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

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Abstract

In a companion paper, we describe the influence of the concentration and the nature of 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}. The same experiments were repeated on a Symmetry-C_{18} column to compare the adsorption mechanisms of this ionic compound on these two very different RPLC systems. Frontal analysis (FA) measurements were first carried out to determine the best isotherm model accounting for the adsorption behavior of propranolol hydrochloride on Symmetry with a mobile phase without salt (and only 25% methanol to compensate for the low retention in the absence of salt). The adsorption data were best modeled by the bi-Moreau model. Large concentration band profiles of propranolol were recorded with mobile phases having 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 by the inverse method (IM) 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}. Increasing the salt concentration increases the two saturation capacities of the adsorbent and the adsorption constant on the low-energy sites. The adsorption constant on the high-energy sites decreases and the adsorbate–adsorbate interactions tend to vanish with increasing salt concentration of the mobile phase. The saturation capacities decrease with increasing radius of the monovalent cation (Na^+, K^+, Cs^+, etc.). Using sulfate as a bivalent anion (Na_2SO_4) affects markedly the adsorption equilibrium: the saturation capacities are drastically reduced, the high-energy sites nearly disappear while the adsorption constant and the adsorbate–adsorbate interactions on the low-energy sites increase strongly. The complexity of the thermodynamics in solution might explain the different influences of these salts on the adsorption behavior.
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1. Introduction

RPLC is now widely applied for the separation and purification of ionic solutes. Numerous studies have been devoted to further our understanding of the chromatographic behavior of ionizable compounds in the presence of salts or buffers in the mobile phase [1–8]. Because some silanol groups are encountered on the surface of most chemically bonded reversed-phase stationary phases, these surfaces are partially charged, depending on the proton concentration (pH) in the mobile phase. As a result and in contrast with what happens in the classical separation mechanisms of neutral analytes that are strictly based on adsorption and/or partition, charge exclusion and ion exchange may contribute significantly to the retention and the separation of ionizable analytes [9].

Despite the large number of publications dealing with the retention of ionizable compounds in buffered or salt-supporting solutions under linear conditions, few efforts have been made to understand the adsorption mechanism of organic ionizable compounds in preparative or under high-concentration conditions [10,11]. To the best of our knowledge, there has yet been no attempt at trying to develop a general understanding of the adsorption isotherm behavior of ionizable compounds in aqueous/organic solvents with and without dissolved salt and to unify the scattered results into a single adsorption mechanism. Yet, without such an

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understanding of the adsorption thermodynamics and the mass transfer kinetics between stationary and mobile phases of ionizable compounds it is difficult to model the behavior of overloaded band profiles in chromatography and to optimize their preparative separations [12]. Measuring the equilibrium isotherm of compounds in a wide concentration range is actually a powerful mean to understand their actual adsorption mechanism under linear conditions because only this adsorption isotherm can reveal the possible heterogeneity of an adsorbent surface and distinguish adsorption sites which act independently, with different adsorption energies, equilibrium constants and possibly adsorbate–adsorbate interactions. As long as measurements are made only under linear conditions, such a deconvolution between the contributions of the different sites is impossible, these individual contributions remain hidden, and only their sum is measured. The details of the adsorbate–adsorbent interactions cannot be understood. This often leads to a fundamental misinterpretation of the retention mechanisms taking place in chromatography because one essential characteristics of all adsorbents is ignored, the heterogeneity of their surface.

We recently attempted to investigate the adsorption and the chromatographic behavior at high concentrations of some ionizable compounds [13]. A first study was carried out on a commercial adsorbent, XTerra-C_{18}, the surface of which exhibits no bonded silanol groups in the pH range 3–11 [14]. Accordingly, none of the interactions observed between charged molecules and this adsorbent surface can be attributed to some strong ion exchange interactions. They must be explained instead by some stronger or weaker dispersive interactions with either the C_{18}-bonded layer or the apolar bare surface of the adsorbent. Some important conclusions could already be drawn from the study of the adsorption data of propranolol, a derivative of naphthalene with a secondary alcohol and a secondary, ionizable amine group ($R'–NH_2^+–R$). First, there is a general adsorption mechanism, valid in a wide range of salt concentrations (0–0.2 mol/l of potassium chloride in aqueous solutions of methanol), that involves two different types of adsorption sites, on each of which adsorbate–adsorbate interactions can take place in the monolayer. Second, the parameters of the isotherm vary with the salt concentration. The saturation capacities of both types of sites increase with increasing salt concentration. The adsorbate–adsorbate interactions vanish rapidly on both types of sites when the salt is introduced in the mobile phase. The equilibrium constant on the low-energy sites increases and that on the high-energy sites decreases with increasing ionic strength of the solution. The high-energy sites adsorption constant increases abruptly by more than 800% when the salt concentration is raised from 0 to 0.002 mol/l and then decreases regularly when the salt concentration increases further.

The increases of the two saturation capacities with increasing salt concentration could be explained by the correlative diminution of the repulsion between adsorbed charged molecules which allows a larger number of molecules to adsorb. On the other hand, the vanishing of the hydrophobic adsorbate–adsorbate interactions on both sites is surprising and this effect needs to be confirmed. The increase of the low-energy adsorption constant could be understood as the result of the formation of an ion-pair complex and the increase of the complex concentration with increasing salt concentration. On the other hand, there are no obvious explanations for the complicated variations of the equilibrium constant on the high-energy sites with the salt concentration. These observations have to be confirmed.

The goal of this work was to perform a similar investigation of the adsorption behavior of the same ionizable compound, propranolol, on an adsorbent having properties most different from those of the completely apolar XTerra-C_{18}. Symmetry-C_{18} (made by the same manufacturer, Waters Corporation, Milford, MA, USA), is a conventional alkyl-bonded, highly pure silica, with some residual silanols. However, these silanols are still inactive in the pH range between 3 and 7, as demonstrated by the lack of retention of the small cation Li^+ on Symmetry-C_{18} [14]. Measurements similar to those made with XTerra-C_{18} were made with Symmetry-C_{18}. Frontal analysis (FA) allowed the accurate determination of the isotherm model best accounting for the adsorption behavior of propranolol in a pure mobile phase, with no salt added. The addition of increasing salt concentrations was considered as a continuous perturbation of the isotherm parameters, allowing the determination of these parameters by recording overloaded band profiles with mobile phases containing different potassium chloride concentrations. Then, the inverse method affords easily the best estimates of the isotherm parameters from these profiles. The results obtained are compared to those derived for XTerra-C_{18} and the validity of the adsorption mechanism proposed previously for XTerra is discussed.

2. Theory

2.1. Determination of the adsorption isotherms by frontal analysis

Frontal analysis [12,15,16] was used to measure the single-component adsorption isotherm data of propranolol on Symmetry-C_{18} with a methanol:water (25:75, v/v) mobile phase containing no salts. This concentration was chosen so that the retention of propranolol be sufficiently large to afford accurate adsorption data. 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 [17].

2.2. Model of isotherm

Among the many models used, the best results were obtained with a model accounting for adsorption behavior on a heterogeneous surface with two types of adsorption

sites and adsorbate–adsorbate interactions on both types of sites. The simplest heterogeneous Moreau model [18] was considered and we used the following extension of the Moreau model, called the bi-Moreau model. This model assumes that a different Moreau model applies to each of these patches, considered as homogeneous and acting independently:

$$q^* = q_{s,1}\frac{b_1C + I_1b_1^2C^2}{1 + 2b_1C + I_1b_1^2C^2} + q_{s,2}\frac{b_2C + I_2b_2^2C^2}{1 + 2b_2C + I_2b_2^2C^2} \quad (1)$$

where q^* and C are the equilibrium concentrations in the solid and liquid phase 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.

The equilibrium constants b_1 and b_2 are associated with the adsorption energies $\varepsilon_{a,1}$ and $\varepsilon_{a,2}$ through the following equation [19]:

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

where $\varepsilon_{a,i}$ is the energy of adsorption, R the universal ideal gas constant, T the absolute temperature and b_0 a preexponential factor that could be derived from the molecular partition functions in both the bulk and the adsorbed phases. b_0 is often considered independent of the adsorption energy $\varepsilon_{a,i}$ [19].

The adsorbate–adsorbate parameter I can be written as [18]:

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

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

2.3. The inverse method

This method consists in adjusting the coefficients of an isotherm model in order to minimize the differences between an experimental band profile and the profile calculated with the equilibrium-dispersive (ED) model and the isotherm selected. The main advantage of the inverse method for isotherm determination is that it requires only the measurement of a few experimental overloaded band profiles [20–23]. This method is described in the companion paper [13]. The abbreviation IM will be used latter in the text to indicate inverse method.

2.4. Modeling of desorption-band profiles in HPLC

The overloaded band profiles of propranolol were calculated, using the equilibrium-dispersive model of chromatography [12,24,25]. 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 umber 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 the time, z the distance along the column, and $F = (1 - \varepsilon_t)/\varepsilon_t$ 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, 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 [12,26] 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 [12,27–29].

3. Experimental

3.1. Chemicals

The mobile phase used in this work was an aqueous solution of methanol (25:75, v/v, for the FA acquisition data and 40:60, v/v, for the acquisition of the overloaded band profiles). Both 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. 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. This 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; and

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

Particle shape	Spherical
Particle size (μm)	5
Pore size[a] (Å)	86
Pore volume[a] (ml/g)	0.90
Surface area[a] (m^2/g)	346
Total carbon (%)	19.6
Surface coverage (μmol/m^2)	3.18
Endcapping	Yes
Total column porosity	0.6044[b], 0.5804[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).

sodium sulfate were all obtained from Aldrich (Milwaukee, WI, USA).

3.2. Columns

The column used in this study (Symmetry®-C_{18}) was given by the manufacturer (Waters Corporation). The column tube dimension was 150 mm × 3.9 mm. The main characteristics of the packing material used are summarized in Table 1. The hold-up time of this column were derived from the retention time of two consecutive thiourea injections. The column porosity remains constant at 0.58039, 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. 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 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 isotherms of propranolol were measured in aqueous solutions of methanol. In the presence of salts, retention was sufficient to afford accurate measurements at a methanol concentration of 40%. In the absence of salt, however, retention was too low and the acquisition of FA data was carried out at a lower methanol content (25%). The solubility of propranolol is approximately 50 g/l in a 25:75 (v/v) methanol:water solution. Accordingly, the maximum concentrations used in FA were 40 g/l to avoid any precipitation in the instrument. Two master solutions were prepared, at 10 and 100% of the maximum concentration. Two consecutive FA runs and a total of 28 data points were then measured, starting from the lowest (first run, 7 points) to the highest concentrations (second run, 21 points). One pump of the HPLC instrument was used to deliver a stream of the pure mobile phase (methanol:water, 25:75, v/v), the second pump for the 100% master solution, the third for the 10% master 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 breakthrough curves 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 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 (10% solution) and 331 nm (100% solution). In each case, the detector response was calibrated accordingly by using the UV absorbance at the plateau observed on the breakthrough curves.

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

After FA data were acquired, the mobile phase was enriched in methanol to 40%, in order to obtain band profiles having a reasonable retention time and which could be measured accurately without having to consume too large a quantity of the mobile phase. Mobile phases with seven salt concentrations were prepared (0, 0.002, 0.005, 0.01, 0.05, 0.1 and 0.2 M). The injections of propranolol were done with 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 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.

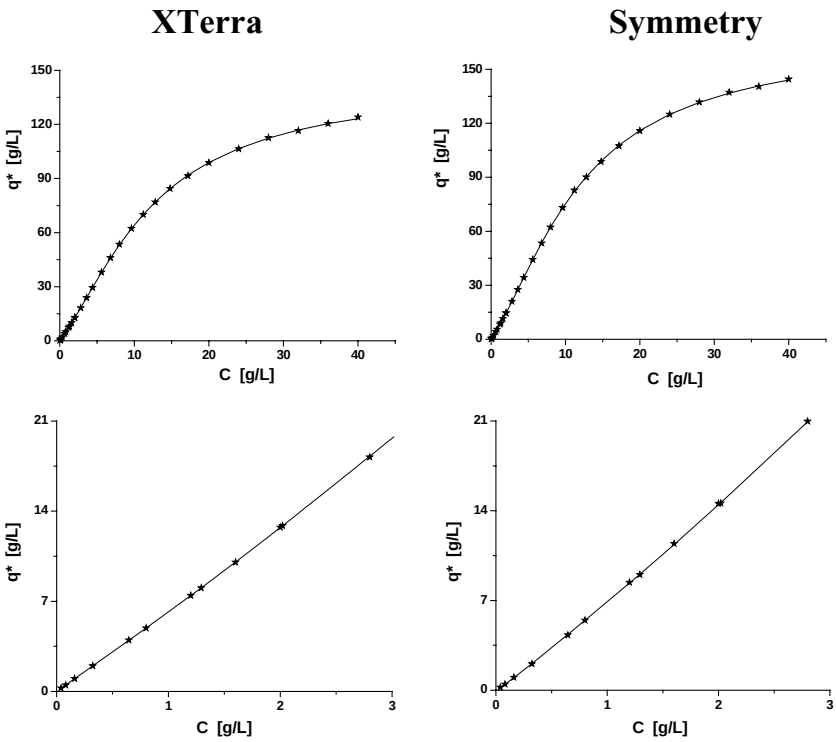


Fig. 1. Adsorption data of propranolol on the XTerra-C_{18} and Symmetry-C_{18} columns measured by FA. $T = 296$ K; mobile phase: 25:75 (v/v) methanol:water. Note the higher capacity of the Symmetry column.

4. Results and discussion

Within the whole concentration range of propranolol in the solution, its pH is slightly acidic and previous work reported that there is no silanol activity whatsoever [14]. The results obtained in this work with Symmetry-C_{18} are compared to those found earlier with XTerra-C_{18} [13]. The main difference between these two adsorbents is the bonding density of the C_{18} chains, 3.18 and 2.17 μmol/m^2 on Symmetry and XTerra, respectively, and the near total lack of Si–OH groups on the surface of the latter adsorbent.

4.1. Adsorption of propranolol on Symmetry-C_{18}

Figs. 1 and 2 show, respectively, the isotherm plot and the Scatchard plot of the adsorption data of propranolol on Symmetry-C_{18} column with a mixture of methanol and water (25:75, v/v) as the mobile phase. They also compare these plots to those obtained with XTerra-C_{18} eluted with the same mobile phase. At high concentrations (top plots), the isotherms obtained on the two columns are very similar, except for the significantly higher overall saturation capacity of the Symmetry column. At low concentrations, by contrast, the adsorption behavior of propranolol is quite different. The isotherm measured on the Symmetry column is always convex downward (i.e., anti-Langmuirian) below $C = 50$ g/l. It is a simple S-shaped isotherm. Although the isotherm on XTerra has also an inflection point around 50 g/l, the curvature of the isotherm changes sign a second time, at very low concentrations, and the isotherm becomes convex upward again.

The best parameters derived for the bi-Moreau isotherms of propranolol on Symmetry are reported in Table 2 and compared to those found on XTerra. We observe that the main adsorption constant, b_1, and the constant of the adsorbate–adsorbate interactions, I_1, are exactly the same on the two columns. However, as expected from the comparison in Fig. 1, the saturation capacity, $q_{s,1}$ is larger on Symmetry (+17%). This result may be explained by the higher content of bonded octadecyl chains (19.6% versus 15.2%) and the higher chain density on Symmetry (3.18 μmol/m^2) than on XTerra (2.17 μmol/m^2). These results suggest that

Table 2
Isotherm parameters

Parameter	Symmetry-C_{18}	XTerra-C_{18}[a]
$q_{s,1}$ (g/l)	168.8	143.8
b_1 (l/g)	0.0325	0.0323
I_1	6.85	6.66
ε_{AA}/RT	1.9	1.9
$q_{s,2}$ (g/l)	0.88	1.84
b_2 (l/g)	0.0521	0.823
I_2	1066	1.37
ε_{AA}/RT	7.0	0.3

[a] Previous results [14]. Mobile phase composition: 25:75 (v/v) methanol:water.

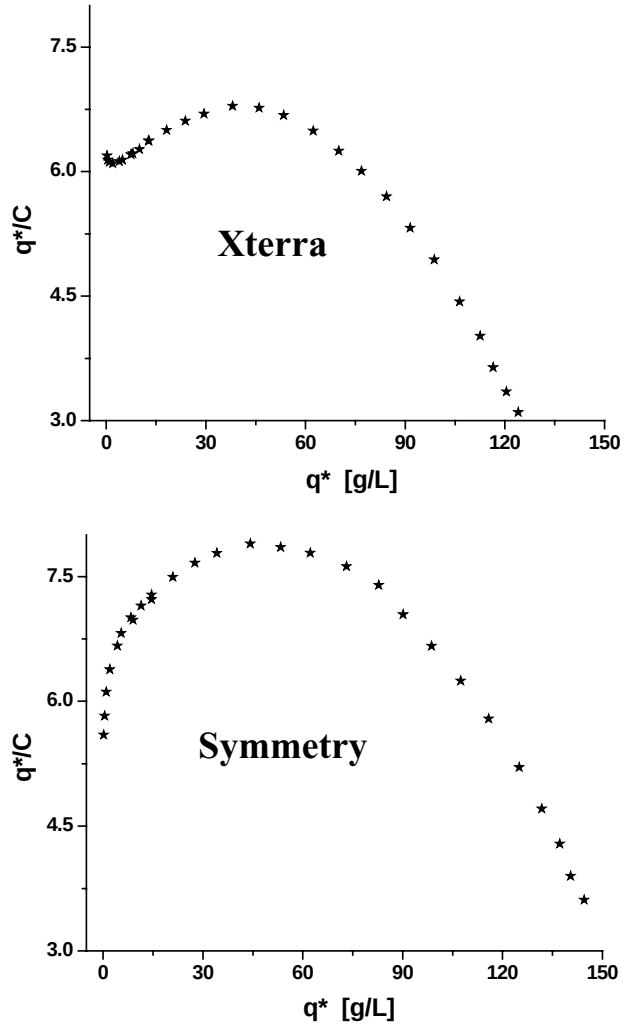


Fig. 2. Scatchard plot representation (q^*/C vs. q^*) of the adsorption data of the XTerra-C_{18} and Symmetry-C_{18} columns presented in Fig. 1. Note the difference in the curvature at very low concentrations.

the adsorption of propranolol on the low-energy sites is the same on both adsorbents but that the density of these sites is higher on Symmetry than on XTerra. The saturation capacity of the high-energy sites is small on Symmetry, less than half that of XTerra. However, the equilibrium constant on these sites is barely higher that on the low-energy sites (+60%) on Symmetry while it was 25 times larger on XTerra. This suggests that propranolol might have access to sites that are more deeply buried or that it adsorbs strongly on the bare surface of XTerra. This cannot take place on Symmetry because of the higher C_{18} chain density that severely hinders the access of the analyte molecules into the alkyl layer, beyond the interface between the bonded layer and the mobile phase.

The most striking and unexpected result in Table 2 is that the adsorbate–adsorbate interaction parameter, I_2, is much stronger on Symmetry than on XTerra. This result explains the marked differences between the isotherms at low concentrations (hence between the Scatchard plots and between the shapes of the overloaded band profiles for an injection during 60 s of a 2 g/l solution) on the two columns (see Fig. 3). A diffuse front boundary and a rear shock layer are observed on the profile obtained with the Symmetry column because the curvature of the isotherm is locally convex downward in the region close to the zero concentration. By contrast, the peak is almost symmetrical on the XTerra column while it was clearly skewed on a Kromasil column, illustrating the marked anti-Langmuirian shape of this last isotherm even at low concentrations [30]. The peak shape is also different on the XTerra column. The initial shock expected for a compound the isotherm of which has an initial negative curvature, i.e., an isotherm chord plot that is decreasing around the origin, cannot be seen in Fig. 3 because the solute dispersion is too high in the connecting tubes that are used when the injection is made with a pump. Finally, Fig. 3 demonstrates the good agreement between the experimental band profiles and the profiles calculated from the isotherm parameters listed in Table 2, the equilibrium-dispersive model of chromatography, and a boundary condition reflecting the actual injection profile at column inlet. Note the difference in the shapes of the profiles obtained with the two columns, due mostly to the different adsorption isotherm behaviors of the high-energy sites of the two adsorbents. The experimental isotherm chord plotted in the same figure explain these different shapes. The profiles obtained at a higher loading (see Fig. 4) demonstrate also a very good agreement between experimental and calculated band profiles.

So, the bi-Moreau model accounts for the adsorption data of propranolol in a non-buffered solution on all three adsorbents studied so far, Kromasil-C_{18} [31], XTerra-C_{18} [13], and Symmetry-C_{18}. This confirms that the adsorption mechanism cannot be based on some ionic interactions with residual silanol groups. XTerra has almost no silanols, while Kromasil and Symmetry have some. However, our investigation of the retention mechanisms of phenol and caffeine have shown that the residual silanols that may exist on these last two adsorbents are either inaccessible or have too weak an acidity to be involved in such interactions with the propranolonium cation (their pK_a must be larger than 6 or 7) [32]. Despite its positive charge, propranolol adsorbs onto the C_{18}-bonded layer, through its large hydrophobic moiety (mainly the naphthyl group). It does so on two different types of sites. The first type corresponds to simple adsorption sites with a low adsorption energy. The second type of sites are certainly related to the heterogeneity of the adsorbent surface that is caused either by the preparation of the stationary phase or by the effect of the mobile phase on the structure of the bonded chains. These sites are more or less buried in the layer, depending on the column. They are relatively few on all three adsorbents studied ($q_{s,2} < 2$ g/l) but their origin is critical for a good understanding of the shape of the band profiles recorded at low concentrations.

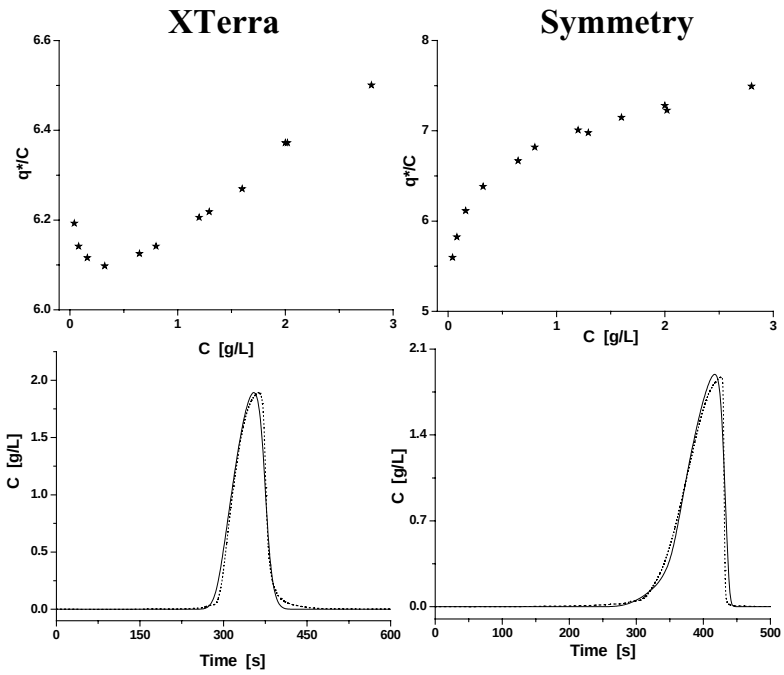


Fig. 3. (Bottom graphs) Comparison between the experimental (dotted line) and simulated (solid line) band profiles of propranolol on the XTerra column and the Symmetry column (methanol:water, 25:75, v/v, 60 s injection of a 2 g/l solution) at low column loading. $T = 296$ K; flow rate: 1 ml/min. Calculation made by using the equilibrium-dispersive model of chromatography. Note the difference in shape of the two profiles in agreement with the shape of the experimental isotherm chords at low concentrations of propranolol in the mobile phase (see upper graphs).

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

In this series of experiments, the methanol fraction in the mobile phase was increased to 40% in order to compensate for the increase in retention due to the presence of salt in the mobile phase and to achieve reasonable retention times. For each value of the salt concentration in the mobile phase (0, 0.002, 0.005, 0.01, 0.05, 0.1 and 0.2 M), two injections of propranolol were made, both lasting 15 s, one at low (1.5 g/l) and the other at high concentration (30 g/l). Their volume was the maximum volume delivered by the auto-sampler (250 μl).

Figs. 5 and 6 report the chromatograms recorded on the Symmetry column, at low and high column loadings, respectively. The inset in Fig. 6 has a different time scale and shows the profiles recorded at low salt concentrations. The shape and position of the band profiles vary continuously from low to high salt concentrations, in the same way as on XTerra-C_{18}. The retention of the band increases with increasing salt concentration but it does not seem that the retention mechanism is fundamentally affected. It is noteworthy that high propranolol concentrations (30 g/l) combined with low ionic strength solution ([KCl] = 0.002 M) lead to very characteristic and unusual band profiles, as was observed on XTerra [13]. The band front begins with a shock layer, then exhibits an intermediate diffuse boundary and finally ends up with another shock layer. Accordingly, the converse situation is seen on the rear front of the profile, two diffuse boundaries are seen, one before, the other one after the shock layer. Because the shape of a band profile is related to that of the isotherm under the same conditions, provided that the column efficiency is sufficiently high [13], we can conclude that these band profiles show that:

- at low salt concentrations, the isotherm is initially convex upward because the molecules of propranolol adsorb first on the high-energy sites which have a relatively low saturation capacity ($q_{s,2} \simeq 1$ g/l when the methanol volume fraction was 25%), hence are rapidly saturated, which exhibit a Langmuirian isotherm behavior, showing that low adsorbate–adsorbate interactions take place on these sites.
- in the intermediate concentration range, the isotherm becomes convex downward because the molecules of propranolol adsorb on the low-energy sites, on which the adsorbate–adsorbate interactions are strong, hence the adsorption isotherm behavior is initially anti-Langmuirian.
- at high concentrations, the overall isotherm is again convex upward because the high-energy sites are fully saturated and the low-energy sites become close to saturation.

The same conclusions were derived from the similar results obtained with XTerra. This confirms our previous statement that whether or not residual silanols exist on the adsorbent surface, these groups are not involved in the retention mechanism of propranolol on the three columns studied. The adsorption data show that the difference between the adsorption energies on the high- and the low-energy sites of Symmetry-C_{18} is very small, less than

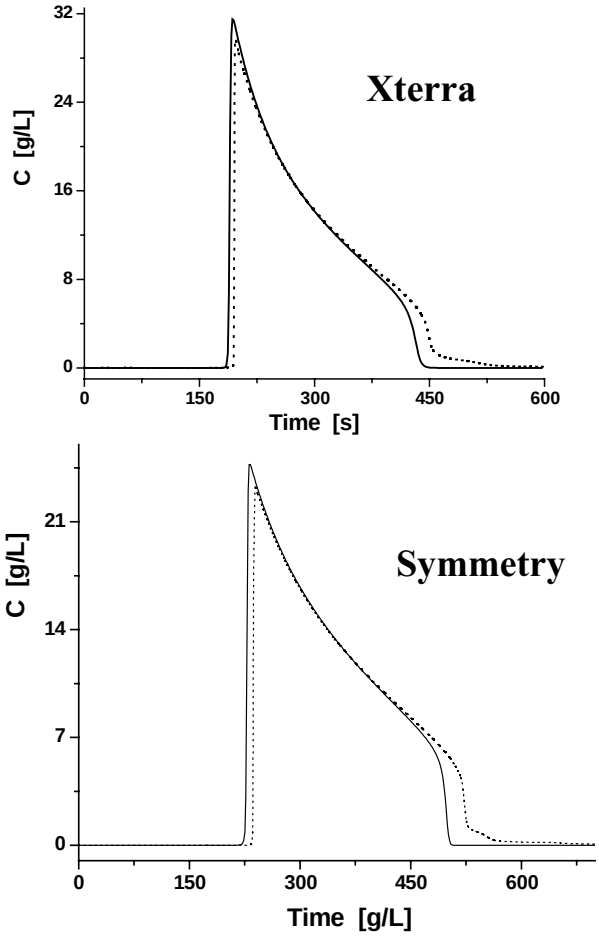


Fig. 4. Comparison between the experimental (dotted line) and simulated (solid line) band profiles of propranolol on the XTerra column and the Symmetry column (methanol:water, 25:75, v/v, 90 s injection of a 40 g/l solution) at high column loading. $T = 296$ K; flow rate: 1 ml/min. Calculation made by using the equilibrium-dispersive model of chromatography.

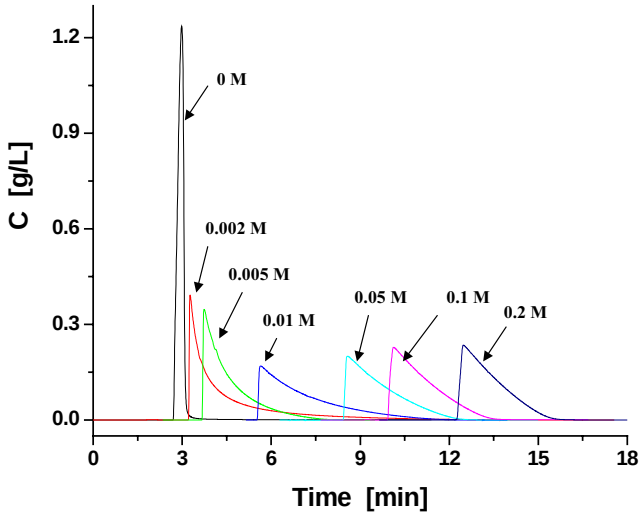


Fig. 5. Evolution of the position and the shape of overloaded band profiles of propranolol (injection of a 1.5 g/l solution during 15 s) as a function of the salt concentration or ionic strength of potassium chloride in the mobile phase (methanol:water, 40:60, v/v) on the Symmetry column. $T = 296$ K; flow rate: 1 ml/min. Note the displacement of the band toward high retention times when the ionic strength solution increases.

half the thermal energy, *RT*. So, either the residual silanols on the surface of Symmetry-C_{18} are very weakly acidic or they are otherwise inactive in solutions with a pH between 4 and 7. This observation is confirmed by a detailed examination of the sets of values of the best isotherm coefficients obtained by IM at each salt concentration. Figs. 7 and 8 show the excellent agreement between the band profiles calculated with the best estimates of the isotherm coefficients and the experimental profiles obtained at high and low column loadings, respectively. The bi-Moreau model describes accurately all the variety of shapes of the band profiles that change continuously with the salt concentration (0–0.2 M). The best parameters are plotted in Figs. 9–14 versus the salt concentration. In order to assign a physical sense to the parameters so derived, it is important that the two sets of six parameters be close whether IM is applied to the high or the low loading chromatograms.

The trends observed for the salt concentration dependence of the six bi-Moreau isotherm coefficients is the same whether the IM method with which they were derived was applied to the high or to the low column-loading profiles. The variation of each parameter as a function of the salt concentration [KCl] is also the very same as the one observed for XTerra. We found that in both cases, both saturation capacities ($q_{s,1}$ and $q_{s,2}$) increase with increasing salt concentration. Also, b_1 increases and b_2 decreases with increasing salt concentration in the mobile phase. It is surprising to note that, as for XTerra, b_2 suddenly jumps from about 1 to nearly 15 l/g within the very narrow range of salt concentration (0–0.002 M). Finally, the coefficients of adsorbate–adsorbate interactions, I_1 and I_2, decrease strongly when salt is added

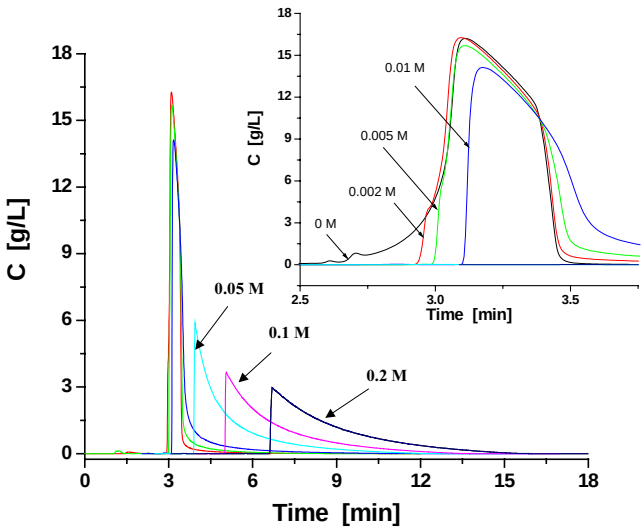
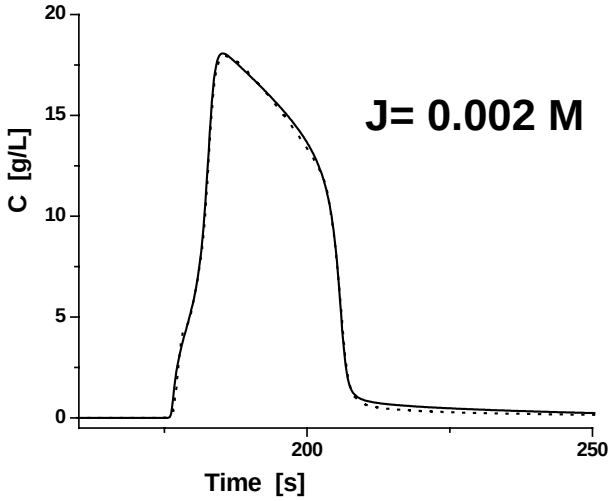


Fig. 6. Same as in Fig. 5, except the injection of a 30 g/l solution of propranolol. Note the change, not only in the position of the bands, but also of the shape of the band profiles.



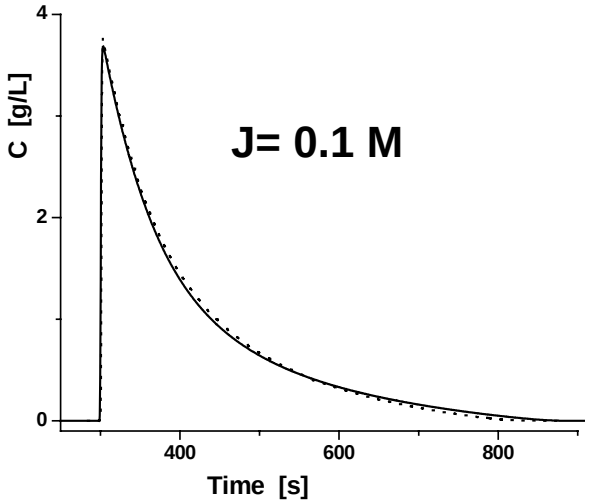
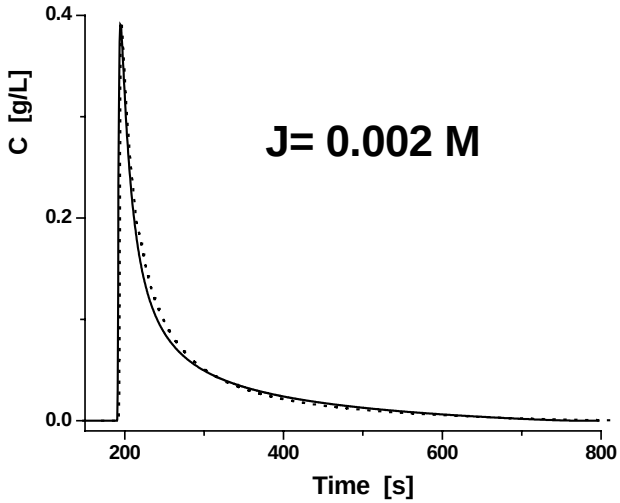


Fig. 7. Comparison between the experimental profiles of propranolol (dotted line) and the best calculated profiles found by the IM (solid line) on the Symmetry column (methanol:water, 40:60, v/v, 15 s injection of a 30 g/l solution) at high column loading for two different concentrations of potassium 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 ($J \leq 0.05$ 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).



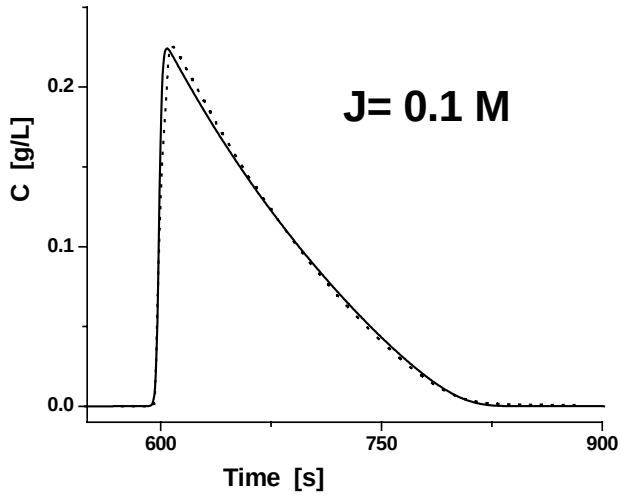


Fig. 8. Same as in Fig. 7, except the injection of a 1.5 g/l solution.

to the mobile phase. Despite the higher number of low- and high-energy sites on Symmetry-C_{18} (20 and 100% more than on XTerra, respectively), these sites seem to have the same characteristics and to describe the same physical environment related to the structure of the C_{18}-bonded architecture.

In conclusion, this work confirms previous conclusions derived from the adsorption behavior of propranolol on other C_{18}-bonded adsorbents. This behavior is described by a bi-Moreau model on both XTerra and Symmetry and the coefficients of this isotherm vary in the same way with the addition of potassium chloride to the mobile phase.

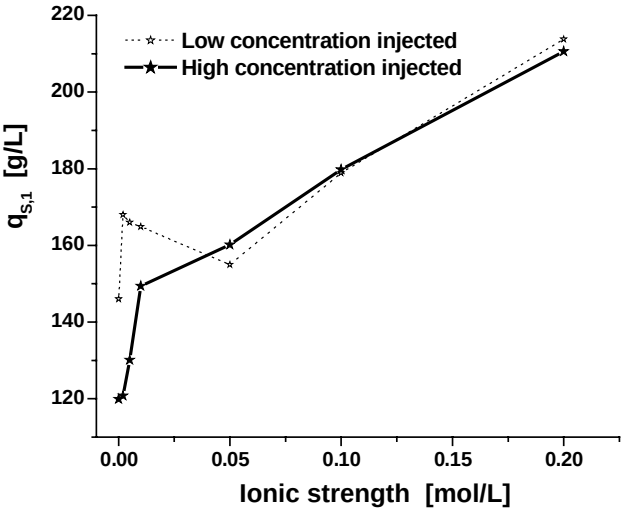


Fig. 9. Best saturation capacity $q_{s,1}$ of the low-energy sites found by using the IM procedure with the high and low loaded band profiles on the Symmetry column. Note the quasi-linear increase of this isotherm parameter with the salt concentration in the mobile phase.

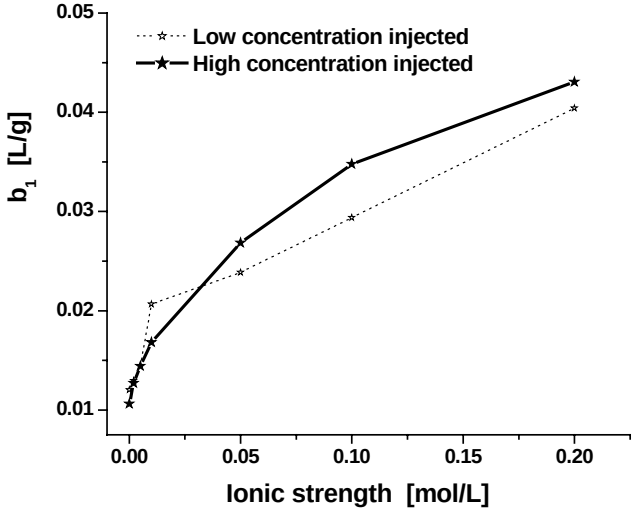


Fig. 10. Best equilibrium constant b_1 of the low-energy sites found by using the IM procedure with the high and low loaded band profiles. Note the increase of the energy of adsorption with the salt concentration.

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

We found in our previous study on XTerra that the ionic strength was not the fundamental factor controlling the adsorption of propranolol from an aqueous solution of methanol. The same results were obtained with Symmetry-C_{18}, as illustrated in Fig. 15. The position and the shape of the band profiles are mildly affected by the nature of the cation used for the salt added to the mobile phase. They are dramatically changed when the anion is changed.

In a first series of experiments, the nature of the monovalent cation was changed, from K^+ (Section 4.1) to Na^+ and Cs^+, and finally to Ca^{2+}, keeping Cl^- as the co-anion

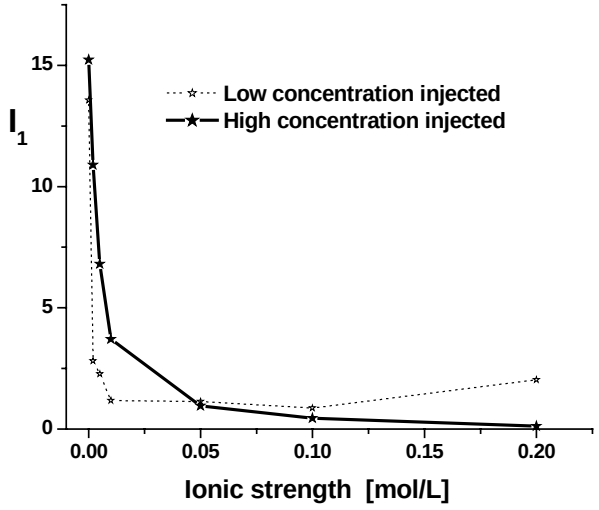


Fig. 11. Best adsorbate–adsorbate interaction parameter I_1 on the low-energy sites found by using the IM procedure with the high and low loaded band profiles. Note the fast decrease of the propranolol–propranolol interactions when the salt concentration increases.

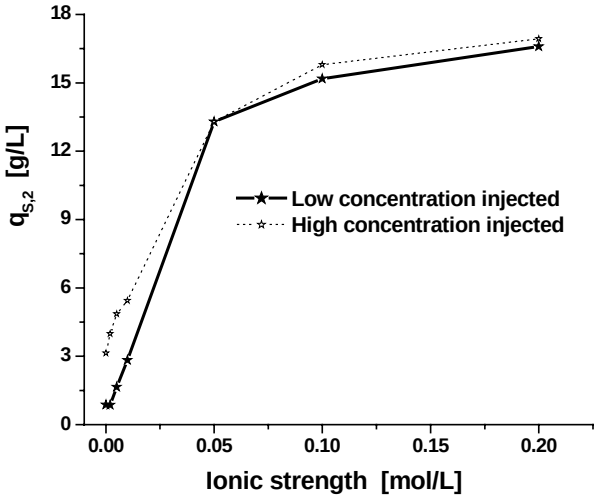


Fig. 12. Best saturation capacity $q_{s,2}$ of the high-energy sites. Note the rapid increase of $q_{s,2}$ then the saturation reached at high salt concentration.

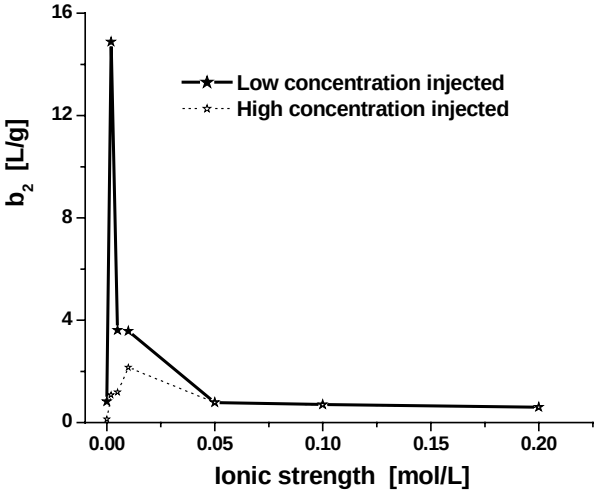


Fig. 13. Best equilibrium constant b_2 of the high-energy sites. Note the rupture found between 0 and 0.002 M.

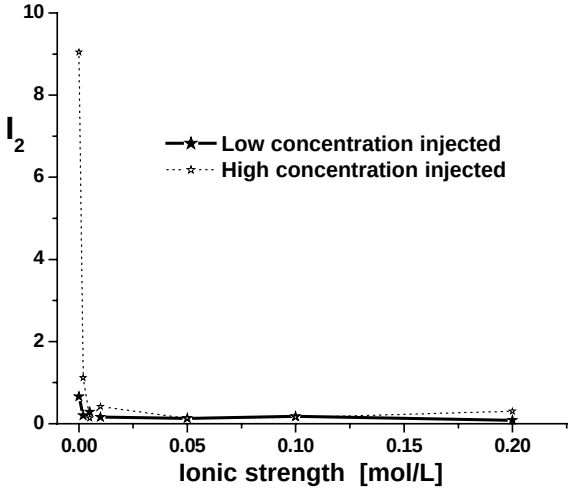


Fig. 14. Best adsorbate–adsorbate interaction parameter I_2 of the high-energy sites.

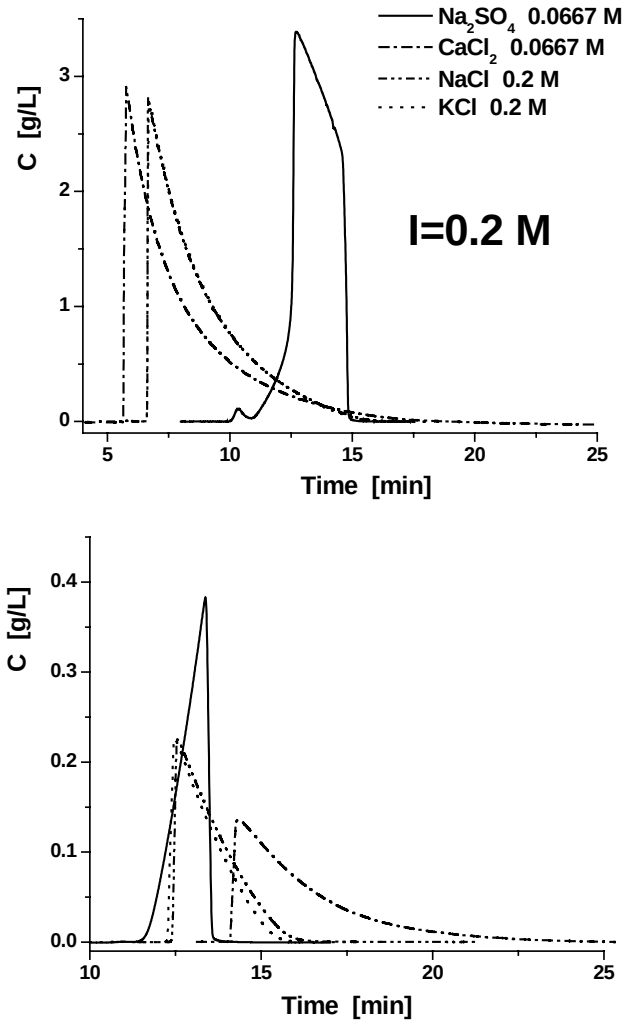


Fig. 15. Evolution of the band profiles of propranolol on the Symmetry column at constant ionic strength $J = 0.2\,\mathrm{M}$ ($J = (1/2)\sum C_i z_i^2$) in the mobile phase (methanol:water, 40:60, v/v) as a function of the size of the cation (NaCl and KCl), the valence of the cation ($CaCl_2$ and KCl)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

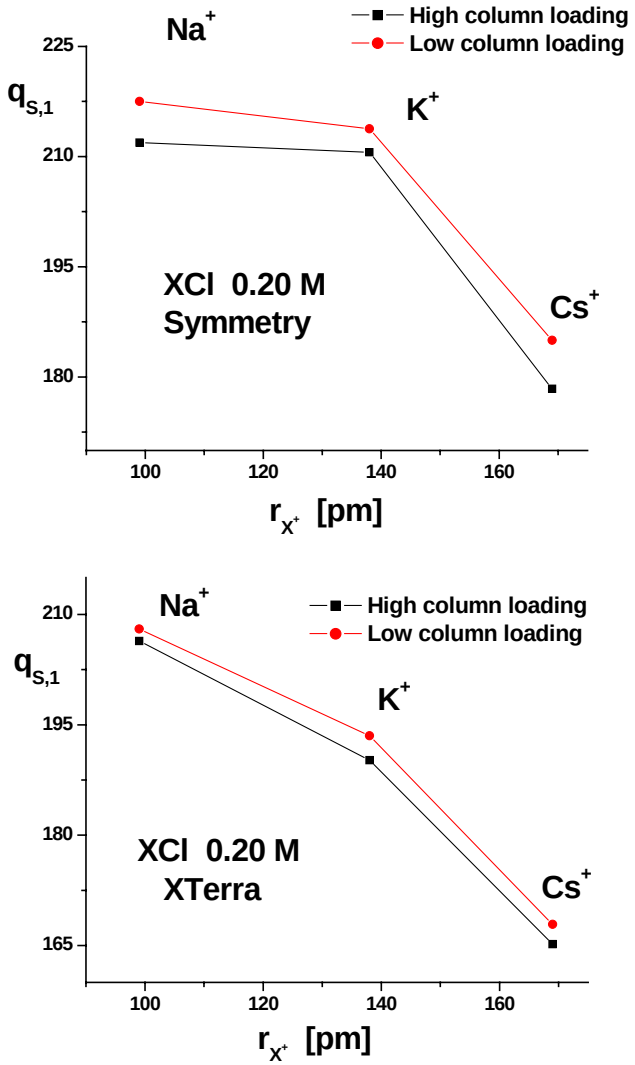


Fig. 16. Effect of the ionic radius of the co-cation X^+ of the chloride salt XCl, dissolved in the methanol:water mixture (40:60, v/v), on the saturation capacity of the low-energy sites $q_{s,1}$ on both the XTerra and the Symmetry columns. Note the decreasing trend of the plots.

and the ionic strength of the solution constant (0.2 M). Fig. 16 shows a plot of the first saturation capacity, $q_{s,1}$, versus the ionic radius of the cation used. It clearly decreases with increasing radius while the adsorption constant remains constant ($b_1 \simeq 0.043$ l/g), suggesting that only steric hindrance from the large cations has an effect. This same phenomenon is observed on both columns. When the bivalent cation Ca^{2+} was used at the same ionic strength (0.0667 M $CaCl_2$), we observed that the high concentrations of propranolol were eluted earlier than with the monovalent cations while the lower concentrations were eluted later. This phenomenon was not observed on XTerra, on which the adsorption constant on the high-energy sites, b_2, was the same for all cations. On Symmetry-C_{18}, b_2 is about twice as large with a Ca^{2+} solution than with a K^+ solution of the same ionic strength. The adsorption constant b_1 is slightly larger with K^+ (0.043 l/g) than with Ca^{2+} (0.038 l/g). These observations suggest that the ionic strength of the solution does not account completely for the influence of the ion concentration on the retention of propranolol.

The shape and the position of the band of propranolol were drastically modified on both XTerra-C_{18} and Symmetry-C_{18} when the monovalent Cl^- anion was replaced with the bivalent anion SO_4^{2-} (as the Na_2SO_4 salt). The effect is illustrated in Fig. 15. The isotherm parameters are considerably changed. The saturation capacity of the low-energy sites, $q_{s,1}$, decreases by around 15% from its value with KCl at the same ionic strength (210 instead of 177 g/l). That of the high-energy sites, $q_{s,2}$, decreases considerably, from 16 to 0.5 g/l. Strong adsorbate–adsorbate interactions ($\varepsilon_{AA} = 1.7 \times RT$) take place now on the low-energy sites whose adsorption energy has markedly increased, from 0.043 to

0.085 l/g. The most probable explanation seems to be that the thermodynamics in solution has been modified and that propranolol might form some multivalent ion pairs with the sulfate anion. In contrast with what was observed with Kromasil-C_{18} and, to a lesser degree, with XTerra, the mass transfer kinetics is not markedly affected and the elution band has no significant tailing.

5. Conclusion

The adsorption behavior of propranolol on Symmetry-C_{18} is in many ways similar to the one previously observed on XTerra-C_{18}. No ionic interactions whatsoever were detected, which confirms the lack of activity of the residual silanol groups found on the surface of the two adsorbents in the pH range studied (between 4 and 6). Only dispersive interactions were found, involving the organic moiety of the solute and the C_{18}-bonded layer on two types of adsorption sites. A slight difference was found between the two adsorbents, regarding the relative and total amounts of these sites. This difference might be related to the difference between the bonding densities of the chains, higher on Symmetry-C_{18} than on XTerra-C_{18}.

When a salt is added to the mobile phase, the solution thermodynamics is changed since the activity coefficients of ionizable compounds are, to a large degree, controlled by the ionic strength of the solution. This does not seem to affect the structure of the C_{18}-bonded layer because the column hold-up volume remains constant whatever the concentration of salt in the mobile phase. However, the adsorption isotherm of propranolol is seriously affected. Based on our results on both Symmetry-C_{18} and XTerra-C_{18}, we can draw the following conclusions regarding the influence of an increase of the salt concentration in the mobile phase on the isotherm parameters of an ionizable compound in RPLC:

- The total saturation capacity increases with increasing salt concentration. The amount of solute adsorbed at equilibrium with a given solute concentration increases. This is consistent with the observations of Hägglund and Ståhlberg [10] showing that the repulsive electrostatic interactions between the polar heads of organic cations decrease.
- The adsorption constant on the low-energy sites (which seems to be a pure adsorption mechanism) increases because a neutral ion pair forms between the propranolol cation and the chloride anion, it is more strongly adsorbed than the cation, and its concentration increases with increasing concentration of KCl. The adsorption constant on the high-energy sites (that seem to partake of the partition mechanism) decreases.
- The adsorbate–adsorbate interactions decrease rapidly when increasing amounts of salts are added into the mobile phase.

Finally, it was also demonstrated that keeping constant the ionic strength was not sufficient to keep constant the isotherm parameters. Some entropic effects are driven by the ionic radius of ions, which would explain the important decrease of the saturation capacity $q_{s,1}$ with increasing ion size (Fig. 16). The large decrease in the saturation capacities in the same time as the binding constants increase when a monovalent ion is replaced with a divalent one remains somewhat mysterious and certainly deserves further investigations.

Acknowledgements

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