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Influence of a buffered solution on the adsorption isotherm and overloaded band profiles of an ionizable compound

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Abstract

The overloaded band profiles and the adsorption isotherms of propranolol were acquired at 23 °C, on the endcapped C_{18}-Kromasil stationary phase, using two aqueous solutions of methanol as the mobile phase. The first solution contained 40% methanol and no buffer. The second contained an aqueous acetate buffer at $C_{buffer} = 0.20$ M and pH = 5.9. In both cases, 33 isotherm data points were acquired by frontal analysis (FA), to achieve an accurate description of the isotherms in the concentration range between 1.54×10^{-3} and 1.54×10^{-1} mol/l of propranolol. The isotherms obtained were best described by a bi-Langmuir and a bi-Moreau isotherm model, depending on whether the mobile phase was buffered or not. This shows that the adsorption of propranolol takes place on two different types of sites, a behavior similar to the one already observed with phenol and caffeine on the same column. The presence of the buffer in the mobile phase drastically changes the adsorption mechanism of propranolol. Weak adsorbate–adsorbate interactions (two and three times *RT* on the low- and the high-energy sites, respectively) take place in the absence of buffer but vanish when the mobile phase is buffered. As expected, the adsorption constants on the abundant low-energy sites with or without buffer are comparable because the mobile phase composition was adjusted to give similar retention times in the two cases. On the other hand, the adsorption of propranolol on the high-energy sites is stronger in presence of the buffer. The difference probably comes from ion-pair formation in the adsorbed phase between the propranolol cation and the acetate anion. The change in total saturation capacity of the adsorbent (22%) compared to that for phenol is explained by the difference in methanol content of the mobile phase.
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1. Introduction

Although reversed-phase liquid chromatography has been by far, and for a long time, the most popular separation mode in analytical applications of high-performance liquid chromatography [1], there remain serious uncertainties regarding its actual mechanism. Alkyl-bonded spherical silica particles and monolithic silica are the most widely used stationary phases in HPLC. These packing materials afford excellent chromatographic reproducibility for the analysis of neutral compounds under analytical [2–6] and overloaded conditions [7–9]. They are used for many biomedical, pharmaceutical and environmental separations. In such applications, however, complex molecules with acido-basic properties are often analyzed. They may strongly interact with the acidic residual silanols of the support [10,11]. Many studies have been carried out to reduce the number of active residual silanols on silica surfaces and to lower the peak asymmetry [12,13]. The most common method is endcapping, that follows the C_{18} derivatization process and ensure their partial removal. A new, less polar silica-methylsilane hybrid surface was proven to have no ion-exchange properties between pH = 2 and 11 [14] and is now available.

At this stage, it is interesting to investigate the influence on column performance of the mobile phase composition, particularly those used to elute acidic or basic compounds. This is currently a topic of high interest [15,16]. In order to perform the best separations of such compounds, the organic content of the aqueous mobile phase, the solution pH, its ionic strength, the temperature, and, even in some

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particular cases, the average column pressure must be optimized. This step may be of critical importance because the chemistry of the adsorbent surface is often sensitive to small changes in the operating conditions. Fornsted et al. [17] have shown that the adsorption constant of several β-blockerson a cellulase protein depends on the solution pH. Hearn and co-workers [18] studied the effect of the ionic strength and the temperature on the adsorption of proteins on immobilised metal ion affinity and dye affinity matrices. Chen et al. [19] demonstrated the effect of the solution pH on the adsorption behavior of phenylalanin anilidine on an imprinted cross-linked polymeric stationary phase. Few systematic studies have been devoted to the strong influence of these experimental parameters on the adsorption behavior of ionic compounds on conventionnal endcapped C_{18}-bonded silica stationary phase until recently [15,16]. In principle, these packing materials are inert within a pH range between 3 and 8 and adsorption on them is not expected to depend much on the mobile phase pH.

In this work, we accurately measured and compared the adsorption behavior of a compound that can exist in solution as a neutral or a positively charged cation, (*R*,*S*)-propranolol, in two mobile phases of different compositions, either a non buffered methanol/water solution (40:60, v/v) or a methanol/water solution (60:40, v/v) made with an acetate buffer whose pH was fixed at 5.9 (with $C_{\text{buffer}} = 0.2\,\text{M}$). Then, we discuss the effect of the buffer on the shape of the overloaded band profiles, the nature of the isotherms measured, the saturation capacity of the column, the adsorption–desorption constants, and the adsorbent surface heterogeneity.

2. Theory

2.1. Determination of the single-component isotherms by frontal analysis (FA)

Among the various chromatographic methods available to determine the adsorption isotherms of single components, frontal analysis is the most accurate [20–22]. Mass conservation of the solute between the times when the new solution enters the column and when the plateau concentration is reached allows the calculation of the adsorbed amount, q^*, of the solute in the stationary phase at equilibrium at the corresponding mobile phase concentration, C. This amount is best measured by integrating the breakthrough curve (equal area method) [23]. The adsorbed amount q^* is given by:

$$q^* = \frac{C(V_{\text{eq}} - V_0)}{V_{\text{a}}} \tag{1}$$

where V_{eq} and V_0 are the elution volume of the equivalent area and the hold-up volume, respectively, and V_{a} is the volume of stationary phase.

2.2. Isotherm models

We tried to use several isotherm models to account for the FA data acquired. These models were derived from the Langmuir, the Moreau, and the BET models.

2.2.1. The bi-Langmuir isotherm model

This model is the simplest possible model that accounts for adsorption behavior on a heterogeneous surface [24]. This surface is considered as paved with two kinds of homogeneous chemical domains, which behave independently. Then, the equilibrium isotherm is the result of the sum of two local Langmuir isotherms:

$$q^* = q_{\text{s},1}\frac{b_1 C}{1 + b_1 C} + q_{\text{s},2}\frac{b_2 C}{1 + b_2 C} \tag{2}$$

As for a Langmuir isotherm, the equilibrium constants b_i are given by the folowing equation [25]:

$$b_i = b_{0,i}\exp\frac{\epsilon_{\text{a},i}}{RT} \tag{3}$$

where $\epsilon_{\text{a},i}$ is the adsorption energy on the sites i and $b_{0,i}$ is a preexponential factor that can be derived, in principle at least, from the molecular partition functions in the bulk and the adsorbed phases for the pure site i. In this model, there are two saturation capacities, $q_{\text{s},1}$ and $q_{\text{s},2}$, and two equilibrium constants, b_1 and b_2, each associated with an adsorption energy, $\epsilon_{\text{a},1}$ and $\epsilon_{\text{a},2}$, through Eq. (3). The energy distribution function is bimodal and both modes are represented by a Dirac δ-function [25].

2.2.2. The monolayer Moreau isotherm model

Moreau et al. [26] described several isotherm models that are extensions of the two-dimensional lattice model with adsorbate–adsorbate interactions to the treatment of multicomponent adsorption. These models were derived using the theory of lattice statistics. This theory assumes that the surface is homogeneous and that the surface coverage is limited to a monolayer. If the adsorbate–adsorbate interactions are limited to those between adjacent pairs of molecules, the model gives for the single component isotherm:

$$q^* = q_{\text{s}}\frac{bC + Ib^2C^2}{1 + 2bC + Ib^2C^2} \tag{4}$$

where q_{s}, b and I are the monolayer saturation capacity, the low-concentration equilibrium constant and the adsorbate–adsorbate interaction parameter. I can be written as [26]:

$$I = \exp\left(\frac{\epsilon_{\text{AA}}}{k_{\text{B}}T}\right) \tag{5}$$

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

2.2.3. The two-layer liquid–solid extended BET isotherm model

For a two-layer adsorption system with adsorbate–adsorbate interactions taking place between molecules belonging to the first and the second layer, the extended liquid–solid BET isotherm derived for two layers gives the following isotherm model [27]:

$$q^* = q_s \frac{b_S C + 2 b_L b_S C^2}{1 + b_S C + b_L b_S C^2} \quad (6)$$

where the parameters q_s, b_S and b_L are related to the monolayer saturation capacity, the low-concentration equilibrium constant on the adsorbent surface and the equilibrium constant on the first adsorbed layer. Note that this isotherm has exactly the same mathematical expression as the Moreau model or as the Ruthven quadratic isotherm model which we have used previously [7] in our study of the reproducibility of the overloaded band profiles on 10 different C_{18}-Kromasil columns.

2.3. Modeling of high-performance liquid chromatography

The overloaded band profiles were calculated using the best isotherm model for the compound studied and the equilibrium-dispersive model (ED) of chromatography [20,28,29]. The ED model assumes instantaneous equilibrium between the mobile and the stationary phase 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 related to the experimental parameters through the following equation:

$$D_a = \frac{uL}{2N} \quad (7)$$

where u is the mobile phase linear velocity, L the column length, and N the number of theoretical plates or apparent efficiency of the column.

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

$$\frac{\partial C}{\partial t} + u\frac{\partial C}{\partial z} + F\frac{\partial q^*}{\partial t} = D_a \frac{\partial^2 C}{\partial z^2} \quad (8)$$

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

2.3.1. Numerical solutions of the ED model

The mass balance equation was integrated numerically using a computer program based on an implementation of the method of orthogonal collocation on finite elements (OCFE) [30–32]. The set of discretized ordinary differential equations was solved with the Adams–Moulton method, implemented in the VODE procedure [33]. The relative and absolute errors of the numerical calculations were 10^{-6} and 10^{-8}, respectively.

2.3.2. Initial and boundary conditions for the ED model

At $t = 0$, the concentration of the solute in the column is uniformly equal to zero, and the stationary phase is in equilibrium with the mobile phase components (methanol and water in this work). The boundary conditions used are the classical Danckwerts-type boundary conditions [20,34] at the inlet and outlet of the column. In all the calculations, the inlet profiles were assimilated to rectangular profiles.

3. Experimental

3.1. Chemicals

Two different mobile phases were used in this work, whether for the determination of the adsorption isotherm data or for the recording of large size band profiles. These solutions were mixtures of HPLC grade water and methanol, both purchased from Fisher Scientific (Fair Lawn, NJ, USA). The composition of the mobile phase was adjusted to obtain a similar retention factor (between 2 and 3) in the two series of experiments. The first solution was of methanol and water (40:60, v/v), without buffer. The second was of methanol and water (60:40, v/v), with an acetate buffer at $C_{buffer} = 0.2$ M and pH = 5.9. The solvents used to prepare the mobile phase were filtered before use on an SFCA filter membrane, 0.2 μm pore size (Suwannee, GA, USA). Uracil, propranolol, glacial acetic acid and sodium acetate were obtained from Aldrich (Milwaukee, WI, USA).

3.2. Materials

A manufacturer-packed, 250 × 4.6 mm Kromasil column was used (Eka Nobel, Bohus, Sweden, EU). This column was packed with a C_{18}-bonded, endcapped, porous silica. This column (Column #E6021) was one of the lot of 10 columns previously used by Kele and Guiochon [3], Gritti and Guiochon [7], and Felinger and Guiochon [8] for their study of the reproducibility of the chromatographic properties of RPLC columns under linear and non-linear conditions. The main characteristics of the bare porous silica and of the packing material used are summarized in Table 1.

The hold-up time of this column was derived from the retention time of small uracil injections. With the two mobile phase compositions considered in this study, the elution time of uracil is nearly the same as that of pure methanol or sodium nitrate. The product of this time and the mobile phase flow rate gives an excellent estimate of the column void volume. The void volume of the column in 40:60 (v/v) and 60:40 (v/v) methanol/water mobile phases are 2.52 and 2.40 cm^3, respectively.

Table 1
Physico-chemical properties of the packed Kromasil-C_{18} (Eka) #E6021column

Particle size (μm)	5.98
Particle size distribution (90:10, ratio (%))	1.44
Pore size (Å)	112
Pore volume (ml/g)	0.88
Surface area (m^2/g)	314
Na, Al, Fe content (ppm)	11, <10, and <10
Particle shape	Spherical
Total carbon (%)	20.00
Surface coverage (μmol/m^2)	3.59
Endcapping	Yes

3.3. Apparatus

The isotherm data were acquired using a Hewlett-Packard (Palo Alto, CA, USA) HP 1090 liquid chromatograph. This instrument includes a multi-solvent delivery system (three tanks of volume 1 dm^3 each), an auto-sampler with a 25 μl loop, a diode-array UV-detector, a column thermostat and a computer data acquisition station. Compressed nitrogen and helium bottles (National Welders, Charlotte, NC, USA) are connected to the instrument to allow the continuous operation of the pump and auto-sampler and solvent sparging. The extra-column volumes are 0.058 and 0.90 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. A relative error of less than 0.4% was obtained. Accordingly, 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. Concentrations measurements by UV spectroscopy

The calibration curves of propranolol in the two mobile phases used are shown in Fig. 1. The UV absorbance of the 33 propranolol solutions used in FA, with concentrations between 0.4 and 40 g/l, in the buffered and the non-buffered solutions, was measured at 330 nm. These calibration data were fitted to a third-degree polynomial and the calibration curve so obtained was used to transform the absorbance signals into concentrations for the overloaded band profiles shown in Figs. 5–8, 10, 12 and 13. The only small difference between the two calibration curves is due to their different content of methanol, 60 and 40%, respectively. The same effect was already noticed in a previous report dealing with gradient elution chromatography of phenol, using methanol/water solutions as the mobile phase [35]. For a given solute concentration, the lower the methanol concentration in the mobile phase, the higher the absorbance. It

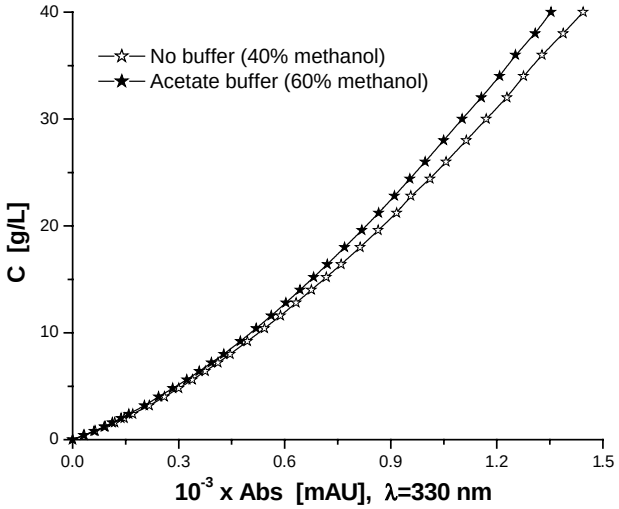

Fig. 1. Comparison between the calibration curves of propranolol (connected filled stars: solution with buffer; connected empty stars: no buffer). Note the slight difference between the curves due to the difference in methanol content in the mobile phase.

seems that the two species, RNH_2^+R' and $RNHR'$, have the same UV absorbance at 330 nm, which is not surprising since the chromophore in propranolol is the naphthyl group and the aliphatic amine does not absorb light at this wavelength.

3.5. Isotherm measurements by frontal analysis

One pump of the HPLC instrument was used to deliver a stream of the pure mobile phase, the second pump, a stream of the concentrated solution of the compound studied in the same mobile phase. Thirty-three data points were acquired during each of the two FA series of runs (one run with the buffer in the mobile phase, the other run without). The concentration of the compound studied (propranolol) in each FA run is determined by the concentration of the mother sample solution (40 g/l) and the flow rate fractions delivered by the two pumps. The breakthrough curves are recorded successively, at a flow rate of 1 $cm^3\,min^{-1}$, with a sufficiently long time delay between each breakthrough curve to allow for the reequilibration of the column with the pure mobile phase. The injection time of the sample varied between 6 and 10 min in order to reach a stable plateau for the component injected at the column outlet. The signal of propranolol was detected with the UV detector at 330 nm.

4. Results and discussion

The importance of the presence of a buffer in the mobile phase on the elution band profile of propranolol is first discussed. Then, the adsorption isotherms of propranolol on the RPLC column are accounted for, using measurements made successively in aqueous solutions of methanol with and without a buffer. The methanol concentration was ad-

justed in order to achieve in each case a value of the retention factor suitable for carrying out accurate measurements of the isotherm data, i.e., of the order of 3.

4.1. Effect of the acetate buffer on the band profiles of propranolol

4.1.1. Ionization of propranolol

Propranolol ($C_{10}H_7$–O–CH_2–CHOH–CH_2–NH–C–$(CH_3)_2$) was dissolved in an aqueous solution of methanol from its protonated acidic form (chloride salt of the amine). At very low concentrations (typically below 0.1 g/l) in a nonbuffered solution, the ammonium group of propranolol dissociates because K_a in pure water is about 9.5 (this value should be corrected to account for the mobile phase being an aqueous solution of methanol). Since the conjugated base is a neutral compound (amine), the dissolution and dissociation of the protonated acid are easily achieved because the base is well solvated in the CH_3OH/H_2O solution. Rived et al. [36] predicted a drop of about 1 unit for the apparent pK_a of protonated amines in aqueous solutions with a volume fraction of methanol equal to 0.4, so $pK_a \simeq 8.5$ in the mobile phase used in this work. In the presence of a buffer at a pH lower than ca. 7.0, there is practically no free amine in solution, the only propranolol species being the ammonium ion. Conversely, the free amine would be the only species in a solution buffered at a pH higher than ca. 10.0, a solution that cannot be used safely with silica-based RPLC packing materials.

The variations of the pH of the two solutions as a function of the propranolol concentration are compared in Fig. 2. This figure shows the pH calculated within the concentration range of the propranolol chloride salt dissolved in the mobile phase for our experiments (1.54×10^{-3} to 1.54×10^{-1} M). This pH remains constant at 5.9 in the solution buffered with a 0.2 M acetate buffer. On the other hand, the pH drops by more than one unit when there is no buffer in the solution. At high concentrations of propranolol chloride the only form present in the solution is the protonated acid form of propranolol. Even at low concentrations, this form is dominant. Also, the ionic strength I of the solution is constantly higher with the buffer (i.e., larger by 0.2 M) and increases from 0.20 to 0.35 M for the buffered solution in the propranolol concentration range studied.

Hence, we may summarize as follows the differences between the two sets of experimental chromatographic conditions regarding the mobile phase:

(1) The methanol concentration in the mobile phase is 40% (v/v) for the unbuffered solution, 60% (v/v) for the buffered solution. This difference arises from the need to adjust this concentration in order to obtain a reasonable retention and a comparable accuracy of the two series

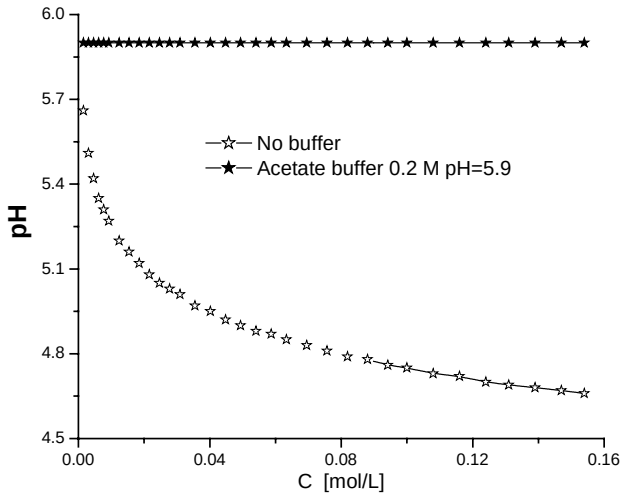

Fig. 2. Comparison between the theoretical pHs of the two mobile phases (connected filled stars: solution with buffer; connected empty stars: no buffer) as a function of the concentration of propranolol dissolved.

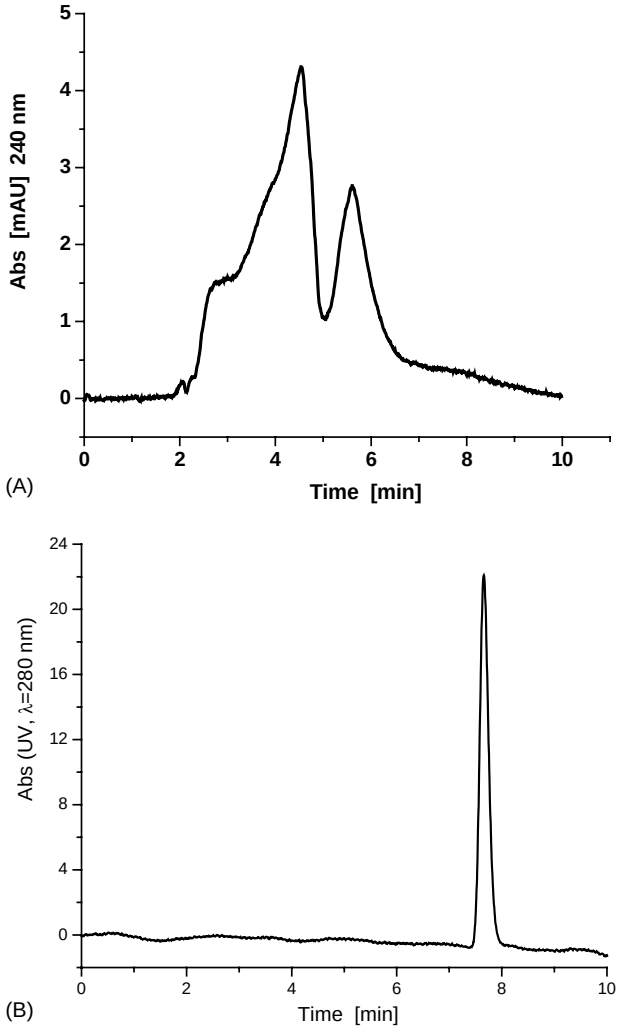

Fig. 3. Effect of the presence of buffer on the analytical chromatograms of propranolol. Injection of 2 μl of a 0.1 g/l propranolol solution. C_{18}-Kromasil column. Flow rate 1 ml/min; $T = 23\,^{\circ}C$. (A) 40:60 (v/v) methanol/water mobile phase; no buffer. (B) 60:40 (v/v) methanol/water mobile phase; buffer acetate, 0.2 M, pH = 5.9. Note the two large diffuse peaks when no buffer is used in the mobile phase.

of FA measurements. Too low a retention was observed with a 60:40 (v/v) CH_3OH/H_2O solution without buffer and too high a retention with a 40:60 (v/v) buffered solution.

(2) The ionic strength of the mobile phase is constantly higher with the buffer whatever the concentration of propranolol salt dissolved (+0.2 M).

(3) The pH of the mobile phase is more acidic without buffer and decreases markedly with increasing concentration of propranolol.

4.1.2. Band profiles of propranolol

Fig. 3 compares the analytical chromatograms of propranolol obtained with a nonbuffered solution (A) and with the acetate buffer at 0.2 M, pH = 5.9 (B). With no buffer in the mobile phase, we observe a complex chromatogram, split into two diffuse bands. Based on the relative absorbance at 240 and 280 nm (Fig. 4), it is clear that propranolol elutes during the whole duration of the complex band in Fig. 3 and that it is under the acidic form, as expected since the solution is prepared from its chloride salt. On the other hand,

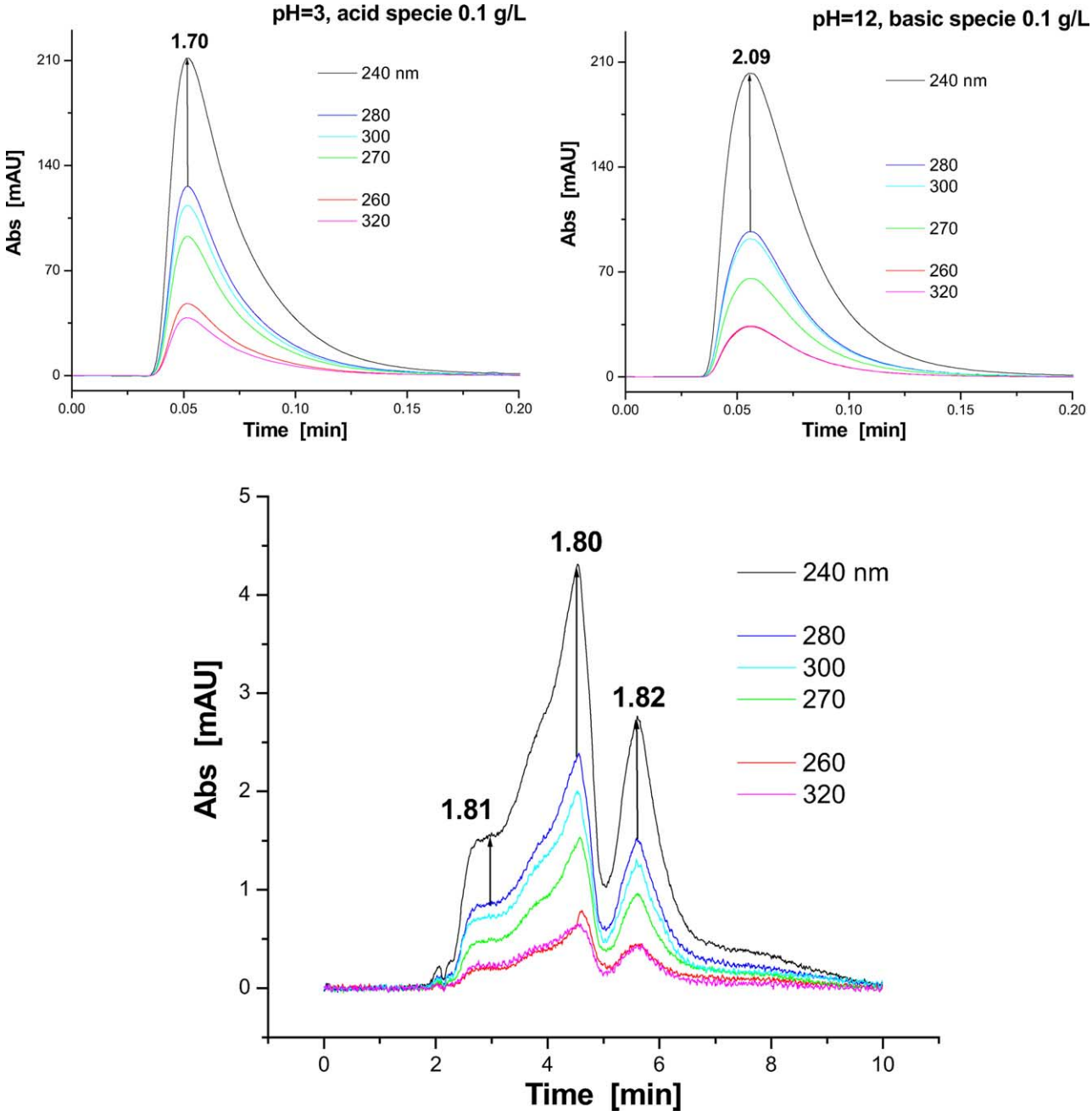

Fig. 4. Elution of propranolol under the same conditions as in Fig. 3A. (a) Signal of the UV detector at 240, 260, 270, 280, 300, and 320 nm for a pulse of 0.3 μg of propranolol in an HCl solution in water/methanol (60:40) at pH = 3.0; (b) same but the solution is at pH = 12 (NaOH solution); and (c) the chromatograms are under the same conditions as in Fig. 3A, but recorded for the different wavelengths.

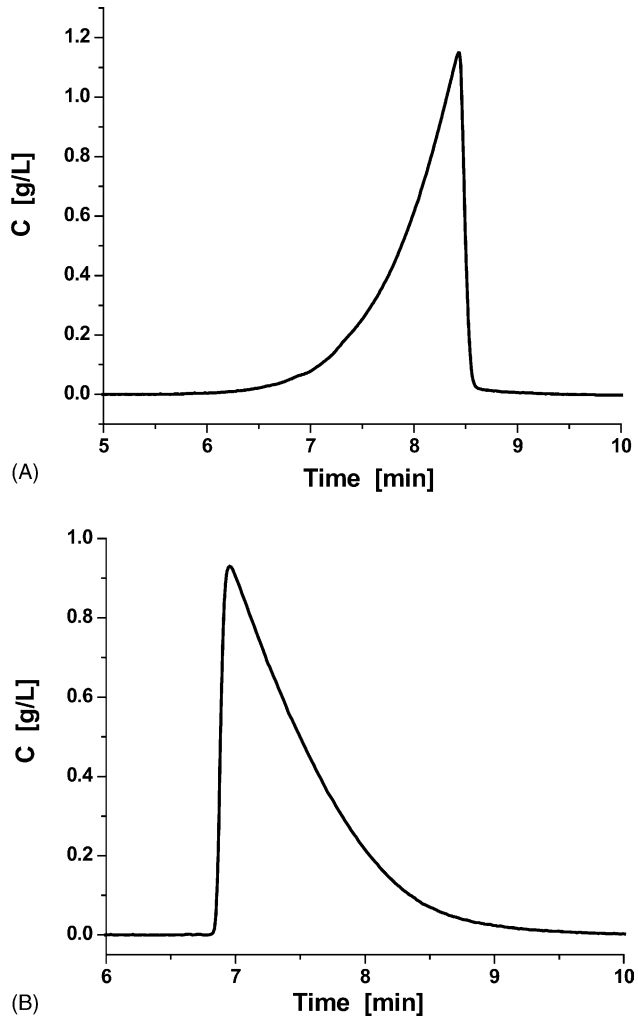

Fig. 5. Comparison between the overloaded band profiles recorded without (A) or with (B) acetate buffer in the mobile phase. Same experimental conditions as in Fig. 1. Injection of a 4 g/l solution during 12 s. Note the opposite shape of the band profiles.

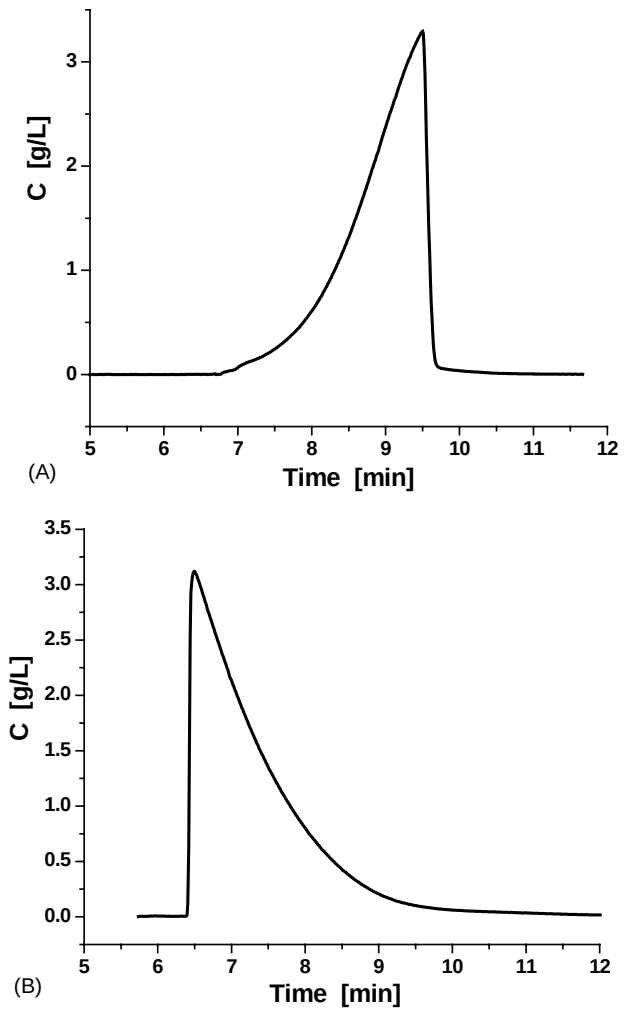

Fig. 6. Comparison between the overloaded band profiles recorded without (A) or with (B) acetate buffer in the mobile phase. Same experimental conditions as in Fig. 1. Injection of a 4 g/l solution during 52 s. Note, as in Fig. 4, the opposite shape of the band profiles.

a quasi-Gaussian peak is recorded when the solution pH is fixed at 5.9. When the sample size is increased, the shapes of the overloaded band profiles are strikingly different with the two solutions (see Figs. 3, 5–8). At moderate concentrations, a diffuse front and a rear shock were observed with the nonbuffered solution (A) while the inverse shape is observed with the buffered solution (B) (Figs. 3, 5 and 6). For the highest amount injected (Figs. 7 and 8), the front part of the band of propranolol begins to exhibit a front shock on the top of the fronting, suggesting a S-shape isotherm, which would be convex downward at low concentration, convex upward at high concentrations when the mobile phase is unbuffered. On the other hand, the elution band profiles of propranolol in a buffered mobile phase appear to be consistent with a convex upward isotherm in the whole concentration range.

The considerable difference between the shapes of these overloaded band profiles suggests that the mechanisms of adsorption of propranolol are different, depending on whether the mobile phase is buffered or not. In the next two sections, we present the result of FA measurements of isotherm data of propranolol in the two mobile phases. Note that, a priori, since the stationary phase is an adsorbent made of a layer of hydrocarbons on silica (trimethylsilyl endcapped surface of C_{18}-bonded silica), its adsorption properties should not depend on the pH or ionic strength of the solution because the accessible surface chemistry is completely apolar, except for possible adsorbate adsorbate interactions. This nonpolar nature of the adsorbent surface was confirmed by the results of previous measurements of the isotherm data of polar compounds like caffeine and phenol [37,38], which do not interact with any “active” or polar sites present on the Kromasil adsorbent.

The comparison between experimental band profiles and the profiles calculated from the FA isotherms using the equilibrium-dispersive model of chromatography will

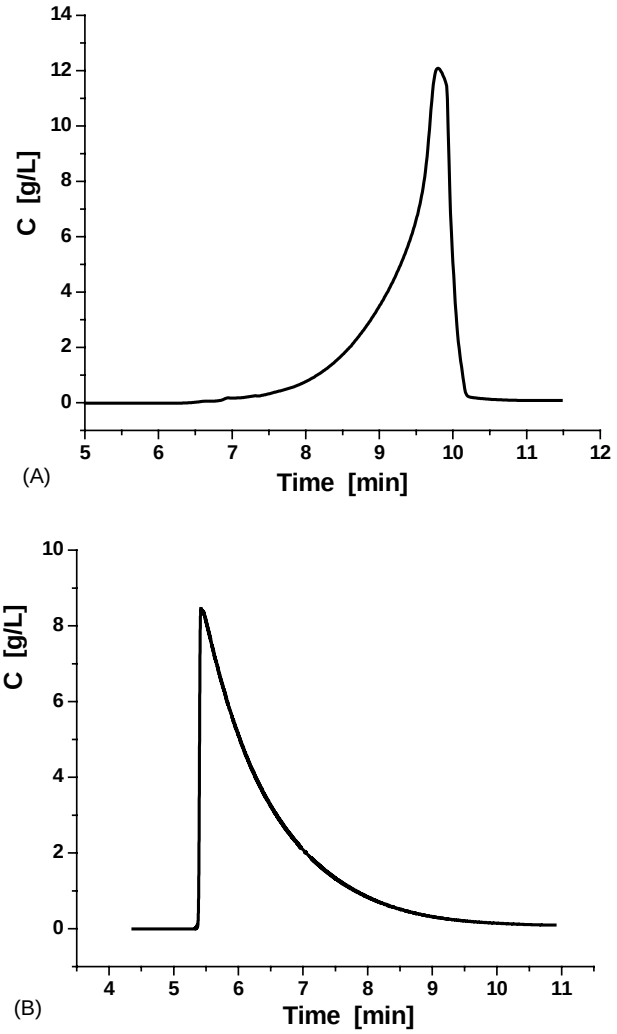

Fig. 7. Comparison between the overloaded band profiles recorded without (A) or with (B) acetate buffer in the mobile phase. Same experimental conditions as in Fig. 1. Injection of a 20 g/l solution during 30 s.

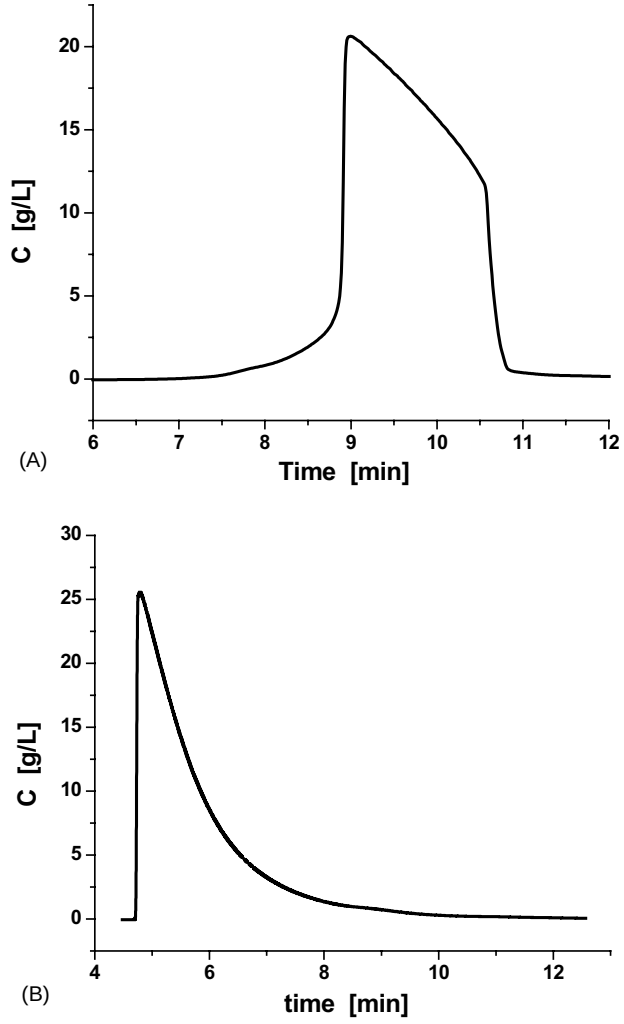

Fig. 8. Comparison between the overloaded band profiles recorded without (A) or with (B) acetate buffer in the mobile phase. Same experimental conditions as in Fig. 1. Injection of a 36 g/l solution during 52 s.

indicate whether a complex kinetics, e.g., improbably related to the acid base equilibrium in the solution or the adsorbed phase or, in otherwise fashion, to the complexity of the mobile phases used) may influence the band profiles. The equilibrium-dispersive model reflects the influence of the equilibrium thermodynamics on the band profiles when the column efficiency is sufficiently large.

4.2. Adsorption of propranolol from a non-buffered solution

4.2.1. Isotherm data measurement and interpretation

The adsorption isotherm data of propranolol was measured between 0.4 and 40 g/l, a dynamic range of 100. Since the front of the experimental breakthrough curves is not a shock layer [20] but a diffuse front, the equivalent area method [23] was used to determine the amount adsorbed on the stationary phase. Fig. 9A shows the experimental data and Fig. 9B confirms the S-shaped isotherm suggested by the shape of the high concentration overloaded profiles (Figs. 7 and 8).

The isotherm data in Fig. 9 were fitted by non-linear regression to a large number of models that may account for S-shaped isotherm data. Among them, the simple Moreau model (Eq. (6)) and the two-layer BET model (Eq. (8)) were found to account best for the data. We summarize here the main results obtained.

4.2.1.1. Moreau model. The Fisher coefficient is 18160 and the best values of q_s, b, and I are 179.8 g/l, 0.0152 l/g, and 5.685, respectively. According to Eq. (7), $\epsilon_{AA} = 1.74\,RT$. This significant intermolecular interaction explains the initial anti-Langmuirian behavior of the adsorption isotherm. At high concentrations, the isotherm shape becomes convex upward because of the finite saturation capacity.

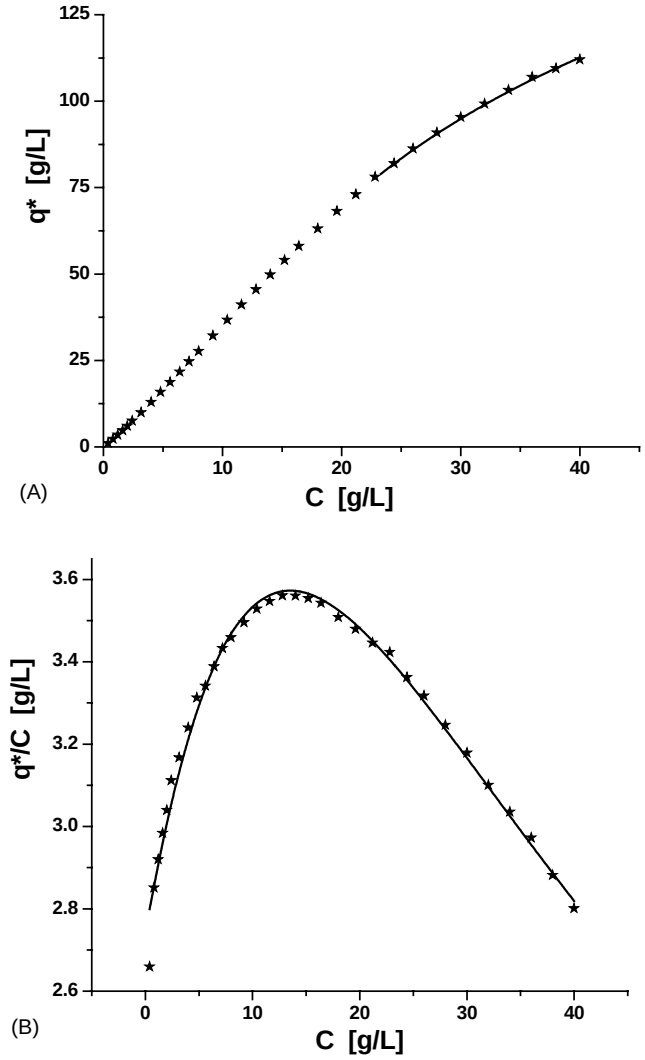

Fig. 9. (A) Experimental isotherm (stars) and best Moreau isotherm (solid line) of propranolol on the packed C_{18}-Kromasil column with methanol/water (40:60, v/v) as the mobile phase ($T = 23\,^{\circ}C$). (B) Experimental isotherm chord q^*/C (stars) and the corresponding Moreau chord (solid line). Note the maximum of the chord at $C \simeq 12$–13 g/l.

The total monolayer saturation capacity of propranolol in the presence of the buffer acetate is about 140 g/l (see later) versus 179.8 g/l with the unbuffered solution, a difference of 20%, significant but moderate. This monolayer saturation capacity is of the same order of magnitude as that of five other low-molecular-mass compounds on the same Kromasil column [7]. These saturation capacities range between 160 and 190 g/l (the values for phenol, ethylbenzene, caffeine, aniline, and theophylline are 166, 167, 171, 184 and 187 g/l, respectively). The saturation capacity found for propranolol without buffer in the mobile phase seems then to be in agreement with what was commonly observed. However, it is somewhat surprising to note the formation of the dimers postulated by the model, $[RNH_3–RNH_3]^{2+}$ being doubly charged. A relatively strong electrostatic repulsion between two positively charged molecules in the adsorbed state would rather lead to a Stahlberg isotherm [39] than to one accounting better for adsorbate adsorbate attraction. Admittedly, intermolecular interactions between the large hydrophobic naphthalenyloxypropyl radical of the propranolol molecule, together with a suitable relative orientation of the two molecules, may compensate for the electrostatic repulsion. This hydrophobic interaction increases with increasing water content of the mobile phase.

4.2.1.2. BET model. This model gives exactly the same Fisher coefficient as the Moreau model because Eqs. (4) and (6) are identical. The difference between the models is the physical meaning ascribed to the parameters. The Moreau model assumes lateral interactions between adsorbed molecules while the BET model assumes that the adsorbate forms successive layers. Hence, the monolayer saturation capacity is twice lower ($q_s = 89.9$ g/l) and the low-concentration equilibrium constant is twice larger ($b_S = 0.0304$ l/g) for the BET than for the Moreau model. The best value of the adsorption–desorption constant on layers made of molecule of propranolol is $b_L = 0.0431$. Accordingly the adsorption of propranolol is stronger on itself than on the adsorbent surface. These best numerical data have a poor physical. First, the monolayer saturation capacity is half the one commonly measured on the same adsorbent. Second, if propranolol adsorb more strongly on itself than on the adsorbent surface, there would be no reason to observe a Langmuirian behavior at high concentration but rather an anti-Langmuirian isotherm or convex downward shape. Third and consequence of the first remark, the adsorption constant of propranolol on the surface ($b_S = 0.0304$ l/g) is about twice those commonly observed for other compound for similar retention factors k ($2.3 \leq k \leq 2.7$). We should not expect large difference between the equilibrium constants of all the compounds since the mobile phase composition was adjusted to get comparable retention factors.

These experimental data can be summarized as follows:

- When adsorbed on C_{18}-bonded Kromasil from a non-buffered aqueous solution of methanol, propranolol forms a monolayer.
- Intermolecular interactions take place in this monolayer, despite the repulsive coulombian interactions. The energy involved in these intermolecular interactions is less than twice the thermal energy, exactly $1.75 \times RT$. Such a value is characteristic of weak interactions like dispersive forces. This suggests some π–π interactions between the naphthalenyl groups of the neighbor molecules. Note that such a behavior is somewhat similar to that of charged surfactant molecules, which have a small ionic head and a large hydrophobic tail. Although the heads repulse each other, the tails associate, forming micelles at sufficiently high concentrations.

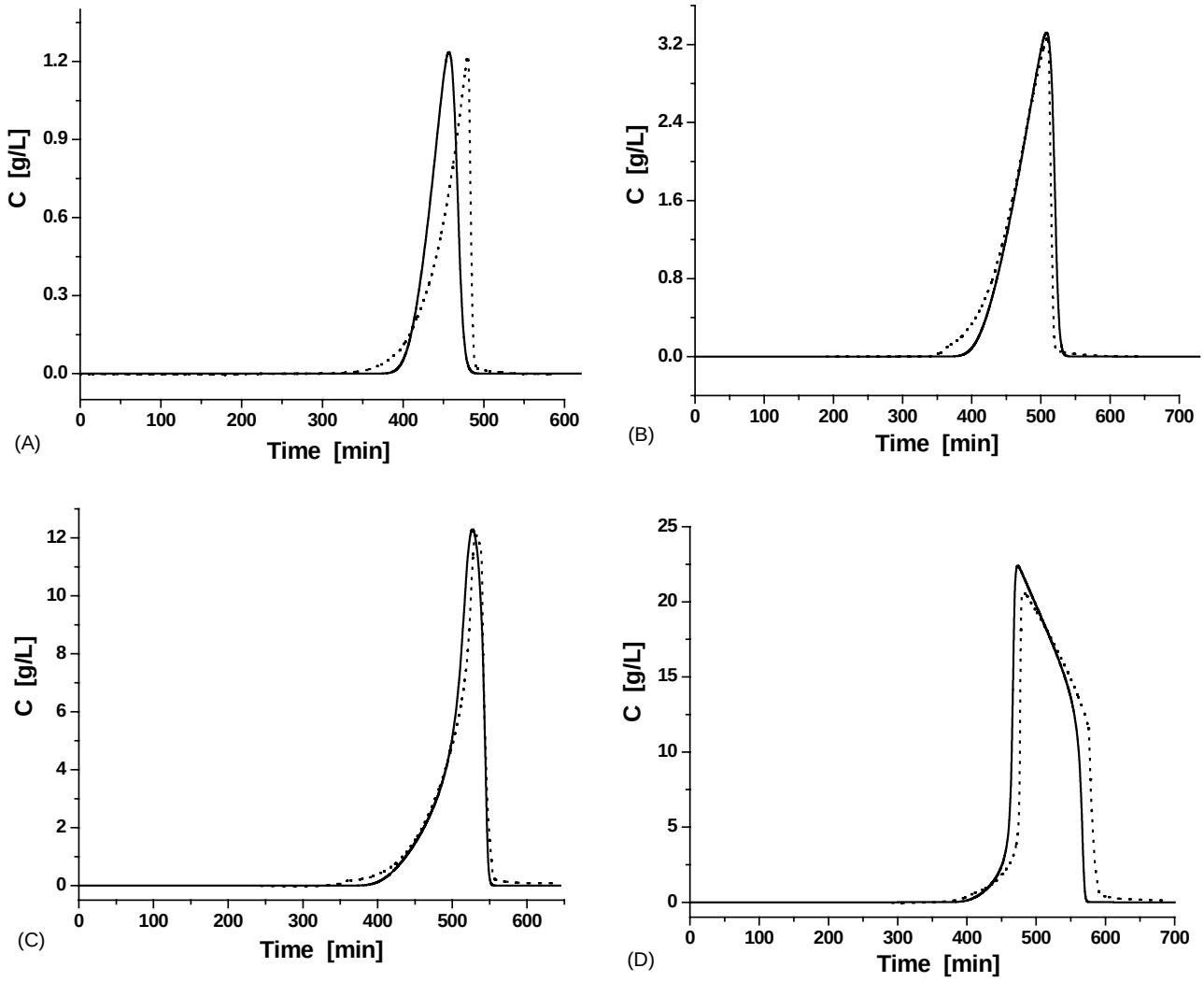

Fig. 10. Comparison between the experimental (dotted line) and calculated (solid line) overloaded profiles of propranolol without buffer in the mobile phase (methanol/water, 40:60, v/v). Four different column loadings: 0.8 mg (A), 1.6 mg (B), 10 mg (C), and 32.4 mg (D).

4.2.2. Thermodynamical consistence of the overloaded band profiles

In the previous section, we derived the best isotherm model accounting for the FA adsorption data of propranolol on the Kromasil column. Fig. 10 compares the experimental overloaded band profiles in Figs. 5–8 and those calculated using the ED model of chromatography. There is an excellent agreement between the two sets of band profiles at high concentrations (Fig. 10B–D), although, at very high concentrations (Fig. 10D), there is a slight difference in the position of the shocks of the front and rear parts of the band (approximately 12 s). However, the agreement is only fair at low concentration. The experimental band is less symmetrical than the calculated one.

The band profiles calculated with the ED model at high concentrations agree well with the experimental ones, validating the isotherm model derived from the FA data and suggesting that the mass transfer kinetics in the column are fast and sufficiently well accounted for by a simple axial dispersion coefficient.

4.3. Adsorption of propranolol from a buffered solution

4.3.1. Isotherm data measurement and interpretation

Fig. 11A shows the adsorption data of propranolol in the presence of an acetate buffer (0.2 M) in a methanol/water mobile phase. The FA data were acquired in the same concentration range as those in Fig. 9, from 0.4 g/l (1.54×10^{-3} mol/l) to 40 g/l (1.54×10^{-1} mol/l). The only difference is the higher methanol concentration of the mobile phase. The isotherm is strictly convex upward, with no inflection point. The best isotherm model accounting for these data is the bi-Langmuir model. This interpretation is supported by the results of the calculation of the adsorption affinity energy distribution (AED) that reflects the heterogeneity of the adsorbent surface energy (Fig. 11B). The method of

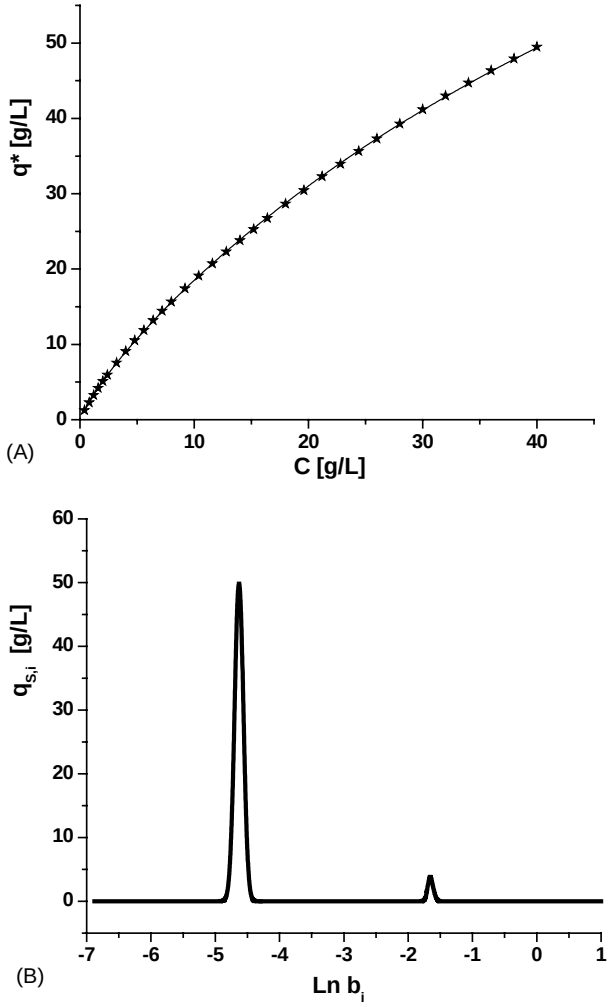

Fig. 11. (A) Experimental isotherm (stars) and best bi-Langmuir isotherm (solid line) of propranolol on the packed C_{18}-Kromasil column with methanol/water (60:40, v/v) as the mobile phase. Buffer acetate, 0.2 M, pH = 5.9 ($T = 23\,^{\circ}C$). (B) Affinity energy distribution (AED) calculated from the raw adsorption data by the expectation-maximization method. Local Langmuir isotherm. Note the bimodal distribution or the existence two types of sites on the adsorbent surface.

calculation of the AED was described elsewhere [7]. The AED of the C_{18}-Kromasil surface for propranolol in a buffered solution is bimodal. We showed earlier [38] that the first type of adsorption sites (abundant, low-energy, large saturation capacity sites) corresponds to the simple adsorption of the solute. The second type of sites seems to correspond to holes or cages penetrating inside the C_{18}-bonded layer and accessible to the solute. These sites (scarce, high-energy, low saturation capacity sites) correspond to a partition mechanism since the solute is partially dissolved inside the thick C_{18}-bonded layer. The best parameters obtained for the parameters $q_{s,1}$, b_1, $q_{s,2}$ and b_2 are 134.0 g/l, 0.0129 l/g, 4.1 g/l and 0.3778 l/g, respectively. This isotherm model is validated by the agreement observed (see Fig. 12) between the experimental overloaded band profiles and those calculated with the equilibrium-dispersive model of chromatography.

The total saturation capacity determined is lower than that measured with the unbuffered solution, 138 instead of ca. 180 g/l. The most probable cause for this difference is the change in the methanol content of the mobile phase. It has already been shown that the total porosity of this Kromasil column decreases with increasing methanol concentration (0.606 and 0.577 with methanol volume fractions of 0.4 and 0.6, respectively) [40]. The C_{18} chains unfold, and, accordingly, the adsorbent specific surface area decreases. The total saturation capacity of this column for phenol decreases from 180 to 140 g/l, a relative decrease of 22%, the same as that observed for propranolol in this work. This result suggests that the decrease observed for the saturation capacity is probably not caused by the higher ionic strength nor by the change in pH. Finally, it is not surprising that the main adsorption constant, b_1 (= 0.0129 l/g) be comparable to the adsorption constant measured for a mobile phase without buffer ($b = 0.0152$ l/g) since the mobile phase concentration in methanol was adjusted to obtain similar retention factors in both situations.

4.4. Surface heterogeneity of the adsorbent

The results presented above seem to be inconsistent. In the former case, the adsorption data of propranolol from an unbuffered solution were accounted for by using a Moreau isotherm model, assuming a homogeneous adsorbent with a single adsorption energy, i.e., a single adsorption constant, b. In the second case, similar data but from a buffered solution were accounted for with a bi-Langmuir isotherm that assumes two types of adsorption sites, hence two adsorption constants (b_1 and b_2) and a heterogeneous surface with two types of patches having different adsorption energies.

A simple approach to reconcile these results would be to extend the homogeneous Moreau model to heterogeneous adsorption onto a surface with two types of patches. So, we consider now the following extension of the Moreau model, a 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:

$$q^* = q_{s,1}\frac{b_1 C + I_1 b_1^2 C^2}{1 + 2b_1 C + I_1 b_1^2 C^2} + q_{s,2}\frac{b_2 C + I_2 b_2^2 C^2}{1 + 2b_2 C + I_2 b_2^2 C^2} \quad (9)$$

The non-linear regression of the adsorption data measured with the unbuffered solution, despite a large number of parameters Eq. (6), still converges toward a new set of six independent parameters. The Fisher value increases from 18160 to 27370. Considering a risk α of 5%, the bi-Moreau model would be statistically better than the Moreau model if the ratio of their respective Fisher coefficients were larger than 1.86. The actual ratio is equal to 1.5, making the comparison inconclusive. The two models are statistically equivalent to

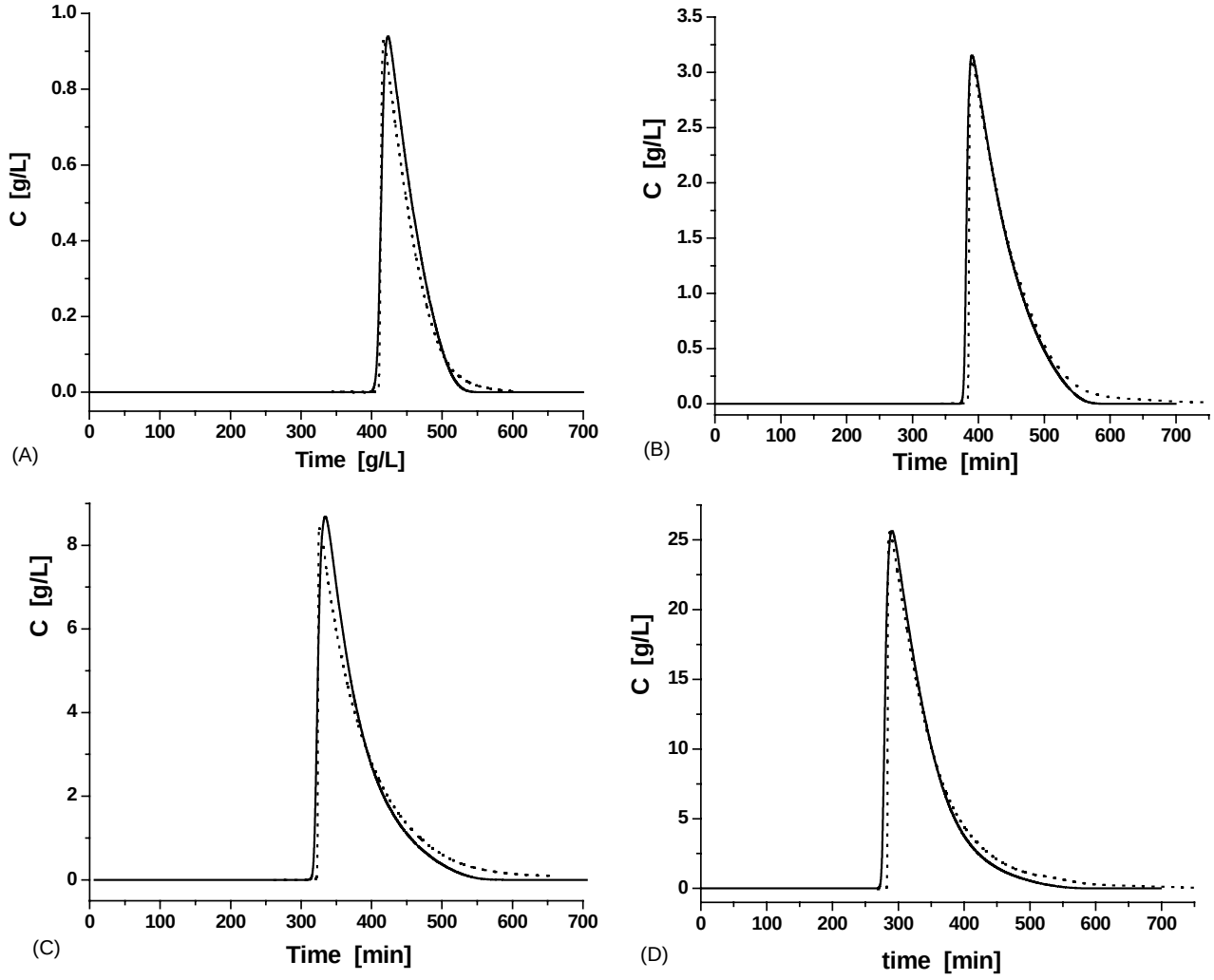

Fig. 12. Comparison between the experimental (dotted line) and calculated (solid line) overloaded profiles of propranolol with acetate buffer (0.2 M, pH = 5.9) in the mobile phase (methanol/water, 40:60, v/v). Same column loadings as in Fig. 9 : 0.8 g (A), 1.6 g (B), 10 g (C) and 32.4 g (D).

describe the experimental data. Table 2 lists and compares the values of the parameters found in each case. As for its adsorption from a buffered solution, the adsorption of propranolol from an unbuffered solution can be described on the basis of an heterogeneous adsorbent. Physically, the numerical values obtained for the parameters of the bi-Moreau model make sense. The total saturation capacity is close to 180 g/l, the value obtained with the simple Moreau model. This capacity is the sum of two terms, one for numerous, low-energy sites ($q_{s,1}$ = 173.9 g/l, b_1 = 0.0135 l/g) and one for the fewer high-energy sites ($q_{s,2}$ = 1.9 g/l, b_2 = 0.0852 l/g). Note that the intermolecular interactions (I_1 and I_2) are higher when the two propranolol molecules are adsorbed on the high-energy sites than if they are adsorbed on the low-energy sites (the differences are three and two times RT, respectively).

Table 2
Comparison of the best fitting parameters accounting for by the adsorption data of propranolol without buffer by using the Moreau and the bi-Moreau isotherm model

Isotherm parameters	Moreau (sites 1)	Bi-Moreau	
		Sites 1	Sites 2
q_s (g/l)	179.8	173.9	1.9
b (l/g)	0.0152	0.0135	0.0852
ϵ/RT	1.75	2.01	3.14

A comparison between the overloaded band profiles calculated with the Moreau (Fig. 10A and B) and the bi-Moreau isotherm models (Fig. 13) and the experimental band profiles recorded at low column loadings (injection of a 4 g/l solution during 12 s) shows an obvious improvement of the degree of agreement. Obviously, since the higher energy sites are occupied first, no significant difference is observed between the profiles calculated with the two models at high column loadings (not shown). These results suggest that the existence on the adsorbent surface of a second type of sites, with a higher energy, must be taken into account and that the bi-Moreau model should be preferred to the simple Moreau model.

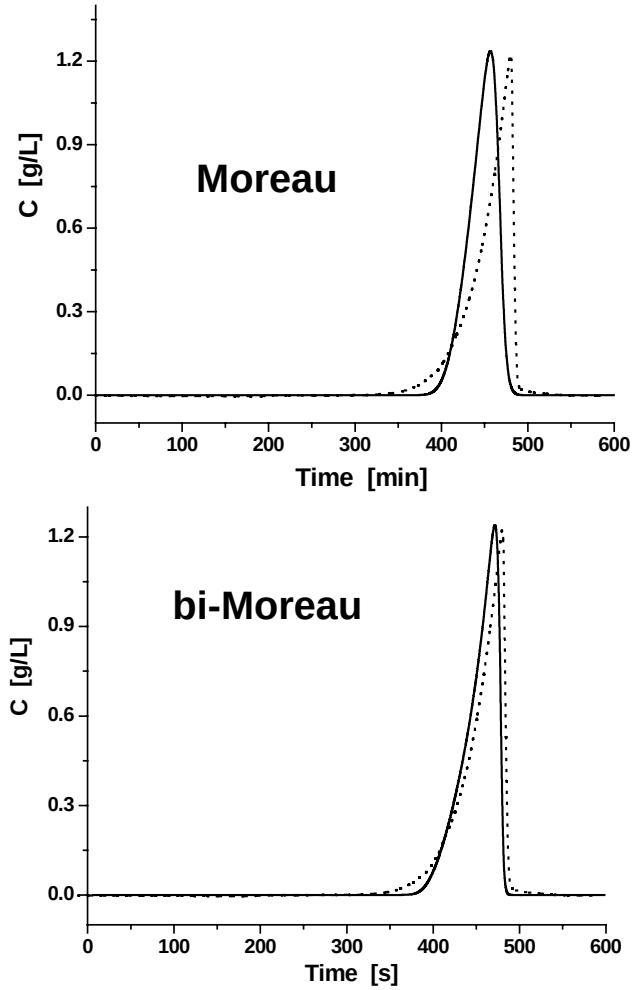

Fig. 13. Comparison between the experimental (dotted line) and calculated (solid line) overloaded profiles of propranolol at low column loading (0.8 g injected) without buffer in the mobile phase (methanol/water, 40:60, v/v). Moreau and bi-Moreau isotherm models. Note the agreement's improvement by using the bi-Mopreau model in the calculation.

5. Conclusion

The surface of the Kromasil column is heterogeneous whether the mobile phase is buffered or not. The propranolol cation may adsorb on either of two types of sites. Our experimental results demonstrate that:

- The presence of a buffer in the mobile phase does not cause nor reduce the degree of heterogeneity of the C_{18}-bonded layer.
- The presence of a buffer in the mobile phase does not influence significantly the saturation capacity of the C_{18}-Kromasil adsorbent. This result does not agree with other, recent results, admittedly acquired under quite different experimental conditions [15,16].
- The presence of a buffer causes an apparent increase of the solute hydrophobicity, a well known effect of salts. To keep nearly constant the retention factor at infinite dilution, the addition of a 0.2 M acetate buffer must be compensated by an increase of the methanol concentration from 40 to 60% (v/v). It is possible that the propranolol cation associates with the acetate anion in the adsorbed phase. Ion-association was mentioned in the literature [41] for propranolol in an aqueous solution with ammonium reineckate, $NH_4[Cr(NH_3)_2(SCN)_4]$, and with sodium cobaltinitrile, $Na_3[Co(NO_2)_6]$.
- The absence of buffer leads to intermolecular interactions between propranolol molecules in the adsorbed phase. These interactions involve an energy of twice to three times *RT*. These interactions vanish when the buffer is added.

Repulsion between ionized compounds at low buffer concentrations and, to the limit, with no buffer in the mobile phase, was recently suggested [15] as a reasonable explanation for the overloading effect observed in this earlier study. In our work, this molecular repulsion has to be counterbalanced by some attractive van der Waals interactions between the hydrophobic parts of the analyte molecules. Only adsorbate–adsorbate interactions can explain the observed anti-Langmuirian behavior of the isotherm and of the overloaded band profiles. Admittedly, it is difficult to endeavor a detailed investigation of molecular interactions when using the mere tools of linear chromatography.

Acknowledgements

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