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Effect of the ionic strength of salts on retention and overloading behavior of ionizable compounds in reversed-phase liquid chromatography I. XTerra-C_{18}

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Abstract

The influence of the salt concentration (potassium chloride) on the retention and overloading behavior of the propranolol cation (R'-NH_2^+-R) on an XTerra-C_{18} column, in a methanol:water solution, was investigated. The adsorption isotherm data were first determined by frontal analysis (FA) for a mobile phase without salt (25% methanol, v/v). It was shown that the adsorption energy distribution calculated from these raw adsorption data is bimodal and that the isotherm model that best accounts for these data is the bi-Moreau model. Assuming that the addition of a salt into the mobile phase changes the numerical values of the parameters of the isotherm model, not its mathematical form, we used the inverse method (IM) of chromatography to determine the isotherm with seven salt concentrations in the mobile phase (40% methanol, v/v; 0, 0.002, 0.005, 0.01, 0.05, 0.1 and 0.2 M). The saturation capacities of the model increase, $q_{s,1}$ by a factor two and $q_{s,2}$ by a factor four, with increasing salt concentration in the range studied while the adsorption constant b_1 increases four times and b_2 decreases four times. Adsorbate–adsorbate interactions vanish in the presence of salt, consistent with results obtained previously on a C_{18}-Kromasil column. Finally, besides the ionic strength of the solution, the size, valence, and nature of the salt ions affect the thermodynamic as well as the mass transfer kinetics of the adsorption mechanism of propranolol on the XTerra column.

Keywords: Adsorption equilibrium; Adsorption isotherm; Moreau isotherm model; Frontal analysis; Overloaded band profiles; Silica gel; Propranolol

1. Intorduction

Reversed-phase liquid chromatography (RPLC) is the technique most widely used to perform biochemical, biomedical, pharmaceutical or environmental separations [1]. The new separation challenges that kept arising from the constantly evolving practical applications encountered in these areas have lead to the use of increasingly complex experimental conditions. Most of the molecules analyzed are ionized or can easily participate into proton exchange with the mobile phase. Most mobile phases are not made from a pure solvent nor a simple aqueous solution but contain complex mixtures of salts and/or buffers. The selection of these additives is an important aspect of the optimization of the experimental conditions of a separation. In most cases, the complexity of the chromatographic system is such that a fundamental understanding of the phenomena that take place in the column is lost. Accordingly, RPLC has become an empirical method, pursuing eclectic separative goals, being practiced with sets of confused and contradictory recipes, so that a sound understanding of the phenomena that take place during the separation process is lost.

Fundamentally, chromatographic separations can be interpreted on the basis of the equilibrium thermodynamics of the solutes between the stationary and mobile phases [2–4]. The independent experimental acquisition of the adsorption isotherm, or the relationship at equilibrium between the amount adsorbed in the stationary phase and the concentration in the mobile phase, allows an accurate prediction of the positions and shapes of the overloaded band profiles recorded in preparative chromatography provided a

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large enough number of plates of the column. The inverse method (IM) [5–7], consisting in determining the adsorption isotherm parameters from the overloaded profiles, cost less from the experimental point of view but assumes always the isotherm model which might be wrong but still lead to a good agreement with the experimental profiles. For instance, we reported the simulation of overloaded band profiles of phenol elued in the gradient mode [8] by using mixtures of methanol and water as the mobile from the measurement of the adsorption isotherm of phenol by frontal analysis (FA) for different composition of methanol in the aqueous mobile phase [9] and by FA by characteristic point (FACP) with pure water [10]. The shape and the position of the band profiles of phenol under gradient elution was then completely understood from the adsorption mechanism determined independently. It was demonstrated that the band profile was the result of the following adsorption mechanism confirmed elsewhere [11]: phenol interacts with the adsorbent on two types of sites, the first one corresponding to the adsorption of phenol on the top of the C_{18}-bonded phase with a low adsorption energy and the second to the partition of phenol within the C_{18}-bonded layer.

In this work, we studied the adsorption behavior of a charged molecule, the β-blocker propranolol ($R\text{-}NH_2^+\text{-}R'$), on a completely apolar adsorbent, the XTerra-C_{18} column, in the presence of a salt of increasing concentrations in a methanol:water mobile phase (composition 40/60, v/v). Our initial goal was to clarify the apparent inconsistency in the adsorption behavior of propranolol from with and without salt in the mobile phase on endcapped Kromasil-C_{18} [12]. A bi-Moreau (a two-sites isotherm with lateral interactions in the adsorbed phase for both sites) and a bi-Langmuir (a two-sites isotherm without lateral interaction in the adsorbed phase) isotherm were the best and simplest isotherm model accounting for by the adsorption of propranolol without and with salt in the mobile phase, respectively. The raised question was to know what was going on when step by step, the concentration of the salt was increased from 0 to 0.2 M. Then, once unified the adsorption behavior and the isotherm model of propranolol both with and without salt in solution, the evolution of the isotherm parameters as a function of the salt concentration (or the ionic strength solution, J) will be estimated from the IM for isotherm determination.

2. Theory

In this work, the equilibrium isotherms of propranolol between an XTerra MS C_{18} column (Waters, Milford, MA) and a methanol:water (25/75, v/v) solution containing different concentrations of several salts were determined. The acquisition of a large number of isotherm data would have been time consuming and would have required large amounts of chemicals. Considerable savings were achieved without loss of accuracy by combining FA and the IM. One isotherm was measured with FA (Section 2.1). It was modeled using a bi-Moreau model (Section 2.2). Assuming that the same isotherm model applies when the experimental conditions are changed without altering profoundly the retention mechanism and that only the numerical values of the model parameters vary, the band profiles of large samples of propranolol were acquired and treated by IM to derive the best values of the coefficients for a variety of salt concentrations (Section 2.3).

2.1. Determination of adsorption isotherms by frontal analysis

FA [2,13,14] was used to measure the single-component adsorption isotherm data of propranolol on the XTerra MS C_{18} column, with a methanol:water solution containing no salts. The derivation of the amount of the studied compound adsorbed on the column at equilibrium with a solution of known concentration is explained in detail elsewhere [15].

2.2. Model of isotherm

The simplest isotherm model for a homogeneous adsorbent surface with lateral, i.e., adsorbate adsorbate, interactions is the Moreau model [16]. This model was considered to describe the adsorption data of propranolol onto the adsorbent surface studied here. The results obtained suggested that the surface was not homogeneous and would be better modeled by assuming that it consists in patches of two different types of sites. So, we considered the following extension of the Moreau model, a model that will be called here the bi-Moreau model. This model assumes that a different Moreau model applies to each type of sites, 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^* and C are the equilibrium concentrations in the stationary and mobile phases, respectively, $q_{s,1}$, $q_{s,2}$, 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 sites 1 and 2, respectively. Note that this model is nearly identical to the Ruthven model developed for adsorption on zeolites [17] and for which the relationships between the coefficients in the numerator and denominator are slightly different.

The equilibrium constants b_1 and b_2 are associated with the adsorption energies $\epsilon_{a,1}$ and $\epsilon_{a,2}$ through the following equation [17]:

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

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 the bulk and the adsorbed phases. b_0 is often considered to be independent of the adsorption energy $\epsilon_{a,i}$ [18].

The adsorbate–adsorbate parameter I can be written as [16]:

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

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

2.3. The inverse method

This method has been developed recently [19]. It consists in calculating the isotherm that best accounts for one or, better, for a series of band profiles obtained upon the injection of a large amount or of increasing amounts of the compound considered. In practice, IM affords the best numerical values of the parameters of an isotherm model that is selected, depending on the shape of the band profile(s) recorded. Its main advantage is that it requires only the measurement of these experimental overloaded band profiles. So, the IM spares much time and chemicals compared to "direct" methods, such as FA, which consists in measuring adsorption isotherm data without assuming any isotherm model and in modeling these data. The isotherm model so obtained is validated according to the degree of agreement between the experimental and calculated overloaded band profiles, the latter chromatograms being calculated with the appropriate program of non-linear chromatography (e.g., the equilibrium–dispersive (ED) model of chromatography, see next section). The measured and calculated band profiles are compared by evaluating the following objective function Obj:

$$\text{Obj} = \sum_{i=1}^{i=N} (C_i^{\text{sim}} - C_i^{\text{exp}})^2 \tag{4}$$

where C_i^{sim} and C_i^{exp} are, respectively, the calculated and measured concentrations at point i among the total of N points recorded. All the experimental chromatograms used in this work contained between $N = 500$ and 1000 points. The isotherm parameters are adjusted to minimize the objective function (Eq. (4)) using an optimization routine.

2.4. Modeling of band profiles in HPLC

The overloaded band profiles of propranolol were calculated, using the ED model of chromatography [2–4]. 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 (mass transfer kinetics) that take place in the chromatographic column. The axial dispersion coefficient is related to the column efficiency under linear conditions through the following equation:

$$D_a = \frac{uL}{2N} \tag{5}$$

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 with a small sample.

In this model, the mass balance equation for a single component is written:

$$\frac{\partial C}{\partial t} + u\frac{\partial C}{\partial z} + F\frac{\partial q^*}{\partial t} - D_a\frac{\partial^2 C}{\partial z^2} = 0 \tag{6}$$

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, $q^* = f(C)$.

2.4.1. Initial and boundary conditions for the ED model

At $t = 0$, the stationary phase is in equilibrium with the pure mobile phase and the concentrations of solute in both phases in the column are uniformly equal to zero. The boundary conditions used are the classical Danckwerts-type boundary conditions [2,20] 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 [2,21–23].

3. Experimental

3.1. Chemicals

The mobile phase used in this work was a mixture of methanol and water, 25:75 (v/v) for FA measurements and 40:60 (v/v) for IM because the retention factors increase considerably in the presence of salt. Both solvents were HPLC grade, purchased from Fisher Scientific (Fair Lawn, NJ, USA). Potassium chloride was dissolved at various concentrations in pure water and methanol was added to that solution to prepare the mobile phase. The solvents used to prepare the mobile phase were filtered before use 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. This is an amino alcohol of structure $C_{10}H_7OCHOHCH_2NHCH(CH_3)_2$. It is used as a β-blocker. It was injected under its protonated form, as the hydrochloride. Thiourea and propranolol; potassium, sodium, and calcium chlorides; and sodium sulfate were all obtained

Table 1
Physico-chemical properties of the C_{18}-bonded packed XTerra column (150 mm × 3.9 mm)

Particle shape	Spherical
Particle size (μm)	5
Pore size[a] (Å)	120
Pore volume[a] (ml/g)	0.64
Surface area[a] (m^2/g)	176
Total carbon (%)	15.2
Surface coverage (μmol/m^2)	2.17
Endcapping	Yes
Total column porosity	0.6384[b], 0.6178[c]

[a] Data for the packings before derivatization.
[b] Data from thiourea injections in a methanol/water mobile phase (25/75, v/v, for FA).
[c] Data from thiourea injections in a methanol/water mobile phase (40/60, v/v, for IM).

from Aldrich (Milwaukee, WI, USA). 3-Phenyl-propan-1-ol was from Fluka/Sigma (St. Louis, MO).

3.2. Columns

The column used in this study (XTerra MS C_{18}, 150 mm × 3.9 mm) was given by the manufacturer (Waters Corporation, Milford, MA, USA). The main characteristics of the packing material used are summarized in Table 1. The hold-up time was derived from the retention time of two consecutive thiourea injections. The column porosity remained constant at 0.6178, whatever the salt concentration in the mobile phase (40:60, v/v). This porosity depends only on the methanol concentration of the mobile phase.

3.3. Instrument

The isotherm data and the overloaded band profiles were acquired using a Hewlett-Packard (now Agilent Technologies, Palo Alto, CA, USA) HP 1090 liquid chromatograph. This instrument includes a multi-solvent delivery system (tank volumes, 1 l each), 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 this contribution. 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 isotherm of propranolol was measured in a mixture of methanol and pure water. The retention was so low with a 40:60 (v/v) solution, in the absence of potassium chloride, that the acquisition of the experimental data was carried out with a lower methanol content, 25%. This was necessary in order to obtain the accurate adsorption data allowing the correct modeling of the FA data. The solubility of propranolol is approximately 50 g/l in a 25:75 (v/v) aqueous solution of methanol. Accordingly, the maximum concentration used in FA was 40 g/l. This avoids any risk of precipitation of the compound in the instrument. Two master solutions were prepared, at 10 and 100% of the maximum concentration. Two consecutive FA runs were then performed, starting from the lowest (first run, 7 points) to the highest concentrations (second run, 21 points), and a total of 28 data points were acquired. One pump (A) of the HPLC instrument was used to deliver a stream of the pure mobile phase (methanol:water, 25:75, v/v) and a second pump (B for the 100% solution, C for the 10% solution) to deliver a stream of the sample solution. The concentration of propranolol in the FA stream is determined by the concentration of the mother sample solution and by the flow rate fractions delivered by the two pumps. The breakthrough curves were recorded at a flow rate of 1 ml/min, with a sufficiently long time delay between each breakthrough curve to allow for the complete re-equilibration of the column with the pure mobile phase between two successive measurements. 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 the feed concentration applied. To avoid recording UV-absorbance signals larger than 1500 mAU and the corresponding signal noise observed at the highest concentrations while keeping a large enough signal at the lowest concentrations, the detector signal was recorded at 325 nm for the 10% solution and at 331 nm for the 100% solution. In each case, the detector response was calibrated accordingly by using the UV absorbance at the plateau observed on each breakthrough curve.

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

After FA was performed, the mobile phase was enriched in methanol (to 40%) in order to obtain measurable and accurate band profiles within a reasonable retention time, thus limiting the amount of mobile phase needed. The addition of potassium chloride into the mobile phase shifts considerably the band position toward high retention times. Solutions with seven different salt concentrations in the mobile phase were prepared, 0, 0.002, 0.005, 0.01, 0.05, 0.1 and 0.2 M. The injections of propranolol were made by using the auto-sampler syringe (250 μl) at two different concentrations, 1.5 and 30 g/l. The band profiles were recorded at

325 and 331 nm after the injections of the 1.5 and 30 g/l solutions, respectively. The overloaded band profiles recorded and used with IM had between 500 and 1000 points.

4. Results and discussion

The goal of our project was to investigate the influence of the buffer composition on the adsorption isotherm of ionic compounds. Initial measurements showed that the ionic strength of the mobile phase also influences this isotherm. Since it is impossible to change the buffer pH without also changing its ionic strength, the addition of a proper amount of an inert salt is required in order to keep the ionic strength of the mobile phase constant. The purpose of this work was to determine whether and to which extent the adsorption behavior of propranolol is affected by the mere addition of a salt in the unbuffered mobile phase. Fig. 1 demonstrates that the addition of significant amounts of a salt ($J = 0.2$ M) has no appreciable effect on the isotherm characteristics of a non-ionic compound, phenyl-3-propan-1-ol.

In a previous work, we reported that the adsorption behavior of propranolol on an endcapped Kromasil-C_{18} column was accounted for by a classical bi-Langmuir model if the mobile phase contained an acetate buffer ($J = 0.2$ M) and by a more unusual bi-Moreau isotherm model in an unbuffered mobile phase [12]. This important difference in adsorption behavior might have been explained by some specific ionic interactions between the positively charged solute and the residual negatively charged surface silanol groups. In order to investigate this possibility, we used an XTerra-C_{18} column, a brand of RPLC stationary phases that exhibits no silanol activity, as demonstrated by the lack of retention of the cation Li^+ in the pH range 3–11 [24]. When propranolol is dissolved in a water solution in the same concentration

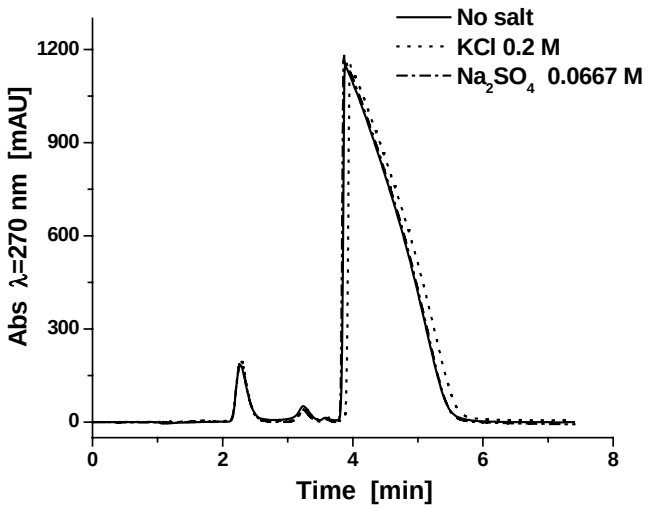

Fig. 1. The addition of a salt to the mobile phase has nearly no effect on the isotherm characteristics of 3-phenyl-propane-1-ol. Sample: 10 mg (250 μl of a 40 g/l solution in the mobile phase). Column: XTerra-C_{18} 150 mm × 3.9 mm. Mobile phase, methanol/water, 50:50 (v/v) with no salt (solid line), 0.2 M KCl (dotted line), or 0.0667 M Na_2SO_4 (solid-dotted line). Flow rate 1 ml/min. Temperature 23 °C.

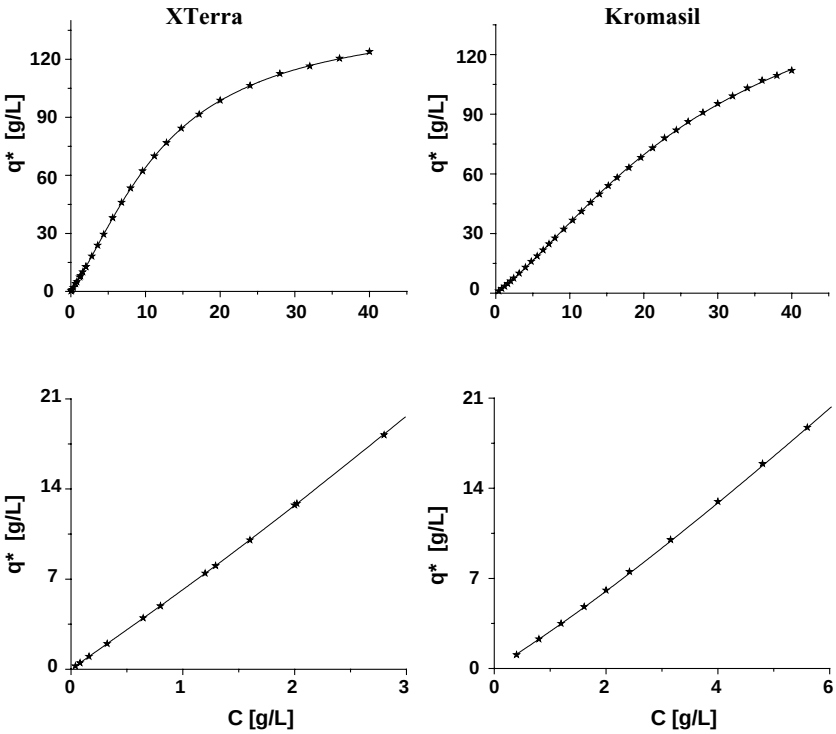

Fig. 2. Adsorption data of propranolol on the XTerra-C_{18} and Kromasil-C_{18} columns measured by FA. $T = 296$ K, mobile phase methanol/water, 25/75 and 40/60 (v/v) with the XTerra and Kromasil columns, respectively. Note the change of the isotherm curvature from low to high concentrations in both cases.

range as the one used in the FA measurement, the solution pH varies by less than 0.5 pH unit, around pH = 5.2 (for propranolol, $pK_a = 9.5$ in pure water, hence, pK_a is approximately 8.8 in a 40:60 (v/v) methanol:water solution [25]). Accordingly, there should be no interactions between propranolol and any ionic exchange or "active" sites on the surface of the XTerra adsorbent.

In this work, we compare the adsorption isotherm behavior of propranolol in a neat aqueous solution of methanol on Kromasil-C_{18} and on XTerra and we discuss the influence of a salt concentration on the equilibrium isotherm of propranolol in the system made of XTerra and a methanol:water solution.

4.1. Adsorption of propranolol on the XTerra-C_{18} column and validation of the bi-Moreau model

Figs. 2 and 3 show the adsorption data (isotherm plots and Scatchard plots, respectively) of propranolol onto the XTerra and the Kromasil columns from a 25/75 and a 40/60 (v/v) solution of methanol in water, respectively. In both cases, the isotherms are clearly convex upward at high concentrations (above ca. 7.5 g/l) and obviously convex downward at low concentrations, below 5 g/l. For XTerra, however, the isotherm appears to be convex upward again at very low concentrations, below ca. 0.2 g/l, as supported by the decreasing Scatchard plot in that low concentration range (Fig. 3). As was done previously with the adsorption data on the Kromasil column [12], the isotherm data for the XTerra column were successfully fitted to the Moreau and the bi-Moreau isotherm models. The regression analysis procedure converged toward a constant set of six independent parameters. The Fisher test value was high, about 40,000. The best parameters for the XTerra column were: $q_{s,1} = 143.8$ g/l, $b_1 = 0.03231$ l/g, $I_1 = 6.66$ (or $\epsilon_{AA} \simeq 1.9RT$), $q_{s,2} = 1.84$ g/l, $b_2 = 0.823$ l/g, $I_2 = 1.37$ (or $\epsilon_{AA} \simeq 0.3RT$). The best parameters obtained with the Kromasil column were : $q_{s,1} = 173.8$ g/l, $b_1 = 0.01349$ l/g, $I_1 = 7.47$ (or $\epsilon_{AA} \simeq 2.0RT$), $q_{s,2} = 1.89$ g/l, $b_2 = 0.08488$ l/g, $I_2 = 23.49$ (or $\epsilon_{AA} \simeq 3.1RT$).

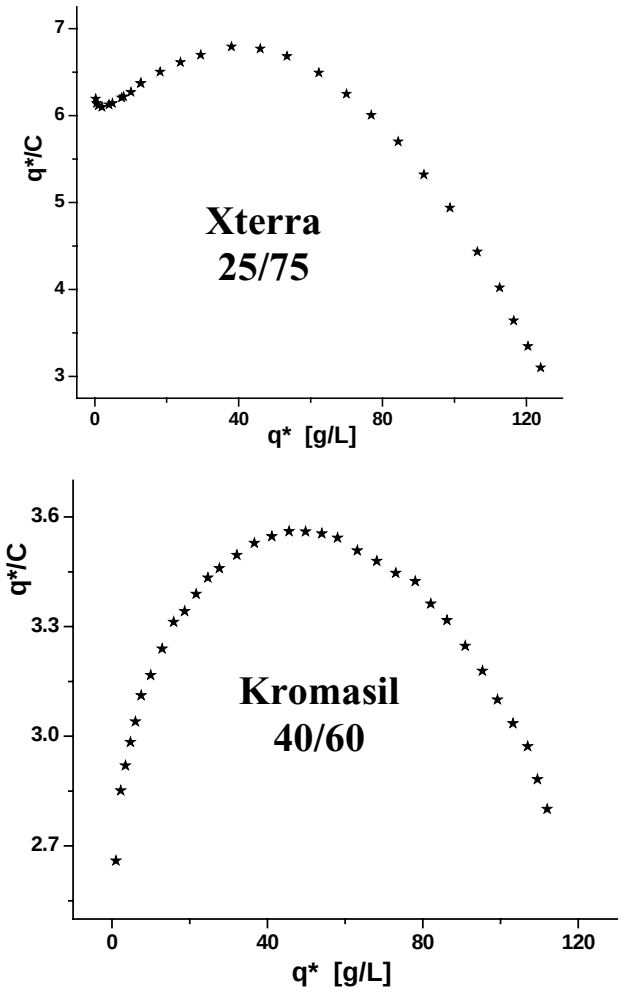

Fig. 3. Scatchard plot representation (q^*/C vs. q^*) of the adsorption data of the XTerra-C_{18} and Kromasil-C_{18} columns presented in Fig. 1. Note the difference in the curvature at very low concentrations.

Comparing these two sets of values, we observe first that the main adsorption constant, b_1, is more than twice larger on XTerra than on Kromasil. This is because propranolol is less soluble in a 25% than in a 40% methanol solution. Secondly, the main saturation capacity, $q_{s,1}$, of the XTerra column is smaller than that of the Kromasil column, probably because the C_{18}-bonded chain density on its surface (2.40 μmol/m^2) is lower than on the Kromasil surface (3.60 μmol/m^2). This difference in chain density arises from the difference in the surface chemistry of the two surfaces. XTerra is a silica–methylsilane hybrid surface with a low density of free silanols. On the more numerous sites of type 1, the intermolecular interactions between propranolol molecules are similar, 1.9 and 2.0 times the thermal energy on the XTerra and the Kromasil columns, respectively. On the other hand, although the saturation capacities of the sites of type 2 ($q_{s,2} \simeq 2$ g/l) on the two columns are close, the equilibrium constant b_2 and the intermolecular interaction parameter I_2 are of different orders of magnitude on the two adsorbents. The adsorption constant b_2 is 10 times larger on XTerra than on Kromasil while the propranolol–propranolol interactions on those sites are 10 times lower on the XTerra column. The difference in the methanol concentrations of the two mobile phases cannot explain such large differences. They must be related to the different chemistry of the two surfaces.

This result explains the large differences between the two isotherm chord plots at low concentrations (Figs. 3 and 4) and between the shapes of the overloaded band profiles recorded at low column loading (Fig. 4). The peak on the XTerra column is almost symmetrical while it is clearly skewed on the Kromasil column, showing that the anti-Langmuirian shape of the isotherm is more pronounced at low concentrations on the Kromasil column than on the XTerra column. In this last case, an initial concentration shock layer would be expected at low concentrations

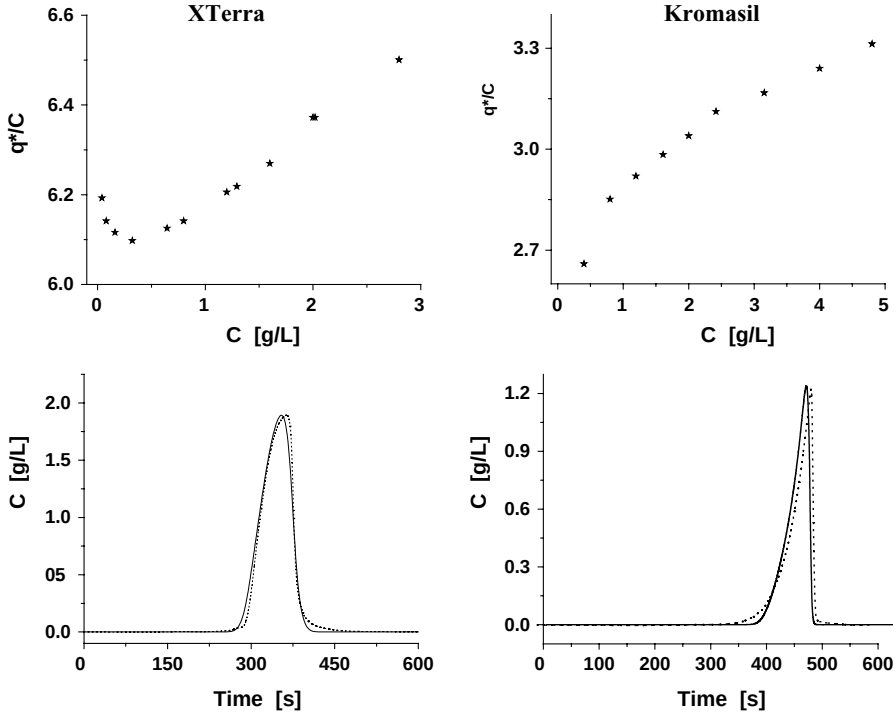

Fig. 4. Adsorption data and band profiles of propranolol. (Top graphs) Scatchard plots of the data in Fig. 3, low concentration range. (Bottom graphs) Comparison between the experimental (dotted line) and simulated (solid line) band profiles of propranolol on the XTerra column (methanol/water, 25/75, v/v; 60 s injection of a 2 g/l solution) and the Kromasil column (methanol/water, 40/60, v/v; 12 s injection of a 4 g/l solution) at low column loading. $T = 296$ K, flow rate 1 ml/min. Calculation made by using the equilibrium–dispersive model of chromatography. Note the difference in shape of the two profiles in agreement with the experimental isotherm chords (see upper graphs).

since the isotherm is initially convex upward, hence has an isotherm chord that decreases with increasing concentration (Fig. 3). This shock layer cannot be seen because the axial dispersion in the column and in the connecting tubes is too important compared to the intensity of the self-sharpening effect at these low concentrations. Finally, Fig. 5 shows an excellent agreement between the experimental band profiles and those calculated from the isotherm parameters aforementioned and the equilibrium-dispersive model of chromatography. Note the important differences in the shapes of the band profiles obtained with the two columns, differences due to the different mobile phase compositions but, more importantly, to the different adsorption behaviors on the high-energy sites of the two surfaces. In fact the band is more retained on Kromasil in methanol:water (40:60) than on XTerra in methanol:water (25:75).

Despite some important quantitative differences between the adsorption isotherms of propranolol on the Kromasil and the XTerra columns, the same bi-Moreau isotherm model accounts as well for the FA adsorption isotherm data on the two columns. It allows an accurate prediction of the overloaded band profiles of this compound on the two columns when no salt is present in the methanol:water mobile phase. It is noteworthy that the same isotherm model accounts as well for two inflection points in the propranolol isotherm on the XTerra column and for only one such point in its isotherm on the Kromasil column.

4.2. Isotherm determination in the presence of salt in the mobile phase by the IM

We assume that the addition of salt (here, potassium chloride) to the methanol/water solution used as the mobile phase does not alter the nature of the isotherm but causes only variations in the numerical values of its parameters. Therefore, we assume that the isotherm behavior remains accounted for by the bi-Moreau model. The basis for this assumption is that the addition of a salt that is entirely dissociated should not affect the dissociation nor the adsorption equilibria of propranolol in the chromatographic system. The retention mechanism should not change abruptly nor the isotherm switch from a model to another one when the salt concentration in the mobile phase is changed. There should be continuity in the behavior of the system.

Seven different potassium chloride concentrations were applied, ranging from 0 to 0.2 M. This time, the IM was used for the determination of the six new sets of isotherm parameters, in order to save time and chemicals. For each salt concentration in the mobile phase (0, 0.002, 0.005, 0.01, 0.05, 0.1 and 0.2 M), two 15 s, 250 μl injections of a propranolol solution were made, at low (1.5 g/l) and high (30 g/l) concentrations. The advantage of performing two injections at very different concentrations is that the high loading-factor profile provides accurate information on the isotherm parameters of the low-energy sites (those that are occupied

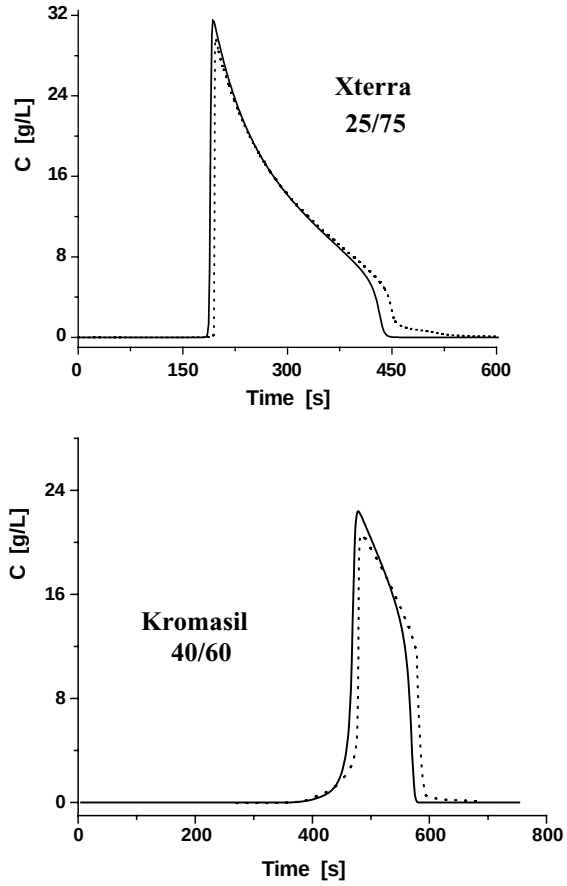

Fig. 5. Comparison between the experimental (dotted line) and simulated (solid line) band profiles of propranolol on the XTerra column (methanol/water, 25/75, v/v; 90 s injection of a 40 g/l solution) and the Kromasil column (methanol/water, 40/60, v/v; 52 s injection of a 36 g/l solution) at high column loading. $T = 296$ K, flow rate 1 ml/min. Calculation made by using the equilibrium-dispersive model of chromatography.

at high concentrations) while the low loading-factor profile provides more accurate data regarding the high-energy site parameters. This is important in the present case in which there is a large difference between the contributions of the two types of sites to the overall Henry constant. It is important to collect data in a wide concentration range in order accurately to estimate the values of the six parameters of the model.

Figs. 6 and 7 show the fourteen chromatograms recorded on the XTerra column, at low (Fig. 6) and high column loadings (Fig. 7). Obviously, the higher the ionic strength of the mobile phase, the higher the band retention, whatever the loading factor. Qualitatively, this behavior is consistent with the adsorption of an ion pair complex formed with a propranolol cation and a chloride anion. The higher the concentration of potassium chloride in the mobile phase, the higher the abundance of neutral propranolol complex, hence a larger retention of the analyte.

In addition to the sharp increase of the retention time, there is an important, progressive change in the shape of the high concentration band profiles. At low potassium chloride concentrations (e.g., at 0.002 M in the insert of Fig. 7), the front of the band exhibits two concentration shock layers separated by a diffuse boundary. The shock layer that was expected in the chromatogram in Fig. 4 because of the strong initial convex upward shape of the isotherm at very low concentrations (Fig. 3) was not actually observed. It is now detectable (Fig. 7). The injection in Fig. 7 was performed with the auto-sampler, the one in Fig. 4 with the pump delivery system which has a larger extra column volume, in which more axial dispersion takes place, smoothing markedly the front of the band profile. This result confirms the validity of the isotherm parameters determined in the

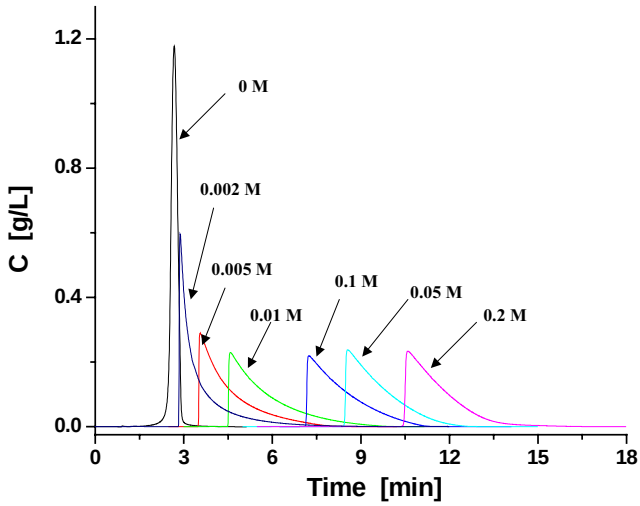

Fig. 6. Evolution of the position and the shape of overloaded band profiles of propranolol (injection of a 1.5 g/l solution during 15 s) as a function of the salt concentration or ionic strength of potassium chloride in the mobile phase (methanol/water, 40/60, v/v). $T = 296$ K, flow rate 1 ml/min. Note that the displacement of the band toward high retention times when the ionic strength solution increases.

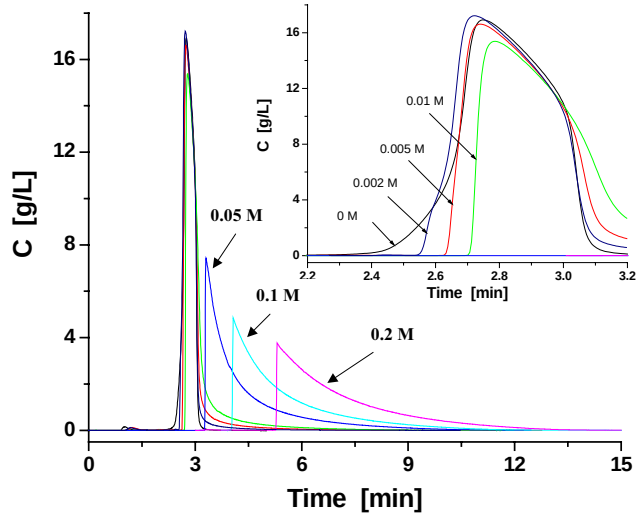

Fig. 7. Same as in Fig. 5 except the injection of a 30 g/l solution of propranolol. Note the change, not only in the position of the bands, but also of the shape of the band profiles.

previous section for XTerra eluted with a mobile phase containing no salt. The curvature of the isotherm is initially convex upward (because the adsorbate–adsorbate interactions taking place on the high-energy sites are quite weak), then it becomes convex downward (because the high-energy sites are saturated and the adsorbate–adsorbate interactions that take place on the low-energy sites are strong), and it finally ends up being convex upward again (when the low-energy sites become close to saturation). This mechanism explains also the curvature of the desorption profile, which is initially diffuse at high concentrations, experiences a shock layer at intermediate concentrations, and finishes with a long tail.

When the potassium chloride concentration becomes large (typically beyond 0.05 M), the band profile becomes more conventional and similar to the profiles obtained for compounds having a convex upward isotherm (e.g., a Langmuir or bi-Langmuir isotherm). Actually, we will show that the bi-Moreau model can also account for this kind of band profiles with appropriate numerical values of the isotherm parameters: the bi-Langmuir isotherm is a particular case of the bi-Moreau isotherm for which there are no adsorbate–adsorbate interactions, i.e., $I_1 = I_2 = 0$.

In order to determine the best numerical values of the bi-Moreau parameters for each salt concentration, we used IM. Fig. 8 shows the agreement between the best calculated band profiles and the experimental band profiles at high loadings, for the seven different mobile phases. An excellent agreement is observed between calculated and experimental profiles. Obviously, the bi-Moreau model predicts as well the overloaded band profiles at very low salt concentrations, where the front and rear parts of the profiles are complex, and at high salt concentrations, where the profile exhibits a front shock and a diffuse rear boundary. As shown in Fig. 8, the fitting procedure gives a nearly perfect agreement for the high concentrations parts of the bands. The prediction of the low concentration part of the band profile and particularly that of its rear boundary is somewhat less satisfactory. This confirms that the parameters of the low-energy sites (i.e., $q_{s,1}$, b_1 and I_1) are assessed with more accuracy than those of the high-energy sites ($q_{s,2}$, b_2 and I_2). The latter parameters are better derived from the profiles obtained with a second injection, performed with a less concentrated solution. Fig. 9 shows the agreement achieved between the best band profiles derived from the IM procedure and the experimental band profiles obtained for a 20 times lower loading. The new set of isotherm parameters is compared to those derived from the high loading-factor injection in Figs. 10–15.

Interesting conclusions can be drawn from the combination of these two series of isotherm parameters. First, the evolution of all six parameters with increasing salt concentration is the same, whether they were derived by IM from the low or from the high concentration band profiles. The results in Figs. 10–15 lead to some meaningful conclusions regarding the adsorption of the propranolol cation on the C_{18}-bonded XTerra column. Regarding the denser

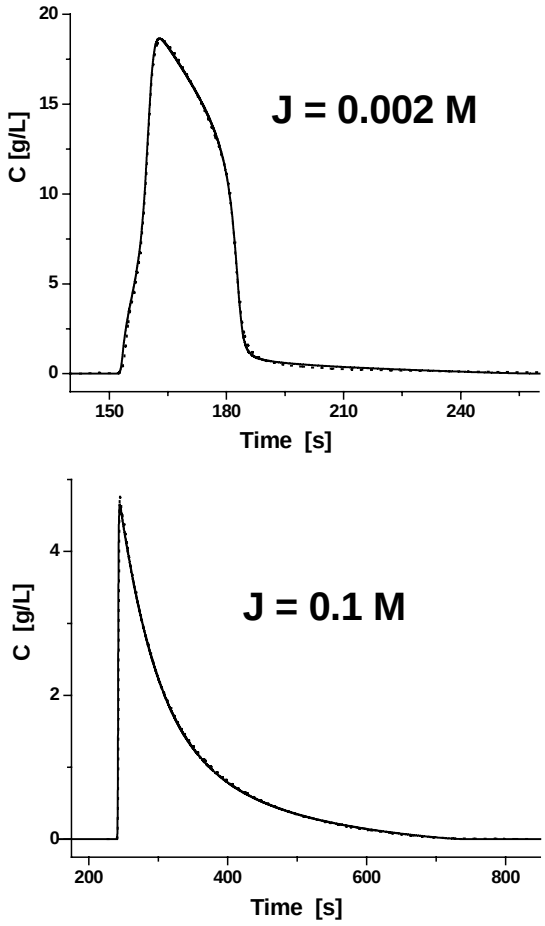

Fig. 8. Comparison between the experimental profiles of propranolol (dotted line) and the best calculated profiles found by the IM (solid line) on the XTerra column (methanol/water, 40/60, v/v; 15 s injection of a 30 g/l solution) at high column loading for different concentrations of potassium chloride 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 ($J = 0.05$ 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 (overestimations compensate further underestimations).

low-energy sites, we can conclude that increasing the ionic strength solution leads to:

- A significant increase of the saturation capacity (by a factor 2 when [KCl] increases from 0 to 0.2 M). A quasi-linear relationship is observed between $q_{s,1}$ and the solution ionic strength.
- A significant increase of the equilibrium constant b_1. It is reasonable to associate this result with the formation of an ion pair complex between the propranolol cation and the chloride anion. A quasi-linear relationship is observed between the reciprocal of b_1 and the logarithm of the solution ionic strength (graph not shown).
- A significant decrease of the adsorbate–adsorbate interactions on these sites (parameter I_1). This interaction energy tends toward zero with increasing solution ionic

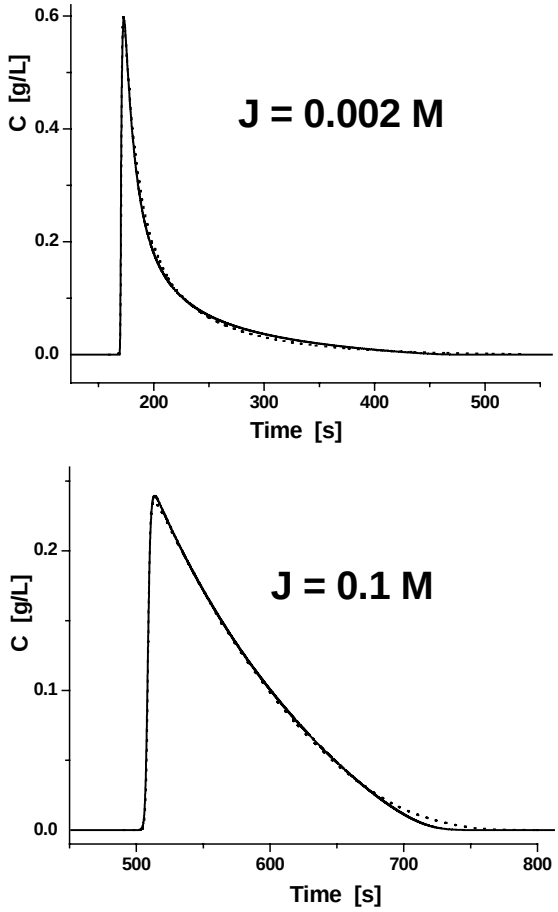

Fig. 9. Same as in Fig. 8 except the injection of a 1.5 g/l solution.

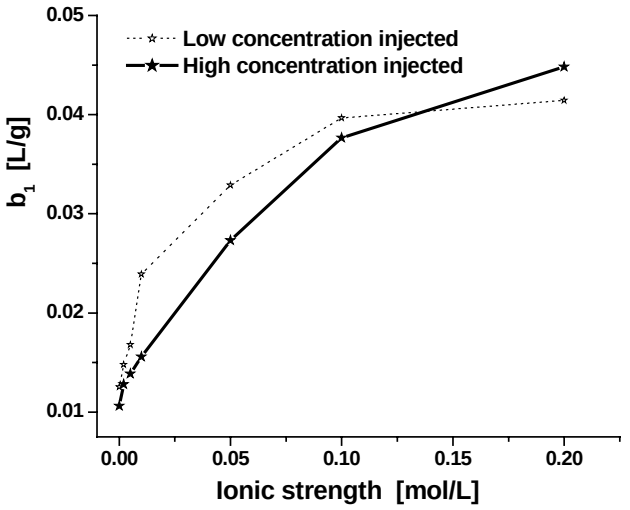

Fig. 11. Best equilibrium constant b_1 of the low energy sites found by using the IM procedure with the high and low loaded band profiles. Note the increase of the energy of adsorption with the salt concentration.

strength, meaning that the bi-Moreau isotherm morphs into a bi-Langmuir isotherm.

As for the less frequent high-energy sites, the increase of the ionic strength solution leads to:

- A large increase of the saturation capacity (by a factor 4 when [KCl] increases from 0 to 0.2 M). The density of these sites tends toward a finite limit at high ionic strength.
- A strong decrease of the equilibrium constant b_2. By contrast with what happens with b_1, a sharp discontinuity is observed when the lowest amount of salt is added to the pure methanol:water solution. A quasi-linear relationship is observed between the reciprocal of b_2 and the logarithm of the solution ionic strength.

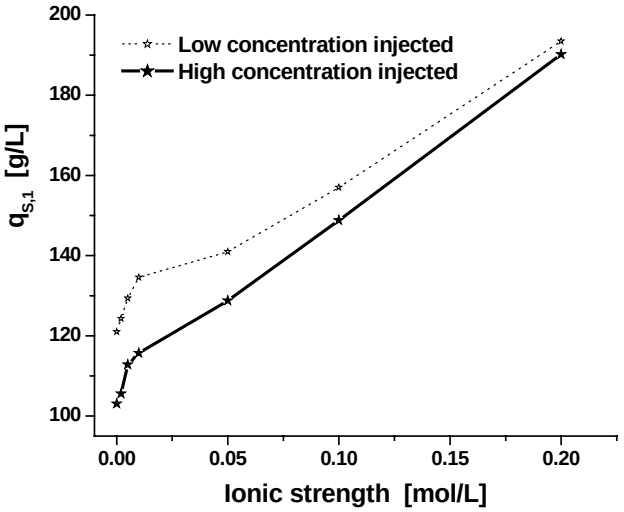

Fig. 10. Best saturation capacity $q_{s,1}$ of the low energy sites found by using the IM procedure with the high and low loaded band profiles. Note that the quasi-linear increase of this isotherm parameter with the salt concentration in the mobile phase.

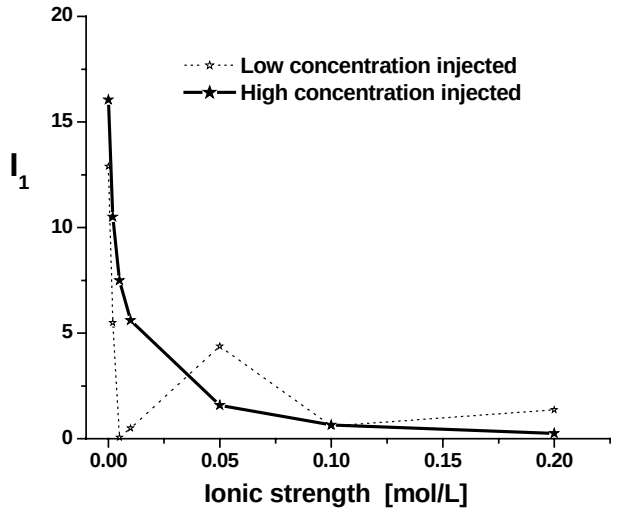

Fig. 12. Best adsorbate–adsorbate interaction parameter I_1 on the low energy sites found by using the IM procedure with the high and low loaded band profiles. Note that the fast decrease of the propranolol–propranolol interactions when the salt concentration increases.

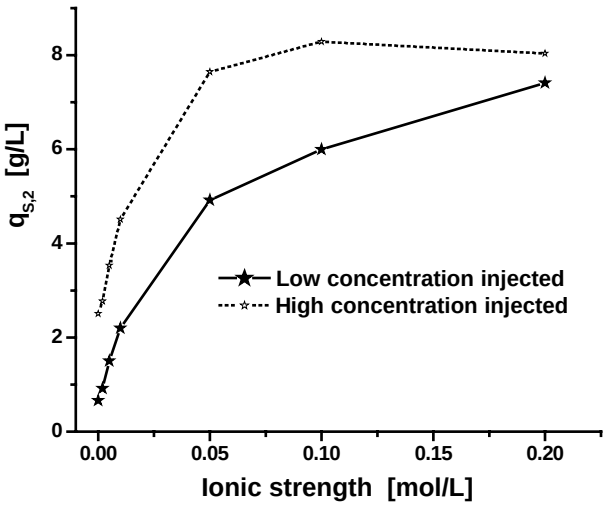

Fig. 13. Same as in Fig. 10 regarding the isotherm parameter $q_{s,2}$. Note that the rapid increase of $q_{s,2}$ then the saturation reached at high salt concentration.

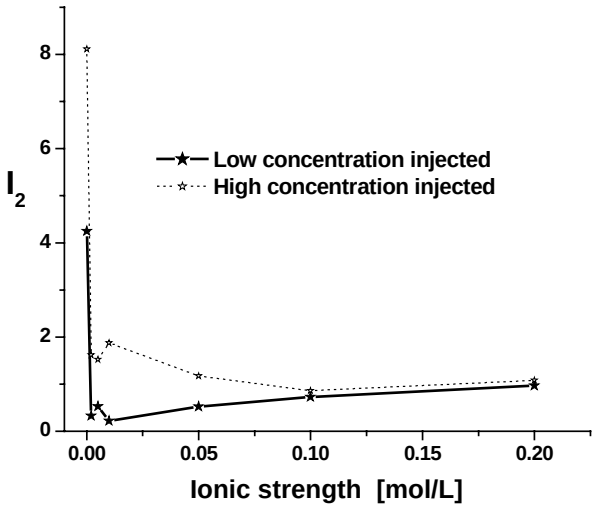

Fig. 15. Same as in Fig. 12 regarding the isotherm parameter I_2.

- A decrease of the adsorbate–adsorbate interactions on these sites. Molecular interactions between propranolol cations are possible because these cations are actually adsorbed as neutral ion pair complexes. The electrostatic repulsion between two propranolol cations is then essentially canceled. This interaction energy becomes close to zero as soon as some salt is added to the mobile phase. However, the high ionic strength limit of I_2 does not seem to be 0 (by contrast with the limit of I_1).

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

A last important issue is whether or not the ionic strength is the fundamental factor controlling the adsorption of propranolol from an aqueous solution of methanol. Accordingly, the nature of the salt was changed while keeping the ionic strength of the solution constant. Fig. 16 summarizes the overloaded band profiles recorded after injection of the same low and high concentration solutions of propranolol as used in the experiments reported in the precedent sections. The ionic strength of the solution was kept constant, at 0.2 M. Obviously, the ionic strength of the solution does not explain the whole variation of the retention time and of the shape of the bands. The nature of the ions used is important.

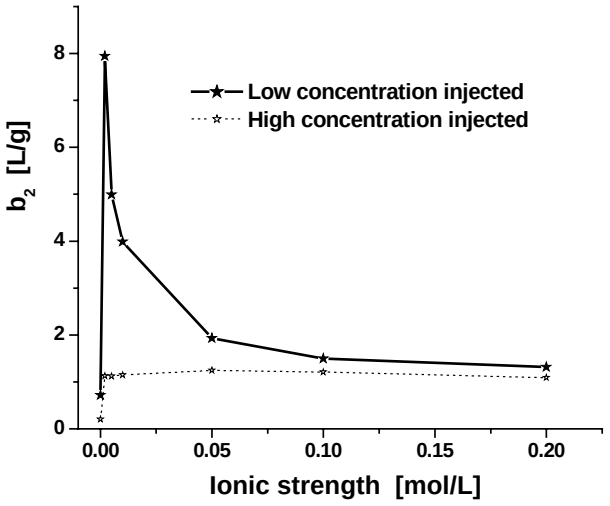

Fig. 14. Same as in Fig. 11 regarding the isotherm parameter b_2. Note that the rupture found between 0 and 0.002 M.

When the monovalent cation potassium was replaced with the smaller monovalent cation sodium, we observed no significant variation of the equilibrium constants b_1 and b_2 (about 0.042 and 1.32 l/g, respectively) and only relatively small variations of the saturation capacities, $q_{s,1}$ increasing from 190 to 208 g/l and $q_{s,2}$ decreasing from 8 to 6 g/l. The adsorbate–adsorbate interactions are still negligible on the first type of sites ($I_1 = 0.076$) and weak on the second type ($I_2 \simeq 1$) as was observed with potassium chloride.

When a bivalent cation like calcium (0.0667 M $CaCl_2$, $J = 0.2$) was used, we observed a significative decrease of the equilibrium constant b_1 (from 0.042 to 0.035 l/g). Thus, it seems that, rather than the ionic strength, the total ion concentration (i.e. $3 \times 0.0667 = 0.2$ M) determines the value of the adsorption constant on the sites of type 1. b_1 was equal to 0.037 l/g with a solution of KCl at 0.1 M ($J =$ 0.1 M). None of the other isotherm parameters is markedly changed.

Finally when a bivalent anion like SO_4^{2-} (0.0667 Na_2SO_4, $J = 0.2$ M) was used, the shape and position of the bands are drastically modified. Strong adsorbate–adsorbate interactions (with ϵ_{AA} increasing from $\simeq$0 to about two times *RT*) now take place on the low-energy sites whose adsorption energy is markedly increased (from 0.042 to 0.075 l/g). The saturation capacity $q_{s,1}$ decreases by 20% of its value,

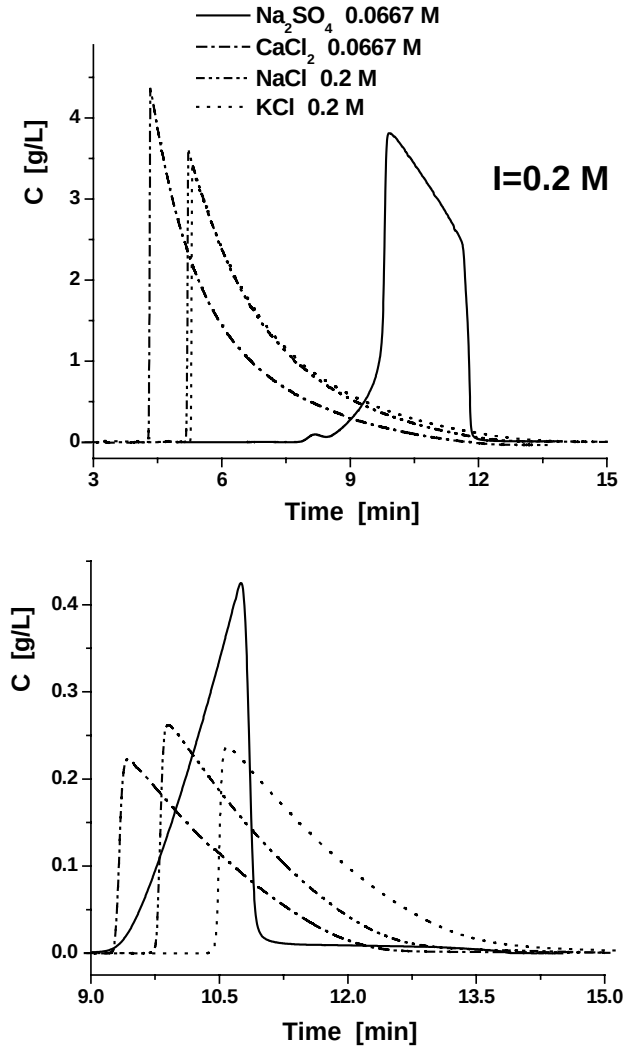

Fig. 16. Evolution of the band profiles of propranolol on the XTerra column at constant ionic strength $I = 0.2$ M ($J = (1/2)\sum C_i z_i^2$) in the mobile phase (methanol/water, 40/60, v/v) as a function of the size of the cation (NaCl and KCl), the valence of the cation ($CaCl_2$ and KCl) and the valence of the anion (NaCl and Na_2SO_4). (Top graph) Injection during 15 s of a 30 g/l propranolol solution. (Bottom graph) Injection during 15 s of a 1.5 g/l propranolol solution. Note in the insert of the lower figure, the long tailing of extra-thermodynamic origin when sodium sulfate is used as the salt.

to 145 g/l. Regarding the high-energy sites, it seems that the desorption kinetics is very slow on these sites. It was impossible for the program to fit correctly the band profiles at low loading. The band tails for as long as 2.5 min. The kinetic origin of this tail was confirmed by repeating the same experiment on a Kromasil-C_{18} column. Fig. 17 looks as if the band of propranolol were split into two overlapping bands. The ED model could not predict this experimental band profiles with any isotherm model. Experiments (not shown) demonstrate that the profile changes significantly with an increase or decrease of the mobile phase velocity, confirming that the effect has, at least in large part, a kinetic origin. We have never seen such a profile yet and find its physical

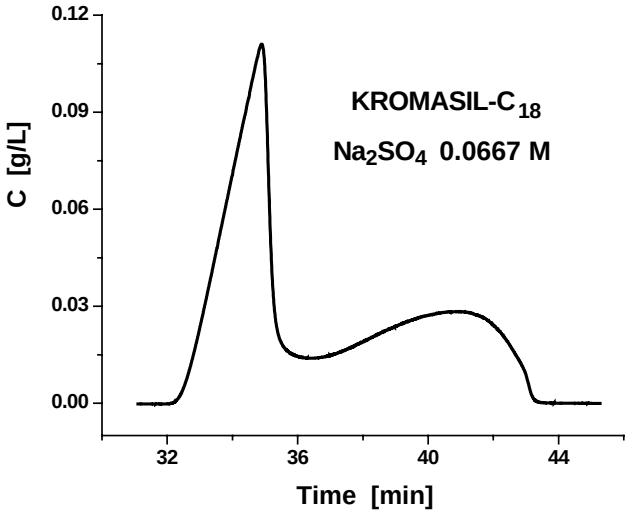

Fig. 17. Band profile recorded with Kromasil C_{18} when sodium sulfate is present in the mobile phase ($J = 0.2$ M). The band profile is split into two overlapped bands, suggesting a very slow desorption kinetics on the high-energy sites 2.

origin puzzling. It deserves further investigations, now in progress.

5. Conclusion

Our results demonstrate clearly that salts have a strong influence on the characteristics of solid–liquid equilibria, at least in the case of ionic compounds. The ionic strength of the solution has a critical impact on the adsorption behavior of ionic species on apolar solid surfaces. Even on a completely apolar chromatographic adsorbent (C_{18}-bonded XTerra column), the concentration of potassium chloride in a methanol:water solution drastically modifies all the isotherm parameters of propranolol. However, the best isotherm model remains the same, whatever the ionic strength of the solution. The adsorption of propranolol is accounted for by a model of the adsorbent surface having two types of adsorption sites, with different adsorption constants and saturation capacities, and on which adsorbate–adsorbate interactions of different energies take place. Adsorbate–adsorbate interactions are possible due to the formation of neutral ion pair complexes in the mobile phase, between the propranolol cation and the chloride anion. This model predicts accurately the low and the high concentration band profiles recorded. The relative values of the parameters of the model change significantly with the salt concentration and this explains the important changes in the band profiles.

Increasing the salt concentration from 0.002 to 0.2 M (i.e., by a factor 100) causes the saturation capacities of the low- and high-energy sites to increase approximately two- and five-fold, respectively; the equilibrium constants of the low- and high-energy sites to increase four-fold and to decrease

four-fold, respectively; and the adsorbate–adsorbate interactions on both sites to drop rapidly to values close to zero, so that, at high ionic strengths, the isotherm becomes equivalent to a simple bi-Langmuir model. However, the ionic strength of the salt is certainly not the only factor that determines the shape and the position of the band profiles of propranolol. The size, the valence, and the charge of the ions dissolved in the solution have an important impact on the isotherm parameters and possibly on the mass transfer kinetics. Considerable work is necessary better to understand the role of the salt on the adsorption mechanism of ionic solutes in RPLC. In forthcoming studies, we will test the validity of these first conclusions by investigating the behavior of other column brands, e.g., Symmetry, and Kromasil. These results seem to complicate the goal of understanding the influence of the solution pH on adsorption phenomena. It is not possible to change the solution pH to any significant extent without modifying either the ionic strength of the solution (to make small pH changes) or the nature of the buffer (to make large pH changes). While the consequences of a change in buffer concentration can be alleviated by keeping the ionic strength constant with the addition of proper amounts of a completely dissociated salt such as KCl, those of changing the buffer ions are more difficult to handle. Any conclusion related to the dependence of the adsorption mechanism(s) on the solution pH must be very careful and should be the subject of extensive experimental verifications.

Acknowledgements

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