	1.40	1.35	2.23	7.89	3.54	6.60	12.71	1.93
	30	0.94	1.41	1.50	2.02	9.30	4.60	5.55	13.70	2.47
	40	0.84	1.42	1.69	1.81	10.44	5.77	4.73	14.56	3.08

[a] For propranolol pH 6.02.

Table 3
BiLangmuir isotherm parameters for *R*- and *S*-alprenolol at different eluent pH ($I = 0.10$)

Type of site	pH	*a*	RSD (%)	*b* (mM^{-1})	RSD* (%)	q_s (m*M*)
R, I	5.01	2.26	1.5	0.136	6.0	16.6
	5.51	2.66	2.8	0.154	9.1	17.3
	6.02	2.98	3.1	0.160	9.9	18.7
S, I	5.01	1.97	1.5	0.100	7.7	19.7
	5.51	2.47	1.0	0.139	4.7	17.8
	6.02	2.95	1.0	0.176	4.5	16.8
R, II	5.01	1.06	2.7	8.21	13	0.13
	5.51	1.85	2.9	6.10	13	0.30
	6.02	3.22	2.1	6.46	9.9	0.50
S, II	5.01	5.37	0.6	8.55	2.7	0.63
	5.51	12.4	0.8	21.0	1.9	0.59
	6.02	24.0	1.0	41.5	1.9	0.58

both β-blocker enantiomers increase with decreasing concentration of the organic modifier and with decreasing ionic strength of the buffer. Then, however, the separation factor remains approximately constant [26,29,30]. In the pH range 4–6, which is most suitable for enantioseparations of β-blockers, the molecule of CBH I (isoelectric point, p$I = 3.9$) has a net negative charge whereas the amine group of the β-blocker is protonated, giving a net positive charge to this molecule. Fig. 1 shows the structures of the three β-blockers studied. The pK_a values are 9.7, 9.65 and 9.45 for metoprolol, alprenolol and propranolol, respectively [36]. Thus, they are fully protonated under the conditions of this study. Their hydrophobicity increases from metoprolol to propranolol.

Table 4
BiLangmuir isotherm parameters for *R*- and *S*-propranolol at different eluent pH ($I = 0.10$)

Type of site	pH	*a*	RSD (%)	*b* (mM^{-1})	RSD* (%)	q_s (m*M*)
R, I	5.01	5.31	1.8	0.201	5.6	26.4
	5.51	6.17	2.1	0.216	6.2	28.6
	6.02	7.02	2.1	0.226	6.3	31.1
S, I	5.01	4.95	1.6	0.178	5.6	27.7
	5.51	6.47	1.6	0.253	5.2	25.6
	6.02	7.28	1.2	0.246	4.1	29.6
R, II	5.01	4.18	2.7	10.9	11	0.38
	5.51	6.37	2.3	10.8	9.9	0.59
	6.02	9.25	2.2	12.0	8.5	0.77
S, II	5.01	8.92	1.2	11.3	4.9	0.79
	5.51	17.3	2.1	24.4	5.3	0.71
	6.02	30.6	1.8	43.0	3.5	0.71

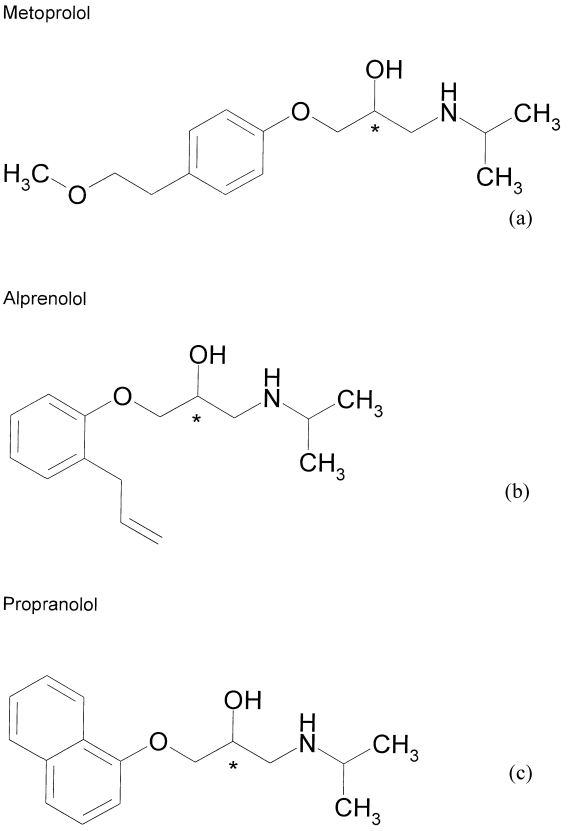

Fig. 1. Structures of the chiral solutes investigated in this study. The chiral centers are marked with asterisks.

4.2. Retention factors and apparent enantioselectivity in linear chromatography

We previously reported that the apparent separation factor of the two propranolol enantiomers increases rapidly with increasing mobile phase pH and column temperature [18]. Van 't Hoff plots of the retention factor of *S*-propranolol versus the column temperature exhibit a progressive change with increasing mobile phase pH, from being exothermic at low pH values to being strongly endothermic at high pH values. The behavior is nearly athermal at pH 5.4 [18]. In the present work, the ionic strength of the

mobile phase ($I = 0.1$) is five times higher than in our previous study (see the reason later). The retention factors of all compounds studied were acquired at three different mobile phase pH values, 5.0, 5.5 and 6.0, and at four temperatures, 10, 20, 30 and 40°C. The data are shown in Table 1.

The retention factors of both enantiomers of the less hydrophobic β-blocker, metoprolol, exhibit the same trend at pH 5.0 and pH 5.5, decreasing slowly with increasing temperature. This indicates a globally exothermic behavior. At pH 6.0, the same behavior is observed for *R*-metoprolol but the retention factor of *S*-metoprolol remains nearly constant (Table 1).

The retention time of *S*-alprenolol is nearly constant at pH 5.0 and increases with increasing temperature at the two higher values of the pH, suggesting an endothermic behavior under these conditions. The relative variation of the retention factor with temperature between 10 and 40°C increases with increasing pH. The retention factors of *R*-alprenolol show the same mild exothermic behavior at all pH values (Table 1).

In spite of the higher ionic strength of the mobile phase, the behavior of the most hydrophobic β-blocker, propranolol, is similar to that observed in our previous study [18]. At pH 5.0, the retention factor of both *R*- and *S*-propranolol exhibits a strong exothermic behavior, although it is less pronounced for *S*-propranolol than for the *R* enantiomer. At pH 5.5, the retention time of *S*-propranolol decreases by less than 10% when the temperature increases from 10 to 40°C, instead of 30% at pH 5.0. At pH 6.0, the retention time of *S*-propranolol increases significantly with increasing temperature. The retention factor of *R*-propranolol decreases in similar fashion with increasing temperature at all three pH values (Table 1).

A first interesting result is that *S*-alprenolol exhibits a stronger endothermic behavior than *S*-propranolol at all pH values. Also, alprenolol is the β-blocker with the highest apparent enantioselectivity (see Table 1). All apparent enantioselectivities increase linearly with increasing temperature at all pH values, but this increase is larger for alprenolol than for propranolol. Since the retention factors of *R*- and *S*-alprenolol have opposite temperature dependencies, the enantioselectivity increases considerably with increasing pH and temperature, from $\alpha_{app} = 1.59$ at pH 5.0 and 10°C to $\alpha_{app} = 5.77$ at pH 6.0 and 40°C (Table 1). A consequence of this rapid increase of the chiral selectivity with increasing pH and temperature is a reversal of the elution order of *R*-propranolol and *S*-alprenolol. At pH 5.0, *R*-propranolol is always eluted first, even at 40°C (Table 1). At pH 5.5, the reversal takes place between 20 and 30°C. At pH 6.0 the reversal takes place at an even lower temperature, between 10 and 20°C.

Thus, examination of the analytical data suggests that the adsorption behavior of the *S*-enantiomers of the β-blockers is exothermic at low pH and endothermic at high pH while that of the *R*-enantiomers is always exothermic. The narrow pH range in which this behavior shifts from exothermic to endothermic depends on the β-blocker. Whether it depends on the hydrophobicity is unclear. The pH range within which the thermal behavior of the retention shifts is around pH 5.5 for *S*-propranolol and around pH 6 for *S*-metoprolol but it is the lowest, around pH 5.0, for *S*-alprenolol, a compound less hydrophobic than propranolol. This result can be explained only after separating the contributions of the enantioselective and the nonchiral sites to the phase equilibrium.

4.3. Adsorption isotherms and enantioselectivity in nonlinear chromatography

4.3.1. Experimental precautions — ionic strength of the mobile phase

In previous studies, we used an acetate buffer (with $pK_a = 4.76$ [37]) at an ionic strength $I = 0.02$ [18]. This buffer has an insufficient buffering capacity for alprenolol at the highest pH. This arose from the combined effect of using a buffer with a low capacity ($I = 0.02$) at pH 6 (1.24 above pK_a) and of alprenolol being available as the hydrogen tartarate salt (an ampholytic protolyte) instead of the chloride (aprotic), as in the cases of metoprolol and propranolol. The only possibility to increase the buffer capacity without changing the type of buffer was to increase its ionic strength, which is why we used buffers at $I = 0.10$. Under these conditions, the passage of the breakthrough fronts of the alprenolol enantiomers did no longer affect significantly the mobile phase pH. The new ionic strength is the reason why we have to rerun the propranolol isotherms instead of comparing the isotherms of Ref.

[18] with new results of metoprolol and alprenolol. Although the presence of adsorbable additives in the mobile phase usually gives rise to system peaks [13], none were observed in this case. The concentrations of all the mobile phases used were 100 m*M* of acetate ions and 100 m*M* of sodium ions. Depending on the pH, they had different concentrations of undissociated acetic acid; at pH 5.0 the mobile phase contained 44.7 m*M* acetic acid, and at pH 6.0, 4.47 m*M*.

4.4. Experimental precautions — range of concentrations

We want to determine whether and how much the saturation capacity (i.e. $q_{i,s}$) and the adsorption energy (derived from b_i) corresponding to each type of adsorption sites vary, first with the hydrophobicity of the solute and second with the pH of the mobile phase. Earlier, we determined in which concentration range it was necessary to acquire isotherm data to determine with an adequate precision the different parameters of a biLangmuir adsorption isotherm for this type of CSP (i.e. an immobilized chiral protein). We found that a dynamic concentration range of at least 4000 was required [18]. The reason is that we need to make some measurements at mobile phase concentrations such that Γ ($=bC$) is very small compared to unity (linear conditions) and other measurements at concentrations for which Γ is sufficiently large compared to 1 (nonlinear conditions) [18]. The former measurements are needed to obtain an accurate estimate of the initial slope of the isotherm, the latter to determine an accurate estimate of the ordinate of the isotherm asymptote, i.e. of the saturation capacity.

With a biLangmuir isotherm there are two different values of Γ for the same concentration, C. In the present case, $\Gamma_I = b_{i,I}\ C_i$ for the nonchiral sites and $\Gamma_{II} = b_{i,II}\ C_i$ for the enantioselective sites. For a Langmuir isotherm, the ideal concentration range extends from $\Gamma < 0.01$ to $0.5 < \Gamma < 1$. A relative concentration range of between 50 and 100 is adequate provided the extreme concentrations are well chosen [13]. However, our previous results showed that for propranolol, the ratio $b_{i,II}/b_{i,I}$ is between 50 and 60 [14,15,18]. Accordingly, measurements must be made in a wide concentration range, of the order of 4000. Data acquisition was carried out in three different concentration ranges, 0.25–5 μ*M*, 5 μ*M* to 0.1 m*M* and 0.1–2.0 m*M*. In the lowest concentration range, the experimental data provide the sum of the two slopes, corresponding to the nonchiral and chiral terms [$a_{i,I} + a_{i,II}$, Eqs. (4) and (5)], the same result as the analytical data. They permit also a check of the linear behavior of the isotherm in this range, i.e. that the concentrations in this range are low enough to allow the determination of the isotherm data under linear conditions [18]. The data in the medium concentration range allow the determination of the values of the coefficient $b_{i,II}$ and give a rough estimate of the value of $q_{i,II,s}$. Finally, the data in the highest concentration range give a confirmation of the estimate of $q_{i,II,s}$ and allow the determination of $b_{i,I}$ and $q_{i,I,s}$ [18].

4.5. Experimental equilibrium isotherms

Figs. 2–4 show the isotherms of the three β-blockers included in this study, metoprolol, alprenolol and propranolol, at the different mobile phase pH values studied, pH 5.01 (Fig. 2), pH 5.51 (Fig. 3) and pH 6.02 (Fig. 4). Figs. 2a, 3a and 4a show the isotherm data in the low concentration range (concentrations from 0 to 5 μ*M*). Figs. 2b, 3b and 4b show the data in the medium concentration range (concentrations from 0 to 0.1 m*M*). Figs. 2c, 3c and 4c show the data in the high concentration range (concentrations from 0 to 1.71 m*M*). The symbols show the experimental data, o, for the *R*-enantiomers, *, for the *S*-enantiomers. The capital letters refer to the β-blockers; A, M and P for alprenolol, metoprolol and propranolol, respectively. The lines show the best biLangmuir isotherms obtained by regression of the experimental adsorption data to Eqs. (1) and (2). The dashed lines correspond to the *R*-enantiomers and the solid lines to the *S*-enantiomers. The best values of the parameters of the isotherm are reported in Tables 2–4 (for metoprolol, alprenolol and propranolol, respectively).

Equilibrium data were not included at pH 5.0 for metoprolol because the adsorption of both enantiomers was too small to be measured accurately (the retention factor of *R*-metoprolol was less than 1) [13]. Fig. 2 shows that the adsorption isotherms of the *R*- and *S*-enantiomers of alprenolol and proprano-

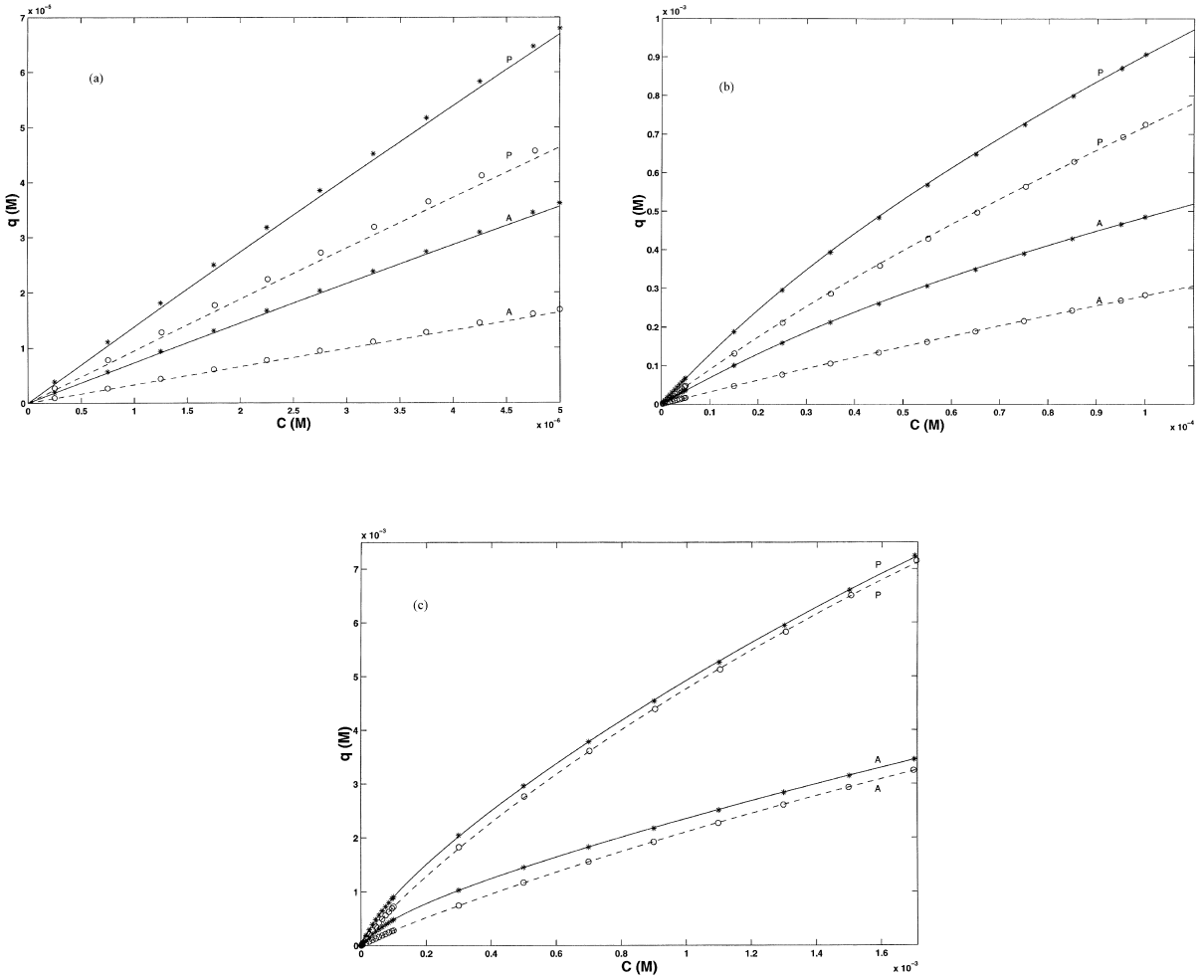

Fig. 2. Single-component equilibrium isotherms for *R*- and *S*-enantiomers of alprenolol and propranolol. Experimental conditions: column, 100×4.6 mm; stationary phase, immobilized CBH I on silica; mobile phase flow-rate, 1.0 ml/min. Symbols: experimental data, o, *R*-enantiomer; *, *S*-enantiomer. Lines: best calculated biLangmuir isotherms (parameters in Tables 3 and 4), dashed lines for the *R*-enantiomer, solid lines for the *S*-enantiomer. Eluent pH: 5.01. Analytes: *R*- and *S*-enantiomers of (A) alprenolol and (P) propranolol; (a) low concentration range, 0–5 m*M*, (b) medium concentration range, 0–0.1 m*M*, (c) high concentration range, 0–1.71 m*M*.

lol at pH 5.0 are linear in the low concentration range, i.e. below 5 μ*M* (Fig. 2a). The initial slopes of the isotherms increase from the alprenolol to the propranolol enantiomers and from the *R*- to the *S*-enantiomers at pH 5.0. The retention times of *R*- and *S*-metoprolol are higher at pH 5.5 and their adsorption data are included in Fig. 3. However, their separation factor is low (Table 1) and the two isotherms are close. At low concentrations, all isotherms are again linear at pH 5.5, or nearly so (Fig. 3a). Their initial slopes are all larger than at pH 5.0, particularly those of the *S*-enantiomers. The least hydrophobic β-blocker, metoprolol, has the lowest slope. Note also that at this pH, *S*-alprenolol is more retained than *R*-propranolol (Fig. 3a). Thus, like the elution order of the compounds (Table 1), the order of increasing initial slope of their isotherms depends as much on the configuration of the chiral center as on the hydrophobicity of the particular β-blocker studied. Similar observations can be made at the highest pH, 6.0 (Fig. 4a). However, the isotherms of *S*-alprenolol and *S*-propranolol exhibit a slight curva-

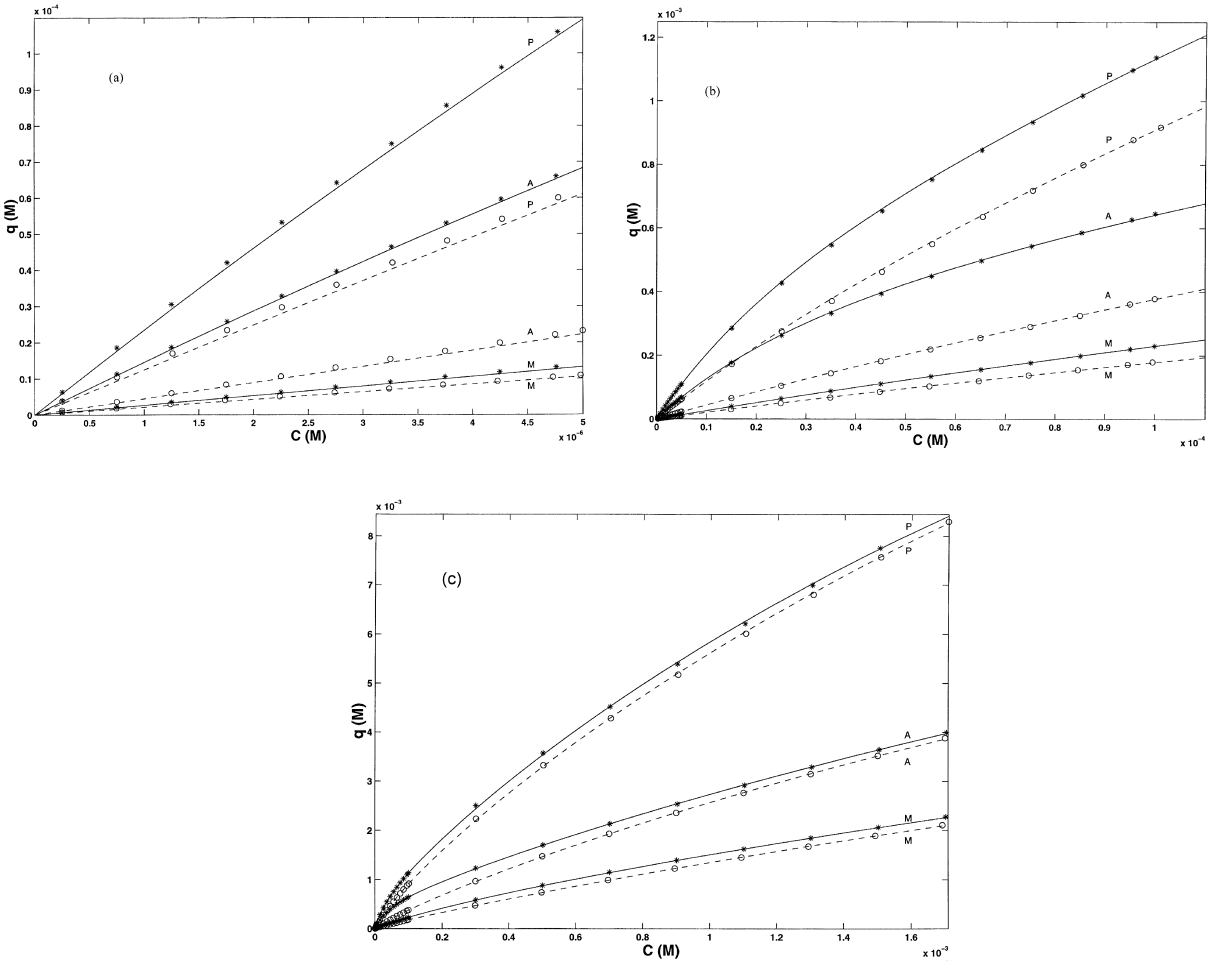

Fig. 3. Single-component equilibrium isotherms for *R*- and *S*-enantiomers of metoprolol, alprenolol and propranolol. Conditions as in Fig. 2. Symbols: experimental data, o, *R*-enantiomer, *, *S*-enantiomer. Lines: best calculated biLangmuir isotherms (parameters in Tables 2–4), dashed lines for the *R*-enantiomer, solid lines for the *S*-enantiomer. Eluent pH 5.51. Analytes: *R*- and *S*-enantiomers of (M) metoprolol, (A) alprenolol and (P) propranolol. (a) Low concentration range, 0–5 μ*M*, (b) medium concentration range, 0–0.1 m*M*, (c) high concentration range, 0–1.71 m*M*.

ture in the low concentration range. The initial slopes of all the isotherms are higher than at the two other pH values, particularly those of the *S*-enantiomers. The elution order of the six compounds is the same at pH 6.0 and at pH 5.5. but the separation factor of *S*-alprenolol and *R*-propranolol is higher at the former pH, in agreement with the results discussed earlier (Table 1).

Figs. 2–4 shows that the isotherms are no longer linear in the intermediate concentration range, i.e. between 0.005 and 0.1 m*M*. The curvature of the isotherms is more important for the *S*-enantiomers, particularly for *S*-alprenolol. At pH 5.5 the curvature of this last isotherm is so strong that it intersects the isotherm of *R*-propranolol, at $C \sim 18$ μ*M* (Fig. 3). The isotherms of the two metoprolol enantiomers seem to remain linear in this concentration range (Figs. 3 and 4). At pH 6.0 the curvature of the isotherm of *S*-alprenolol is unusually strong. This isotherm intersects that of *R*-propranolol at $C \sim 27$ μ*M* (Fig. 4). Although the isotherm of *S*-propranolol is more strongly curved at pH 6.0 than at pH 5.5 and

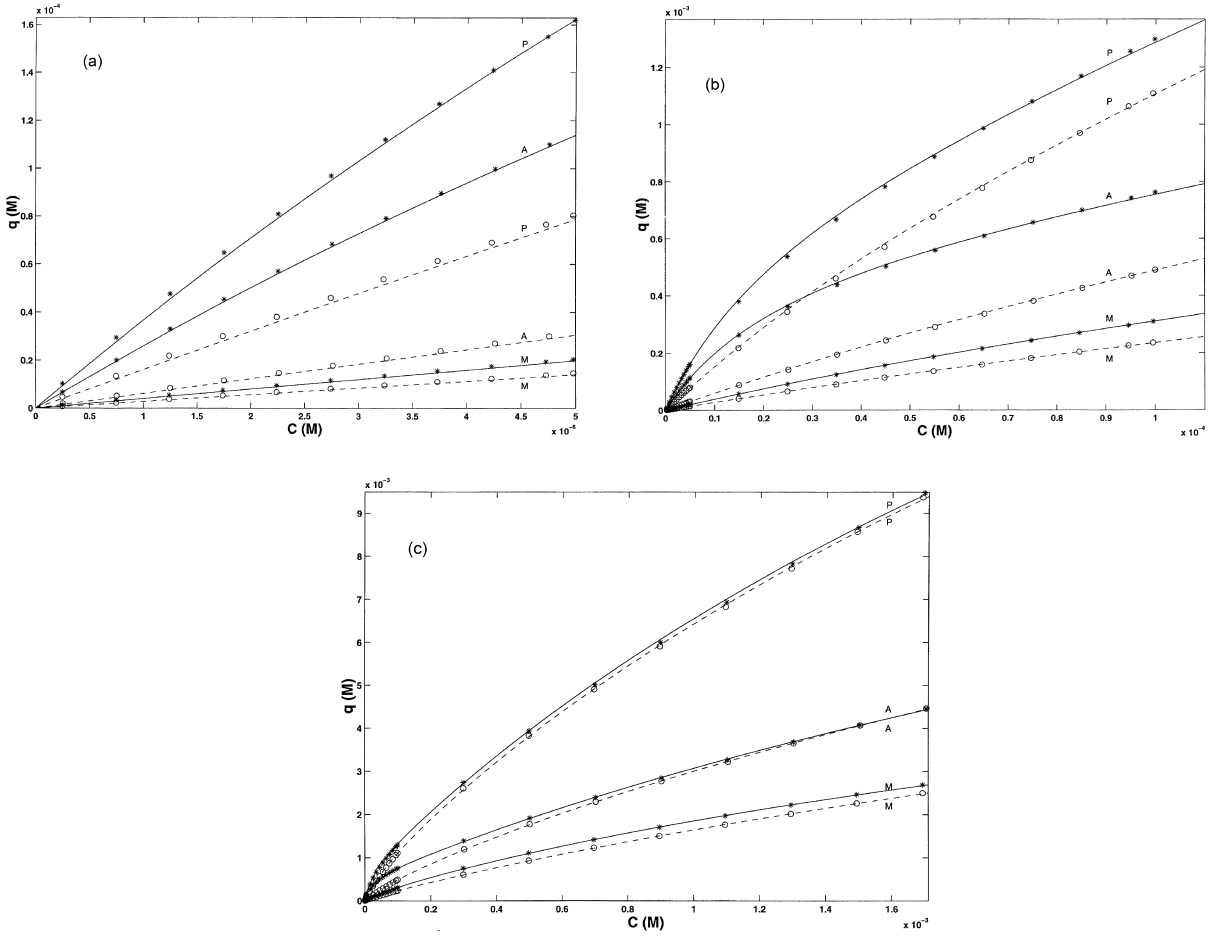

Fig. 4. Single-component equilibrium isotherms for *R*- and *S*-enantiomers of metoprolol, alprenolol and propranolol. Conditions as in Fig. 2. Symbols: experimental data, o, *R*-enantiomer, *, *S*-enantiomer. Lines: best calculated biLangmuir isotherms (parameters in Tables 2–4), dashed lines for the *R*-enantiomer, solid lines for the *S*-enantiomer. Eluent pH: 6.02. Analytes: *R*- and *S*-enantiomers of (M) metoprolol, (A) alprenolol and (P) propranolol. (a) Low concentration range, 0–5 μ*M*, (b) medium concentration range, 0–0.1 m*M*, (c) high concentration range, 0–1.71 m*M*.

5.0 (Figs. 2–4), it is always less curved than the isotherm of *S*-alprenolol at the same pH.

Figs. 2c, 3c and 4c show the isotherms of all the compounds studied in the high concentration range. The two isotherms of each pair of enantiomers become close and tend to be parallel (a characteristic feature of the biLangmuir model for enantiomers). The figures show that these isotherms are ordered, first, by the nature (i.e., the hydrophobicity) of the β-blocker, second, by the pH of the mobile phase, and, third, by the configuration of the chiral center. The resolution between the enantiomers of each pair disappears at high concentrations. These effects are due to the complete saturation of the selective sites [Eqs. (1) and (2)]. The isotherms of the two metoprolol enantiomers are significantly curved only at high concentrations.

4.6. Isotherm modeling — discrimination between different possible models

The experimental adsorption data of the β-blockers were fitted to three isotherm models, the biLangmuir [Eqs. (1) and (2)], the Langmuir and the

Freundlich isotherm equations [13]. Successful completion of a regression requires weighing the data points when the dynamic range is broad, as is the case here. For proper weighing of our experimental data, we used a weight equal to $1/q_{pred}$, where q_{pred} is the stationary phase concentration predicted by the model. This assumes that the relative error is constant, an assumption supported by all results obtained. Neither the Langmuir nor the Freundlich model fitted the data as well as the biLangmuir one. This was obvious from a visual comparison of the experimental data and the best isotherms (not shown).

That the biLangmuir model fits our data best is confirmed by the values of the residuals (Table 5) and by the conclusion of an *F*-test on the three models. In all cases, the values of the *F*-test were larger than 68 when comparing the results of the biLangmuir model to those of the Freundlich model and larger than 370 when comparing them to those of the Langmuir model. The critical value in this case (29 data points, 4 degrees of freedom for the biLangmuir model, 2 for each of the other two models) was 3.4. This result confirms the validity of our assumption of a two-site surface. Although such a surface is heterogeneous, it does not verify the Freundlich model which assumes infinite retention at infinite dilution, a result falsified by our experimental findings (note the obviously linear region of the isotherms at low concentrations). Finally, note that, although possible in principle, a triLangmuir model had to be ruled out. In spite of the acquisition of data corresponding to a wide range of concentrations, the regression did not give meaningful results. The nonlinear regression program issued a warning suggesting that there are too many parameters in the fitting equation and the estimates of the errors on some of the parameters are huge.

4.7. BiLangmuir parameters

The best values of the parameters of the biLangmuir model are listed in Tables 2–4. Each set of data was fit to Eqs. (1) and (2) using eight independent parameters in these equations. This procedure allowed the validation of the isotherm model since no assumption is made regarding the number and nature of the adsorption sites. Still the best estimates of the type-I parameters (a_I, b_I, $q_{I,s}$) obtained for each enantiomeric pair are close, with differences not exceeding a few percents. This demonstrates the validity of the model in our specific case. Because of the low retention factors of the two metoprolol enantiomers ($k' \sim 1$, see Table 1), their adsorption is low and the results do not have the same precision as for the other two β-blockers [13].

The nonchiral equilibrium constants (a_I-terms) of the three β-blocker pairs increase regularly with increasing mobile phase pH. For metoprolol, the coefficient a_I is 34% larger at pH 6.0 than at pH 5.5 (Table 2). For alprenolol, it is 40%, and for propranolol, 39% larger at pH 6.0 than at pH 5.0 (Tables 3

Table 5
Residuals in the modeling of the equilibrium data (sum of the weighed squared residuals)

	R (pH 5.0)	*S* (pH 5.0)	*R* (pH 5.5)	*S* (pH 5.5)	*R* (pH 6.0)	*S* (pH 6.0)
Metoprolol						
BiLangmuir	N.a.	N.a.	$5.99\cdot10^{-7}$	$3.77\cdot10^{-7}$	$6.33\cdot10^{-7}$	$9.87\cdot10^{-7}$
Freundlich	N.a.	N.a.	$3.86\cdot10^{-6}$	$2.03\cdot10^{-5}$	$1.28\cdot10^{-5}$	$3.48\cdot10^{-5}$
Langmuir	N.a.	N.a.	$1.87\cdot10^{-5}$	$2.68\cdot10^{-5}$	$2.62\cdot10^{-5}$	$5.39\cdot10^{-5}$
Alprenolol						
BiLangmuir	$4.43\cdot10^{-7}$	$3.01\cdot10^{-7}$	$8.25\cdot10^{-7}$	$5.52\cdot10^{-7}$	$1.14\cdot10^{-6}$	$1.14\cdot10^{-6}$
Freundlich	$1.03\cdot10^{-5}$	$6.65\cdot10^{-5}$	$2.46\cdot10^{-5}$	$8.51\cdot10^{-5}$	$4.16\cdot10^{-5}$	$8.28\cdot10^{-5}$
Langmuir	$2.26\cdot10^{-5}$	$2.18\cdot10^{-4}$	$4.31\cdot10^{-5}$	$6.25\cdot10^{-4}$	$9.00\cdot10^{-5}$	$1.30\cdot10^{-3}$
Propranolol						
BiLangmuir	$2.01\cdot10^{-6}$	$1.42\cdot10^{-6}$	$2.85\cdot10^{-6}$	$4.52\cdot10^{-6}$	$3.67\cdot10^{-6}$	$3.98\cdot10^{-6}$
Freundlich	$4.25\cdot10^{-5}$	$7.51\cdot10^{-5}$	$6.54\cdot10^{-5}$	$5.81\cdot10^{-5}$	$8.29\cdot10^{-5}$	$3.40\cdot10^{-5}$
Langmuir	$1.14\cdot10^{-4}$	$3.46\cdot10^{-4}$	$1.94\cdot10^{-4}$	$7.64\cdot10^{-4}$	$3.09\cdot10^{-4}$	$1.54\cdot10^{-3}$

and 4). The coefficient b_I is 74% larger at pH 6.0 than at pH 5.5 for metoprolol (Table 2). For alprenolol b_I increases 42% (Table 3) and for propranolol 24% (Table 4) between pH 5.0 and pH 6.0. The monolayer capacity of the nonchiral interactions, i.e. $q_I = a_I/b_I$, is nearly independent of the pH for metoprolol (Table 2) and alprenolol (Table 3) but increases slightly, by 12%, for propranolol (Table 4). So, for the two less hydrophobic β-blockers, metoprolol and alprenolol, the increase of the coefficient a_I with increasing pH can be explained mainly by an increase of the interaction energy. By contrast, for the more hydrophobic propranolol, the increase of a_I with increasing pH must be explained by an increase of both the interaction energy and the column saturation capacity, even if the former effect dominates. This is at variance with the results obtained at $I = 0.02$, a case in which the increase of the a_I term was mainly due to an increase of the saturation capacity with increasing pH [18].

For both enantiomers of each pair of β-blocker, the coefficients a_{II} increase rapidly with increasing pH. As was expected from the data on the retention factor under linear conditions (Table 1), the effect is stronger for the *S*-enantiomers, especially in the case of alprenolol and propranolol. The coefficients a_{II} of *R*- and *S*-metoprolol increase moderately, by 47 and 59%, respectively, when the pH increases from 5.5 to 6.0 (Table 2). By contrast, when the pH increases from 5.0 to 6.0, the coefficients a_{II} of *R*- and *S*-alprenolol increase three and nearly 4.5 times, respectively (Table 3). For *R*- and *S*-propranolol, the relative increases are comparable, slightly more than twice and nearly 3.5 times, respectively (Table 4).

The coefficients b_{II} of the three *R*-enantiomers are practically independent of the pH, within the precision of the measurements (Tables 2–4). This result is in agreement with our previous results on propranolol at a lower ionic strength [18]. This means that, for the *R*-enantiomers, the increase in a_{II} originates from an increase of the saturation capacity, $q_{R,II,s}$. For *R*-metoprolol, this capacity increases by 87%, for *R*-propranolol, it increases approximately twofold and, for *R*-alprenolol, it does so fourfold in the pH range studied (Tables 2–4).

The b_{II} coefficients of the three *S*-enantiomers increase still more rapidly with increasing pH. When the pH increases from 5.5 to 6.0, the b_{II} coefficient increases twice for *S*-metoprolol (Table 2). When the pH increases from 5.0 to 6.0 the b_{II} coefficient increases nearly five times for *S*-alprenolol and nearly four times for *S*-propranolol (Tables 2 and 4). The saturation capacities of the *S*-enantiomers are independent of the pH (Tables 2–4). This behavior is the same for all β-blockers. It is in agreement with our previous observations for propranolol at $I = 0.02$ [18].

In conclusion, it seems that, for the three β-blockers studied here, the number of chiral sites increases with increasing eluent pH for the *R*-enantiomers but remains constant for the *S*-enantiomers. By contrast, the interaction energy of the chiral sites remains constant for the *R*-enantiomers but increases considerably for the *S*-enantiomers. Under the same conditions, the monolayer capacity for the *R*-enantiomers seems to tend toward the value observed for the more strongly adsorbed *S*-enantiomers.

4.8. BiLangmuir parameters and characteristics of the isotherms

The accuracy of the isotherm parameters depends strongly on the width of the concentration range in which measurements of the amount adsorbed at equilibrium are made [18]. The data in the low concentration range give the sum of the nonchiral and the chiral *a* terms, i.e. $a_{i,I} + a_{i,II}$ [18]. This is also what linear chromatography gives, the total retention factor. The intermediate concentration range provides the interaction energies of the type-II sites, i.e. the values of the $b_{i,II}$terms, and an estimate of the monolayer capacities of the chiral sites, i.e. the of the $q_{i,II,s}$ terms. The data in the high concentration range give the values of the total monolayer capacity, i.e. $q_{i,I,s} + q_{i,II,s}$, and the parameters of the type-I sites, i.e. $b_{i,I}$ and $q_{i,I,s}$ [18].

In the low concentration range (Figs. 2a, 3a, and 4a), the isotherms are linear. Their initial slopes (equal to $a_{i,I} + a_{i,II}$, Tables 2–4) are larger for the *S*- than for the *R*-enantiomer. They increase with increasing hydrophobicity of the β-blocker, metoprolol < alprenolol < propranolol, and with increasing pH. The resolution of the *S*- and *R*-enantiomers under linear conditions increases with increasing pH. The only significant change in the elution order of the six compounds is the reversal in the elution order of *S*-alprenolol and *R*-propranolol between pH 5.00 and pH 5.50 (Figs. 2 and 3).

This reversal results from the different behavior of the initial slopes of the nonchiral and chiral isotherms. The nonchiral contributions increase only slightly, between 34 and 40% for the different β-blockers, with increasing pH. By contrast, the chiral contribution increases several fold. The initial slope of the isotherm of *S*-alprenolol increases more rapidly than that of *R*-propranolol because of the large increase of the chiral contribution of *S*-alprenolol. The sum $a_I + a_{II}$ for *S*-alprenolol is 7.34 at pH 5.0 and 26.91 at pH 6.0 because the a_{II} term increases 4.5 times in this pH range. The corresponding sum for *R*-propranolol is 9.49 at pH 5.0 and 16.27 at pH 6.0 because the increase of the a_{II} term of the *R*-enantiomer is only 2.2 times. The a_{II} term for *S*-propranolol also increases less than that of *S*-alprenolol, which explains why the resolution of these two compounds decreases with increasing pH (see Tables 3 and 4).

In the medium concentration range the *S*-alprenolol and *R*-propranolol isotherms intersect at $C = 0.28$ and 0.17 m*M* (Figs. 3 and 4), at pH 5.5 and 6.0, respectively. In this concentration range, progressive saturation of the isotherms of type-II sites is taking place. This is why the isotherm data in this range provide the values of the $b_{i,II}$terms and an estimate of the monolayer capacities of the chiral sites, $q_{i,II,s}$ [18]. In this concentration range, an increase of $q_{i,II,s}$ at constant $b_{i,II}$ results in steeper isotherms whereas an increase of $b_{i,II}$ at constant $q_{i,II,s}$ results in more strongly curved isotherms [18].

When the pH increases from 5.0 to 6.0, the isotherms of both *S*-alprenolol and *S*-propranolol become more curved, the former more so than the latter (Figs. 2–4). This trend is due to the rapid increase of $b_{i,II}$ for these two *S*-enantiomers, nearly five and four times for *S*-alprenolol (Table 3) and *S*-propranolol (Table 4), respectively. At pH 6.0, the surface coverages are Θ_{II} ($= q_{II}/q_{II,s}$) = 82 and 83% at $C_m = 0.11$ m*M* for *S*-alprenolol and *S*-propranolol, respectively, and the sites are nearly saturated. The *S*-alprenolol isotherm is markedly more curved than that of *S*-propranolol (Figs. 2–4) although its value of $b_{i,II}$ is slightly lower, which should give it a stronger curvature [18]. The explanation is that the relative contribution of the type-II sites to the total adsorption is larger for alprenolol (89%) than for propranolol (81%) [the relative chiral contribution = $a_{II}/(a_I + a_{II})$]. Also, the relative proportion of sites of type-II to the total number of sites is larger for alprenolol (3.4%) than for propranolol (2.7%). So, the effect of a stronger curvature in the case of propranolol is drowned in the larger nonchiral contribution.

The other four isotherms appear nearly linear in the intermediate concentration range. This is essentially an optical illusion due to the relatively small amounts of solutes adsorbed at equilibrium and to the ordinate scale of the figure. The surface coverage of type-II sites is $\Theta_{II} = 55$, 43, 47 and 46% at $C_m = 0.11$ m*M* for *R*-propranolol, *R*-alprenolol, *R*-metoprolol, and *S*-metoprolol, respectively. Those values of the chiral site surface coverages are quite significant and account for the curvature. The relatively large nonchiral contribution taking place in each case also tends to obscure the phenomenon.

The isotherm data in the high concentration range give access to the chiral monolayer capacity, $q_{i,II,s}$, and to the parameters of the type-I sites, $b_{i,I}$ and $q_{i,I,s}$ [18]. In this concentration range the type-II sites, which are responsible for the resolution between the isotherms, are completely saturated. Thus, all the isotherms in Figs. 2–4 are grouped by pairs corresponding to those of the two enantiomers at the same pH. The contribution of the chiral type-II sites is limited to a small shift of the horizontal asymptote of the isotherm. The effect of the pH on the adsorption properties of type-I sites is seen in Figs. 2–4. The amount of solute adsorbed at the highest concentration and the corresponding isotherm slope increases with increasing pH. This is due to the increase of the interaction energy of the nonchiral type-I sites, for metoprolol and alprenolol (Table 2–3), and the increase of both the interaction energy and the monolayer capacity of the nonchiral type-I sites for propranolol (Table 4). The steepness of the isotherms in this range seems to depend more on the type of β-blocker than on the pH, which indicates that hydrophobicity is the determining parameter for adsorption on these sites, not the pH which is of secondary importance.

4.9. Comparison of linear and nonlinear data

In a previous study [15] we found that the adsorption of *S*-propranolol on the chiral sites was endothermic while the adsorption of *R*-propranolol

on these sites and the adsorption of both compounds on the nonchiral sites are exothermic. In this study we showed that the shift from exothermic to endothermic behavior takes place at a lower pH for the less hydrophobic β-blocker alprenolol than for the more hydrophobic propranolol and at the highest pH for the least hydrophobic metoprolol. This observation seems in contradiction with our conclusion that the reason for the endothermic behavior is the hydrophobic interaction between *S*-propranolol and CBH I [15]. We can now explain this apparent contradiction. The results of the isotherm modeling (isotherms in Figs. 2–4, best biLangmuir parameters in Tables 2–4) show that this contradiction is due to the fact that linear chromatographic data ignore the existence of a mixed retention mechanism. The shift from exothermic to endothermic behavior takes place at a higher pH for *S*-propranolol than for *S*-alprenolol because of the difference between the relative contributions of the chiral and nonchiral interactions for alprenolol and propranolol. The relative contribution of chiral interactions to retention can be quantified with the ratio $a_{II}/(a_I + a_{II})$. This ratio is lower for *S*-propranolol than for *S*-alprenolol, especially at low pH values. For example, the relative chiral contributions are 84 and 64% at pH 5.0, 89 and 81% at pH 6.0 for alprenolol and propranolol, respectively. The relative chiral contributions for metoprolol are 49% at pH 5.5 and 51% at pH 6.0. Accordingly, the balance between the exothermic, nonchiral contribution to retention and the endothermic, chiral contribution is such for *S*-alprenolol that the chiral contribution dominates at lower pH than for *S*-propranolol, the endothermic behavior of the latter being diluted with a larger amount of nonchiral interactions.

5. Conclusions

The strong influence of the pH on the adsorption of propranolol on CBH I was previously demonstrated [18]. Because the adsorption data fit well to the biLangmuir isotherm, it was possible in this work to determine separately the thermodynamic parameters characterizing the adsorption of the enantiomers of three different β-blockers on the chiral and the nonchiral sites. Knowing these contributions permitted the independent study of the influence of the nature of the β-blocker and the energy of the ionic binding on these chiral and nonchiral interactions.

For all β-blockers, the retention of the *S*-enantiomer on the chiral sites increases with increasing pH because the adsorption energy also increases with the pH. The parallel increase of the monolayer capacity of these sites explains why the *R*-enantiomers are more strongly adsorbed at high pH values. While, for the two least hydrophobic β-blockers, the increase of the nonselective adsorption energy with increasing pH is the only cause of their retention increase, for propranolol, the same increase is also an effect of a higher monolayer capacity. The more hydrophobic the β-blocker, the higher the monolayer capacities on both the chiral and the nonchiral sites.

Finally, linear retention data show that the retention of the *R*-enantiomer of all β-blockers are exothermic. That of the *S*-enantiomer is exothermic at low pH values, endothermic at high pH values, and athermal at some intermediate pH. This last pH is close to 6 for the least hydrophobic β-blocker, metoprolol. It is somewhere between 5.5 and 6.0 for the most hydrophobic, *S*-propranolol. However, it is 5.0 for *S*-alprenolol. This apparent contradiction — that the retention behavior of the less hydrophobic alprenolol is endothermic at lower pH values than that of the more hydrophobic propranolol, although the hydrophobicity plays a significant role in the interactions of the β-blockers with CBH I — is due to the different influences of the pH on the enantioselective and on the nonselective interactions. This difference cannot be taken into account when linear data are discussed and this causes a model error, a mixed retention mechanism being wrongly treated as a single mechanism. The separate determination of the contributions of the two mechanisms that is allowed by the measurement of the isotherm data and their modeling clarifies the situation. The relative contribution to the retention of *S*-alprenolol of its adsorption on the exothermic nonchiral sites is smaller than those of both *S*-propranolol and *S*-metoprolol at all pH values at which measurements were carried out. However, the pH at which the endothermic chiral adsorption balances exactly the exothermic nonchiral adsorption depends on the compound studied.

Acknowledgements

This work was supported in part by grant CHE-97-01680 from the National Science Foundation and by the cooperative agreement between the University of Tennessee and Oak Ridge National Laboratory. We acknowledge the support of Maureen S. Smith in solving our computational problems. The authors thank the Swedish Natural Science Research Council (NFR) and AstraZeneca R&D AB (Mölndal, Sweden) for the financial support of this project.

References

[1] W.H. Pirkle, J.M. Finn, J. Org. Chem. 46 (1981) 2935.
[2] W.H. Pirkle, T.C. Pochapsky, G.S. Mahler, D.E. Corey, D.S. Reno, D.M. Alessi, J. Org. Chem. 51 (1986) 4991.
[3] Y. Okamoto, M. Kawashima, K. Yamamoto, K. Hatada, Chem. Lett. (1984) 739.
[4] D.W. Armstrong, S. M Han, Enantiomeric separations in chromatography, CRC Crit. Rev. Anal. Chem. 19 (1988) 175.
[5] S. Allenmark, Chromatographic Enantioseparation, Ellis Horwood, Chichester–New York, 1991.
[6] S. Allenmark, S. Andersson, J. Chromatogr. A666 (1994) 167.
[7] J. Hermansson, J. Chromatogr. 325 (1985) 379.
[8] I. Wainer, S.A. Barkan, G. Schill, LC–GC 4 (1986) 422.
[9] J. Hermansson, Trends Anal. Chem 8 (1989) 251.
[10] S.C. Jacobson, S. Golshan-Shirazi, G. Guiochon, J. Am. Chem. Soc. 112 (1990) 6492.
[11] S.C. Jacobson, S. Golshan-Shirazi, G. Guiochon, AIChE J. 37 (1992) 836.
[12] F. Charton, G. Guiochon, J. Chromatogr. 630 (1993) 21.
[13] G. Guiochon, S.G. Shirazi, A.M. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, Boston, MA, 1994.
[14] T. Fornstedt, G. Zhong, Z. Bensetiti, G. Guiochon, Anal. Chem. 68 (1996) 2370.
[15] T. Fornstedt, P. Sajonz, G. Guiochon, J. Am. Chem. Soc. 119 (1997) 1254.
[16] T. Fornstedt, P. Sajonz, G. Guiochon, Chirality 10 (1998) 375.
[17] T. Fornstedt, G. Guiochon, in: P. Lundahl, A. Lundqvist, E. Greijer (Eds.), Quantitative Analysis of Biospecific Interactions, Harwood Academic, Amsterdam, 1998.
[18] T. Fornstedt, G. Götmar, M. Andersson, G. Guiochon, J. Am. Chem. Soc. 121 (1999) 1164.
[19] V. Schurig, M. Juza, J. Chromatogr. A 757 (1997) 119.
[20] M.C. Ringo, C.E. Evans, Anal. Chem. 70 (1998) 315A.
[21] T.A.G. Noctor, G. Felix, I.W. Wainer, Chromatographia 31 (1991) 55.
[22] I.W. Wainer, J. Chromatogr. A. 666 (1994) 221.
[23] J. Yang, D.S. Hage, J. Chromatogr. A 725 (1996) 273.
[24] B. Loun, D.S. Hage, Anal. Chem. 66 (1994) 3814.
[25] S. Jönsson, A. Schön, R. Isaksson, C. Pettersson, G. Pettersson, Chirality 4 (1992) 505.
[26] M. Hedeland, S. Jönsson, R. Isaksson, C. Pettersson, Chirality 10 (1998) 513.
[27] T.C. Pinkerton, W.J. Howe, E.L. Ulrich, J.P. Comiskey, J. Haginaka, T. Murashima, W.F. Walkenhorst, W.M. Westler, J.L. Markley, Anal. Chem. 67 (1995) 2354.
[28] P. Jadaud, S. Thelohan, G.R. Schonbaum, I.W. Wainer, Chirality 1 (1989) 38.
[29] I. Marle, P. Erlandsson, L. Hansson, R. Isaksson, C. Pettersson, G. Pettersson, J. Chromatogr. 586 (1991) 233.
[30] R. Isaksson, C. Pettersson, G. Pettersson, S. Jönsson, J. Ståhlberg, J. Hermansson, I. Marle, Trends Anal. Chem. 13 (1994) 431.
[31] Anon. (FDA), Chirality 4 (1992) 338.
[32] R. Howe, R.G. Shanks, Nature 210 (1966) 1336.
[33] C. Vandenbosch, D.L. Massart, W. Lindner, Anal. Chim. Acta 270 (1992) 1.
[34] H. Henriksson, J. Ståhlberg, R. Isaksson, G. Pettersson, FEBS Lett. 390 (1996) 339.
[35] H. Henriksson, J. Ståhlberg, A. Koivula, G. Pettersson, C. Divne, L. Valtcheva, R. Isaksson, J. Biotechn. 57 (1997) 115.
[36] C. Hansch, P. Sammes, J.B. Taylor, C.J. Drayton, in: 1st ed., Comprehensive Medicinal Chemistry, Vol. 6, Pergamon, Oxford, 1990.
[37] 1st ed., IUPAC Chemical Data Series, Vol. No 23. Ionisation Constants of Organic Acids in Aqueous Solution, Pergamon, Oxford, 1979.