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Effect of the pH, the concentration and the nature of the buffer on the adsorption mechanism of an ionic compound in reversed-phase liquid chromatography II. Analytical and overloaded band profiles on Symmetry-C_{18} and Xterra-C_{18}

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Abstract

In a previous report, the influence of the pH, the concentration, and the nature of the buffer on the retention and overloading behavior of propranolol ($pK_a = 9.45$) was studied on Kromasil-C_{18} at $2.75 < pH < 6.75$, using four buffers (phosphate, acetate, phthalate, and succinate), at three concentrations, 6, 20, and 60 mM. The results showed that the propranolol cation was eluted as an ion-pair with the buffer counter-anion. A similar study was carried out with Symmetry-C_{18} and Xterra-C_{18}. Two additional buffers, formate and citrate, were also used. Propranolol elution band profiles were recorded for a small (less than 1 μg) and a large (375 μg) sample size. The results are similar to those obtained with Kromasil and confirm earlier conclusions. The buffer concentration, not its pH, controls the retention time of propranolol, in agreement with the chaotropic model. The retention factor depends also on the nature of the buffer, particularly on its valence, and on the hydrophobicity of the basic anion. With the monovalent anions $HCOO^-$ (pH 3.75), $H_2PO_4^-$ (pH 2.75), $HOOC–Ph–COO^-$ (pH 2.75), $HOOC–CH_2–CH_2–COO^-$ (pH 4.16), CH_3COO^- (pH 4.75) and $HOOC–CHCOOH–COO^-$ (pH 3.14), at moderate loadings, and for the two larger buffer concentrations, the band profiles are well accounted for by a simple bi-Langmuir isotherm model (no adsorbate–adsorbate interactions). By contrast, these profiles are accounted for by a bi-Moreau isotherm model (i.e., with significant adsorbate–adsorbate interactions) with the bivalent anions $^-OOC–Ph–COO^-$ (pH 4.75), $^-OOC–CH_2–CH_2–COO^-$ (pH 5.61), HPO_4^{2-} (pH 6.75), and $HOOC–CHCOO^-–COO^-$ (pH 4.77) and with the trivalent anion $^-OOC–CHCOO^-–COO^-$ (pH 6.39). The best values of the isotherm parameters were determined using the inverse method. The saturation capacity and the equilibrium constant on the low-energy sites increase with increasing buffer concentration, a result consistent with the formation in the mobile phase of a hydrophobic complex between the propranolol cation and the buffer anion. With bivalent and trivalent anions, adsorbate–adsorbate interactions are strong on the low-energy sites but they remain negligible on the high-energy sites. The density of the high energy sites is lower and the equilibrium constant on the low-energy sites are both higher with the bivalent and the trivalent buffer anions than with the univalent buffer anions. These results are consistent with the formation of a 2:1 and a 3:1 propranolol–buffer complex with the bivalent and the trivalent anions, respectively.

Keywords: pH effects; Mobile phase composition; Band profiles; Adsorption isotherms; Stationary phases, LC; Propranolol

1. Introduction

In previous reports, we investigated the adsorption behavior at high concentrations of some ionizable compounds in RPLC. This topic is of interest since many low molecular weight pharmaceuticals are ionized in aqueous solutions, many of them because they have amine groups. There is a dearth of literature in this field [1–3]. Most of the relevant publications deal with the retention of ions under analytical conditions and discuss the simultaneous influence of the concentrations of several or numerous buffers [4–12]. Due to the complexity of such mobile phases, which contain

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the studied ionizable compound(s), their co-ions, and the buffer's ions, it is not easy to sort out the mutual influence of all these ions on their adsorption behavior and premature conclusions regarding the form under which the analyte is adsorbed on the stationary phase, possible ion-ion interactions, and equilibria in the mobile phase are not exceptional. Actually, the solvated compound and any neutral ion-pair that can possibly form between one or more anions and one or more cations may contribute to the overall retention of an ionic compound. This description arises from the theory of chaotropicity [13,14], which, so far, has been applied only under linear conditions. To understand the behavior of organic ions in preparative RPLC, a better, more detailed understanding of such complex systems is necessary. This requires the measurement of the adsorption isotherms of these ions, the key to the prediction of overloaded band profiles and of the optimization of chromatographic processes. The influence of many experimental parameters, like the nature of the buffer or of supporting salts in the mobile phase, of their concentration, their pH, and even of the valence of these ions have to be understood from a thermodynamic viewpoint.

Recently, we measured by frontal analysis (FA) the adsorption properties of the propranolonium cation and showed them to depend strongly on the presence of supporting salts or of buffers in an aqueous-organic mobile phase and on their concentration [15]. The adsorption behavior changes from that described by a classical langmuir isotherm in the presence of an acetate buffer to that accounted for by a more complex S-shaped isotherm in the absence of a buffer. The main equilibrium constant was lower in the absence of a buffer while the saturation capacity was little modified. The initial conclusions were: (i) propranolol adsorbs as a more hydrophobic form when the mobile phase contains a concentrated buffer (0.2 M), (ii) whether the mobile phase contains a buffer or not, propranolol must adsorb as a neutral species, otherwise the column saturation capacity would have been significatively lower, since then there would have been important cation-cation repulsions in the adsorbed phase.

It was also demonstrated that the saturation capacity of the column (Kromasil [16], Symmetry [17] and Xterra [18,19]) for propranolol chloride and the associated equilibrium constant increase largely with the concentration of supporting salt (KCl) present in the mobile phase. This result was easy to interpret since the excess of chloride in the mobile phase displaces the equilibrium between the solvated propranolol cation and the propranolol-chloride ion pair towards the formation of more neutral ion pair species. The concentration of the neutral ionic complex of propranolol and a buffer anion, complexes which do not repulse each other, increases, the stationary phase can adsorb more sample. Moreover, at constant ionic strength, different salts lead to different band profiles, showing that the adsorption properties of the positively charged molecule depend not only on the compound studied but also on the supporting salt used [16–19]. Most importantly, the type of isotherm obtained (langmuirian, S-shaped, anti-langmuiriran, ...) could be correlated to the valence of the anion of the supporting salts [20]. Monovalent anions lead to convex upward isotherms while bivalent and trivalent anions were likely to generate S-shaped and anti-langmuirian isotherms, respectively.

These first results were obtained with neutral salts. Later they were extended to various buffer systems, at pH values between 2.75 and 6.75. In these solutions, propranolol exists quantitatively in its protonated form. A fist work carried out with Kromasil-C_{18} [21] confirmed what had been observed with neutral salts. Increasing the buffer concentration (i.e., the basic anion concentration) leads to an increase of the concentration of the neutral ion-pair species, hence of the column saturation capacity and of the adsorption equilibrium constant. The results of the perturbation on a plateau method complete those derived from overloaded band profiles measured for different concentrations of different buffers of different valences [20] to demonstrate that the isotherm shape measured on Kromasil-C_{18} is more likely langmuirian, S-shaped, or anti-langmuirian if the anion is monovalent, bivalent and trivalent. respectively. This observation needed confirmation.

In this study, we report on the results of similar measurements and observations on the retention mechanism of propranolol at high concentrations on two other columns, Symmetry-C_{18} and Xterra-C_{18}. Eleven different buffer systems were used. The effects of the buffer concentration, of its nature and of the valence of its anions on the retention of propranolol chloride and on its adsorption parameters determined by the inverse method of isotherm determination are discussed and compared to the precedent results found on Kromasil-C_{18}.

2. Theory

2.1. Models of isotherm used

The isotherm model used in this study is consistent with the information that is already available regarding the surface hetrogeneity of the RPLC adsorbents [22–28]. It is an extension of the Moreau isotherm model [29], the bi-Moreau model, that was already used to account for the adsorption behavior of propranolol on Kromasil-C_{18}. The equation of this isotherm is:

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

where q^* and C are the equilibrium concentrations of the compound considered in the adsorbed and and in the liquid phases, respectively, and $q_{s,1}$, $q_{s,2}$, b_1, b_2, I_1 and I_2 are the monolayer saturation capacities, the low-concentration equilibrium constants, and the adsorbate–adsorbate interaction parameters on the sites of types 1 and 2, respectively.

Note that the bi-Moreau model morphs into the bi-Langmuir model when $I_1 = I_2 = 0$.

The equilibrium constants b_1 and b_2 are associated with the adsorption energies $\epsilon_{a,1}$ and $\epsilon_{a,2}$, respectively, through a relationship that is discussed elsewhere [30]. The adsorbate–adsorbate interaction parameter, I, are related to the interaction energy between two molecules of A adsorbed on close sites [30]. Note that the bi-Langmuir model is the limit case of the bi-Moreau model when the adsorbate–adsorbate interaction parameter, I, tends toward 0. Thus, in the work reported here, we adopted the bi-Moreau isotherm model as the initial model in the applications of the IM method.

2.2. The inverse method of isotherm determination

This method consists in the numerical adjustment of the coefficients of an isotherm model in order to minimize the differences between a recorded experimental band profile and the profile calculated for the same sample, using the equilibrium-dispersive model of chromatography (see next section) and the isotherm model selected. The main advantage of the inverse method of isotherm determination is that it requires the measurement of only one or a few experimental overloaded band profiles [31–34]. Accordingly, the method is fast and it requires small amounts of solvent and sample. This method was described previously [18]. It gives results that are in excellent agreement with those of FA [34].

2.3. Modeling of band profiles in HPLC

The overloaded band profiles of propranolol were calculated using the equilibrium-dispersive model (ED) of chromatography [35–37] (see the mass balance equation in reference [21]). At $t = 0$, the concentrations of the solute and the adsorbate in the column are uniformly equal to zero (except in stair-case FA) and the stationary phase is in equilibrium with a stream of the pure mobile phase. The boundary conditions used are the classical Danckwerts-type boundary conditions [35,38] at the inlet and outlet of the column. The ED model was numerically solved using the Rouchon program based on the finite difference method [35,39–41].

3. Experimental

3.1. Chemicals

The mobile phases used in this work were all buffered aqueous solutions of methanol (40:60, v/v). Both water and methanol were of HPLC grade, purchased from Fisher Scientific (Fair Lawn, NJ, USA). The buffers were first prepared in pure water (all pH values reported in the text are those measured in pure water) and methanol was added thereafter to the buffer solution to prepare the final mobile phase (see Table 1). The buffer concentrations given in the text are reported to the mobile phase mixture. Prior to their use, the solvents were filtered on an SFCA filter membrane, 0.2 μm pore size (Suwannee, GA, USA). Thiourea was chosen to measure the column hold-up volume. The solute studied was propranolol, 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 formate, potassium acetate, potassium hydrogenphthalate, potassium dihydrogen phosphate, disodium succinate and trisodium citrate; 1 M hydrochloric acid, formic acid 96%, acetic acid 99.5%, succinic acid and citric acid were all obtained from Aldrich (Milwaukee, WI, USA).

3.2. Columns

The two 150 mm × 3.9 mm columns used in this study were packed one with Symmetry-C_{18} and the other with Xterra-C_{18}). They were gifts from the manufacturer (Waters, Milford, MA, USA). The main characteristics of these packing materials are summarized in Table 2. The Symmetry column was one of the lot of ten columns previously used to test the column-to-column and batch-to-batch reproducibility under linear conditions [42]. The void volumes of the Symmetry and the Xterra columns were derived from the average of the retention times of two consecutive thiourea injections. They are 1.040 and 1.107 mL, respectively. The column porosities remained constant, whatever the buffer used and its concentration in the mobile phase. They were found to depend only on the methanol concentration of the mobile phase (40%, v/v).

3.3. Apparatus

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 (volume of each tank, 1 L), an auto-sampler with a 250 μL sample loop, a diode-array UV detector, a column thermostat and a data station. Compressed nitrogen and helium bottles (National Welders, Charlotte, NC, USA) are connected to the instrument to allow the continuous operations of the pump, the auto-sampler, and the solvent sparging. The extra-column volumes are 0.058 and 0.93 mL as measured from the auto-sampler and from the pump system, respectively, to the column inlet. All the retention data were corrected for these contributions. The flow-rate accuracy was controlled by pumping the pure mobile phase at 23°C and 1 mL/min during 50 min, from each pump head, successively, into a volumetric glass of 50 mL. The relative error was less than 0.4%, so that we can estimate the long-term accuracy of the flow-rate at 4 μL/min at flow rates around 1 L/min. All

Table 1
Preparation of the different buffers

Buffer	Acid solution	Base solution	Volume water (mL)	Volume acid (mL)	Volume base (mL)	pH	Volume MeOH (mL)	C_{Buffer} (mM)
Phosphate I	HCl 0.1 M	KH_2PO_4 0.1 M	0	56	300	2.75	237	50.6
			200	26	100		217	18.4
			270	14	30		209	5.7
Phosphate II	KH_2PO_4 0.1 M	NaOH 0.5 M	0	300	28	6.75	219	54.8
			200	100	7.8		205	19.5
			270	30	2		201	6.0
Phthalate I	HCl 0.1 M	$KH_5C_8O_4$ 0.1 M	0	226	375	2.75	400	37.5
			300	45	64		273	9.4
			540	25	22		391	2.2
Phthalate II	$KH_5C_8O_4$ 0.1 M	NaOH 0.1 M	0	300	109	4.75	273	44.0
			200	100	30		220	18.2
			270	30	7.5		205	5.9
Succinate I	$C_4H_6O_4$ 0.1 M	$Na_2C_4H_4O_4$ 0.1 M	0	250	99	4.16	233	60.0
			180	60	21		174	18.6
			235	20	6		174	6.0
Succinate II	$C_4H_6O_4$ 0.1 M	$Na_2C_4H_4O_4$ 0.1 M	0	51	253	5.61	203	60.0
			200	20	80		200	20.0
			270	7.5	25		202	6.4
Formate	CH_2O_2 0.1 M	$KCHO_2$ 0.1 M	0	175	175	3.75	233	60.0
			200	60	60		214	22.5
			300	20	20		227	7.1
Acetate	$C_2H_4O_2$ 0.1 M	$KC_2H_3O_2$ 0.1 M	0	175	175	4.75	233	60.0
			230	60	60		217	21.2
			310	20	20		205	7.2
Citrate I	$C_6H_8O_7$ 0.1 M	$Na_3C_6H_5O_7$ 0.1 M	0	250	63	3.14	209	60.0
			210	80	19		206	19.2
			280	25	5.5		207	5.9
Citrate II	$C_6H_8O_7$ 0.1 M	$Na_3C_6H_5O_7$ 0.1 M	0	125	196	4.77	214	60.0
			218	40	63		214	19.3
			280	16	20		211	6.8
Citrate III	$C_6H_8O_7$ 0.1 M	$Na_3C_6H_5O_7$ 0.1 M	0	11	200	6.39	141	59.9
			140	4.5	70		143	20.8
			190	2.2	25		145	7.5

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 overloaded band profiles of propranolol

Two types of propranolol injections were made with the auto-sampler (maximum volume 250 μL). Ten μL of a 0.1 g/L solution and 250 μL of a 1.5 g/L solution were successively injected to record an analytical and a moderately overloaded band profile under each set of experimental conditions, respectively. The samples were dissolved in the buffer solution used as the mobile phase in the LC experiments (methanol–water mixture containing the buffer). These profiles were recorded at 310 and 325 nm. Segments of these elution profiles having between 500 and 1000 points were used to perform the IM calculations.

Table 2
Characteristics of the $C_1$8-bonded Symmetry and Xterra columns

	Symmetry	Xterra
Particle size (μm)	5	5
pore size (Å)	86	120
Pore volume[a] (mL/g)	0.90	0.64
Surface area[a] (m^2/g)	346	176
Particule shape	Spherical	Spherical
Total carbon (%)	19.6	15.2
Surface coverage (μmol/m^2)	3.18	2.40
Total porosity[b]	0.5804	0.6178
Endcapping	Yes	Yes

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

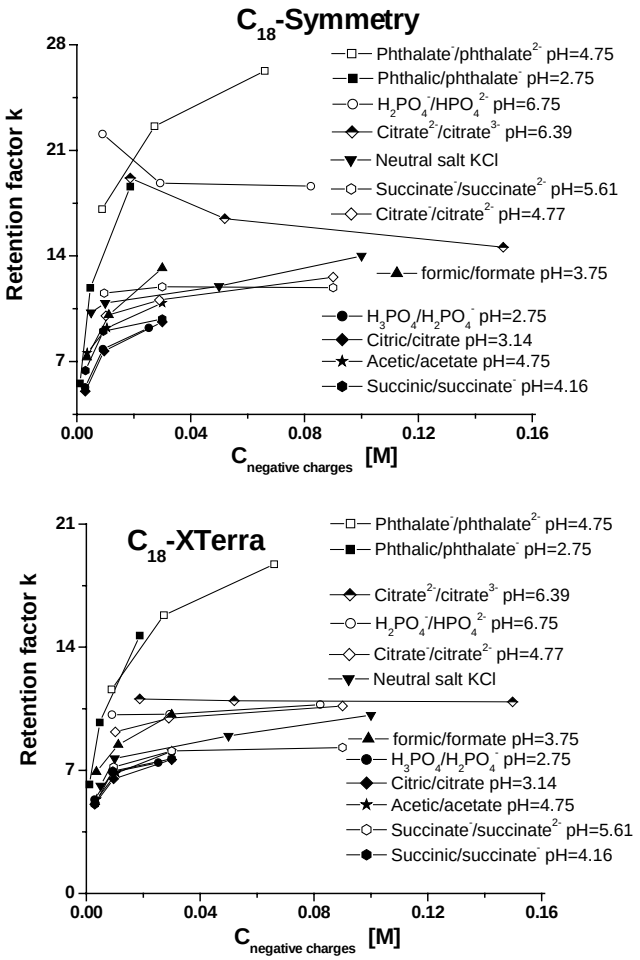

Fig. 1. Evolution of the retention factor of propranolol as a function of the concentration of negatives charges coming from the buffer anions for eleven buffered mobile phase and one neutral salt on the Symmetry and Xterra columns. $T = 296$ K. Note the systematic increasing trend for Xterra and some exceptions for the Symmetry columns at the two highest pH (phosphate II, pH 6.75 and citrate III, pH 6.39).

4. Results and discussion

4.1. Linear chromatography

Fig. 1 summarizes the concentration dependence of the retention factors of propranolol measured on Symmetry (top) and Xterra (bottom) under the different sets of experimental conditions investigated in this study (different buffers, different pH, and different buffer concentrations). In this figure, the retention factors are plotted versus the concentration of the anions generated by the buffer. This parameter was shown to be the one that controls the retention factor of propranolol for any buffer [21]. The data in Fig. 1 were measured for one neutral salt (KCl), six buffers having a monovalent anion ($HCOO^-$, $H_2PO_4^-$, CH_3COO^-, $HOOCC_2H_4COO^-$, $HOOCCHCOOHCOO^-$ and $HOOCC_6H_4COO^-$), four buffers having a bivalent anion (HPO_4^{2-}, $^-OOCC_2H_4COO^-$, $^-OOCCHCOOHCOO^-$ and $^-OOCC_6H_4COO^-$) and one buffer having a trivalent anion $^-OOCCHCOO^-COO^-$. These results call for the following three general comments.

(1) For all salts, except the phosphate and citrate buffers at high pH (6.75 and 6.39, respectively), the retention factor of propranolol increases rapidly with increasing buffer concentration at low concentrations and tends toward a limit at high concentrations. Despite the fact that only three data points were acquired, this behavior is consistent with the theory of chaotropicity [13,14]. This theory assumes that, in the mobile phase, the counter-anions form some association complex with the oppositely charged propranolol cation. This association is promoted by the strong electrostatic attraction between these ions which tends to displace the surrounding water molecules. It follows that the apparent hydrophobicity of the analyte affects its affinity for the C_{18}-bonded stationary phase, hence its retention factor. When the counter-anion concentration exceeds largely that of the analyte, practically all the water-solvated propranolol cations are turned into the ion complex and the retention factor measured is that of this complex. At lower buffer concentrations, the retention factor is a weighted average of those of the complex and the free cation. In the case of the phosphate and citrate buffers at pH 6.75 and 6.39, the converse result is obtained on Symmetry and the retention factor decreases with increasing concentration of these two buffers. This suggests that the complexes formed between the cation and the anions of these two buffers are less retained than the free solvated propranolol cation on Symmetry. An ion-exchange mechanism might take place at pH exceeding 6, which could explain this result. On Xterra, the retention factor remains constant, whatever the concentration of the phosphate or citrate buffer. No ion-exchange mechanisms are expected on Xterra. This behavior will be discussed later when analyzing the profiles of mildly overloaded bands and determining the best isotherm parameters and particularly the Henry constant, H (with $k' = FH$, F being the phase ratio).

(2) The retention factor of propranolol depends hardly on the pH of the mobile phase. The main reason is probably that, in the pH range investigated (2–7), propranolol ($pK_a = 9.45$ [14]) is present essentially as a cation, not as the basic, neutral form. This neutral form should be more retained, as observed elsewhere [12]. In addition, the two stationary phases studied have a reduced number of accessible silanols, due to the endcapping of Symmetry and the nature of the adsorbent in Xterra. The pH is clearly not a fundamental parameter regarding the adsorption of a charged compound at a pH far removed from its pK_a.

(3) For the buffers that have a similarly poor hydrophobicity (formate, acetate, succinate, and citrate), the retention of propranolol depends essentially on the

valence of the buffer. Note that the retention factors with all the monovalent buffers formate, acetate, succinate and citrate are comparable (Fig. 1). This means that the contribution of the buffer anion to the overall hydrophobicity and the retention factor of the ion pair is weak and that the retention factor depends mainly on the hydrophobicity of propranolol. When a more hydrophobic buffer is used, e.g., phthalate, the retention factor jumps up and the difference between the monovalent and the bivalent phthalate buffers becomes minor. For a given buffer and number of negative charges of the anion, the retention factor is always larger with the bivalent than with the monovalent buffers (see Fig. 1, plots for the succinate and citrate buffers). Similarly, the retention factor is larger in solutions containing the trivalent citrate anion than in those of the bivalent anion.

There are two possible simultaneous explanations for this effect:

(a) The presence of high valence anions reduces the abundance of the free solvated propranolol cation which is poorly retained in the mobile phase because the cation has a stronger affinity for multivalent anions (equilibrium displacement towards the formation of neutral, high stoichiometric ion pair complexes).
(b) The adsorption of high stoechiometric complexes (e.g., 2:1 or 3:1 ion pairs) is stronger than that of two or three 1:1 complexes, respectively.

This issue will be discussed again later with the analysis of the isotherm parameters.

The obviously different behavior of Symmetry and Xterra with phosphate II and citrate III as buffers are explained by different surface properties of these two materials at pH close to neutrality. If fixed negative charges appear on the Symmetry surface, strong ion-exchange interactions could take place with the solvated propranolol cation which would explain a higher retention time of propranolol at low than at high buffer concentrations. By contrast, the retention on Xterra is certainly only controlled by the adsorption of the neutral ion pair complexes.

4.2. Slight overloaded band profiles and isotherm determination

Figs. 2–12 show the experimental overloaded band profiles (dotted lines) recorded when successively using in the mobile phase the eleven different buffers studied on Symmetry and Xterra. The solid lines in these figures show the best calculated band profiles obtained at the end of the application of IM to derive the values of the best isotherm parameters. The figures are listed according to the valence of the basic anion (Figs. 2–7: monovalent anions, Figs. 8–11: bivalent anions, Fig. 12: trivalent anions).

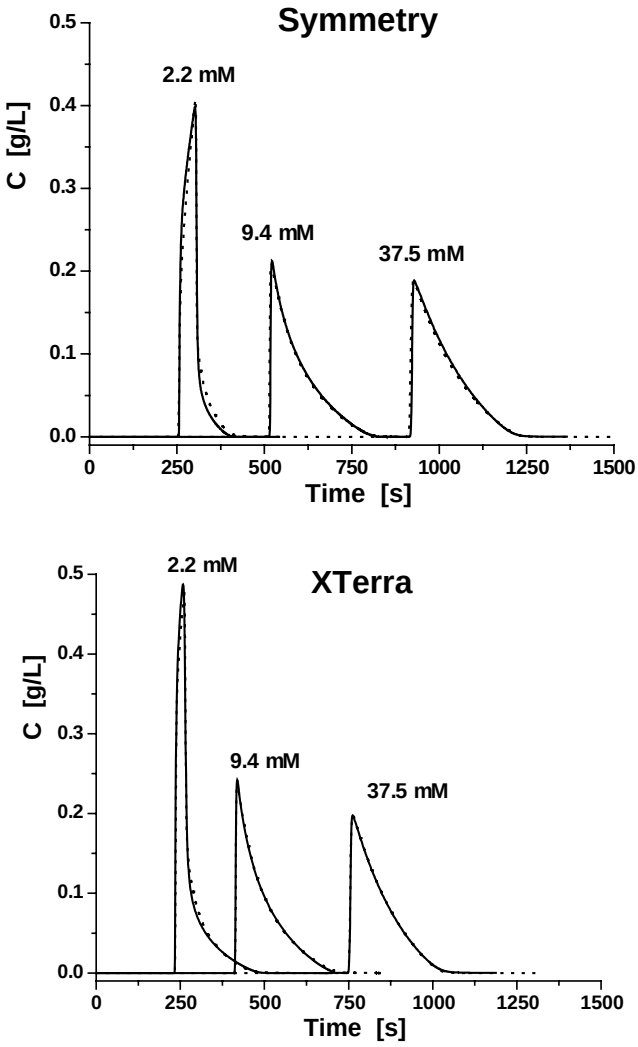

Fig. 2. Experimental (dotted lines) and best calculated (IM, solid lines) band profiles of propranolol on the Symmetry and Xterra column after injection of 250 μL of a 1.5 g/L solution of propranolol chloride for three different buffered mobile phase (methanol-water, 40:60, v/v). Buffer: phthalate I at pH 2.75. $T = 296$ K, flow rate 1 mL/min. Note the apparition of adsorbate–adsorbate interactions at low buffer concentrations, according to the change in the shape of the band profile. The best isotherm parameters derived by the IM are listed in Table 3.

4.2.1. Buffers with monovalent basic anion

The general effect of the buffer concentration on overloaded band profiles can be seen on each figure. For all six monovalent buffers ($2.75 < \text{pH} < 4.16$), the retention time of the band always increases with increasing buffer concentration, on both columns. This result is consistent with the diminution of the concentration of free propranolol cations in the mobile phase and with the correlated increase of that of neutral ion pairs, since the cation is poorly retained and the ion pairs are strongly retained. The overloaded band profiles are always accounted for by the bi-Moreau isotherm model but with no adsorbate–adsorbate interactions ($I_1 = I_2 = 0$), except at very low buffer concentrations, in the

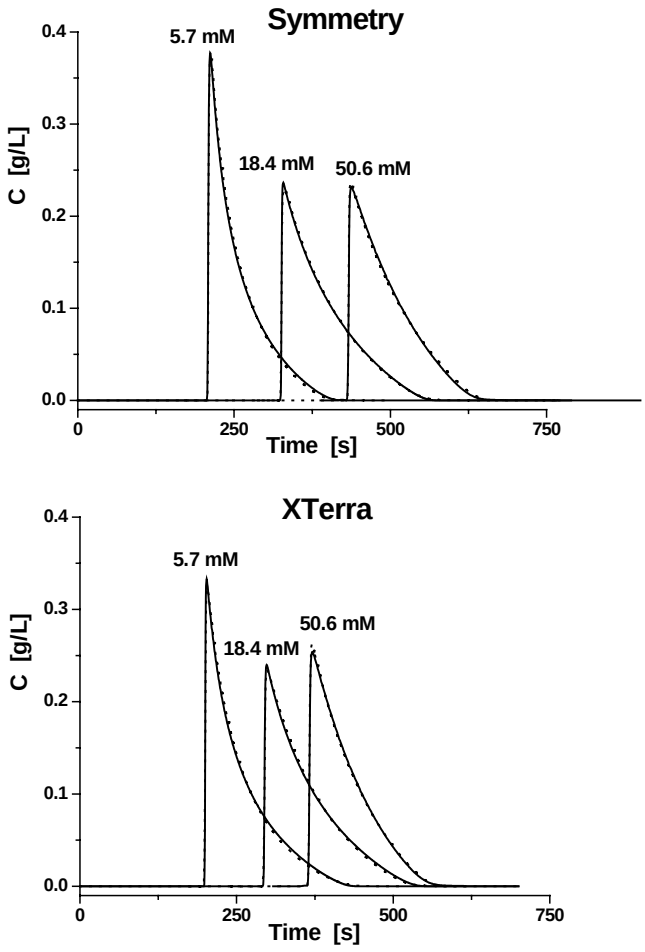

Fig. 3. Same as in Fig. 2 except buffer: phosphate I at pH 2.75.

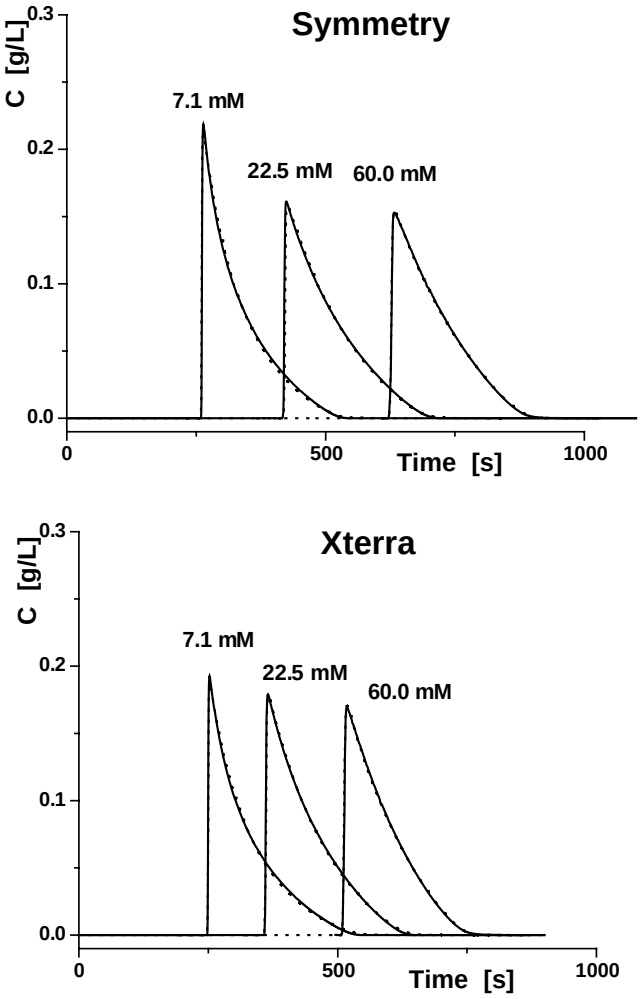

Fig. 4. Same as in Fig. 2, except buffer: formate at pH 3.75.

case of the phthalate, succinate and citrate buffers (see the shape of the band profile in Figs. 2, 5 and 7), for which I_1 was assumed to be different from zero. This is not surprising since, when the buffer concentration tends towards zero, the isotherm should converge to that of propranolol chloride with no salts or buffer in the mobile phase. This particular isotherm was best described by the bi-Moreau model [15] with adsorbate–adsorbate interaction parameters different from zero. The results are then consistent.

The best isotherm parameters obtained by IM, using the two-sites bi-Moreau model are listed in Table 3. The average saturation capacities, $q_{s,1}$, of the most abundant type 1 sites calculated for Symmetry and Xterra over the three buffer concentrations (≃6, 20, and 60 mM) vary between 85 and 144 g/L for the former and between 72 and 131 g/L for the latter adsorbent. Symmetry has a higher saturation capacity than Xterra, as expected because it has a higher carbon content and surface coverage (see Table 2), and because a similar result had been observed with FA measurements made without or with neutral salts in the mobile phase [17–19]. The highest values are obtained with the phthalate buffer for which the propranolol cation has the strongest affinity. The decrease in concentration of free propranolol cations that repulse each other when adsorbed on the stationary phase, explains the increase of the column saturation capacity. This is consistent with the ion-exclusion mechanism, as was pointed out by Hägglund and Ståhlberg [1–3]. Charged adsorbed solutes tend to repulse other charged sample molecules from the vicinity of the surface and diminish the saturation capacity. The low-energy equilibrium constant b_1 is almost the same for all buffers (≃0.05, 0.04 and 0.02 L/g at 6, 20, and 60 mM, respectively) and on both adsorbents, except with phthalate (I) (0.10, 0.05 and 0.01 L/g) because, in this case, the high hydrophobicity of the 1:1 ion pair complex is increased by the large nonpolar benzene ring in the buffer anion. All the other buffers are poorly hydrophobic so that they contribute little to enhance the ion-pair hydrophobicity and b_1 remains nearly constant. As expected from previous results on Kromasil, b_1 increases with increasing buffer concentration, in agreement with the theory of chaotropicity.

The properties of the second type of sites on both adsorbents are very similar. The saturation capacity of the high-energy sites, $q_{s,2}$, increases with increasing buffer con-

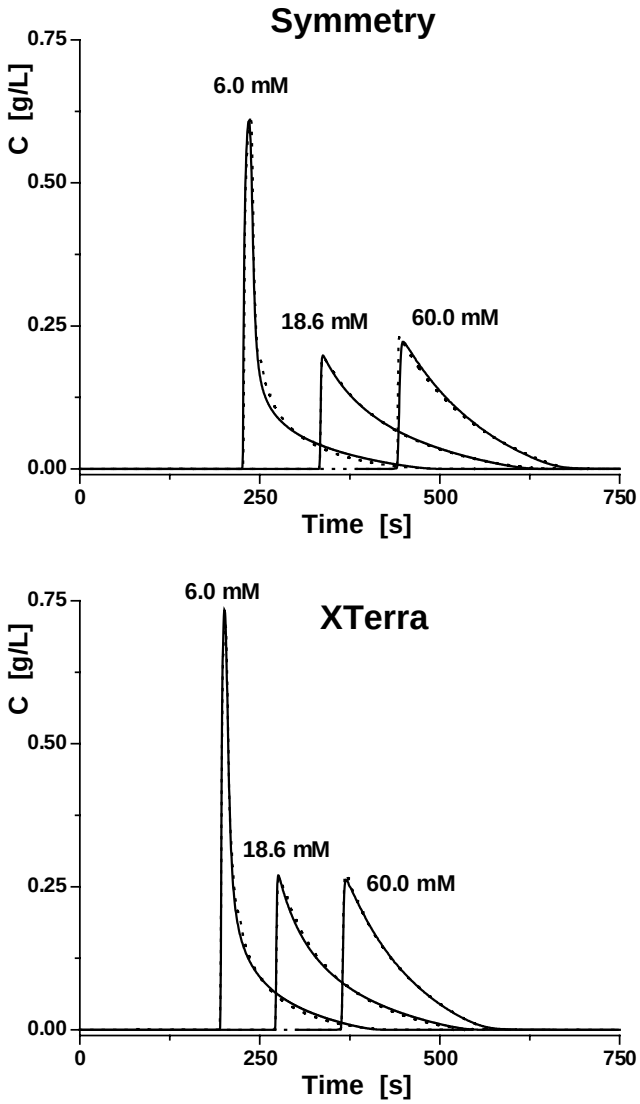

Fig. 5. Same as in Fig. 2, except buffer: succinate I at pH 4.16.

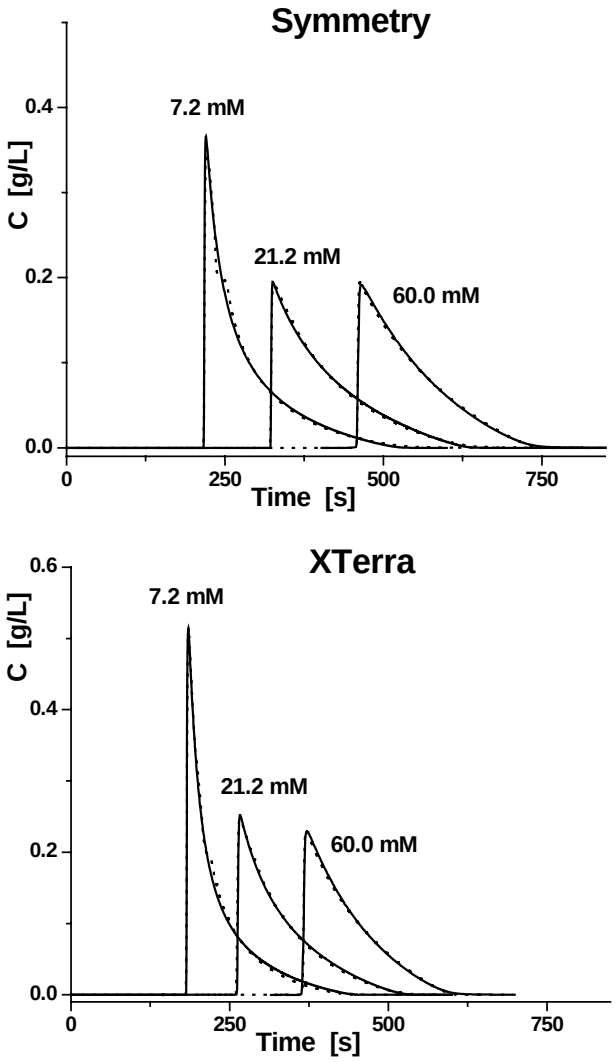

Fig. 6. Same as in Fig. 2, except buffer: acetate at pH 4.75.

centration, which is consistent with the adsorption of a neutral ion pair. It always ranges between 1 and 5 g/L, on the two adsorbents, as well as on Kromasil-C_{18}, previously studied [21]. Depending on the buffer concentration, the saturation capacity of type 2 sites accounts for between 1 and 10 % of the total saturation capacity. Compared to the values of the parameters obtained previously for Kromasil [21], the high-energy equilibrium constant are much lower on Symmetry and Xterra. The difference between the energies $\epsilon_{a,2}$ and $\epsilon_{a,1}$ of the two types of sites varies between approximately 15 kJ/mol at low buffer concentrations and 8 kJ/mol at high buffer concentrations. This similarity seems to rule out the most plausible physical interpretation of the nature of the higher-energy type of sites, as ion-exchange sites. It would be inconsistent with the lack of ionic activity of Xterra below pH 10. Finally, the equilibrium constant, b_2, decreases with increasing buffer concentration on all three adsorbents, a result that is consistent with the experimental band profiles (Figs. 2–12) the tailing of which are more and more pronounced when the buffer concentration decreases. There does not seem to be any simple explanation for this result.

To summarize, the band profiles recorded with monovalent buffers can be accounted for by a simple bi-Langmuir isotherm model, at least at moderate to high buffer concentrations. For a given buffer concentration, the saturation capacity of the low-energy, more abundant type 1 sites depends on the nature of the buffer. The higher the affinity of the analyte cation for the buffer anion, the higher the saturation capacity because the concentration of the neutral ion-pair relative to that of the cation increases with increasing affinity. The adsorption constant b_1 depends on the nature of the buffer. The higher its hydrophobicity, the higher b_1. The saturation capacity of the high-energy type 2 sites increases with increasing buffer concentration. It can account for up to 10% of the total saturation capacity. The equilibrium constant on the high-energy sites decreases with in-

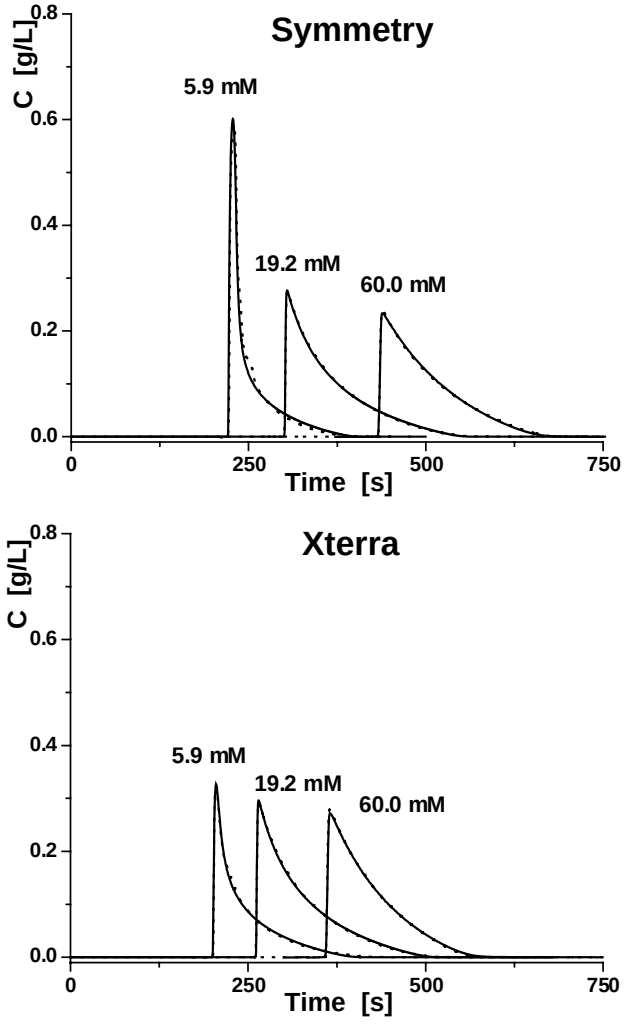

Fig. 7. Same as in Fig. 2, except buffer: citrate I at pH 3.14.

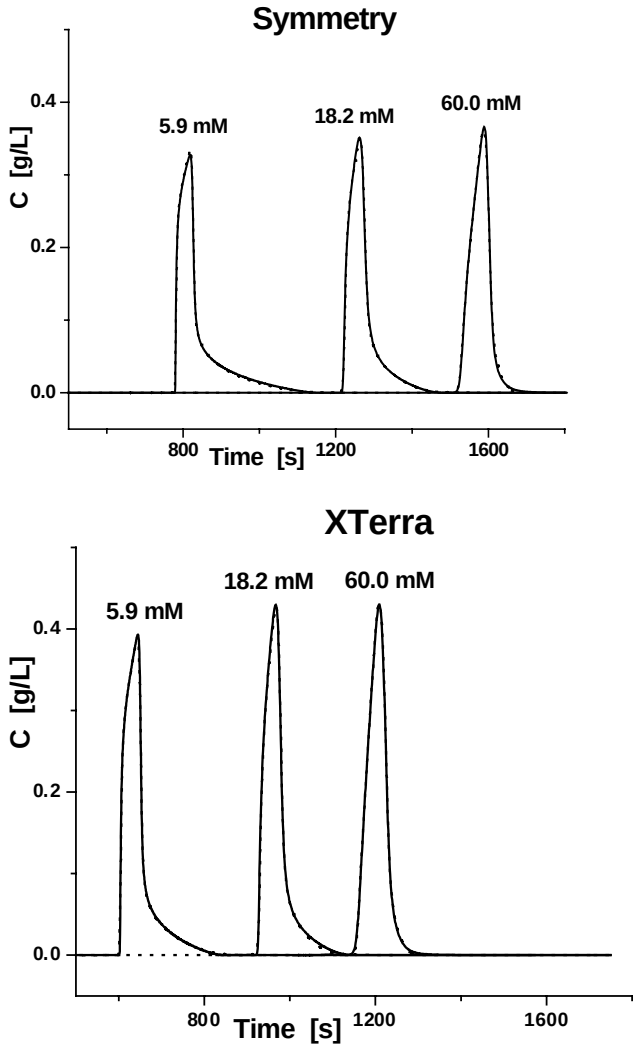

Fig. 8. Same as in Fig. 2, except buffer: phthalate II at pH 4.75. Note by comparison to the monovalent buffers in Figs. 2–7, the change in the shape of the band profiles at high buffer concentrations.

creasing buffer concentration. Although this result could be explained by an ion-exchange mechanism on Kromasil-C_{18} and Symmetry-C_{18} at a pH close to neutrality, it cannot be on Xterra. However, the similar behavior of the three columns casts serious doubts on the validity of this mechanism.

4.2.2. Buffers with bivalent basic anions

There were four buffers of this type, phthalate II, succinate II, phosphate II, and citrate II. The experimental and best calculated band profiles are shown in Figs. 8–11. These profiles are quite different from those obtained with the buffers having monovalent anions (Figs. 2–7). The maximum band concentrations are much higher, suggesting that the isotherm behavior deviates less from that of a straight line. The front part of the band is no longer a simple shock layer but includes a shock layer followed by a diffuse boundary. Similarly, the rear part of the profile exhibits a shock layer in several cases. The bi-Moreau isotherm model allows the successful calculation of profiles nearly identical to the experimental band profiles. Most other isotherm models tried would not. Its critical feature is an adsorbate–adsorbate interaction parameter different from zero on the low-energy adsorption sites (I_1), which accounts for the initial curvature of the isotherm toward the mobile phase concentration axis (i.e., an anti-langmuirian behavior at low concentrations). By contrast, I_2 was zero for all buffers, except for the phthalate II buffer.

The saturation capacity of the low-energy type of sites, $q_{s,1}$, varies between 136 and 140 g/L on Symmetry-C_{18} and between 110 and 129 g/L on Xterra-C_{18}. This means that $q_{s,1}$ is nearly independent of the nature of the buffer when this buffer is made of a monovalent and a divalent anions. These values are very close to those found for neutral analytes in mobile phases of similar composition (30–60% methanol in water) [43,44]. As expected, the saturation capacity is higher on Symmetry than on Xterra.

The adsorption constant b_1 is always about twice what it is with the corresponding monovalent buffer. The dif-

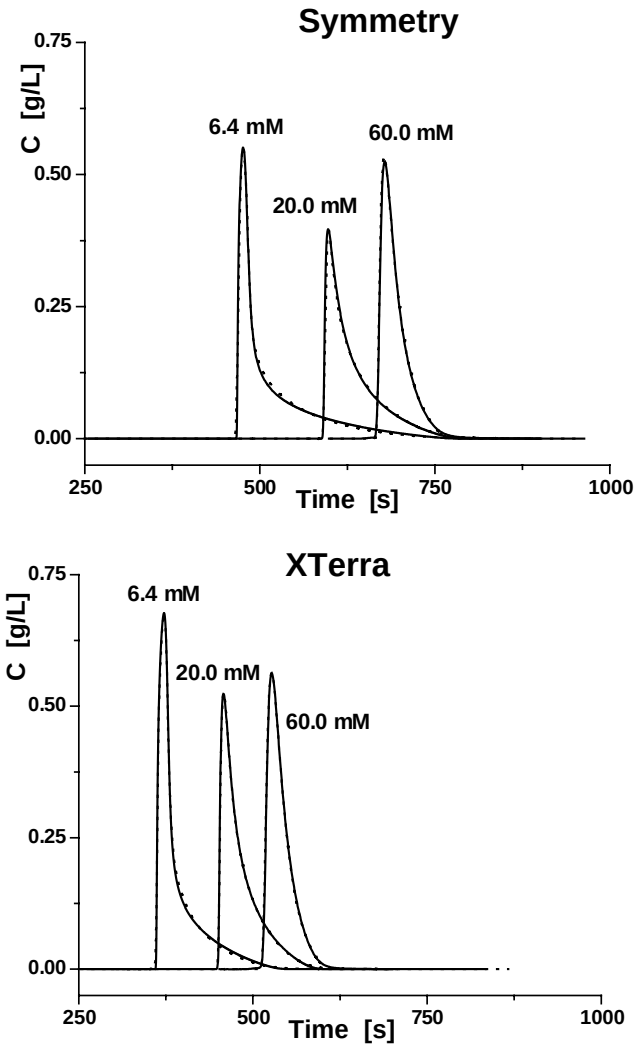

Fig. 9. Same as in Fig. 2, except buffer: succinate II at pH 5.61.

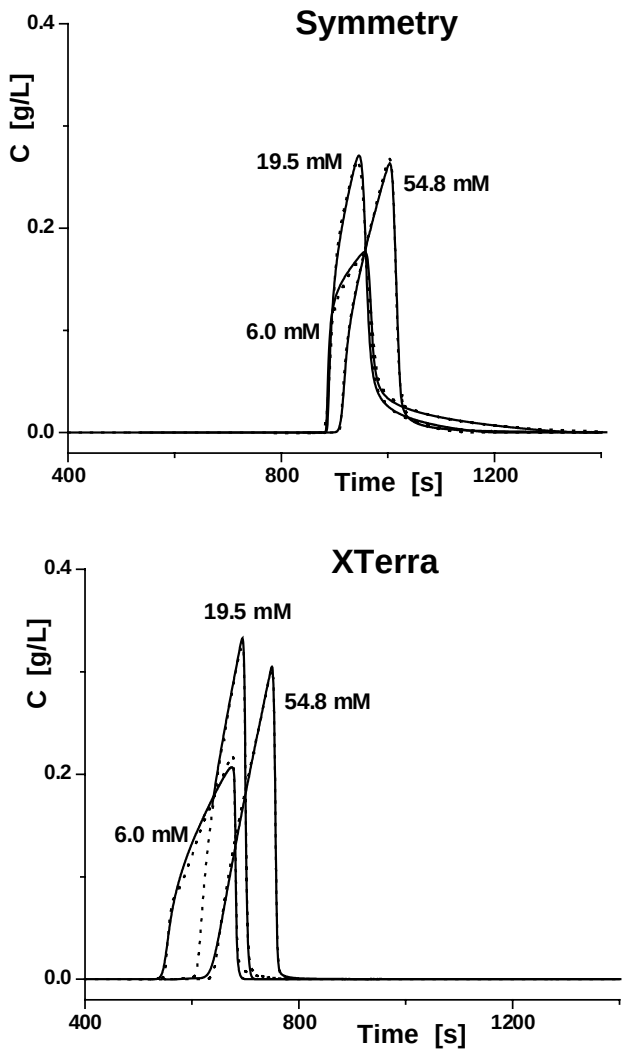

Fig. 10. Same as in Fig. 2, except buffer: phosphate II at pH 6.75.

ference is in part explained by the fact that the fraction of solvated propranolol cations remaining in solution is much lower when the cation can form an ion pair with either the acidic (monovalent) or the basic anion (divalent). The free cation is weakly retained compared to the ion complexes.

Surprisingly, the saturation capacity of the high-energy type 2 sites is lower when the buffer of the mobile phase is bivalent than when it is monovalent. It varies between 0.3 and 2.4 g/L and represents less than 2% of the total saturation capacity. This trend was also observed on Kromasil-C_{18} [21] and is not an isolated case. The high-energy type 2 sites might be specific to the adsorption of the solvated propranolol cation. If this is true, their number should be still lower with a trivalent buffer (see next section).

The values obtained for the adsorption constant b_2 on the two columns are comparable, except for the phosphate buffer for which b_2 is much higher on Symmetry than on Xterra. This explains why the plots of the retention factor with this buffer versus the buffer concentration are so different for the two columns in Fig. 1. On Symmetry, the band tailing is important at low buffer concentrations and the retention time under analytical conditions (i.e., at infinite dilution) is markedly higher than at high buffer concentrations. The evolution of b_2 with the buffer concentration is complex. Since no tailing could be observed on Xterra, it is possible that, at pH 6.75 ion-exchange interactions could take place between the propranolol cation and some groups at the surface of Symmetry-C_{18}.

Another important change arising from the use of a divalent rather than a monovalent buffer is that significant adsorbate–adsorbate interaction now take place on type 1 sites, at any buffer concentration. These interactions decrease with increasing buffer concentration. This result has already been observed with the mere addition of a supporting salt, KCl, to a mobile phase having the same composition [17–19]. The adsorbate–adsorbate interactions on the high-energy type 2 sites are often negligible.

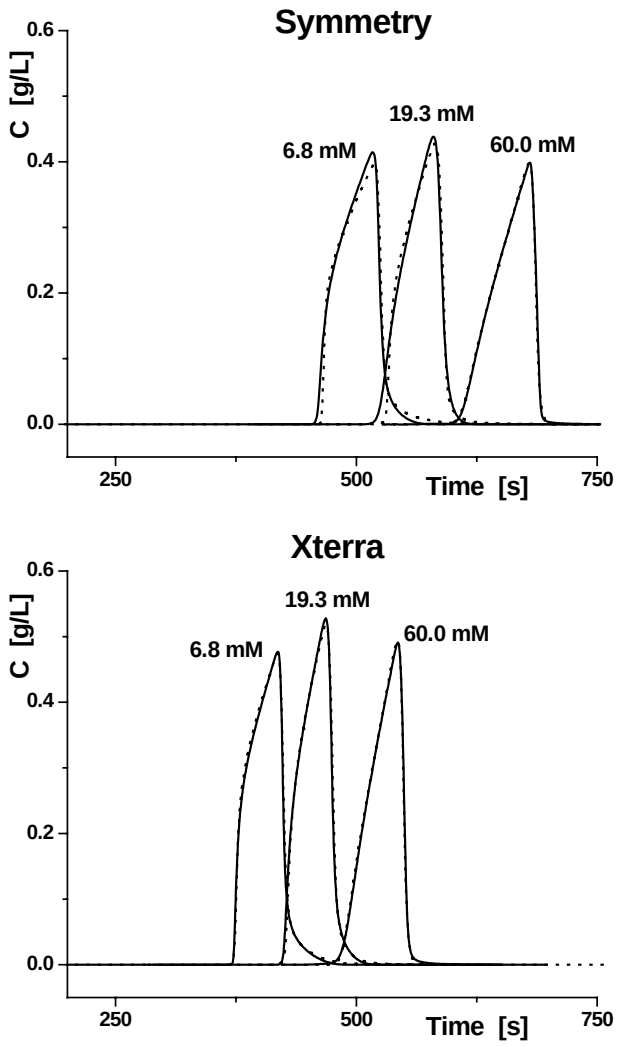

Fig. 11. Same as in Fig. 2, except buffer: citrate II at pH 4.77.

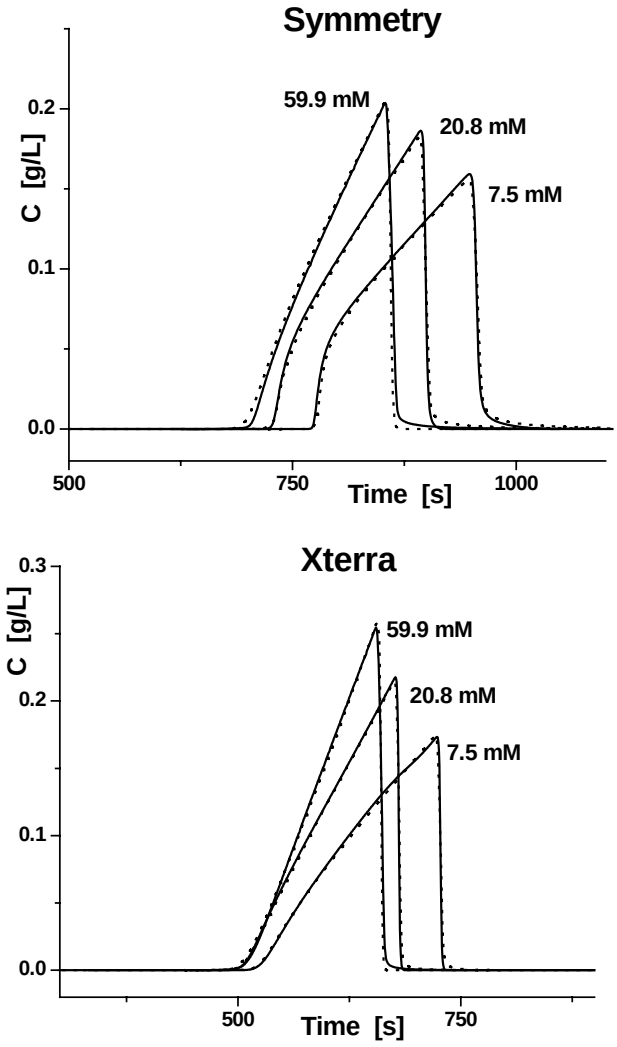

Fig. 12. Same as in Fig. 2, except buffer: citrate III pH 6.39. Note by comparison to the monovalent and bivalent buffers in Figs. 2–11, the even more pronounced anti-langmuirian shape of the band profiles.

In summary, compared to that of monovalent buffers, the use of the corresponding bivalent buffer results in an increase of the column saturation capacity and of the adsorption equilibrium constants and in the apparition of significant adsorbate–adsorbate interactions.

4.2.3. Buffers with trivalent basic anions

The only easily available and convenient buffer made with a triacid is the citrate buffer III. Fig. 12 shows the experimental and the best calculated band profiles. Their shape suggests a strong anti-langmuirian behavior. This observation is confirmed by the IM results (Table 3). The average saturation capacity is 147 g/L for Symmetry-C_{18} and 119 g/L for Xterra-C_{18}. These values are close to those measured with the corresponding bivalent buffers and suggest that propranolol adsorbs only as a neutral ionic complex. The adsorption constant b_1 is barely larger than that measured with citrate II while the saturation capacity of the high-energy sites is almost zero (<0.5 g/L), as expected (see previous section). The main change in the isotherm parameters is the value of the adsorbate–adsorbate interaction parameter, I_1, that is twice larger than with citrate II at high buffer concentrations (20 and 60 mM). This observation is explained by the fact that a trivalent anion can form a complex with up to three propranolol molecules, which has an effect equivalent to that of an increase of the adsorbate–adsorbate interaction parameter in the mobile phase (the concentration of propranolol in the adsorbed phase can increase up to three times faster than that in the mobile phase). Finally, with the citrate III buffer as with the phosphate II buffer, a much higher (nearly three times) adsorption equilibrium constant, b_2, was measured on Symmetry than on Xterra resulting in a longer band tailing with Symmetry.

Table 3
Best isotherm parameters estimated by the inverse method (IM) for isotherm determination

Buffer	pH	C_{Buffer} (mM)	$q_{s,1}$ (g/L)		b_1 (L/g)		I_1		$q_{s,2}$ (g/L)		b_2 (L/g)		I_2	
			Sym	Xtra	Sym	Xtra	Sym	Xtra	Sym	Xtra	Sym	Xtra	Sym	Xtra
Phosphate I	2.75	50.6	138	120	0.042	0.043	0	0	4.9	4.5	1.4	1.4	0	0
		18.4	108	105	0.035	0.034			3.5	3.4	1.9	2.2		
		5.7	101	114	0.025	0.020			1.7	2.1	2.7	2.9		
Phosphate II	6.75	54.8	144	125	0.117	0.099	6.3	8.2	0.3	0.4	20	6.6	0.5	0.7
		19.5	134	128	0.113	0.084	7.0	10.1	0.7	0.5	12	5.8	0.3	1.7
		6.0	129	131	0.072	0.048	43	62.1	2.0	1.0	9.0	5.8	0.1	1.4
Phthalate I	2.75	37.5	169	154	0.106	0.096	0.0	0.1	3.4	4.5	2.1	1.7	0	0
		9.4	156	128	0.047	0.047	4.7	4.4	3.4	3.4	2.6	2.6		
		2.2	108	112	0.009	0.004	622	1710	1.8	2.4	3.3	3.7		
Phthalate II	4.75	44.0	147	150	0.205	0.171	3.4	3.1	0.4	0.2	9.3	11.3	1.7	2.2
		18.2	130	130	0.149	0.132	6.0	5.1	1.9	1.0	5.6	7.4	0.8	1.0
		5.9	114	106	0.073	0.074	22	16.2	2.3	1.4	6.4	7.5	0.5	1.0
Succinate I	4.16	60.0	153	117	0.042	0.047	0	0	3.7	3.8	1.8	1.6	0	0
		18.6	125	125	0.036	0.031	0	0	2.3	2.2	3.2	3.2		
		6.0	120	102	0.017	0.021	38	15.6	1.4	1.4	4.7	4.1		
Succinate II	5.61	60.0	140	114	0.094	0.094	2.2	2.2	0.9	1.0	1.9	1.7	0	0
		20.0	140	109	0.080	0.077	2.2	2.8	1.2	1.7	3.5	2.1		
		6.4	137	107	0.053	0.045	6.3	11.0	1.5	2.5	5.3	2.5		
Formate	3.75	60.0	119	106	0.071	0.067	0	0	6.0	5.8	1.5	1.5	0	0
		22.5	88	77	0.055	0.055	0	0	4.1	4.2	2.2	2.1		
		7.1	57	58	0.044	0.044	8.1	4.1	2.4	2.0	3.1	3.7		
Acetate	4.75	60.0	107	90	0.052	0.052	0	0	5.1	4.8	1.7	1.6	0	0
		21.2	87	74	0.043	0.045			2.9	2.6	2.9	2.8		
		7.2	61	52	0.050	0.049			1.3	1.2	5.1	5.1		
Citrate I	3.14	60.0	119	90	0.056	0.062	0	0	3.1	3.8	2.0	1.6	0	0
		19.2	107	83	0.041	0.045	0	0	1.9	2.2	3.1	2.9		
		5.9	118	98	0.019	0.016	23.8	52.5	1.2	1.7	3.9	3.5		
Citrate II	4.77	60.0	143	117	0.078	0.078	6.0	5.9	0.6	0.8	2.4	1.9	0	0
		19.3	139	114	0.063	0.060	7.6	9.0	1.1	1.3	2.1	2.4		
		6.8	139	111	0.046	0.045	16.6	19.2	1.4	1.5	2.6	2.8		
Citrate III	5.61	59.9	148	121	0.085	0.084	13.7	12.0	0.5	0.5	4.0	1.5	0	0
		20.8	143	116	0.090	0.085	15.5	16.7	0.3	0.2	12.0	5.5		
		7.5	149	119	0.088	0.085	21.0	21.4	0.3	0.2	20.2	6.0		

Optimization made on a band profile recorded after the injection of a 1.5 g/L solution of propranolol chloride during 15 s.

5. Conclusion

The parameters of the adsorption equilibrium isotherms of propranolol from various buffer solutions onto Symmetry-C_{18} and Xterra-C_{18} were determined by the IM method, using overloaded band profiles. This allowed the systematic investigation of the influence of the nature and the concentration of the buffer. Buffers made with monovalent, bivalent and trivalent anions were used. The results obtained confirm previous results obtained by the same method with Kromasil-C_{18}. They show that propranolol, which is ionized as a cation in the mobile phases, forms ionic complexes with the various anions afforded by the different buffers and that it may adsorb under different forms, depending on the valence of the buffer anions used.

The adsorption mechanism is well described by an isotherm model including two adsorption sites. The first of the two terms of the corresponding isotherm equation is a Moreau isotherm, the second turns out to be a Langmuir isoterm. The saturation capacities on both sites increase with increasing buffer concentration, because the concentration of the neutral ionic complexes increases in the same time. The adsorption constant on the low-energy sites also increases in the same time because the contribution to the overall equilibrium constant of the solvated propranolol cation is lower than that of the neutral complexes. The adsorbate–adsorbate interaction parameter on the high-energy sites is negligible. That on the low-energy sites increases with increasing buffer concentration. The adsorption equilibrium constant on the high-energy sites increases with increasing buffer concentration in most cases. The origin of these high-energy sites is probably related to ion-exchange sites, as suggested by the long tail of the band at pH 6.75 (phosphate II) and 6.39

(citrate III) on Symmetry-C_{18} while there is no such tail on Xterra-C_{18}.

The valence of the buffer is a critical factor in the adsorption mechanism. For polyacids, the buffers made with the low valence anion have a saturation capacity that is smaller than for the corresponding buffers made with the high valence anion, because the concentration of the free propranolol cation in the mobile phase is higher with the former anion. The saturation capacity which is relatively low with monovalent buffers is larger with the bivalent anions and still larger with the trivalent anion. Then it is close to the values of the saturation capacities measured for neutral compounds. Furthermore, with high valence anions, the adsorbate–adsorbate interaction parameter in the stationary phase is higher, the isotherm becomes convex downward. at least at low concentrations, and the band profile has a characteristic anti-langmuirian behavior. Our experimental results show that, in contrast to what has recently been concluded by several other authors, the saturation capacities of Symmetry-C_{18} and Xterra-C_{18} (and probably that of numerous other RPLC stationary phase) for ionic species in buffer solutions remains of the same order of magnitude as that of neutral compounds, as long as the buffer concentration in the mobile phase remains sufficiently high. This should be expected since most of these organic ionic species are present in solution as neutral ion-pair complexes. However, the loadability may not be as good as that obtained for the unprotonated form of propranolol at basic pHs.

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