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Effect of the ionic strength of the solution and the nature of its ions on the adsorption mechanism of ionic species in RPLC III. Equilibrium isotherms and overloaded band profiles on Kromasil-C_{18}

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Abstract

In two companion papers, we have described the influence of the concentration and the nature of completely dissociated salts dissolved in the mobile phase (methanol:water, 40:60, v/v) on the adsorption behavior of propranolol (R′–NH_2^+–R, Cl^-) on XTerra-C_{18} and on Symmetry-C_{18}. The same experiments were repeated on a Kromasil-C_{18} column to compare the adsorption behavior of this ionic compound on these three different RPLC systems. The adsorption data of propranolol hydrochloride were first measured by frontal analysis (FA) using a mobile phase without salt. These data fit best to the Bi-Moreau model. Large concentration band profiles of propranolol were recorded with mobile phases containing increasing KCl concentrations (0, 0.002, 0.005, 0.01, 0.05, 0.1 and 0.2 M) and the best values of the isotherm coefficients were determined using the numerical solution of the inverse problem of chromatography. The general effect of a dissociated salt in the mobile phase was the same as the one observed earlier with XTerra-C_{18} and Symmetry-C_{18}. However, obvious differences were observed for the shape of the band profiles recorded at low column loading (1.5 g/L, 250 μL injected). A long shoulder is visible at all salt concentrations and the band broadening is maximum at low salt concentrations. A slow mass transfer kinetics on the high-energy sites of the bi-Moreau model might explain this original shape. Five other salts (NaCl, CsCl, KNO_3, $CaCl_2$ and Na_2SO_4) were also used at the same ionic strength ($J = 0.2$ M). As many different band profiles were observed, suggesting that specific solute–salt interactions take place in the adsorbed phase.

Keywords: Ionic strength; Adsorption equilibrium; Adsorption isotherms; Frontal analysis; Moreau isotherm model; Overloaded band profiles; Kromasil-C_{18}; Propranolol

1. Introduction

The adsorption behavior of the complex molecules of ionizable organic compounds has become of high interest since most RPLC separations now made concern these molecules that are widely used in pharmaceutical and biomedical applications. These compounds are often charged molecules under the experimental conditions selected for their elution and the addition of salts and/or buffers into the mobile phases is essential to achieve proper chromatograms exhibiting good resolution and peak shapes compatible with accurate quantitation. Few works have been devoted to the study of ionizable compounds under overloaded column conditions [1–3] and most investigations of the chromatographic behavior of these ionizable compounds are restricted to analytical (i.e., highly dilute) conditions [4–11]. The mere acquisition of the elution times of small pulses of ionizable compounds does not give much useful information on their adsorption behavior beyond an estimate of their overall retention. No information can be obtained on the column saturation capacity, the adsorption equilibrium constant, nor the surface-adsorbate adsorption energy distribution. These critical parameters can be derived from measurements of the equilibrium isotherm

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in the widest possible concentration range (i.e., from 0 to near saturation of the mobile phase). Examples of the use of isotherm data to determine the characteristics of several coexisting retention mechanisms for neutral, low-molecular mass molecules like phenol or caffeine on various brands of RPLC stationary phases can already be found in the literature [12–17].

The same approach can be extended readily to the investigation of the adsorption behavior of ionizable molecules when there are salts dissolved in the mobile phase. It is remarkable that the adsorption behavior of the propranolonium cation on a given C_{18}-bonded RPLC column, is very different whether the mobile phase contains a buffer or does not while the adsorption behavior of a neutral compound of similar structure (e.g., 3-phenyl-propan-1-ol) is independent on the presence of salt in the mobile phase [18]. Because of the shape of the different isotherms obtained, it was concluded that some adsorbate–adsorbate interactions take place for propranolol on Kromasil-C_{18} in the absence of a buffer and that the presence of a buffer prevents these interactions and increases the apparent hydrophobicity of propranolol. More recently, it was shown that a general model of adsorption isotherm, the Bi-Moreau model (a simple extension of the Moreau model [19] to certain types of heterogeneous surfaces), could explain both behaviors of propranolol, in the presence and in the absence of a buffer in the mobile phase, on both Xterra [20] and Symmetry-C_{18} RP-HPLC [21]. The continuous evolution of the parameters of the Bi-Moreau model in a concentration range of the salt between 0 and 0.2 M allowed to refine the adsorption mechanism. Increasing the ionic strength concentration in the mobile phase led to the increase of the total saturation capacity, consistent with the previous observations of Stählberg [3] because the repulsive electrostatic interactions in the adsorbed monolayer decrease when the salt concentration increases. The adsorption constant on the low-energy sites of the bi-Moreau model increases with increasing salt concentration, consistent with the "salting out" and/or "common ion" effects. Finally, the adsorbate–adsorbate interactions between the organic moitie of propranolol (e.g., a naphthyl group) decreases rapidly with increasing salt concentration.

These conclusions are provisional. Further investigations and ample additional experimental data are required to generalize them. We report in this work on the results of investigations and measurements similar to those previously carried out [20,21], but performed on another RPLC packing material, Kromasil-C_{18}. This material is an endcapped, bonded silica material with a higher surface coverage density than the two materials previously studied. As in our earlier studies, frontal analysis allowed the derivation of the best isotherm model for the adsorption behavior of propranolol from a methanol:water mobile phase (40/60, v/v) without salt to the packing material. The addition of salt was considered as a perturbation of the adsorption behavior that is insufficient to change the retention mechanism(s) but merely modifies the values of the isotherm parameters. The inverse method (IM) allows a simple, fast, but accurate derivation of the best estimates of these parameters from the fitting of the band profiles recorded at the different salt concentrations [30]. The results are then compared with those obtained with Xterra and Symmetry and discussed.

2. Theory

2.1. Determination of the adsorption isotherms by frontal analysis (FA)

Frontal analysis (FA) [22–24] was used to measure the single-component adsorption isotherm data of propranolol on Kromasil-C_{18} with a methanol:water (40/60, v/v) mobile phase containing no salt. This methanol concentration was chosen so that the retention of propranolol be sufficiently large to afford accurate adsorption data ($k \geq 2$). The derivation of the amount of the studied compound adsorbed on the column at equilibrium with a solution of known concentration is explained in details elsewhere [25].

2.2. Model of isotherm

Previous studies suggest that, although heterogeneous, the surfaces of many modern RPLC stationary phases consist in patches of two or a few different and rather homogeneous surfaces (i.e., types of sites), having different adsorption energies. The isotherm models used here to account for the experimental adsorption behavior of propranolol correspond to this general description. They consist in the sum of two (or a few, at most) terms, each of them corresponding to the isotherm model for the homogeneous surface of one of the types of sites involved. These models are the Langmuir model, when there are no adsorbate–adsorbate interactions or these interactions are negligible, the Moreau model when these interactions are significant. The simplest heterogeneous Moreau model [19] was considered and we used here the following extension of the Moreau model, the bi-Moreau model. This model assumes that a different Moreau model applies to each of two types of patches, considered as homogeneous and acting independently:

$$q^* = q_{s,1}\frac{b_1 C + I_1 b_1^2 C^2}{1 + 2b_1 C + I_1 b_1^2 C^2} + q_{s,2}\frac{b_2 C + I_2 b_2^2 C^2}{1 + 2b_2 C + I_2 b_2^2 C^2} \quad (1)$$

where $q_{s,1}$, $q_{s,2}$, b_1, b_2, I_1 and I_2 are the monolayer saturation capacities, the low-concentration equilibrium constants, and the adsorbate–adsorbate interaction parameters on the sites of types 1 and 2, respectively.

The equilibrium constants b_1 and b_2 are associated with the adsorption energies $\epsilon_{a,1}$ and $\epsilon_{a,2}$ through the following classical equation [26]:

$$b_i = b_0\, e^{\epsilon_{a,i}/RT} \quad (2)$$

where $\epsilon_{a,i}$ is the energy of adsorption, R is the universal ideal gas constant, T is the absolute temperature and b_0 is a pre-exponential factor that could be derived from the molecular partition functions in both the bulk and the adsorbed phases. b_0 is often considered to be independent of the adsorption energy $\epsilon_{a,i}$ [26].

The adsorbate–adsorbate parameter I can be written as [19]:

$$I = \exp\left(\frac{\epsilon_{AA}}{RT}\right) \quad (3)$$

where ϵ_{AA} is the interaction energy (by convention $\epsilon_{AA} \geq 0$) between two neighbor adsorbed molecules of A.

2.3. The inverse method of isotherm determination

This method consists in adjusting the coefficients of an isotherm model in order to minimize the differences between a recorded experimental band profile and the profile calculated with the equilibrium-dispersive model of chromatography and the isotherm model selected. The main advantage of the inverse method for isotherm determination is that it requires the measurement of only a few experimental overloaded band profiles [27–30]. Accordingly, the method is fast and requires little amounts of solvent and sample. This method was described previously [20]. It gives results that are in excellent agreement with those of FA [30].

2.4. Modeling of band profiles in HPLC

Overloaded band profiles of propranolol were calculated, using the equilibrium-dispersive model (ED) of chromatography [22,31,32]. The ED model assumes instantaneous equilibrium between the mobile and stationary phases and a finite column efficiency originating from an apparent axial dispersion coefficient, D_a that accounts for the dispersive phenomena (molecular and eddy diffusion) and for the non-equilibrium effects that take place in a chromatographic column. The axial dispersion coefficient is:

$$D_a = \frac{uL}{2N} \quad (4)$$

where u is the mobile phase linear velocity, L the column length, and N the number of theoretical plates or apparent efficiency of the column, measured under linear conditions, i.e., with a small sample size. In this model, the mass balance equation for a single component is written:

$$\frac{\partial C}{\partial t} + u\frac{\partial C}{\partial z} + F\frac{\partial q^*}{\partial t} - D_a\frac{\partial^2 C}{\partial z^2} = 0 \quad (5)$$

where q^* and C are the stationary and mobile phase concentrations of the adsorbate at equilibrium, respectively, t is the time, z the distance along the column, and $F = (1 - \epsilon_t)/\epsilon_t$ is the phase ratio, with ϵ_t the total column porosity. q^* is related to C through the isotherm equation, $q^* = f(C)$ (see Eq. (1)).

2.4.1. Initial and boundary conditions for the ED model

At $t = 0$, the concentrations of the solute and the adsorbate in the column are uniformly equal to zero (except in staircase FA), and the stationary phase is in equilibrium with a stream of the pure mobile phase. The boundary conditions used are the classical Danckwerts-type boundary conditions [23,33] at the inlet and outlet of the column.

2.4.2. Numerical solutions of the ED model

The ED model was solved using the Rouchon program based on the finite difference method [22,34–36].

3. Experimental

3.1. Chemicals

The mobile phase used in this work was the same aqueous solution of methanol (40:60, v/v) for the acquisition of the FA data and for the acquisition of the overloaded band profiles. Both water and methanol were of HPLC grade, purchased from Fisher Scientific (Fair Lawn, NJ, USA). Potassium chloride was dissolved at the appropriate concentration in pure water and methanol was added to that solution to prepare the mobile phase. The salt concentrations given in the text are reported to the mobile phase mixture. Prior to their use, the solvents were filtered on an SFCA filter membrane, 0.2 μm pore size (Suwannee, GA, USA). Thiourea was chosen to measure the column hold-up volume. Propranolol was the solute used in this study. It is an amino alcohol of structure $C_{10}H_7OCHOHCH_2NHCH(CH_3)_2$. It was injected under its protonated form, as the hydrochloride. Thiourea and propranolol; potassium, sodium and calcium chlorides; potassium nitrate and sodium sulphate were all obtained from Aldrich (Milwaukee, WI, USA).

3.2. Columns

The column used in this study (Kromasil-C_{18}, # E6022) was given by the manufacturer (Eka Nobel, Bohus, Sweden, EU). The tube dimensions are 250 × 4.6 mm. The main characteristics of the packing material used are summarized in Table 1. This column was one of the lot of ten columns previously used to test the column-to-column and batch-to-batch reproducibility under linear [37] and non-linear conditions [38,39]. The hold-up time of this column were derived from the retention times of two consecutive thiourea injections. The column porosity remains constant at 0.5916, whatever the salt concentration in the mobile phase (40:60, v/v). The porosity depends only on the methanol concentration of the mobile phase.

3.3. Apparatus

The isotherm data and the overloaded band profiles were acquired using a Hewlett-Packard (Palo Alto, CA, USA) HP 1090 liquid chromatograph. This instrument includes a

Table 1
Physico-chemical properties of the packed Xterra (Waters), Symmetry^R (Waters) and Kromasil-C_{18} (Eka) columns

	Kromasil	Symmetry^R	XTerra
Particle size (μm)	6	5	5
pore size (Å)	110	90	125
Pore volume[a] (mL/g)	0.88	0.90	0.70
Surface area[a] (m^2/g)	314	346	175
Particule shape	Spherical	Spherical	Spherical
Total carbon (%)	20.0	19.6	15.5
Surface coverage (μmol/m^2)	3.6	3.2	2.5
Total porosity[b]	0.5916	0.5804	0.6178
Endcapping	yes	yes	yes

[a]Data for the packing before derivatization.
[b]Data from injection of the non-retained thiourea compound in a methanol:water mobile phase (40/60, v/v).

multi-solvent delivery system (volume of each tank, 1 L), an auto-sampler with a 250 μL sample loop, a diode-array UV-detector, a column thermostat and a data station. Compressed nitrogen and helium bottles (National Welders, Charlotte, NC, USA) are connected to the instrument to allow the continuous operations of the pump, the auto-sampler, and the solvent sparging. The extra-column volumes are 0.058 and 0.93 ml as measured from the auto-sampler and from the pump system, respectively, to the column inlet. All the retention data were corrected for these contributions. The flow-rate accuracy was controlled by pumping the pure mobile phase at 23 °C and 1 mL/min during 50 min, from each pump head, successively, into a volumetric glass of 50 mL. The relative error was less than 0.4%, so that we can estimate the long-term accuracy of the flow-rate at 4 μL/min at flow rates around 1 mL/min. All measurements were carried out at a constant temperature of 23 °C, fixed by the laboratory air-conditioner. The daily variation of the ambient temperature never exceeded ±1 °C.

3.4. Measurements of the adsorption isotherm of propranolol by FA

The adsorption isotherms of propranolol were measured in an aqueous solution of methanol (40% methanol volume). The solubility of propranolol is approximately 50 g/L in this solution. Accordingly, the maximum concentration used in FA was 40 g/L, to avoid any precipitation in the instrument. One master solutions was prepared, at 40 g/L, and diluted to prepare two secondary solutions. A total of 33 data points were then measured, covering a dynamic range of 100 (from 0.4 to 40 g/L). One pump of the HPLC instrument was used to deliver a stream of the pure mobile phase (methanol:water, 40:60, v/v), the second pump a stream of the pure master or secondary solution. The concentration of propranolol in the FA stream is determined by the concentration of the sample solution and the flow rate fractions delivered by the two pumps. The breakthrough curves were recorded at a flow rate of 1 mL min^{-1}, with a sufficiently long time delay between successive experiments, to allow for the complete reequilibration of the column with the pure mobile phase. The injection time of the sample was fixed at 6 min for all FA steps in order to reach a stable plateau at the column outlet whatever feed concentration was used. To avoid recording any UV-absorbance signal larger than 1500 mAU and the corresponding signal noise at high concentrations while keeping a large enough signal at the lowest concentrations, the signal was detected at 325 nm (1.5 g/L solution) and 331 nm (30 g/L solution). In each case, the detector response was calibrated, accordingly.

3.5. Measurements of the overloaded band profile of propranolol in presence of salt in the mobile phase

After FA data were acquired, mobile phases derived from the same aqueous solution but containing seven different salt concentrations were prepared (0, 0.002, 0.005, 0.01, 0.05, 0.1 and 0.2 M). The injections of propranolol were made with the auto-sampler syringe (250 μl), at two different concentrations, 1.5 g/l and 30 g/l. The band profiles were recorded at 325 and 331 nm after injections of the 1.5 and 30 g/l solutions, respectively. Segments of the elution profiles having between 500 and 1000 points were used to perform the IM calculations [30].

4. Results and discussions

The main differences between the three endcapped packing materials, Kromasil-C_{18}, Symmetry-C_{18} and XTerra-C_{18}, are their surface coverage densities, 3.6, 3.2, and 2.5 μmol/m^2, respectively (data from the manufacturers), and the nature of the underlying silica. No experimental data were available regarding the silanol activity of Kromasil. The silanol activity reported for the other two adsorbents is none or practically none [40], at least in the pH range investigated in this study, e.g. between 4 and 6.

4.1. Comparison of the adsorption behavior of propranolol on Kromasil-C_{18}, Symmetry-C_{18} and XTerra-C_{18} in the same methanol:water mixture, without salt

As demonstrated above and previously [18,20,21], the isotherm model that accounts best for the adsorption data of propranolol on these three adsorbents is the bi-Moreau model. The isotherm model most often referred to in the literature dealing with the chromatography of ionizable compounds and the most generally accepted model is the Stählberg isotherm model which is an extension of the Langmuir model to ionizable adsorbates. The fundamental reason for the interest that this model attracts is that it takes into account the electrostatic repulsion between the charged adsorbate

molecules. To this purpose, it incorporates the theoretical expression of the electrostatic surface potential with respect to the solution of the general Poisson–Boltzmann equation [1,2].

However, the Stählberg isotherm cannot be applied to the experimental data obtained in our cases because it can lead only to strictly convex upward, i.e., to langmuirian isotherms. By contrast, the experimental isotherm data of propranolol on the three adsorbents studied exhibit inflection points a feature that cannot be inconsistent with the Stählberg model. The main flaw of this model is that, although there is electrostatic repulsion between the ionic groups of two neighbor adsorbate molecules, there is also attractive adsorbate–adsorbate interactions, e.g., between the large naphthyl groups, a situation that is reminiscent of the interactions between surfactant molecules. The existence of large hydrophobic moieties in the structure of ionic species like sodium dodecyl sulphate (SDS) and the interactions between these groups explains the formation of micelles at concentrations exceeding the critical micellar concentration (CMC). This physical phenomenon is consistent with the anti-Langmuirian behavior observed for the isotherm data in the initial range of concentrations, far below the saturation capacity of the adsorbents (mobile phase concentrations in the range 0–7.5 g/L for Symmetry and XTerra).

The bi-Moreau model is the only general model that is able to account for the adsorption data and the band profiles of propranolol that were measured or recorded in the whole concentration range of potassium chloride, from very low to high concentrations, and for the three adsorbents studied. The degree of heterogeneity of the surface that is suggested by this model is consistent with the results found otherwise for a neutral compound of similar molecular weight, caffeine. The adsorption data of caffeine on those columns was always described by a bi-Langmuir model, not a simple unimodal isotherm model [12,16]. The bi-Langmuir model is the limit of the bi-Moreau model when the adsorbate–adsorbate interactions tend toward 0.

The parameters of the bi-Moreau isotherm are the two saturation capacities $q_{s,1}$ and $q_{s,2}$, the two equilibrium constants b_1 and b_2, and the two adsorbate–adsorbate interaction parameters, I_1 and I_2. Table 2 summarizes the values of these parameters derived from the measurements made by frontal analysis and by the inverse method on Kromasil-C_{18}. For reasons that will be clarified later, the best parameters derived by IM were estimated only from the high concentration band profiles (i.e. 30 g/L). There is a good agreement between the values of the parameters afforded for propranolol in the pure aqueous solution by the two independent methods, which confirms the validity of the isotherm parameters determined by the inverse method. The best values of the isotherm parameters obtained for propranolol in the same mobile phase, on Symmetry and XTerra, were estimated as the arithmetic mean of the parameters derived by IM from the high and the low column-loading band profiles. Some comments regarding the values of the parameters are in order.

– The saturation capacity of the low-energy adsorption sites, $q_{s,1}$, decreases in the order Kromasil (170 g/L) ≥ Symmetry (130 g/L) ≥ XTerra (110 g/L). This result seems to be correlated to the surface coverage density of the C_{18}-bonded chains (3.6, 3.2 and 2.5 μmol/m^2, respectively) and to the specific surface area of the underlying solid (314, 346 and 175 m^2/g, respectively). The higher the chain density and the specific surface area, the higher the number of adsorption sites. In addition, the adsorption constant b_1 on these sites is of the same order of magnitude on all three columns (≃0.0125 L/g). The strength of the adsorbate–adsorbate interactions I_1 and I_2 are relatively weak and never exceed a few times *RT*.
– The saturation capacity of the high-energy adsorption sites $q_{s,2}$ represents only a few percent of the total saturation capacity. It oscillates around 2 g/L for all three adsorbents. While no significative differences were observed between the columns regarding the values of the parameters b_1 and I_1, the intensity of the adsorption energy on sites 2 varies markedly from on adsorbent to the next. First, the equilibrium constant, b_2, is about five times higher on Symmetry and Xterra than on the Kromasil column. Accordingly, the difference of adsorption energies on the sites of types 1 and 2 never exceeds 9 kJ/mol for Symmetry and Xterra and only 5 kJ/mol on Kromasil. A possible explanation would be that the molecules of propranolol penetrate more easily the network of the C_{18} chains when the surface density of these chains is low, as it is the case for Xterra and Symmetry.

However, for all columns, the weak difference between the energies of adsorption on the two types of sites is consistent only with a structural origin of the high energy sites, not with the involvement of strong electrostatic or hydrogen bonding interactions with some free silanol groups hidden within the C_{18}-bonded layers.

4.2. Evolution of the overloaded band profiles of propranolol on Kromasil-C_{18} with increasing salt concentration in the mobile phase

At each salt concentration (0, 0.002, 0.005, 0.01, 0.05, 0.1 and 0.2 M), two injections of propranolol were made, both lasting for 15 s, one with a low (1.5 g/l), the other with a high concentration (30 g/l) solution. Their volume was the maximum volume delivered by the auto-sampler (250 μl).

As mentioned earlier, the isotherm parameters were derived by applying the inverse method only to the high concentration profiles ($C = 30$ g/L). The reason for not considering the low concentration profiles in this case is given in Fig. 1 which shows these profiles for the seven salt concentrations used in this study. The most striking feature of these profiles is the shoulder on the rear front of the profiles. This shoulder decreases with increasing salt concentration but persists even at 0.20 M. When $C_{salt} = 0$, a plateau concentration is observed on the rear of the profile, at 0.0032 g/L. It lasts for

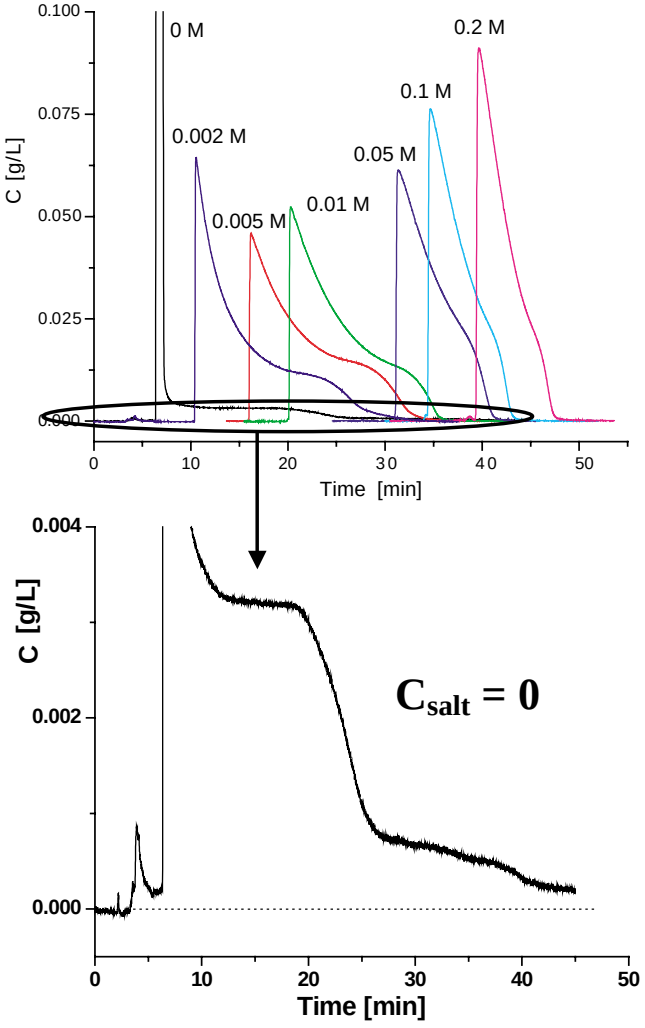

Fig. 1. (Top) Evolution of the position and the shape of overloaded band profiles of propranolol (injection of a 1.5 g/l solution during 15 s) as a function of the salt concentration or ionic strength of potassium chloride in the mobile phase (methanol:water, 40/60, v/v) on the Kromasil column. $T = 296$ K, flow rate 1 ml/min. Note the displacement of the band toward high retention times when the ionic strength solution increases and the shoulder at the end of all the bands. (Bottom) Evidence of the "tag-along" effect on the band profile recorded without salt in the mobile phase.

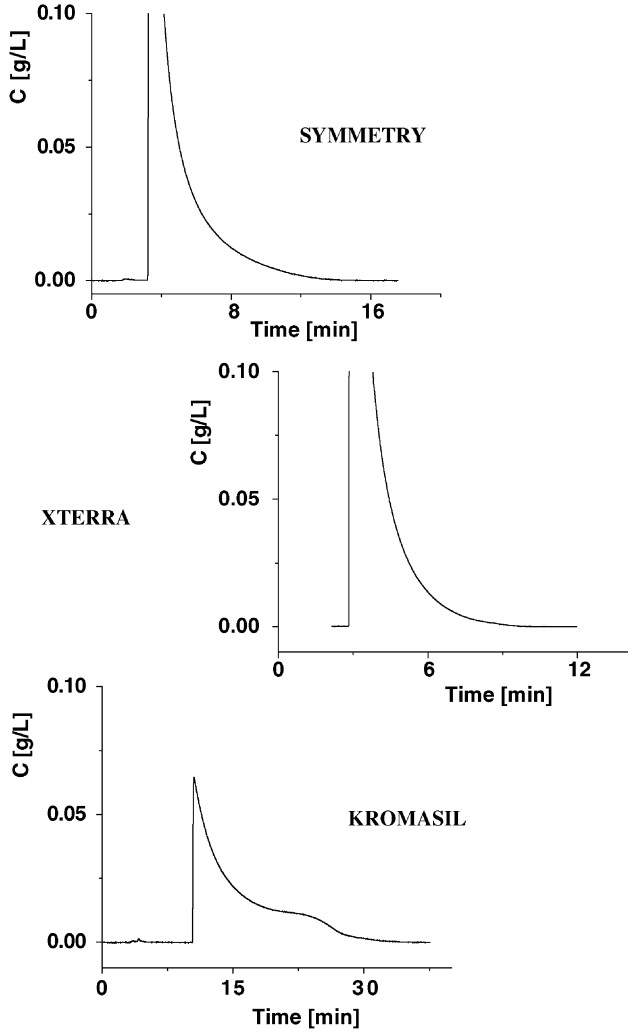

Fig. 2. Comparison of the band profile of propranolol (injection of a 1.5 g/l solution during 15 s) with a concentration of potassium chloride of 0.002 M between the three C_{18}-bonded columns, Symmetry, XTerra MS and Kromasil. Same other conditions as in Fig. 1. Note the absence of shoulder on the Symmetry and XTerra columns.

about ten minutes. When the potassium chloride concentration in the mobile phase is increased, the length of this plateau decreases and it is progressively transformed into a shoulder. This surprising phenomenon was not observed on XTerra and Symmetry, under the same experimental conditions (Fig. 2). It does not seem to have a thermodynamic origin but is probably related to a slow desorption kinetics of propranolol from sites deeply buried in the stationary phase or from some active sites on the underlying layer of alkyl chains on the surface. The desorption kinetics becomes faster when the salt concentration increases and a nearly conventional band tailing is observed at 0.2 M (Fig. 1). Fig. 2 compares the low concentration parts of the band profiles (at $C \leq 0.1$ g/L) recorded on Symmetry, XTerra and Kromasil. The profile on the last of these columns is much broader than those observed on the other two columns. No such shoulder were ever observed with the first two columns. This strong tailing could be related to the high density of the surface coverage of Kromasil by the bonded alkyl chains, which would cause a slow molecular diffusion of propranolol from the high-energy, deeply buried sites back to the mobile phase. However, a similar shoulder was never observed on the same Kromasil column with neutral compounds like caffeine whose adsorption from an aqueous solution of methanol is described by a bi-Langmuir model [12,16]. Accordingly, the origin of this shoulder should be related to some ion-exchange interactions between accessible negatively charged sites on the surface and the propranolonium cation. This conclusion need to be confirmed by an estimate of the Kromasil silanol activity, regarding the retention of Li^+, for instance.

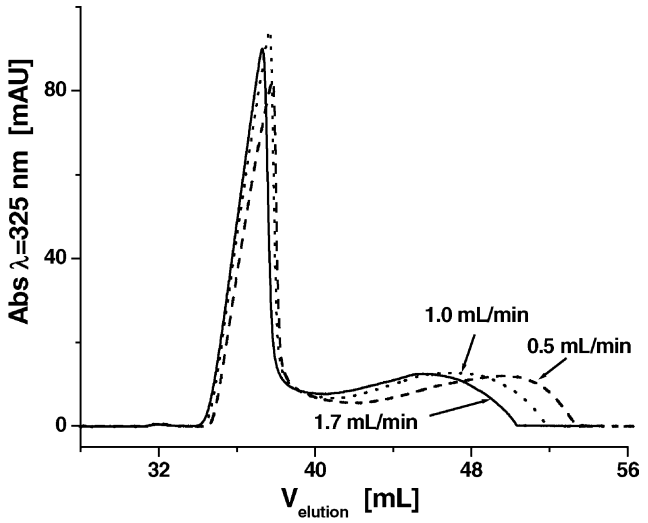

Fig. 3. Chromatograms of propranolol (injection of a 1.5 g/l solution during 15 s) on the Kromasil-C_{18} with a concentration of sodium sulphate of 0.0667 M in the mobile phase (methanol:water, 40/60, v/v) as a function of the mobile phase velocity (0.5, 1.0 and 1.7 mL/min). Note the kinetic effect observed on the second part of the band.

This shoulder may even become a second, partially separated band. Fig. 3 shows the chromatogram recorded on the same column, with a mobile phase of similar composition but with sodium sulphate instead of potassium chloride, at the same ionic strength, 0.2 M. Then, a second band appears and the molecules eluted in this second band are the same as those eluted in the first one. Band profiles were recorded at different mobile phase velocities (0.5, 1.0 and 1.7 mL/min) and plotted as a function of the elution volume (Fig. 3). The shape of the first part of the band is not significatively altered and seems to be controlled mainly by the nonlinear thermodynamics. The differences observed in the second part of the band confirm that the kinetic of convection of the band affects its profile.

Although we expended on this shoulder phenomenon, the actual point is that, due to the existence of a shoulder on the profiles of propranolol bands, the isotherm parameters cannot be determined by applying the inverse method of chromatography to the low concentration profiles because the profiles used in this method should not display any significant extra-thermodynamic broadening beyond that caused by a moderate degree of apparent dispersion [30]. Instead of the simple equilibrium-dispersive model of chromatography, a more sophisticated model of chromatography including slow mass transfer kinetics on a small fraction of the surface area of the stationary phase would be necessary but we do not have any good model for this kinetics yet.

Therefore, the determination of the best parameters of the bi-Moreau model for propranolol on Kromasil-C_{18} were based on the band profiles recorded upon injection of the higher concentration solution at C = 30 g/L (Fig. 4). Fig. 5 compares the experimental profiles and those calculated with the bi-Moreau model that exhibit the best agreement with these experimental profiles, for all the salt concentrations investigated. The numerical values of the isotherm parameters are compared to those previously acquired on Xterra and Symmetry in Table 2. Figs. 6–11 show the evolution of the different parameters as a function of the salt concentration for the three columns. The similarity of the corresponding curves suggests that the mechanism of retention is the same on all three C_{18}-bonded stationary phases. Some general conclusions and relationships regarding the dependence of these parameters on the salt concentration can be derived.

– Regarding the most abundant sites of type 1, their saturation capacity, $q_{s,1}$ increases linearly with increasing concentration of potassium chloride in range investigated. A constant difference of 25 g/L is observed between the specific surface area of Symmetry and Xterra. Kromasil has by far the highest number of sites 1, again because of its high surface coverage.
– The sites of type 2 are much less abundant. They represent about 5% of the total saturation capacity. Their saturation capacity, $q_{s,2}$, also increases with increasing salt concentration. It nearly reaches a saturation level at 0.2 M of potassium chloride. This saturation capacity increases in the order Xterra ≤ Symmetry ≤ Kromasil. Since the same order is observed as for $q_{s,1}$, it is likely that $q_{s,2}$ is also related to the surface coverage density of the columns (i.e. to the properties connected to the C_{18} chains) not to

Table 2
Comparison between the bi-Moreau isotherm parameters (two-sites 1 and 2) of propranolol measured by frontal analysis (FA) and/or the Inverse Method (IM), on the Kromasil-C_{18}, the Symmetry-C_{18} (IM) and on the Xterra-C_{18} column (IM) with a mixture of methanol and water (40/60, v/v) as the mobile phase

	KROMASIL				SYMMETRY		XTERRA	
	FA		IM[a]		IM[b]		IM[b]	
	Sites 1	*Sites 2*	Sites 1	*Sites 2*	Sites 1	*Sites 2*	Sites 1	*Sites 2*
q_S [g/L]	174	*1.9*	170	*2.4*	133	*2.4*	113	*1.7*
b [L/g]	0.0135	*0.0849*	0.0130	*0.0687*	0.0113	*0.473*	0.0116	*0.461*
I [L^2/g^2]	7.47	*23.5*	7.56	*5.66*	14.4	*1.32*	14.0	*5.94*
ε/RT	2.01	*3.14*	2.02	*1.73*	2.67	*0.28*	2.64	*1.78*

[a] Parameters calculated from the analysis of the high loading band profile (30 g/L).
[b] Parameters calculated from the analysis of both the high and low loading band profiles (arithmetic average, 30 g/L and 1.5 g/L) [21,22].

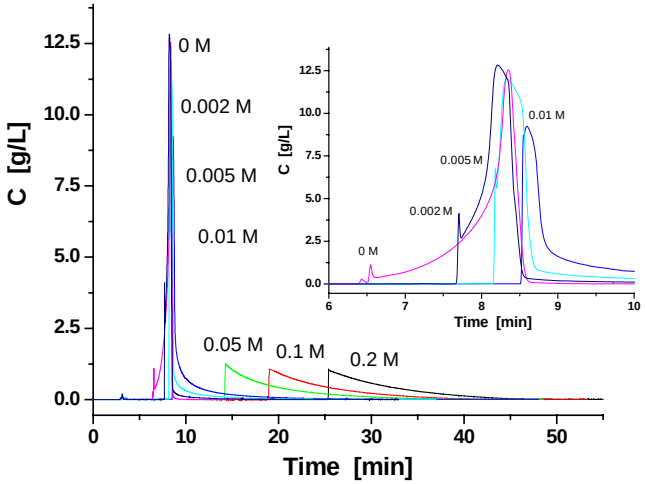

Fig. 4. Evolution of the position and the shape of overloaded band profiles of propranolol (injection of a 30 g/l solution during 15 s) as a function of the salt concentration or ionic strength of potassium chloride in the mobile phase (methanol:water, 40/60, v/v) on the Kromasil column. $T = 296$ K, flow rate 1 ml/min. Note the displacement of the band toward high retention times when the ionic strength solution increases. (Insert) Enlargement for the four first salt concentration. Note the progressive growth up of the front chock.

the properties of the bare silica (i.e., density of accessible free silanols). We may write the following expression for $q_{s,1}$ and $q_{s,2}$ as a function of the concentration of added salt C_S (Figs. 12 and 15):

$$q_{S,i} = q_{i,0} + \frac{\kappa_{i,1} C_S}{1 + \kappa_{i,2} C_S} \tag{6}$$

with $\kappa_{i,2} = 0$ for $i = 1$.

– The equilibrium constant b_1 increases similarly with increasing salt concentration for the three adsorbents. The physical origin of the sites of type 1 is the same. It is probably related to the simple adsorption of the hydrophobic part of the propranolonium ion on the C_{18}-bonded layer. Note that Cl^- is the co-ion in the propranolol solution in the mobile phase. According to the common ion effect (i.e., to Le Chatelier principle), the solubility of the salt decreases. The solubility product K_S is written:

$$K_S = \gamma_+ \gamma_- [\text{propranolol}^+]_S([Cl^-]_S + [Cl^-]_{KCl}) \tag{7}$$

where γ_+ and γ_- are the activity coefficients of the propranolonium and chloride ions, respectively. $[\text{propranolol}^+]_S$ is the saturated concentration of propranolonium, $[Cl^-]_S$ and $[Cl^-]_{KCl}$ are the concentrations of the chloride ion coming from the dissociation of propranolonium chloride and from potassium chloride, respectively. Since

$$[\text{propranolol}^+]_S = [Cl^-]_S$$

and according to Eq. (7), the concentration of a saturated solution of propranolol in the presence of an added salt containing the same anion is:

$$[\text{propranolol}^+]_S = \sqrt{C_S^2 + K_S^{''}} - C_S \tag{8}$$

where $K_S^{''} = K_S \gamma_+ \gamma_-$. From Eq. (8), it is obvious that the addition of the common ion, Cl^-, leads to a decrease of the solubility of the propranolol salt. The equilibrium constant b_1 increases and can be fitted to the following relationship (Fig. 13):

$$b_1(C_S) = b_{1,0} + \frac{\beta_{1,1} C_S}{1 + \beta_{1,2} C_S} \tag{9}$$

– The variation of the equilibrium constant b_2 as a function of the concentration of KCL is not clearly established. It seems to be more complex than that of b_1. First, without addition of salt in the mobile phase, b_2 is minimum for all the columns. After addition of the smallest amount used in this work, 0.002 M of KCL, to the mobile phase, b_2 jumps abruptly to the highest value measured, which is confirmed by the brutal change observed in the band profile. There is no band tailing when the mobile phase contains no salt but a very long tailing for a concentration of 0.002 M KCL (see Fig. 5 for the Kromasil column). Then, b_2 decreases rapidly with increasing KCl concentration and tends toward a plateau. The curve decay is well fitted for the concentrations of KCl different from zero by (Fig. 16):

$$b_2(C_S) = b_{2,0} + \frac{\beta_{2,1}}{C_S} \tag{10}$$

– The interaction parameter I_1 decreases similarly with increasing salt concentration for all columns. The addition of salt into the mobile phase reduces progressively the adsorbate–adsorbate interactions. This parameter was fitted to (Fig. 14):

$$I_1(C_S) = \frac{\zeta_{1,1}}{1 + \zeta_{1,2} C_S} \tag{11}$$

– The interaction parameter I_2 can be considered as constant over the whole range of salt concentration (Fig. 17). However, I_2 is significatively larger when $[KCl] = 0$.

$$I_2(C_S) = \zeta_{2,0} \tag{12}$$

The numerical values of the parameters in Eqs. (6) and (9)–(12) are listed in Table 3. These equations describe a possible mathematical model for the overall adsorption isotherm of propranolol as a function of its concentration C and of the concentration of KCl, C_S, in the mobile phase. The validation of this empirical model will be discussed in a further work dealing with the calculation of the overloaded band profiles of propranolol under conditions of a decreasing gradient of KCl from 0.20 to 0.002 M.

4.3. Influence of the valence of the salt on the overloaded band profiles obtained at constant ionic strength

Previous work on Symmetry and XTerra showed that the retention and shape of the band profiles of large samples of propranolol are not determined only by the ionic strength

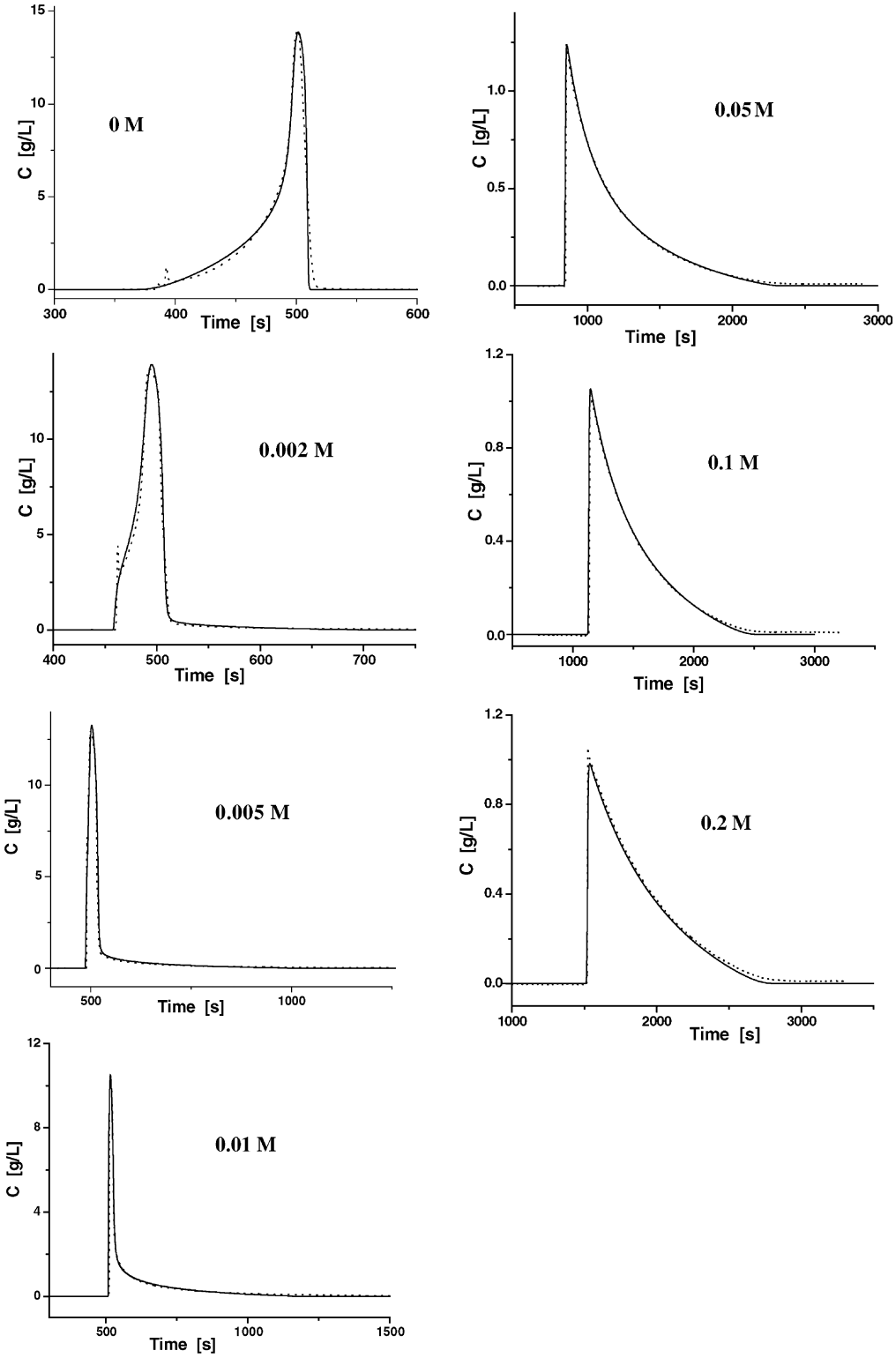

Fig. 5. Comparison between the experimental profiles of propranolol (dotted line) and the best calculated profiles found by the IM (solid line) on the Kromasil column (methanol:water, 40/60, v/v, 15 s injection of a 30 g/l solution) at high column loading for all the different concentrations of potassium chloride salt in the mobile phase. $T = 296$ K, flow rate 1 ml/min. The Bi-Moreau model was used in the IM. Note that the simple Bi-Langmuir would have failed to describe the band profiles at low ionic strength solution ($J \leq 0.01$ M). However, note that the best profile found by the program lead to a certain disagreement between the calculated and experimental profile for the lowest concentrations (overestimatations compensate further underestimations).

Table 3
Best numerical values of the parameters $q_{i,0}$ (g/L), $\kappa_{i,1}$ (L^2/g^2), $\kappa_{i,2}$ (L/g), $b_{i,0}$ (L/g), $\beta_{i,1}$ (L^2/g^2), $\beta_{1,2}$ (L/g), $\zeta_{1,1}$, $\zeta_{1,2}$ (L/g) and $\zeta_{2,0}$ of the empirical models (equations 18, 21–24) that describe the evolution of the 6 parameters of the bi-Moreau isotherm as a function of the added concentration C_S of potassium chloride in the methanol:water mobile phase (40/60, v/v) measured on the C_{18}-bonded Kromasil, Symmetry and Xterra columns

Isotherm parameters	Parameters' expression	Kromasil	Symmetry	XTerra
$q_{s,1}$	$q_{1,0}$	181.3	143.6	116.5
	$\kappa_{1,1}$	547.6	345.6	375.3
	$\kappa_{1,2}$	0	0	0
$q_{s,2}$	$q_{2,0}$	2.65	1.64	1.47
	$\kappa_{2,1}$	421.4	371.7	261.5
	$k_{2,2}$	18.5	18.7	36.2
b_1	$b_{1,0}$		0.01218	
	$\beta_{1,1}$		0.6032	
	$\beta_{1,2}$		15.12	
b_2	$b_{2,0}$	0.8219	0.5539	1.4807
	$\beta_{2,1}$	0.00094	0.01436	0.0065
I_1	$\zeta_{1,1}$		12.61	
	$\zeta_{1,2}$		180.11	
I_2	$\zeta_{2,0}$	0.06152	0.1731	0.5491

of the solution. The nature of the cation of salt added to the mobile phase plays a minor role on the adsorption behavior. The larger the radius of the cation, the smaller the saturation capacity $q_{s,1}$. However, by contrast, the band profiles are dramatically affected when the anion is changed. For instance, replacing the monovalent chloride by the bivalent sulphate anion reverses the shape of the adsorption and desorption profiles (the diffuse profiles are switched to shocks and *vice-versa*).

The same general remarks can be made concerning Kromasil. Although all chromatograms were recorded with mobile phases having the same ionic strength (0.2 M), each salt gives a different band profile at low and at high column loadings (Fig. 18). The adsorption isotherm of propranolol depends obviously on the nature of the salt. There is a common feature for all the salts, all the low-loading band profiles exhibit a hump similar to the one previously described in Section 4.1. This hump was absent from the profiles recorded on XTerra and Symmetry under the same conditions (same mobile phase). Thus, its presence must be related to some specific surface properties of Kromasil.

A surprising result was observed with sodium sulphate. The band shape is reversed, the front is a diffuse boundary and the rear is a shock layer, a behavior that is typical of an anti-Langmuirian isotherm. The IM results (not shown) demonstrate that the bi-Moreau isotherm model still applies.

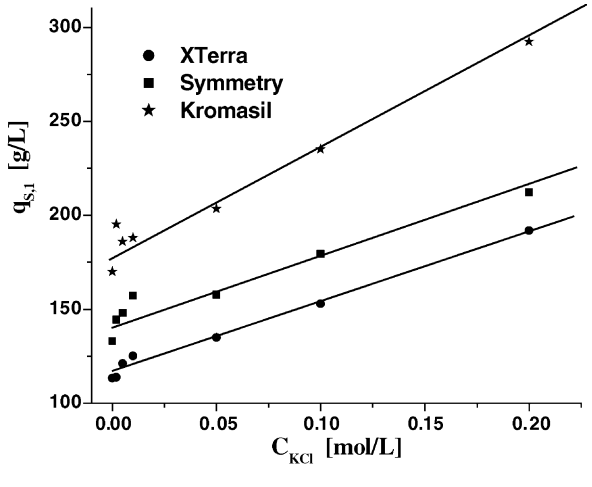

Fig. 6. Plots of the best saturation capacities of the low-energy sites ($q_{s,1}$) in the Bi-Moreau isotherm found by the IM on the C_{18}-bonded Kromasil, Symmetry and XTerra columns as a function of the concentration of potassium chloride in the mobile phase (methanol:water, 60/40, v/v). Note, within the range of concentration investigated from 0 to 0.2 M, the quasi-linear increase of $q_{s,1}$ and the higher values in the order Kromasil ≥ Symmetry ≥ XTerra.

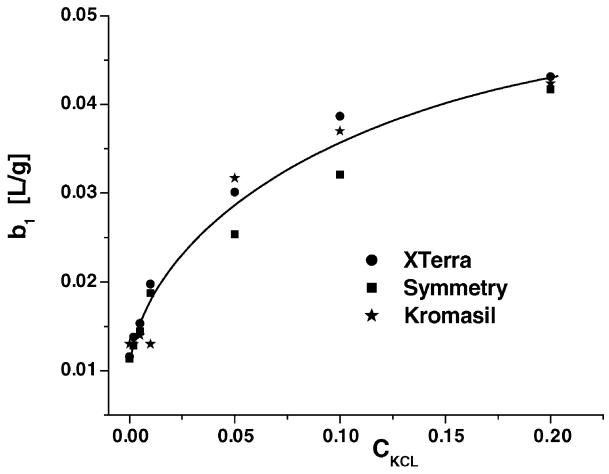

Fig. 7. Plots of the best equilibrium constant (b_1) between the low-energy sites and the mobile phase found by the IM on the C_{18}-bonded Kromasil, Symmetry and XTerra columns as a function of the concentration of potassium chloride in the mobile phase (methanol:water, 60/40, v/v). Note the increasing trend and the quasi-identical behavior of the three curves.

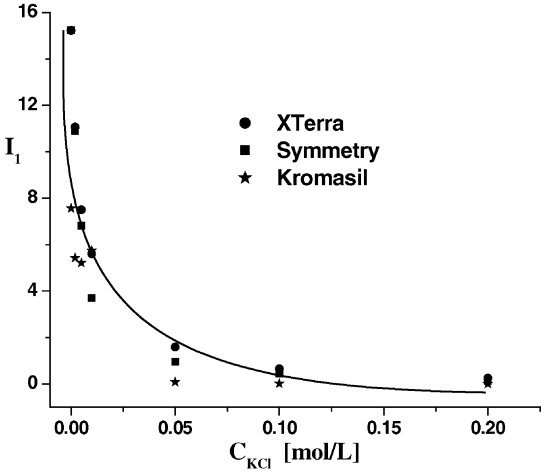

Fig. 8. Plots of the best interaction parameter (I_1), between two molecules of propranolol adsorbed on the low-energy sites, found by the IM on the C_{18}-bonded Kromasil, Symmetry and XTerra columns as a function of the concentration of potassium chloride in the mobile phase (methanol:water, 60/40, v/v). Note the decreasing trend and the quasi-identical behavior of the three curves.

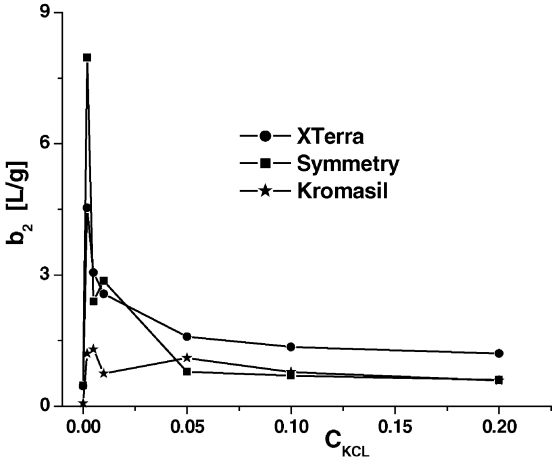

Fig. 10. Same as in Fig. 7 except the equilibrium constant between the high-energy sites and the mobile phase. Note the quasi-stationary values of b_2 within the highest range of the salt concentration ($C_{KCl} \geq 0.05$ M).

The total saturation capacity of the column (225 g/L) is lower than it is with a salt of monovalent ions like KNO_3 (340 g/L), KCl (310 g/L), NaCl (300 g/L) and CsCl (280 g/L). It is comparable to the total saturation capacity found with a bivalent cation like $CaCl_2$, 220 g/L. The equilibrium constant b_1 is also larger with Na_2SO_4 (0.075 L/g versus about 0.045 L/g with all the other salts studied). The striking difference with the other salts, however, is that the adsorbate–adsorbate interactions on the sites of type 1 become most important when sodium sulphate is dissolved in the mobile phase (I_1 = 5.9 instead of 0.0001 with KCl). This difference suffices to explain the antilangmuirian behavior of the isotherm, the corresponding shape of the band profiles, and the long, diffuse front of the adsorption profile. The last important difference is that, in the presence of Na_2SO_4, the saturation capacity of the sites of type 2 is very small, 2 g/L instead of 21 g/L with KCl.

A possible simple explanations of the following unexpected results is that (1) the diminution of the saturation capacity $q_{s,1}$ (300 to 225 g/L); (2) the increase of the equilibrium constant b_1 (0.045 to 0.075 L/g); (3) the increase of adsorbate–adsorbate interaction I_1 (∼0–6); (4) the strong decrease of the saturation capacity $q_{s,2}$ (21–2 g/L); and (5) the same intensity of the equilibrium constant b_2 (≃0.6 L/g) could be due to the formation in the adsorbed phase of a large adsorbate complex made of two molecules of propranolol bound to one sulphate ion. The decrease of the saturation ca-

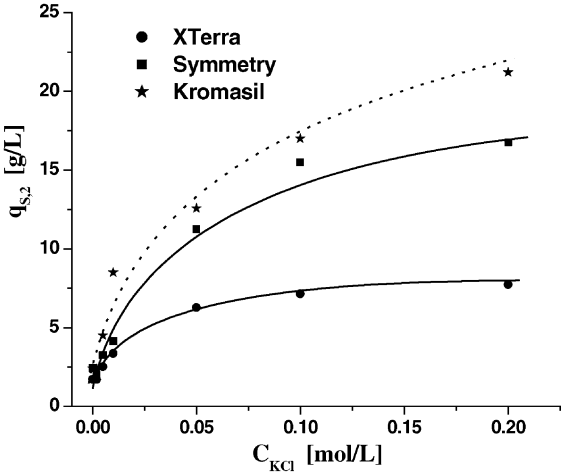

Fig. 9. Same as in Fig. 6 except the saturation capacities of the high-energy sites. Still, note the increasing trend, but the almost saturation of $q_{s,2}$ for the highest concentration of potassium chloride used (0.2 M).

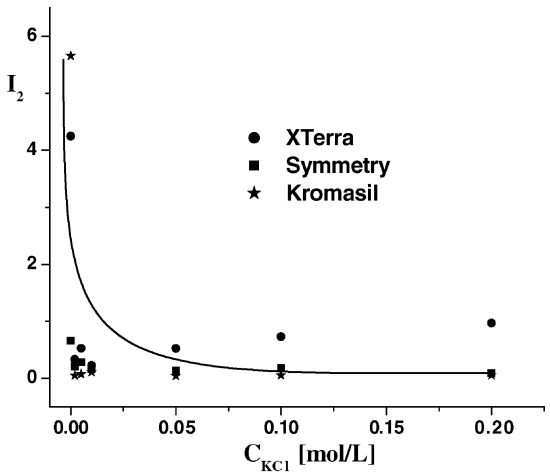

Fig. 11. Same as in Fig. 8 except the interaction parameter I_2 on the high-energy sites. Note the rapid decreasing trend of the curves.

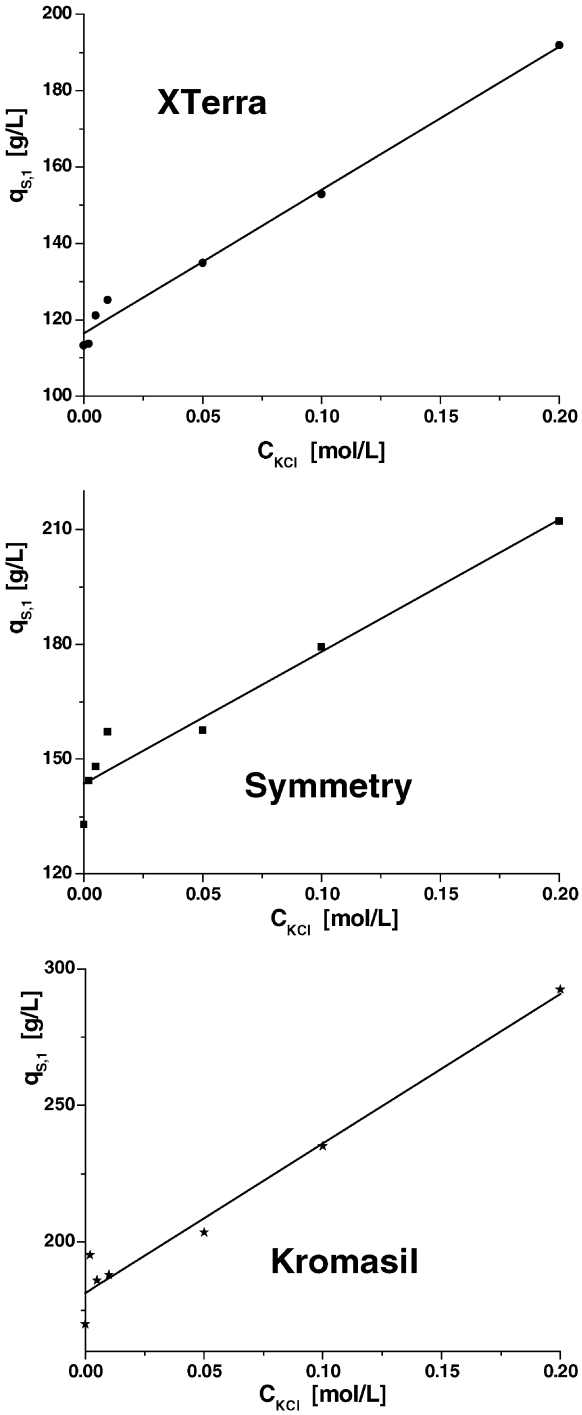

Fig. 12. Best fitting of the $q_{s,1}$ data curves using Eq. (6).

pacities of the two types of sites would be caused by the large steric hindrance of this large complex the size of which does not allow an important mass of propranolol to be in contact with the C_{18} chains (especially at the buried sites of type 2). Because the adsorbate species would be neutral, its adsorption energy is stronger (the adsorbate can penetrate deeper in the C_{18} layer at the interface with the liquid phase) and the partition equilibrium would remain constant (because the

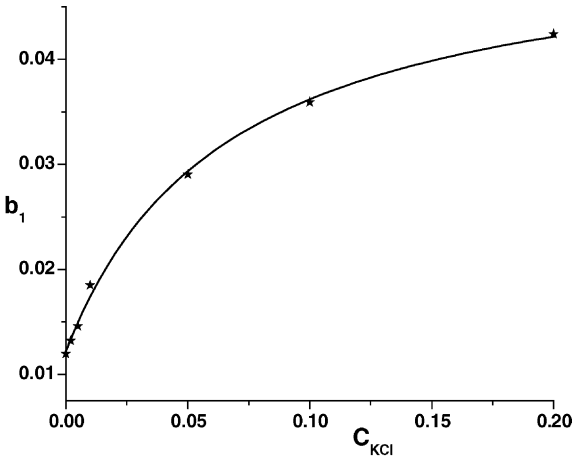

Fig. 13. Best fitting of the b_1 data curves (average of data measured on the Kromasil, Symmetry and XTerra column) using Eq. (9).

adsorbate is already completely buried inside the C_{18} layer). Adsorbate-adsorbate interactions are much stronger because SO_4^{2-} aggregates the two propranolol molecules by some ionic bound. This description of the adsorbed phase has the advantage of being fully consistent with the observations. It also explains why the effect observed with the sulphate ion is not encountered with any monovalent anion (Cl^- and NO_3^-) nor with a bivalent cation (e.g., Ca^{2+}).

We measured also the influence of potassium borate, using the bivalent anion $B_4O_7^{2-}$ (with $[K_2B_4O_7] = 0.0667$ M). While the pH of the mobile phase containing sodium sulphate was about 4, the pH of the mobile phase containing $K_2B_4O_7$ was 9. The retention volume of propranolol in this mobile phase was about 130 hold-up volumes (see Fig. 19). By contrast, its retention volume in the sulphate solution was only 12 hold-up volumes. In addition to a ten-fold higher retention volume, we observed that the band profile becomes langmuirian and that the adsorbate–adsorbate interactions vanish.

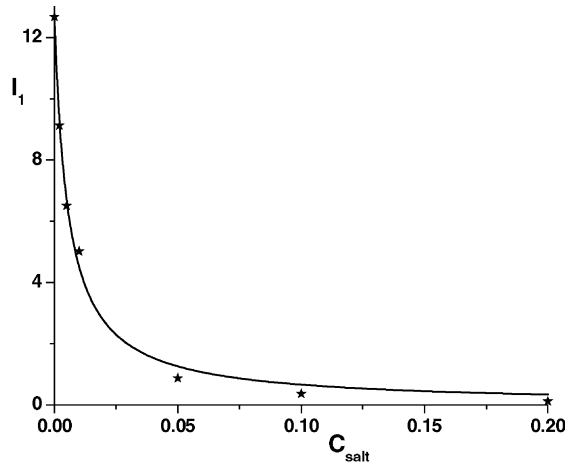

Fig. 14. Best fitting of the I_1 data curves (average of data measured on the Kromasil, Symmetry and XTerra column) using Eq. (11).

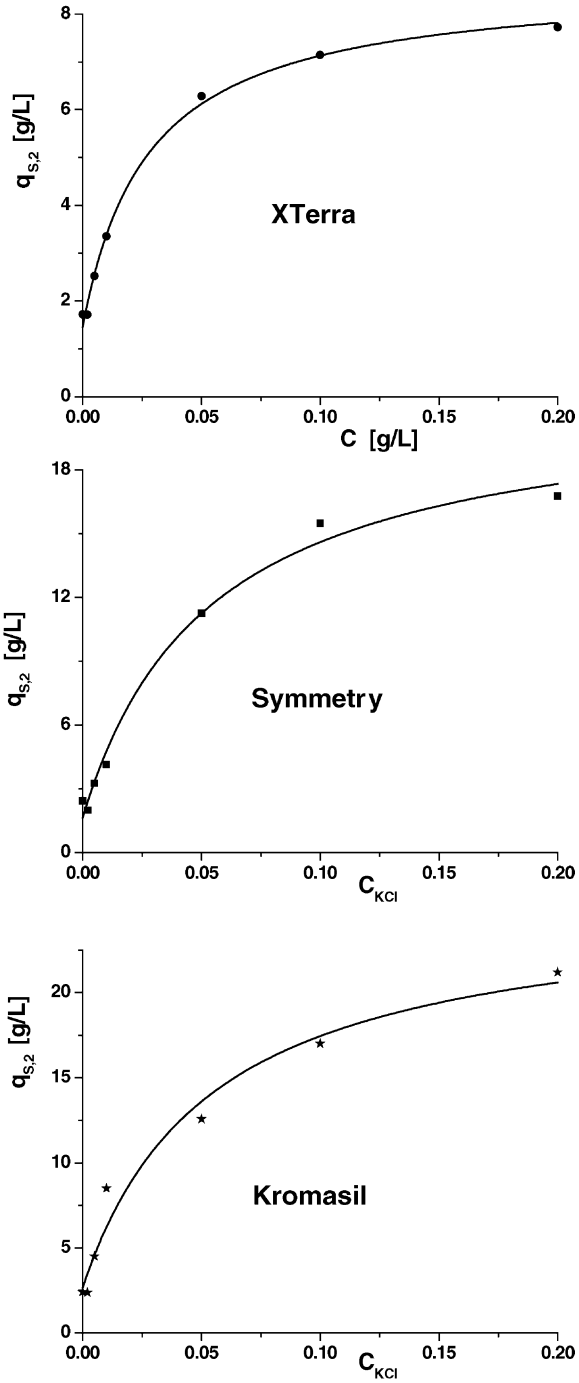

Fig. 15. Best fitting of the $q_{s,2}$ data curves using Eq. (6).

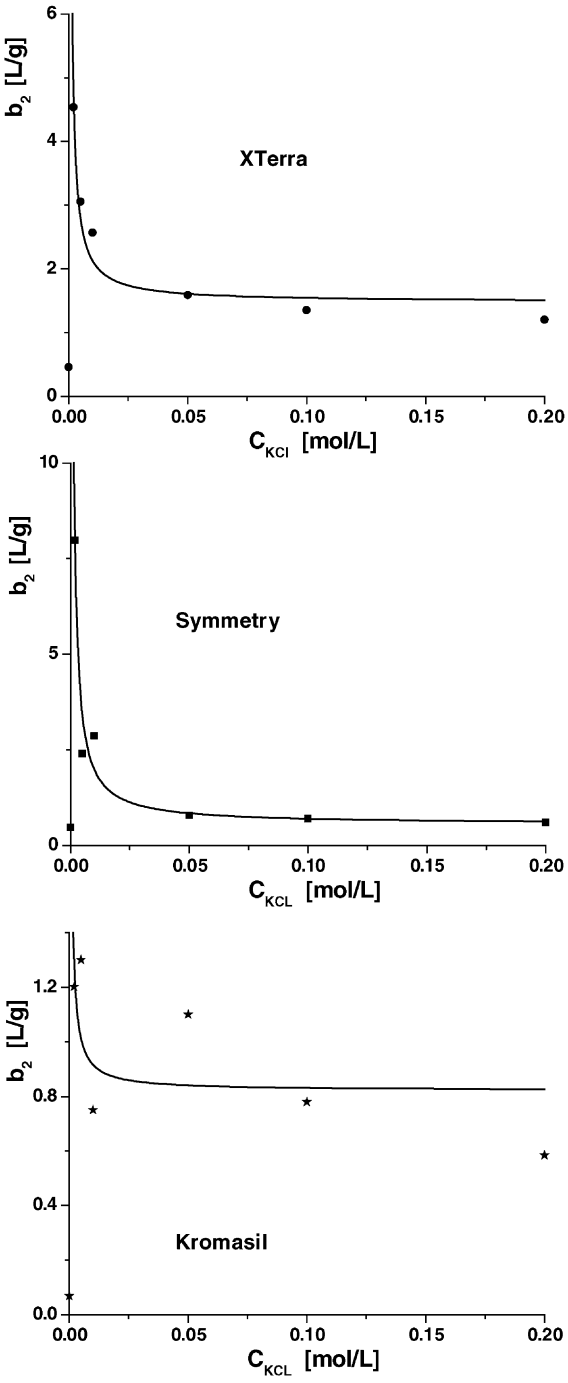

Fig. 16. Best fitting of the b_2 data curves using Eq. (10).

Two phenomena may explain the considerable increase of the retention volume

1. A strong ionic interaction of the adsorbate with the silanol groups that are nonprotonated at this high pH cannot be neglected on the conventional Kromasil-C_{18} phase.
2. The fraction of propranolol that is protonated in the solution decreases (p$Ka \simeq 9$) and the retention factor of the unprotonated form (neutral) of propranolol is larger than that of the protonated form that is dominant at neutral or acidic pH.

The same experiment gives similar results on XTerra MS and Symmetry-C_{18}, with retention volumes of propranolol of approximately 85 and 130 column hold-up volumes, respectively, with potassium borate and 10–12 column hold-up volumes with sodium sulphate. This result allows the elimination

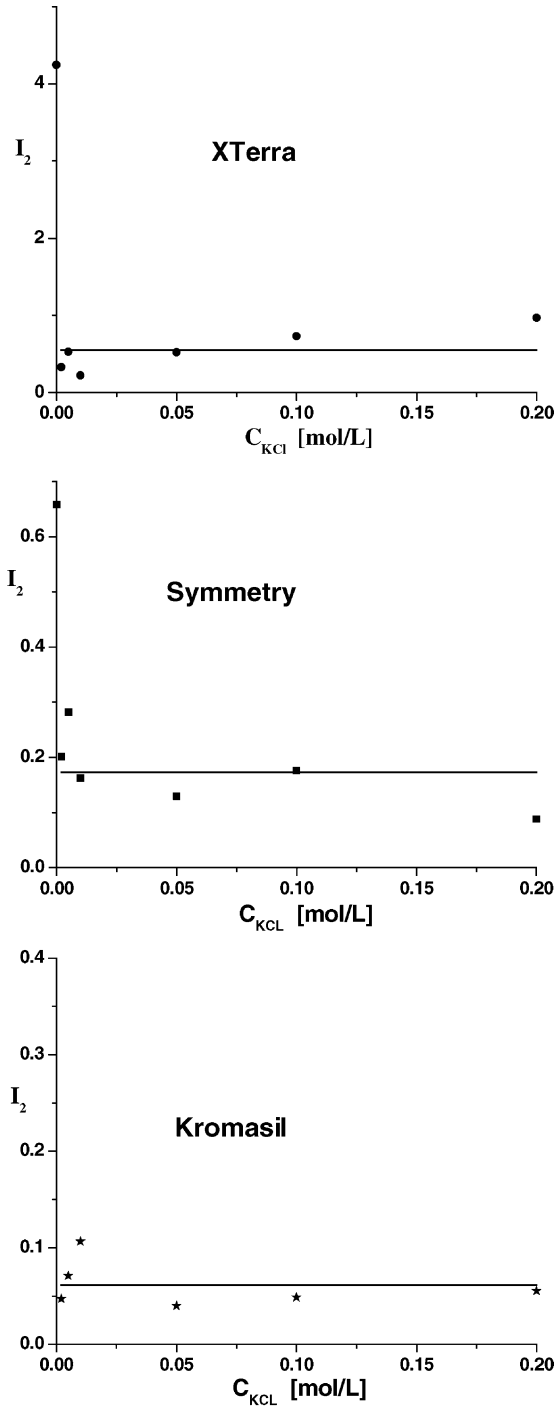

Fig. 17. Best fitting of the I_2 data curves using Eq. (12).

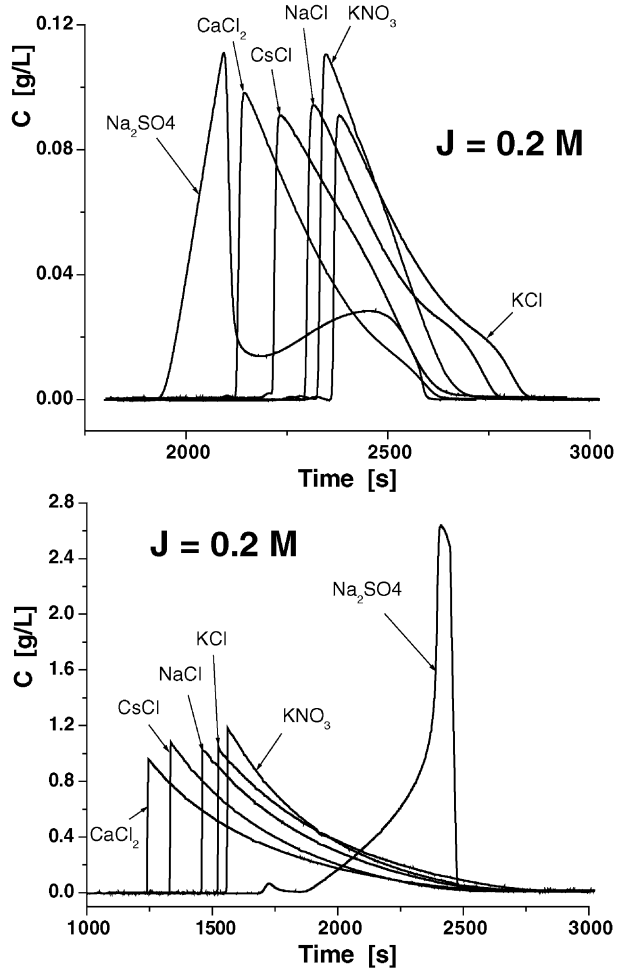

Fig. 18. (Top) Evolution of the band profiles of propranolol on the Kromasil column at constant ionic strength $J = 0.2$ M ($J = 1/2 \sum C_i z_i^2$) in the mobile phase (methanol:water, 40/60, v/v) as a function of the size of the cation (NaCl and KCL), the size of the cation (KNO_3 and KCL), the valence of the cation ($CaCl_2$ and KCl) and the valence of the anion (NaCl and Na_2SO_4). (Bottom) Injection during 15 s of a 1.5 g/l propranolol solution; injection during 15 s of a 30 g/l propranolol solution.

of the first possibility because XTerra has a very small silanol activity at pH 9. This is demonstrated by the constant retention of bretylium (mobile phase acetonitrile:water, 50/50, v/v) [40] and of lithium (mobile phase methanol: bufered water [0.001 M Acetate], 60/40, v/v) [41] in a wide range of pH, from acidic to above 11. Accordingly, the considerable increase of retention associated with the substitution of the borate to the sulphate anion can be due only to the deprotonation of the acid form of propranol to its basic (i.e., neutral) form at pH = 9. The ratio between the retention factors of the two propranolol species is approximately 8.5, 11, and 11 for Xterra, Symmetry and Kromasil, respectively. These ratios are of the same order of magnitude as those reported by Neue and al. [40] who studied the effect of the pH on the retention of acidic and basic forms of compounds on XTerra. The increase in retention associated with the substitution of sulphate with borate is due to the drastic change in the degree of deprotonation of propranolol, not to the increase of the interactions between the positively charged solute molecules and the unprotonated surface silanol which has a smaller effect, although it explains the difference observed between the behavior of Symmetry and Kromasil (which both contain many more residual silanols than XTerra).

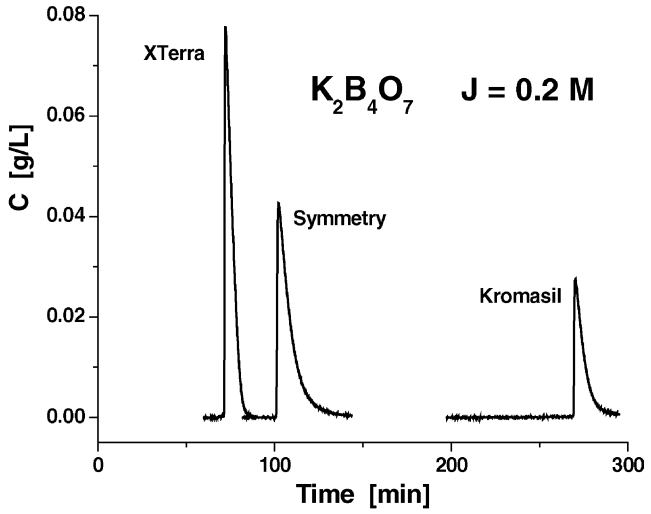

Fig. 19. Band profiles of propranolol (injection during 15 s of a 1.5 g/l solution) on the Kromasil column using potassium borate (bivalent anion) as the salt dissolved in the mobile phase (methanol:water, 40/60, v/v). Ionic strength $J = 0.2$ M. Note, by comparison to the use of the Na_2SO_4 salt in Fig. 18, the strong increase of the retention as well as the langmuirian shape of the band with no more shoulder.

5. Conclusion

The influence of the ionic strength of the mobile phase and of the nature of the salt used on the adsorption behavior of propranolol is quite similar on Kromasil-C_{18} and on XTerra-C_{18} and Symmetry-C_{18}. The phenomenon is well described by assuming that there are two different types of adsorption sites, as suggested earlier by independent frontal analysis data. As shown earlier, this behavior cannot be explained by ion exchange interactions between the cation propranolol$^+$ and the free unprotonated Si–O$^-$ groups at the surface of the adsorbent for several reasons.

– The surface coverage of endcapped Kromasil is high (3.6 versus 3.2 and 2.5 μmol/m^2 for Symmetry and XTerra, respectively) and the access for a two-rings molecule to free silanol groups which are buried within the C_{18} chains is but limited.
– The pH of the mobile phase is imposed by the dissociation equilibrium of propranolol (pH $\simeq$ 5). This pH is below the threshold of activation of the silanol groups on bonded phases (this pH is around 6–8).
– The difference between the adsorption energies on the sites of types 2 and 1 is small (only a few kJ/mol for Kromasil). This would not be consistent with an ion-exchange reaction.

We must conclude that only dispersive interactions may take place between the molecules of propranolol and the surface of Kromasil-C_{18}. This result is consistent with similar results obtained with neutral compounds like caffeine and phenol. The essential difference between the behaviors of neutral and ionizable molecules is that the latter may interact in the adsorbed phase with other ionized molecules of the same species, especially when the solution contains no supporting salt, like it was in the present study. Also, the saturation capacities, the adsorption constants and the adsorbate–adsorbate interaction coefficients depend strongly on the salt concentration of the mobile phase.

The set of experimental results obtained on three different bonded phases (XTerra, Symmetry and Kromasil) leads to the following general conclusions regarding the influence of the concentration of aprotic ions.

- The amount of ionizable molecules that the stationary phase can adsorb at saturation on either types of sites increases with increasing concentration of salt. The maximum is reached at lower concentration for sites 2 (less numerous but with a higher adsorption energy) than sites 1 (predominant). This observation is consistent with the progressive decrease of the repulsive interactions between the polar heads of the adsorbed organic cations.
- The equilibrium constant on the low-energy adsorption sites increases because the solubility of propranolol decreases. There are two reasons. First, the "common ion effect" due to the addition of potassium chloride to a solution of propranolonium chloride. Second, the "salting out effect" has an impact on the naphthyl group of propranolol by enhancing the naphthyl-naphthyl interactions. The equilibrium constant on the high-energy sites is much less sensitive and remains constant above 0.05 M.
- The adsorbate–adsorbate interactions decrease rapidly when the supporting salt is added to the mobile phase. The simple bi-Langmuir isotherm model may well describe the band profiles recorded at high salt concentrations.

However, the concentration of salt added to the mobile phase or the ionic strength of the solution are not the only parameters controlling the general adsorption behavior of ionizable compounds on endcapped C_{18}-bonded stationary phases. No two salts among those studied, KCl, NaCl, CsCl, KNO_3, $CaCl_2$, and Na_2SO_4 give the same band profiles for propranolol at the same solution ionic strength while they all give exactly the same band profiles for neutral analytes like 3-phenyl-propan-1-ol. The specific salt-solute electrostatic interactions seem to be responsible for these differences.

Finally, our results demonstrate that the acquisition of accurate equilibrium isotherm data in a wide concentration range, for selected compounds in chromatographic systems, allows a detailed investigation of the retention mechanisms of these compounds and permits conclusions that linear retention data cannot afford. The usefulness of this approach, already established regarding chiral separation mechanisms, will be illustrated in a forthcoming series of papers dealing with the influence of the anions of various buffers on the retention of propranolol.

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