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Systematic errors in the measurement of adsorption isotherms by frontal analysis Impact of the choice of column hold-up volume, range and density of the data points

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Abstract

Besides the accuracy and the precision of the measurements of the data points, several important parameters affect the accuracy of the adsorption isotherms that are derived from the data acquired by frontal analysis (FA). The influence of these parameters is discussed. First, the effects of the width of the concentration range within which the adsorption data are measured and of the distribution of the data points in this range are investigated. Systematic elimination of parts of the data points before the calculation of the nonlinear regression of the data to the model illustrates the importance of the numbers of data points (1) within the linear range and (2) at high concentrations. The influence of the inaccuracy of the estimate of the column hold-up volume on each adsorption data point, on the selection of the isotherm model, and on the best estimates of the adsorption isotherm parameters is also stressed. Depending on the method used to measure it, the hold-up time can vary by more than 10%. The high concentration part of the adsorption isotherm is particularly sensitive to errors made on $t_{0,exp}$ and as a result, when the isotherm follows bi-Langmuir isotherm behavior, the equilibrium constant of the low-energy sites may change by a factor 2. This study shows that the agreement between calculated and experimental overloaded band profiles is a necessary condition to validate the choice of an adsorption model and the calculation of its numerical parameters but that this condition is not sufficient.

Keywords: Adsorption isotherm; Frontal analysis; Systematic errors; Column hold-up volume; Data acquisition; Isotherm modeling; Overloaded band profiles

1. Introduction

Many reviews have discussed the retention mechanisms that take place in reversed-phase liquid chromatography (RPLC) [1–3]. Most of these studies are based on the use of linear chromatography data, e.g., on the measurement of the retention times of impulses and the determination of the retention factors of series of analytes. The study of the influence on these retention factors of different parameters, e.g., the nature and concentration of the organic modifier, the stationary phase chemistry (e.g., monomeric or polymeric bonding, endcapping), and the temperature, has brought conclusions that are now classical. For instance, it is widely accepted that the retention factors of analytes follow the Van't Hoff Law and the Linear Solvation Strength Model (LSSM) [4] with respect to the influence of the temperature and the concentration of the organic modifier in an aqueous mobile phase, respectively. An abundant literature is devoted to the study of the influence of the temperature on the retention behavior of compounds and to the derivation and interpretation of such thermodynamic properties as the changes in enthalpy (ΔH) and entropy (ΔS) associated to the transfer of the analyte from the mobile to the stationary phases. The type of bonding used (whether its process involves the use of a mono- or a trichlorooctadecylsilane as the reagent) is important since there are obvious differences in the selectivity of monomeric and polymeric phases.

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Unfortunately, the accumulation of measurements of retention data has not shed much light on the interactions involved between the molecules of analytes and the stationary phase in RPLC. It is generally assumed that the stationary phase is a homogeneous and flat surface. Although we know that the interface is thick at the molecular level, most models consider it as devoid of structure and complexity. Yet, we know that no actual surface is homogeneous and that the complexity of their preparation process makes the surface of RPLC materials prone to be highly heterogeneous [5]. A new approach based on the acquisition of retention data in a wide range of concentrations, from very low (the Henry domain) to very high (so as to achieve solid phase concentrations as close as possible to the saturation capacity) allows the identification of several types of adsorption sites. The simultaneous presence of these different types illustrates clearly the heterogeneity of the surface of alkyl-bonded silica. This conclusion applies to all brands of packing materials used in chromatography [6]. Further investigations have shown that the low-energy adsorption sites are located at the interface between the alkyl-bonded layer and the mobile phase while the high-energy sites are inside the hydrophobic layer. The difference in adsorption energy between these two types of adsorption sites is of the same order of magnitude as the energy involved in weak dispersive interactions, markedly less than 10 kJ/mol. However, "supersites" were also found, with adsorption energies more than 20 kJ/mol higher than that on the low-energy sites [3,7]. Their density on the surface is very low.

All these critical conclusions were based on the measurement of accurate adsorption isotherm data by the frontal analysis (FA) method. The adsorption isotherm is built step by step, concentration after concentration, and results from measurements performed on long series of injections of breakthrough curves. Obviously, the larger the concentration range investigated and the larger the number of data points acquired, the more accurate the determination of the best final isotherm model and of its isotherm parameters. The eventual limitations on precision and accuracy come essentially from the accuracy and precision of the instrumentation itself (flow rate, flow mixer, thermostat) and from the overall reproducibility of the different unit functions of the apparatus. Experimentally, the limits arise also from the amount of chemicals available (preparation, price) and the needs to perform the whole series of data acquisition within a reasonable lapse of time. To a lesser extent, the precision of the measurements of adsorption isotherms depend also on the determination of the extra-column volume and, most importantly, on that of the hold-up volume. In a recent publication, Sajonz [8] studied the influence of the hold-up time on the accuracy of the adsorption isotherm and, ultimately, on that of the predictive calculations of chromatographic band profiles which is directly related. He showed how a wrong estimate of the hold-up volume could affect the adsorption isotherm parameter and lead to an inconsistent trend for the amount adsorbed at high concentrations. Sajonz showed how bad could be the consequences of an error on the hold-up volume in preparative chromatography where nonlinear isotherms are often encountered. However, his work was based only on calculations simulating the measurements, not on actual experimental results. He assumed a true column void volume, a true Langmuir isotherm model, and calculated the breakthrough curves using the ideal model (i.e., assuming that the column efficiency is infinite) [9]. These curves were used to derive isotherm data points using erroneous values of the hold-up volume [8]. The isotherm obtained by this process was compared to the true isotherm.

The aim of this work is to assess the actual error made in the realistic situation when the true parameters of the chromatographic system (the true column void volume, the true extra-column volume, and the true adsorption isotherm) are unknown. The breakthrough curves are not calculated but measured and the isotherm model is derived from these experimental curves. More particularly, the effect of the number of data points acquired on the determination of the best adsorption isotherm was studied. The distribution of these points in the concentration range is another important factor. How many points are needed in the low- and in the high-concentration ranges? When are more data needed in the intermediate concentration range? Incorrectly designed or planned experiments are frequent and the limitations of the frontal analysis method are often misunderstood. It is useful to be aware of the error that can be made if the data points are acquired in a narrow concentration range or with too few data in the low concentration range. Also critical are the influence on the adsorption isotherm parameters of the accuracy of the hold-up volume and the influence of this parameter on the results of isotherm modeling. The adsorption of phenol on a highly efficient column will be used as a case in point because the large solubility of phenol in methanol/water solutions allows measurements of isotherm data points at concentrations close to column saturation.

2. Theory

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

The adsorption data of phenol that are used in this work to illustrate our approach were acquired by the frontal analysis method [1]. The details and systematic steps followed with this method are described elsewhere [3]. The amount adsorbed on the stationary phase is simply derived from the integral mass conservation, which can be expressed by unit volume of the adsorbent in contact with the liquid phase

$$q^*_{\mathrm{vol}} = \frac{F_{\mathrm{v}}(t_{\mathrm{shock}} - t_{\mathrm{ext}} - t_0)C}{\pi r^2_{\mathrm{in}} L - F_{\mathrm{v}} t_0} \quad (1)$$

where F_{v} is the mobile phase flow rate, t_{shock} the elution time of the front shock of the breakthrough curve, t_{ext} the extra-column volume (measured from the elution time of the inflection point of the same breakthrough curve injected with no column), t_0 the hold-up time, r_{in} the internal radius of the column tube and L the length of the column.

Eq. (1) describe the excess amount of solute adsorbed in the column if $V_0 = F_{\mathrm{v}} t_0$ is assumed to be the total free volume accessible to the analyte. They represent the total amount adsorbed

if V_0 represents the volume of the non-adsorbed bulk mobile phase in the column. In practice, t_0 is measured with a chemical marker that may be slightly adsorbed on the packing material and/or partially excluded from very narrow spaces present near the surface of the adsorbent. In addition, the compressibility of the mobile phase [10] tends to generate a higher value for t_0 than the one expected if the solvent were not compressible. For all these reasons, the final adsorption data calculated with Eq. (1) cannot be referred as the true "excess adsorption data" or "total adsorption data". Corrections are needed. Without them a systematic error is made.

All the adsorption data are calculated here according to Eq. (1), and expressed in amount adsorbed per unit volume of the solid adsorbent.

2.2. Model of isotherm

The adsorption of phenol on the Gemini-C_{18} column was best described by a bi-Langmuir isotherm model. Similar conclusions were found on many other brands of C_{18}-bonded columns [6] (e.g., Kromasil, Luna, Hypersil, Symmetry). This isotherm model is the simplest model that accounts for the adsorption of chemicals on heterogeneous surfaces. In the present case, the adsorbent surface is paved with two types of adsorption sites, type 1 and type 2. According to precedent results [3,6], sites of type 1 correspond to the adsorption of the compound studied at the interface between the top of the C_{18}-bonded layer and the bulk mobile phase while sites of type 2 are adsorption sites located deeper in the hydrophobic alkyl layer. The difference between the adsorption energies on the sites of types 2 and 1 is usually of the order of 5 kJ/mol.

The bi-Langmuir model is:

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

where $q_{s,1}$, $q_{s,2}$, b_1 and b_2 are the monolayer saturation capacities and the equilibrium constants for sites of types 1 and 2, respectively.

The equilibrium constants b_1 and b_2 are associated with the adsorption energies $\epsilon_{a,1}$ and $\epsilon_{a,2}$, through the following equation [11]:

$$b_i = b_0\, e^{(\epsilon_{a,i}/RT)} \quad (3)$$

where $\epsilon_{a,i}$ is the energy of adsorption on sites of type i, R is the universal gas constant, T is the absolute temperature and b_0 is a pre-exponential factor that could be derived from the molecular partition functions in both the bulk and the adsorbed phases. b_0 is often considered to be independent of the adsorption energies [11], $\epsilon_{a,i}$, so that it is possible to assess the energy difference between sites of types 2 and 1.

2.3. Calculation of the adsorption energy distribution

The calculation of the AED uses the expectation-maximization method (EM) developed by Stanley et al. [12]. The advantage of this method is that it does not assume a priori any energy distribution. It converged toward the most likely energy distribution according to the local isotherm chosen in the calculation. A more detailed description and application of this method to the raw adsorption data is given in reference [3].

2.4. Modeling of band profiles in HPLC

The calculation of breakthrough curves was carried out using the equilibrium-dispersive model of chromatography [1,13,14]. This model assumes instantaneous equilibrium between the mobile and the stationary phases and a finite column efficiency originating from an apparent axial dispersion coefficient, D_a, that accounts for the dispersive phenomena (molecular and eddy diffusion) and for the non-equilibrium effects that take place in a chromatographic column. In the present case, the solid–liquid equilibrium is governed by a bi-Langmuir model, as described above. The column efficiency was fixed at 3000 and 1500 plates for the calculation of the elution profiles of the low- and the high-concentration breakthrough curves.

The axial dispersion coefficient is related to the column efficiency by:

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

where u is the mobile phase linear velocity and L the column length.

2.4.1. Initial and boundary conditions for the ED model

At $t = 0$, the concentrations of the solute and the adsorbate in the column are uniformly equal to zero (except in staircase-mode 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 [1,15] at the inlet and outlet of the column.

2.4.2. Numerical solutions of the ED model

The ED model was solved using the Rouchon program based on the finite difference method [1,16–18].

3. Experimental

3.1. Chemicals

The mobile phase used in this work was a mixture of methanol and water at 30% methanol (v/v). Both water and methanol were of HPLC grade, purchased from Fisher Scientific (Fair Lawn, NJ, USA). 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. Phenol was the only solute used. Thiourea and phenol were obtained from Aldrich (Milwaukee, WI, USA).

3.2. Columns

The column used in this study (Gemini-C_{18}) was a gift from the manufacturer (Phenomenex, Torrance, CA, USA). The tube dimensions are 150 mm × 4.6 mm. The volume of the steel tube

Table 1
Physico-chemical properties of the column used (provided by the manufacturer)

	C_{18}-Gemini
Column dimension (mm × mm)	150 × 4.6
Particle size (μm)	5
Mesopore size (Å)	110
Specific surface (m^2/g)	375
Bonding process	Monomeric
Carbon content (%)	14
Surface coverage (μ mol/m^2)	n.a.
Endcapping	Yes

is 2.4929 mL. The main characteristics of the packing material are summarized in Table 1. The column hold-up volume was derived from the elution volumes of three consecutive thiourea injections (1.7395 mL). The column porosity was then 0.6978.

3.3. Apparatus

The breakthrough curves and the corresponding retention times of the front shocks necessary to calculate the adsorption data were acquired using a Hewlett-Packard (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–vis detector, a column thermostat and a data station. The extra-column volumes are 0.035 and 0.845 mL, as measured from the auto-sampler and from the pump system, respectively, to the detector cell. All the retention data were corrected for these contributions. The flow-rate accuracy was controlled by pumping the pure mobile phase at 22 °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 0.1%, so we estimate the long-term accuracy of the flow-rate at 1 μL/min at flow rates around 1 mL/min. The temperature was controlled by the thermostat at ±0.1 K.

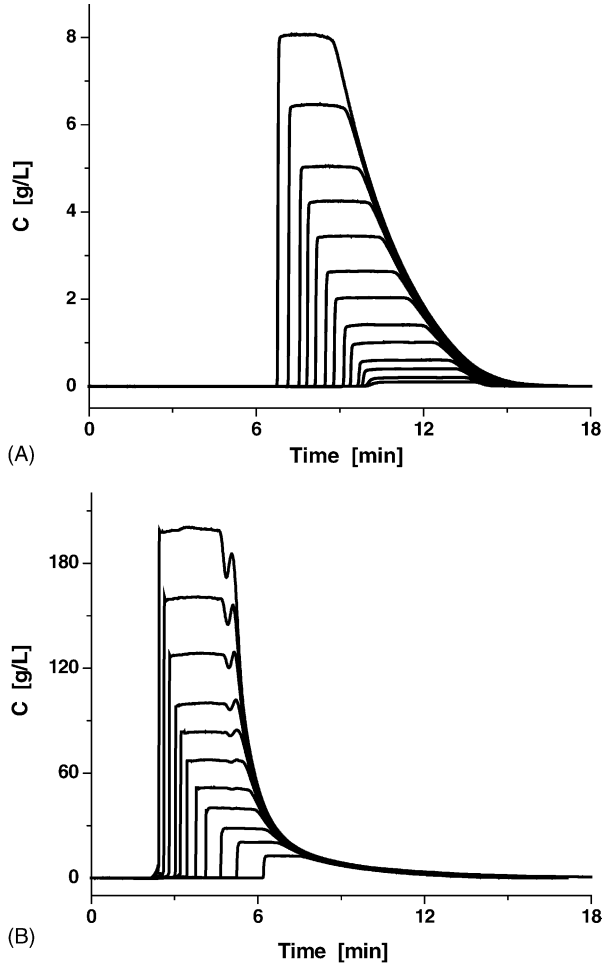

Fig. 1. Experimental breakthrough curves recorded for two different ranges of concentration of phenol: (A) 0.1–8.0 g/L and (B) 8–200 g/L. C_{18}-Gemini column; methanol/water (30/70, v/v) as the mobile phase; flow rate = 1 mL/min; T = 295 K.

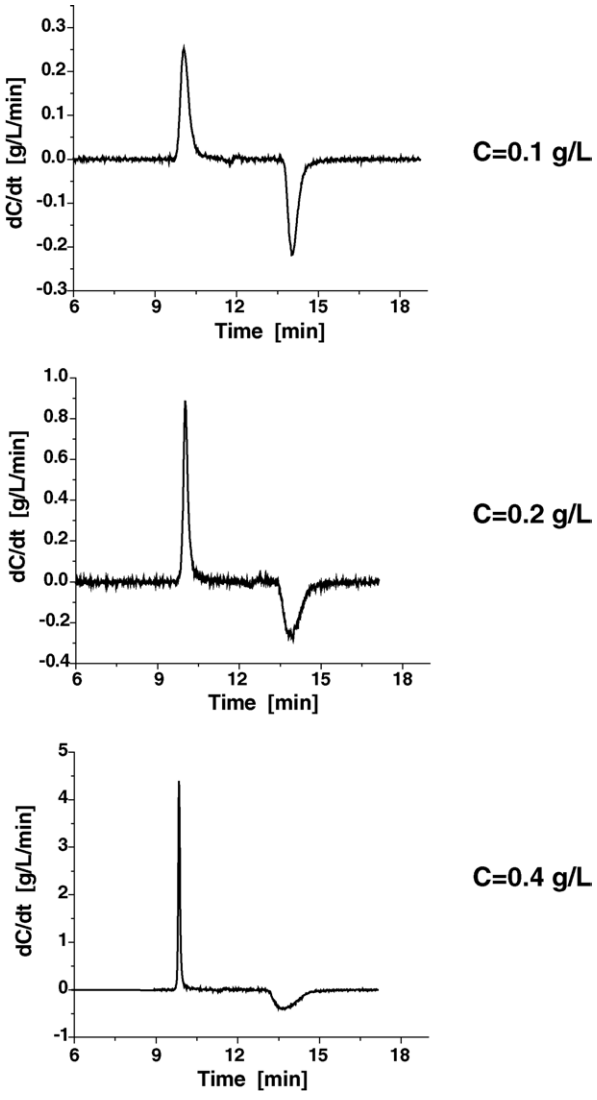

Fig. 2. Derivatives of the breakthrough curves for plateau concentrations of 0.4, 0.2, and 0.1 g/L. Note the evolution of the symmetry of the derivative of the breakthrough curve, revealing the linear range of the adsorption isotherm for concentrations inferior to 0.1 g/L.

3.4. Measurements of the breakthrough curves of phenol

The measurements of the breakthrough curves of phenol were made using the multi-solvent delivery system (maximum volume 100 μL). Two series of FA run were carried out, one covering the high-concentration range (8–200 g/L), the other the low-concentration range (0.1–10 g/L). Accordingly, two mother solutions of phenol at 10 g/L (pump C) and 200 g/L (pump B) were prepared in the methanol/water mobile phase (pump A). The stream to the column is a mixture of the streams of pumps A and B or C. The total flow rate remains constant. The ratio of the pump flow rates determined the concentration of the stream to the column. A 4 mL plug of mixture is sent to the column, after what a stream of pure mobile phase is resumed. Each breakthrough curve was recorded until the elution of the pure mobile phase was resumed. An estimate of the maximum volume of mobile phase required to elute entirely the injected plug is given by the sum of the analytical retention volume of phenol (about 10 mL), the volume of the plug (4 mL), the extra-column volume (≃1 mL). For safety sake, 20 min were chosen as the maximum elution time for each breakthrough curves at a flow rate of 1 mL/min. Two series of flow rate fractions, {1, 2, 4, 6, 10, 14, 20, 26, 34, 42, 50, 64, 80, and 100%} and {4, 6, 10, 14, 20, 26, 34, 42, 50, 64, 80, and 100%} were successively applied to pumps C and B, respectively, the complementary flow rate being delivered by pump A. The two series of breakthrough curves are shown in Fig. 1. The lowest concentration injected was chosen so that a symmetrical breakthrough curve was observed (qualitative estimate) or that the derivative of the curve $C(t)$ relatively to the time, t, shows a positive and a negative peak of the same or nearly the same amplitude (quantitative estimate, see Fig. 2). Then, it was deemed unnecessary to inject lower concentration plugs because there is no influence of the concentration on breakthrough curves in the initial, linear part of the adsorption isotherm. More measurements would give redundant experimental data. Note that the breakthrough curves at the highest concentrations (between 60 and 200 g/L) have some anomalies, the unexpected apparition of an "extra peak". The only explanation that we found is a defectuous operation of the mixing system of the HPLC apparatus. During the injection of the concentration plug, a small volume of pure mobile phase (pump A) is injected, creating the vacancy observed on the breakthrough curves in Fig. 1B.

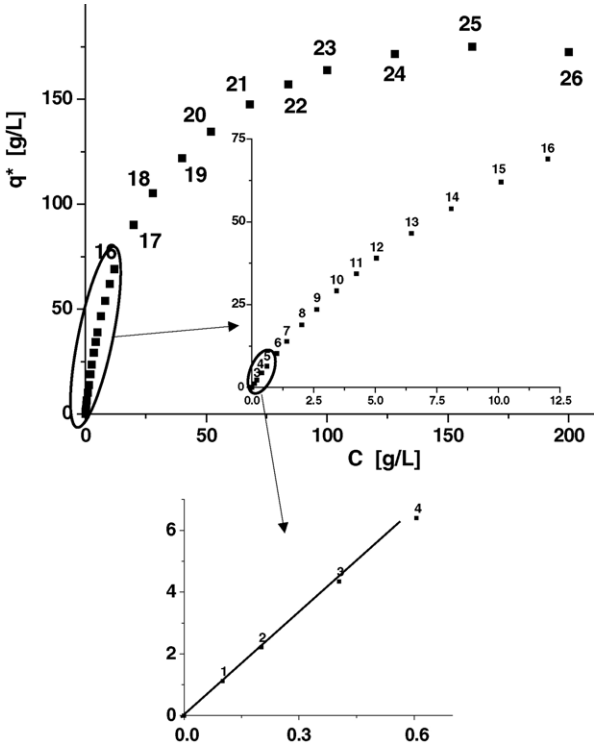

Fig. 3. The 26 adsorption data of phenol calculated from Eq. (1) assuming t_0 as the elution time of thiourea. The inserts zoom on the distribution of the point at low concentrations. Same experimental conditions as in Fig. 1.

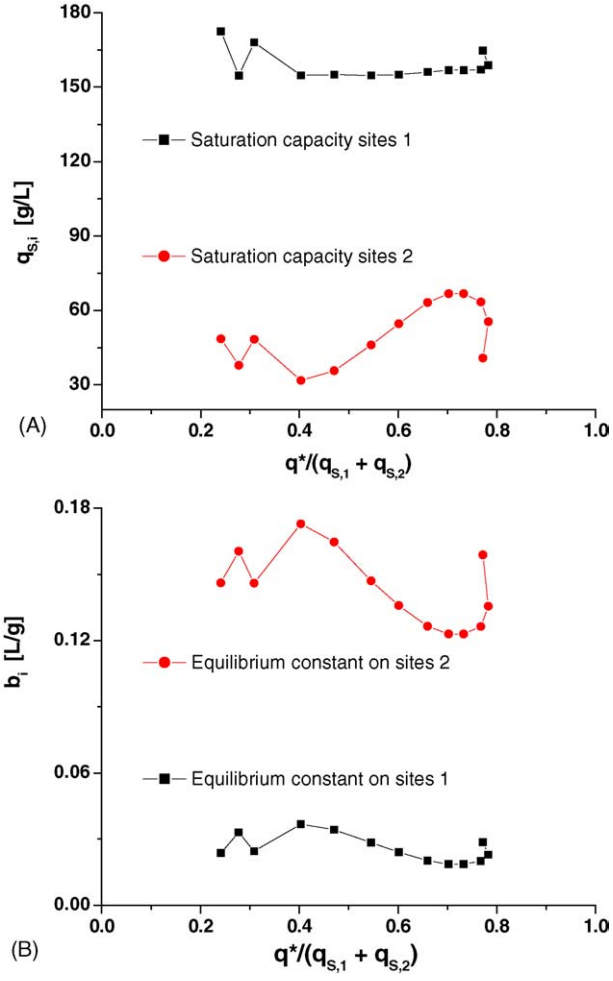

Fig. 4. Evolution of the best estimated bi-Langmuir parameters from the multi linear regression analysis of the adsorption data as a function of the surface coverage of the adsorbent corresponding to the highest concentration applied in FA: (A) saturation capacities and (B) associated equilibrium constants. Note the high degree of dependence of the parameters of both sites with the highest concentration used in the FA run.

4. Results and discussion

In this work, we deliberately choose to study the adsorption behavior of phenol on a RPLC C_{18}-bonded column (Gemini) with a solution of methanol and water (30/70, v/v) as the mobile phase for several reasons. First, the solubility of phenol in water is very high (>200 g/L), which allows measurements up to solid-phase concentrations that approach closely the saturation capacity of the column. Thus, the adsorption isotherm can be measured over a very large range of surface coverage and that makes more precise the determination of the best isotherm model. Furthermore, methanol/water-C_{18}-bonded phase systems tend to generate a strictly convex upward isotherm behavior that can be modeled by a multi-Langmuir isotherm model [19]. Accordingly, it is possible to interpret the adsorption data as the sum of several distinct local Langmuir isotherms and to calculate the AED distribution based on the EM method.

4.1. Adsorption isotherm parameters and number of points acquired by FA

The way in which adsorption isotherm data are usually measured may affect drastically our conclusions, e.g., the nature of the selected adsorption isotherm model and the best values of the isotherm parameters. A usual mistake made in FA experiments consists in an inadequate planning of the series of injected breakthrough curves. The concentration distribution of the data points does not always cover properly the most important domains of the adsorption isotherm. For instance, an insufficient number of

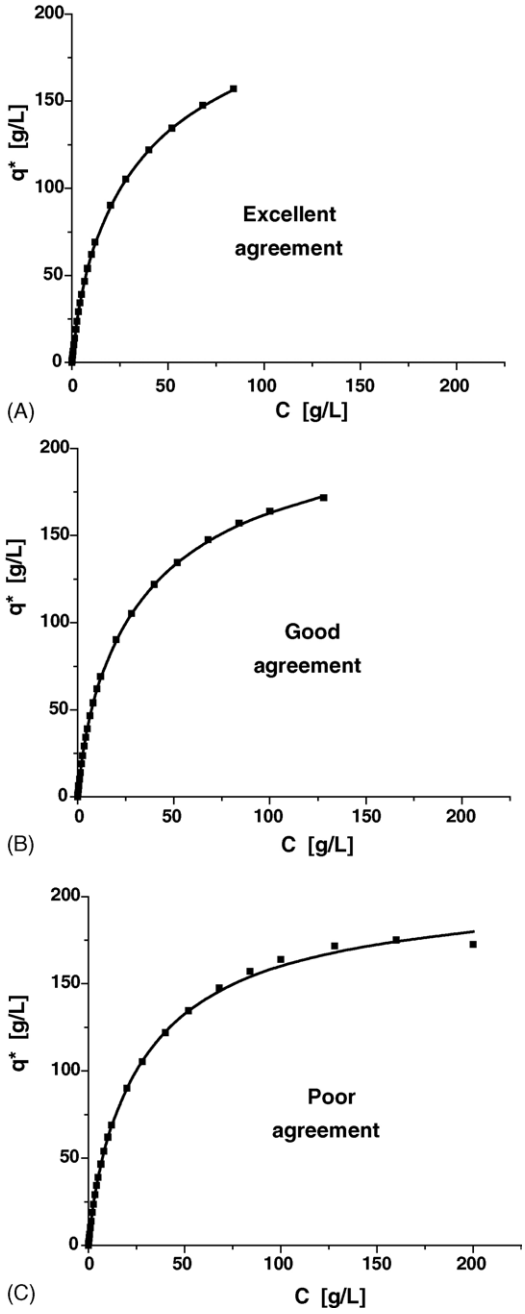

Fig. 5. Agreement between the adsorption data and the best bi-Langmuir model when the five (A) and two (B) highest concentration data points are removed from the initial full data set (C). Note the poor agreement with the full data set because of an experimentally erroneous estimation of the column hold-up volume.

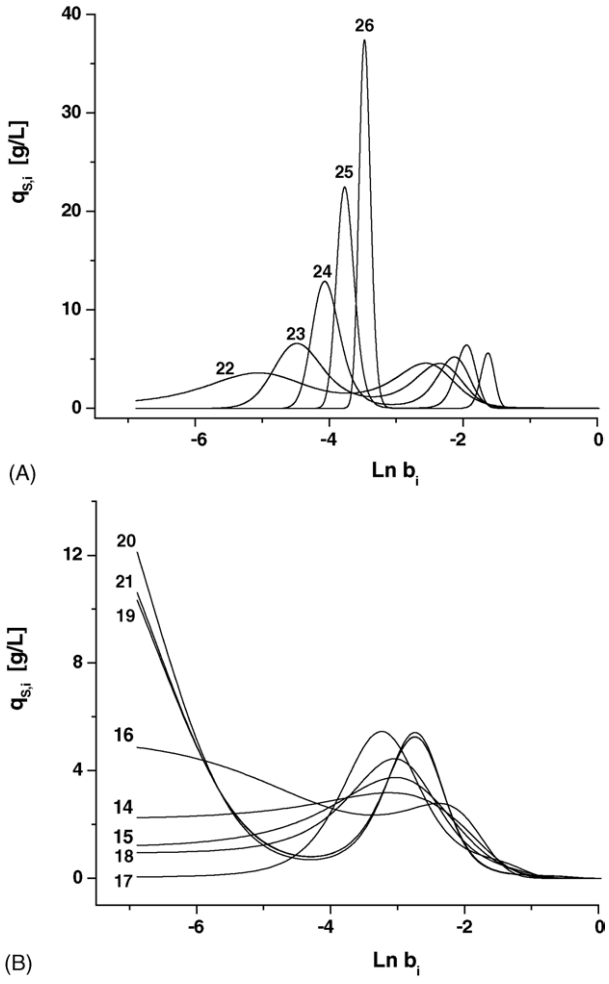

Fig. 6. Calculation of the AED from the raw adsorption containing the first N points recorded: (A) $22 < N < 26$ and (B) $14 < N < 21$. The EM parameters (number of iterations, energy grid) are given in the text. Note that the convergence disappears when too few the number of adsorption data at high concentrations is.

data points may be collected in the low-concentration domain, close to the Henry domain. Conversely, high-concentration data may be missing, for the lack of solubility of the studied compound (about which nothing can be done) or for any spurious, inexcusable rational (e.g., lack of understanding of the problem, laziness, excessive cost of chemicals). The importance of acquiring data points in these opposite ranges is now investigated.

4.1.1. Importance of the high-concentration data

Fig. 3 shows the typical plot of the isotherm data acquired for phenol on Gemini. A series of 26 adsorption data points were measured. The calculation of the amount adsorbed was done following Eq. (1) and the hold-up time, t_0, was chosen arbitrary, as the elution time of thiourea a compound that is generally assumed to be non retained (V_0 = 1.7395 mL). We know however, that thiourea is weakly retained [20,21].

It is important to notice an unusual property of the high concentration data, the amount adsorbed, q^*, in equilibrium with a concentration of phenol of 200 g/L is lower that with a concentration of 160 g/L. Excess isotherm may show such a behavior. For instance, the excess isotherm of acetonitrile or methanol in water on alkyl-bonded phases shows a maximum for a concentration in the organic modifier of about 7 mol/L [22]. This situation is rarely observed at such low mobile phase concentrations (here the phenol concentration is barely 2 mol/L) because the concentration in the adsorbed phase is usually much higher than the concentration in the mobile phase and the excess isotherm

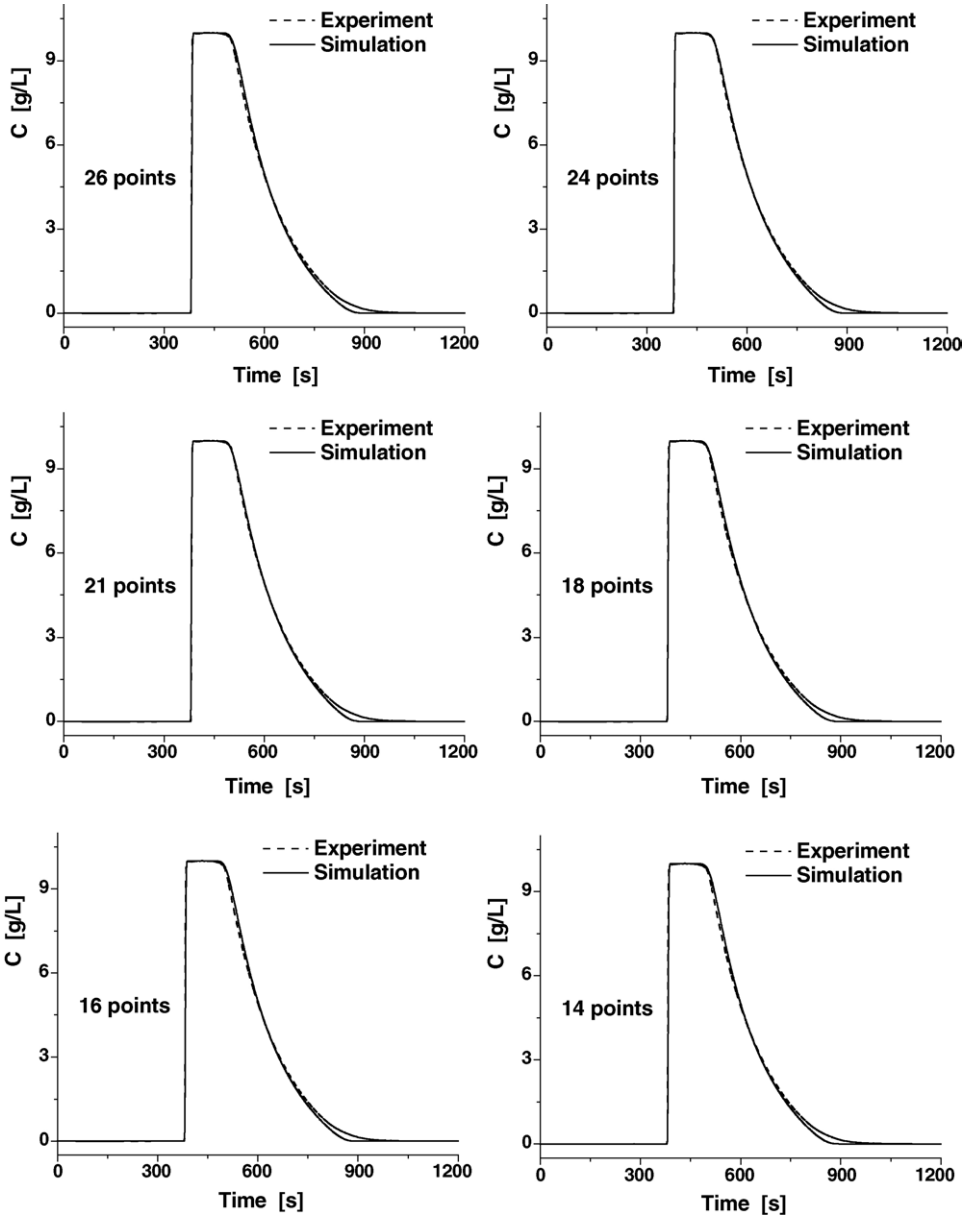

Fig. 7. Comparison between the experimental and simulated breakthrough curves of phenol for low and high concentrations (10 and 160 g/L, respectively). The six plots correspond to six different simulations using the best isotherm parameters that fit the data containing all the data points, and the first 24, 21, 18, 16, and 14 data points. Note the systematic excellent agreement.

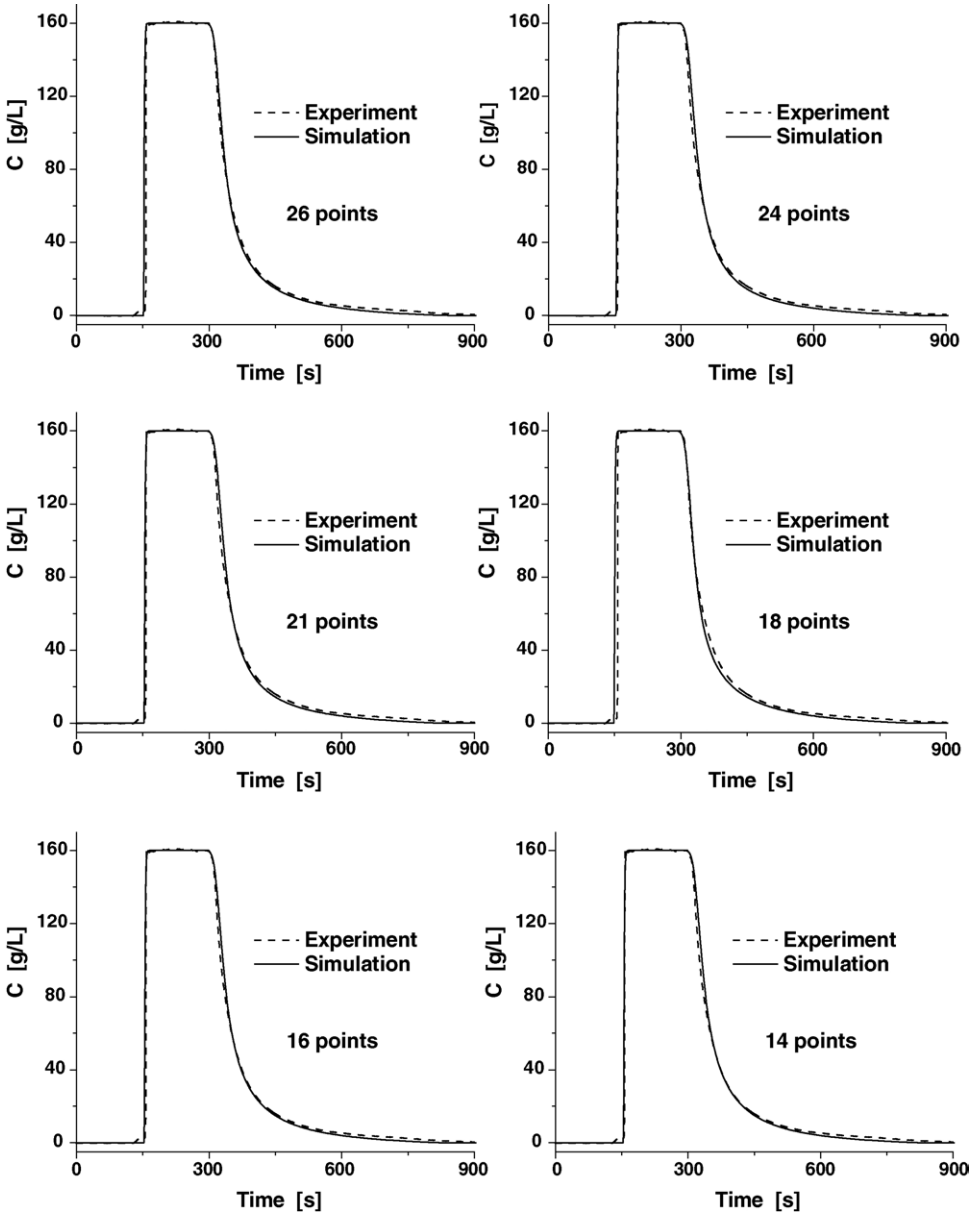

Fig. 7. (*Continued*).

remains very close to the total adsorption isotherm in the low concentration range. Between 160 and 200 g/L (i.e., between 1.7 and 2.1 mol/L) of phenol, the adsorbent is close to saturation and the excess quantity ($q^* - C$) begins to decrease with increasing C. In other words, this result suggests that the value chosen for V_0 represents most probably the total free volume of eluent in the chromatographic column, which leads to the measurement of the excess adsorption isotherm rather than that of the total adsorption isotherm. This issue will be discussed later in this work (Section 4.2), where the effect of t_0 on the adsorption data is discussed.

The best fit of the adsorption data given in Fig. 3 comforts the choice of the bi-Langmuir adsorption isotherm model for this system, when the complete set of data points is taken into account (26 points). This confirms anterior findings regarding the adsorption behavior of this same compound on other brands of monomeric C_{18}-bonded columns [6]. The saturation capacities of the two types of sites, $q_{s,1}$ and $q_{s,2}$, are 1.76 and 0.43 mol/L, respectively, and the difference between the adsorption energies on the sites of types 2 and 1 is 4.2 kJ/mol ($\Delta E = RT(\ln b_2 - \ln b_1)$).

Fig. 4 shows the evolution of the best values of the parameters of the bi-Langmuir isotherm obtained by multi-linear regression analysis when more and more high-concentration data points are successively omitted. The maximum adsorbate concentration, q^*_{max}, of the range covered by the data points used for the fitting decreases (except after the removal of point #26) when the data points are dropped one by one from the data set. The curves are plotted versus the fractional surface coverage, θ:

$$\theta = \frac{q^*(C_{max})}{q_{s,1} + q_{s,2}} \quad (5)$$

The actual saturation capacity of the column $q_{s,1} + q_{s,2}$ was estimated to be equal to the highest sum of the values of $q_{s,1}$ and $q_{s,2}$. It was obtained from the fits of the data corresponding to the first 22 and 23 data points. At a fractional surface coverage of about 0.25, when only the first 14 data points of the set remained under consideration, the fit of the data to the four-parameter bi-Langmuir model does not converge anymore. Too much information is missing in the high-concentration range. Up to a coverage of 0.40 (with the lowest 17 data points), the MLRA gave a solution but the trends of the four parameters become erratic (Fig. 4). In this particular case, no satisfactory isotherm fit could be achieved if the surface coverage was less than 40%. When the high surface coverage data are included, a regular trend is observed in the parameters, up to the data point #23, and the isotherm parameters are stable. The higher range corresponds to a fractional surface coverage of about 70%. The best parameters found for $q_{s,1}$, $q_{s,2}$, b_1, and b_2 are 156.9 g/L, 66.9 g/L, 0.0186 L/g, and 0.123 L/g, respectively. These values are quite different from those obtained with the full set of 26 data points, e.g., 164.8 g/L, 40.8 g/L, 0.0286 L/g, and 0.159 L/g. When the mobile phase concentration becomes too important, significative differences arise between the excess and the total adsorption isotherm. As indicated earlier, our data are likely excess adsorption data while the bi-Langmuir isotherm is a total adsorption isotherm model. It increases monotonically with increasing concentrations while the experimental isotherm decreases at high concentration. The fit becomes poor when the points 24, 25, and 26 are taken into account. This is illustrated in Fig. 5. The corresponding parameters become erroneous, as shown in Fig. 4 with the sudden change in their evolution. The difficulties encountered in fitting accurately the high-concentration data arise in part from the use of a wrong estimate of the column hold-up volume (see later, Section 4.2). They are certainly due also in a large part to the fact that a major consequence of the solution and the adsorbed phases becoming concentrated is neglected in our isotherm model. The concentration dependence of the activity coefficients in both phases

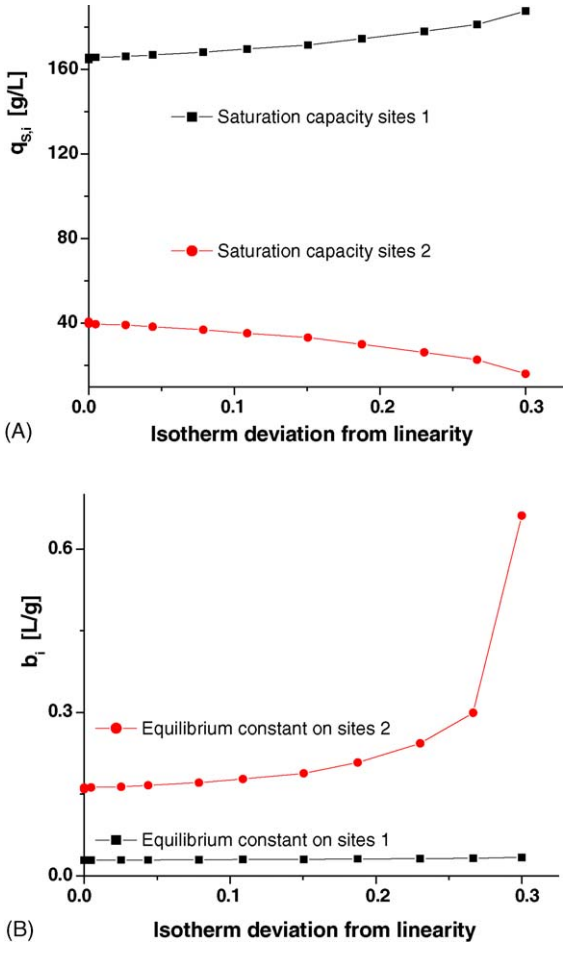

Fig. 8. Evolution of the best estimated bi-Langmuir parameters from the multi linear regression analysis of the adsorption data as a function of the isotherm deviation from linearity calculated from the first data point taken into account in the fitting (see in the text). All the high-concentration data points were conserved: (A) saturation capacities and (B) associated equilibrium constants. Note the high degree of dependence of the parameters of the second site.

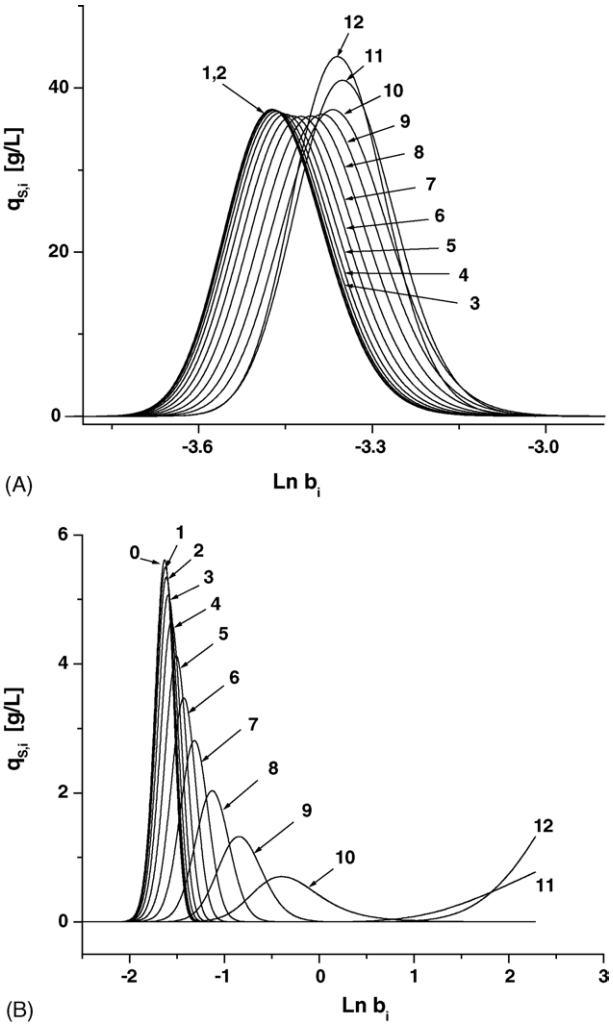

Fig. 9. Calculation of the AED from the complete raw adsorption data from which the first N points were removed. $1 < N < 12$. Same EM parameters as in Fig. 4: (A) low-energy adsorption band and (B) high-energy adsorption band. Note that the convergence of the high-energy band shifts and even disappears when too many adsorption data are missing in the low concentration range. The low-energy band is poorly affected since the high concentration data points were conserved.

is certainly significant [23] and is not accounted for in our isotherm model. A more complex expression should be considered instead.

As a result, the fitting of the adsorption data and the best parameters derived are very sensitive to the number of data points recorded in the high-concentration range. A surface coverage of at least 40% is required to achieve a satisfactory fit. The progressive convergence of the AED with increasing number of high-concentration data points is illustrated in Fig. 6. In these calculations, the number of iterations was kept constant at one hundred millions, the energy grid contained 200 points and the range of the equilibrium constant b was [0.001; 10] in L/g. The bimodal distribution becomes more and more obvious when the number of high-concentration data points increases. In these conditions, 22 data points (i.e., a 70% fractional coverage) are necessary to obtain the actual convergence of the AED to a bimodal distribution. The exercise shows also that the equilibrium constant cannot be accurately determined because the bands are constantly shifting from low to high values when the number of data points increases. Certainly, the equilibrium constants would spread less if the number of iterations were higher.

The estimates of the amount adsorbed in equilibrium with concentrated solutions are very sensitive to the choice of the

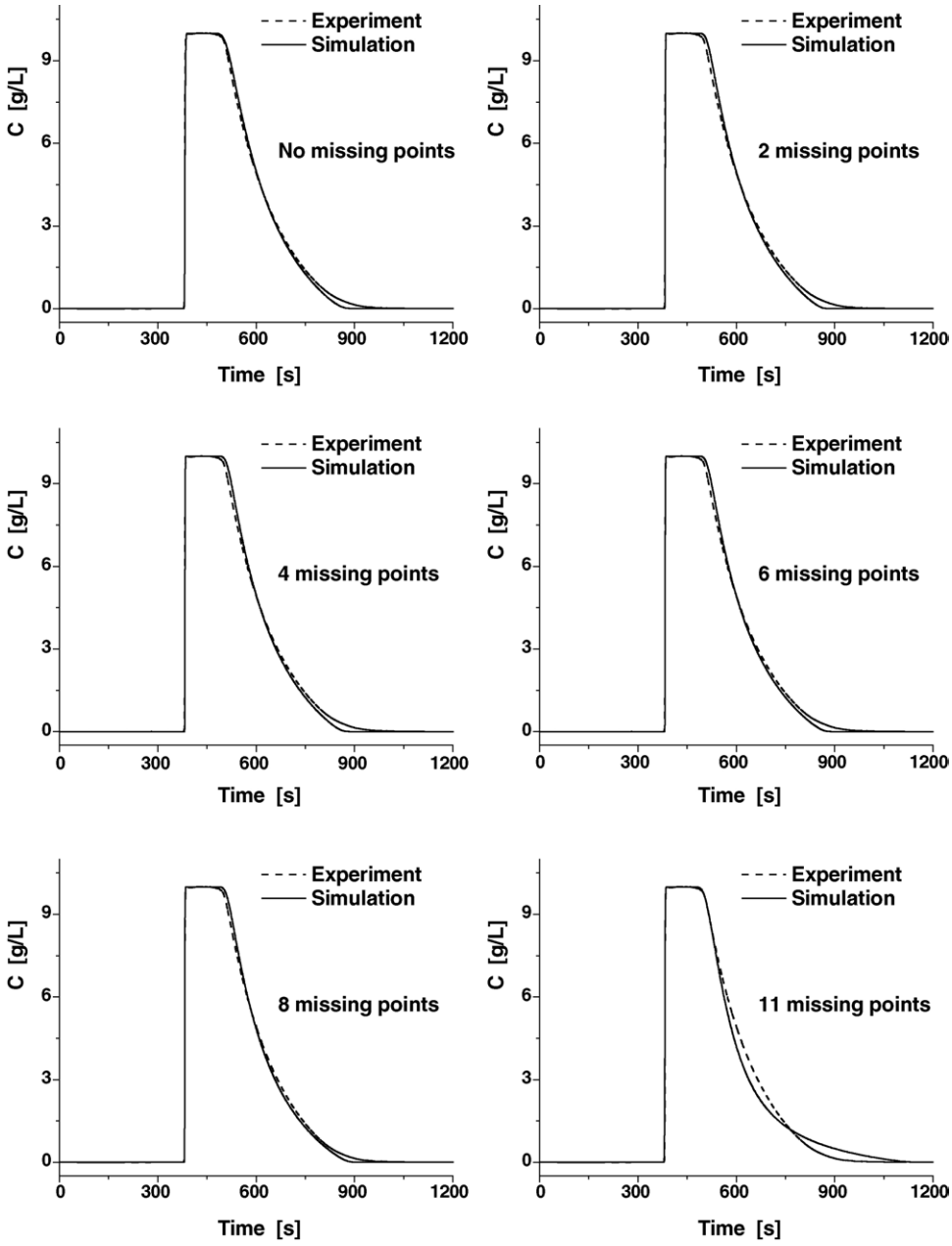

Fig. 10. Comparison between the experimental and simulated breakthrough curves of phenol for low and high concentrations (10 and 160 g/L, respectively). The six plots correspond to six different simulations using the best isotherm parameters that fit the data containing all the data points, all but the 2, 4, 6, 8, and 11 first data points in the low concentration range. Note the systematic and excellent agreement, except for the low concentration band profiles when the first 11 data points were removed.

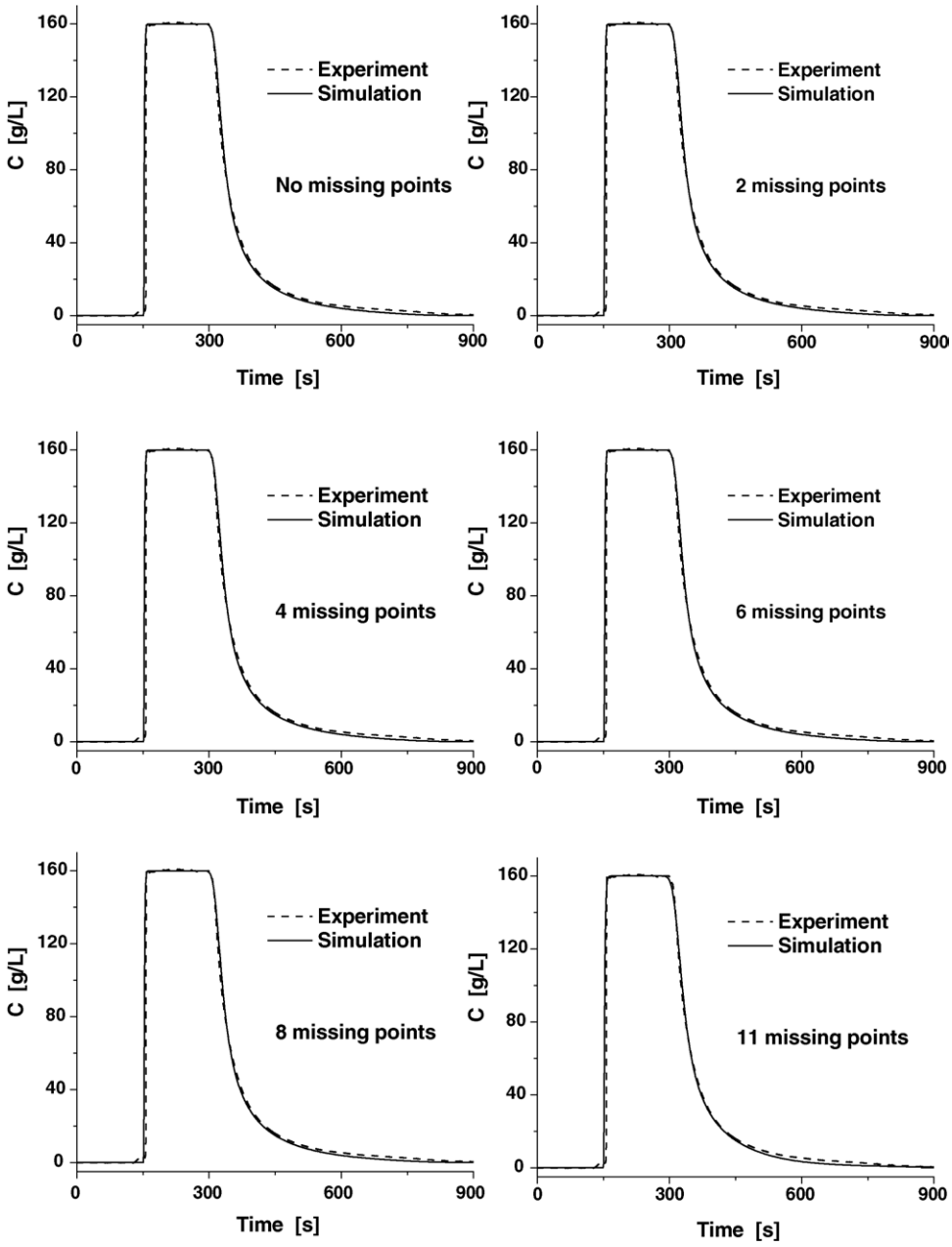

Fig. 10. (*Continued*).

value of the hold-up volume, V_0. The retention time of the front shock of the breakthrough curve is often less than twice the hold-up volume, t_0. So, the data may not correspond exactly to the total adsorption isotherm considered, as in the isotherm model. Accordingly, measurements at high surface coverages should be avoided and 70% may be the upper limit. Actually, for most compounds used in RPLC, such high surface coverages are rarely reached because of their limited solubility in the mobile phase. The problem discussed here does not occur frequently. Most often, the problem encountered in FA measurements comes from the low value of the maximum surface coverage that can be achieved. Although the fitting process is successful and the agreement between experimental and calculated band profiles is excellent (see Fig. 7), this does not guarantee that the isotherm parameters derived are the true ones (if there are any true isotherms). Whatever the number of high-concentration data points considered in this study, an excellent agreement between experimental and calculated breakthrough curves is observed as well for low- and for high-concentrations plugs injected. Other similar studies have also demonstrated that, when high-concentration data are omitted, a wrong isotherm model could be obtained that would fit the remaining adsorption data as well as the true isotherm model [24].

4.1.2. *Importance of the low-concentration data*

The same approach was followed, this time by eliminating progressively the adsorption data at low concentrations from

the set used to calculate the regression. Fig. 8 shows plots of the best values of the bi-Langmuir isotherm parameters versus the deviation of the isotherm from linear behavior, defined as

$$D(C) = 1 - \frac{q^*(C)}{HC} = 1 - \frac{C_{\min} q^*}{q^*(C_{\min})C} \quad (6)$$

where H is the Henry constant (initial slope of the adsorption isotherm) and $C_{\min}$ is the lowest concentration used in the FA runs (in this study $C_{\min}$ was 0.1 g/L). The fitting of the adsorption data was not possible (b_2 tends towards infinite) when the first 12 data points were removed, e.g., when the deviation of the isotherm from linear behavior, $D(C)$, exceeds 0.3.

The variations of the four parameters are monotonous and affect essentially the parameters of the high-energy adsorption sites ($q_{s,2}$ and b_2), as expected since low-concentration data were removed. $q_{s,1}$ and b_1 increase by merely 13 and 18%, respectively while the saturation capacity $q_{s,2}$ decreases by more than a factor two 2 (from 40 to 16 g/L) and the equilibrium constant b_2 by a factor 4 (0.16–0.66 L/g). This clearly demonstrates the importance of measuring adsorption isotherm data down to concentrations for which the isotherm behavior is linear. If data are missing in this region, the accurate determination of the isotherm parameters and particularly that of the high-energy type of sites becomes erroneous.

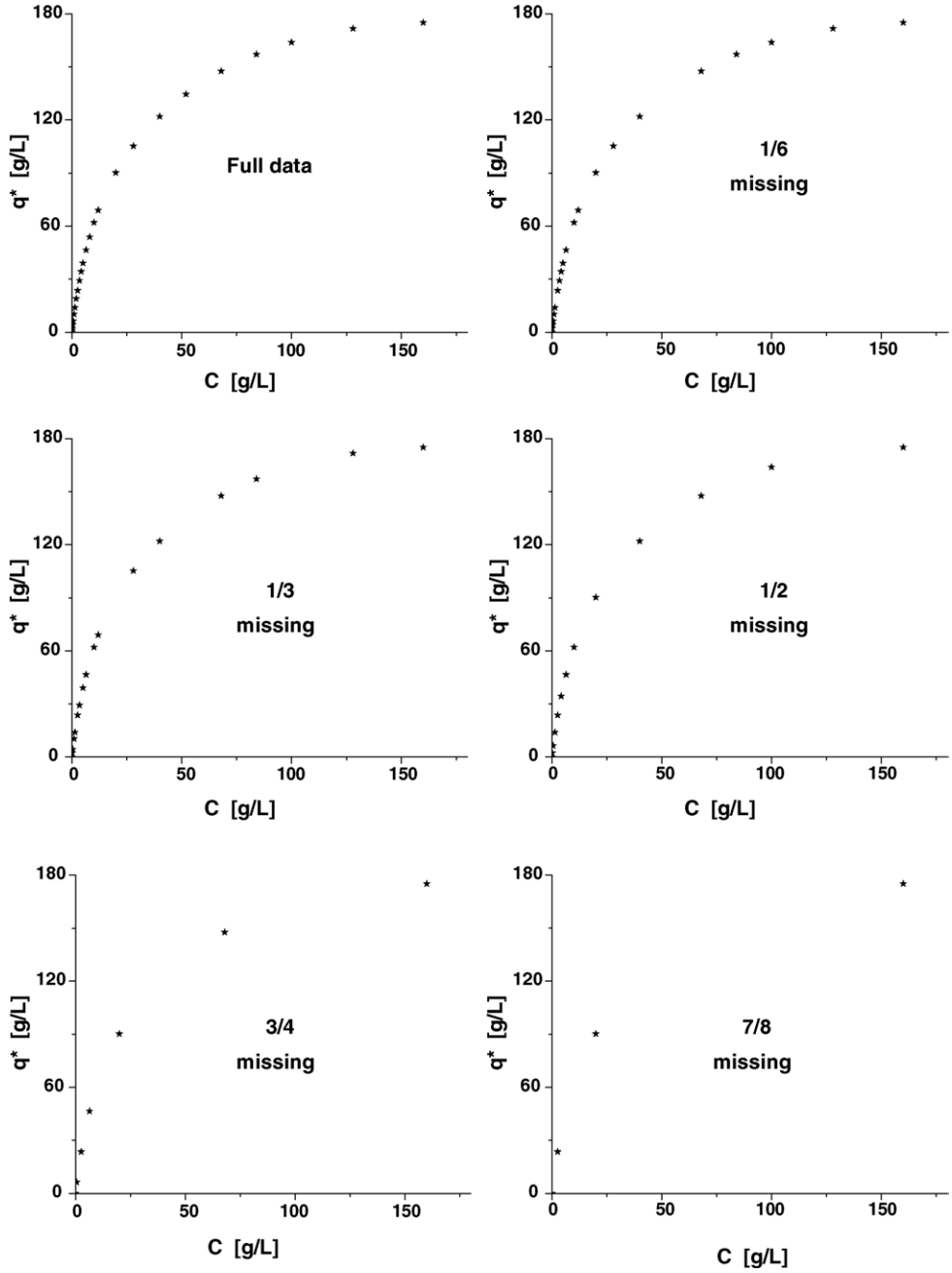

Fig. 11. Distribution of the experimental data points after removal of a fraction of the adsorption data. The complete set of adsorption data contained the first 24 points recorded. The five other graphs show the distribution of the points when 1 out of 6, 1 out of 3, 1 out of 2, 3 out of 4, and 7 out of 8 data points are omitted.

This result is confirmed by the data in Fig. 9, where the evolution of the calculated AED is shown as a function of the number of data points removed from the low end of the data set. The band of the low-energy sites (those occupied at high concentrations) is poorly affected and shifts by less than 0.2 logarithm unit. The situation is quite different for the high-energy band which shifts slowly at first toward the higher energies (by 0.2 logarithm unit when the first five data points are deleted), then rapidly (1 unit for nine data points) and finally tends to disappear and the convergence of the AED becomes impossible. This is the most frequent observation made on the treatment of the FA data by the AED program. The divergence of the AED in the high range of equilibrium constants always suggests the need for additional data points in the low-concentration range of the adsorption isotherm.

Fig. 10 compares the experimental breakthrough curves with curves calculated using an isotherm model, the parameters of which were derived from the fitting of a truncated data set. Despite the isotherm fit lacking sufficient data at low concentrations, the agreement between the calculated and the experimental breakthrough curves remains very good even at low concentration (Fig. 10) until eight points were removed from the set. Not surprisingly, the absence of the low-concentration data does not affect the agreement between the calculated and the experimental profiles at high concentrations (Fig. 10).

4.1.3. Dilution of the isotherm data

Another potential source of errors in the determination of adsorption isotherms by FA may come from the acquisition of too small a number of data points. Coupled with the use of a poorly precise and reproducible apparatus, this may lead to considerable errors of measurement if not to large interpretation errors. To check the importance of the density of the data points, we determined the best values of the isotherm parameters by fitting the data when 1/8, 1/6, 1/4, 1/3, 1/2, 2/3, 3/4, 5/6, and 7/8 of the first 24 data points acquired were eliminated. The last data point, i.e., the point measured for the concentration of 160 g/L, was kept in all cases. Fig. 11 shows the six different distributions of the adsorption data for which the isotherm parameters (Fig. 12) and the AED (Fig. 13) were calculated.

Clearly culling the data points homogeneously has little effect on either the isotherm parameters or the AED until few data point remain. With the six data points in Fig. 11E, the AED is only modestly affected and the parameters have changed little (Figs. 12 and 13). Eliminating up to 1/3 of the data points has almost no effect (Fig. 12). The AEDs (Fig. 13) converge toward a bimodal distribution with only six data points (Fig. 11E). This means that, if the measurements are precise and spread over a wide concentration range, accurate results can still be obtained. Note, however, that the accuracy begins to decrease markedly when more than half the data points are removed from the data set. The values of the isotherm parameters and the two bands of

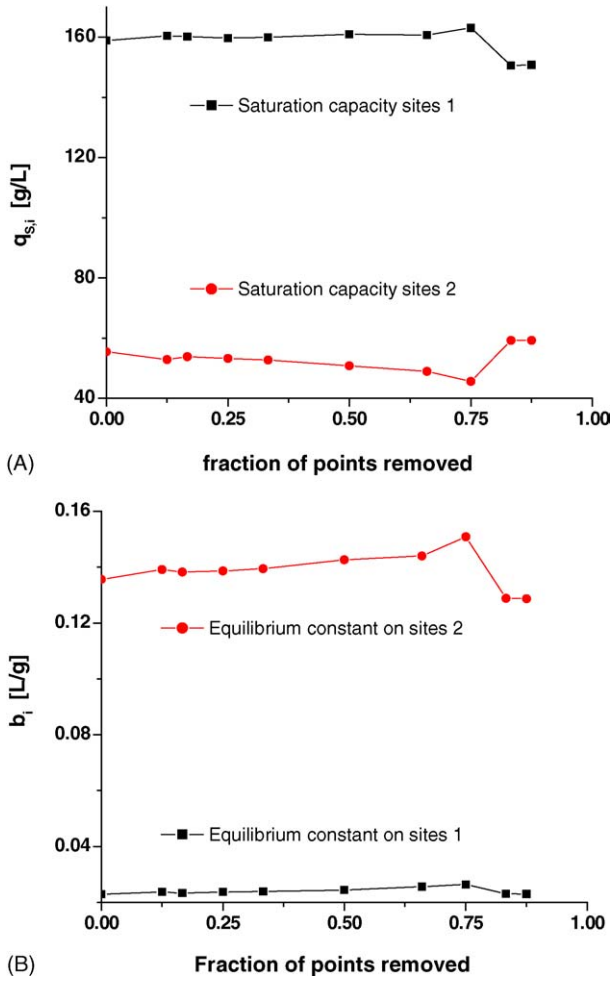

Fig. 12. Evolution of the best estimated bi-Langmuir parameters from the multi linear regression analysis of the adsorption data as a function of the fraction of adsorption data points omitted (from 0 to 82.5%): (A) saturation capacities and (B) associated equilibrium constants. Note the reasonable stability of the isotherm parameters suggesting a excellent precision of the FA measurements.

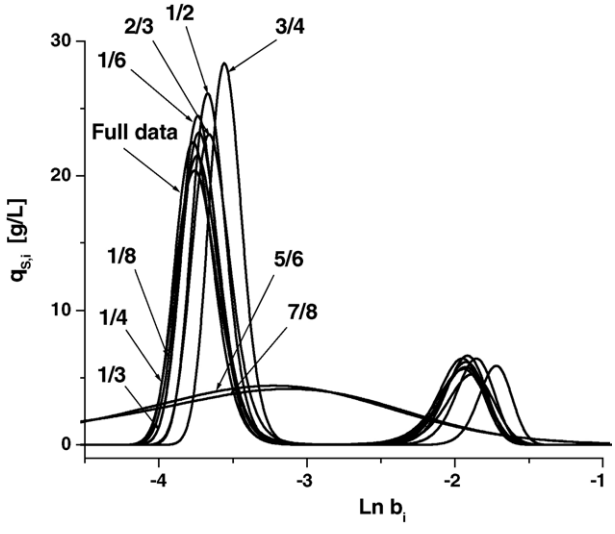

Fig. 13. Calculation of the AED from the complete raw adsorption data from which various fractions of data points were removed. The ratio on the figure indicates the fraction of points omitted. Same EM parameters as in Fig. 4. As in Fig. 12, note the poor sensitivity of the isotherm parameters to the dilution of the data points and the loss of the AED convergence when the remaining experimental points are less than 6.

the AED deviate progressively and with only four data points, the AED has only one energy mode. Despite the high precision of the measurement made, it is difficult accurately to determine the four parameters of the isotherm model with four data points.

As in the two precedent sections, the agreement at low and at high column loadings between the calculated and the experimental breakthrough curves remains excellent even when the isotherm is derived from the three data points in Fig. 11F (not shown). A correct prediction of the band profiles could be obtained from an isotherm obtained by modeling a few adsorption data points, probably less than 10 if the measurements are very precise. Obviously, as the best model of isotherm is unknown, the acquisition of a significant number of data points, a number larger than the 8 or 10 required in this case, is strongly recommended.

4.2. Adsorption isotherm parameters and hold-up time t_0

The calculation of the adsorption data, hence the derivation of the best adsorption isotherm and the calculation of its parameters depend on the determination of the hold-up column volume of the chromatographic support (see Eq. (1)). The value calculated for the amount of compound adsorbed at equilibrium is essentially controlled by the term $(t_{shock} - t_0)$, which becomes small and highly sensitive to errors made on t_0 when the shock of the breakthrough curves elutes rapidly, close to the hold-up time. Even if the mobile phase composition is selected so that the retention factor k'_0 is sufficiently large, the shock will get close to the unretained peak when the concentration becomes high and the adsorbent surface gets close to saturation. Then an accurate measurement of the hold-up time, t_0, becomes crucial for the determination of correct adsorption isotherm data.

In this work, we measured the hold-up volume from the elution time of thiourea, a compound that is usually recommended as a good hold-up time marker in RPLC [25]. However, we know that the elution volume of thiourea is only an approximation of the true void volume (e.g., of the volume inside the column that is available to the solute). Thiourea is slightly retained on C_{18}-bonded phases. The value of V_0 derived from its retention time appears larger than the expected hold-up volume derived from

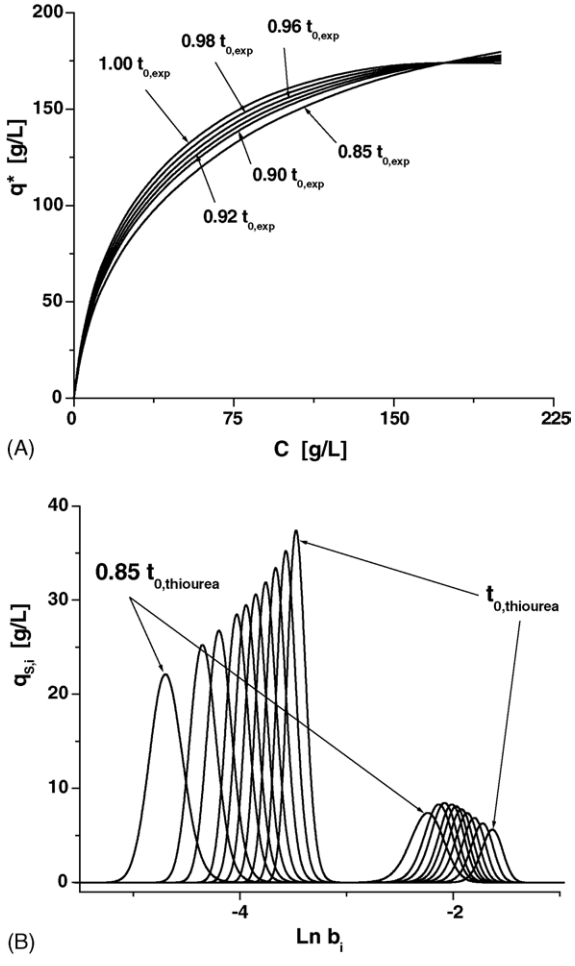

Fig. 14. (A) Evolution of the experimental adsorption curve when the experimental hold-up time is underestimated by a factor 0.98, 0.96, 0.92, 0.90, and 0.85. (B) Effect of the variation of the hold-up volume from 0.85 $t_{0,exp}$ to $t_{0,exp}$ (the successive fraction corresponding to the AED from the left to the right are: 0.85, 0.90, 0.92, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, and 1.00). Note the severe displacement of the low-energy bands towards the lower energies.

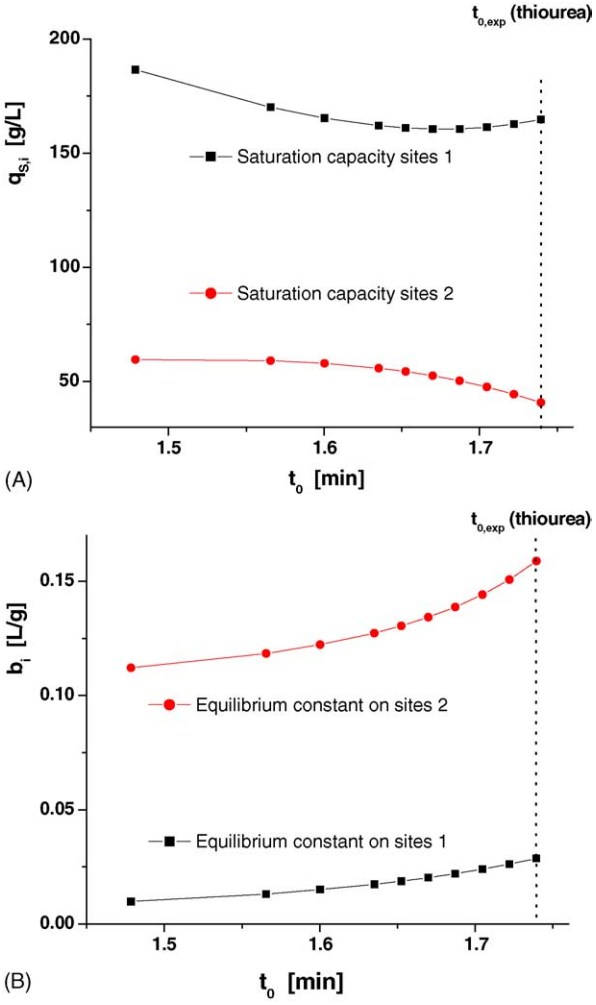

Fig. 15. Evolution of the best estimated bi-Langmuir parameters from the multi linear regression analysis of the full 26 adsorption data as a function of hold-up time used in Eq. (1): (A) saturation capacities and (B) associated equilibrium constants. Note the strong influence of t_0 on the isotherm parameters.

pycnometric data. Recently, it was shown that the elution volume of thiourea measured with a 30% methanol aqueous mobile phase on an endcapped Symmetry-C_{18} column was 1.06 mL while the total volume accessible to the mobile phase measured by pycnometry was only 0.91 mL. In other words, the column void volume measured from the elution time of thiourea was overestimated by about 14%, quite a significant difference. The value measured for the hold-up volume is method dependent and the influence of changes in t_0 should be taken into account in the determination of the adsorbed amount.

Fig. 14A shows the evolution of the adsorption data calculated from Eq. (1), using the same breakthrough curves (Fig. 1), hence the same retention times for the shock of the breakthrough curves, but assuming different values for the hold-up time. These values decrease from the retention time measured for thiourea ($t_{0,exp}$) to 85% of this value, with intermediate steps at 99, 98, 97, 96, 95, 94, 92, 90, and 85%. This choice was based on our knowledge, from pycnometry measurements, that thiourea is slightly retained but that its retention factor is less than 0.15 [21]. In the interval from 85 to 100% of $t_{0,exp}$, the retention factor of thiourea would vary from 0.176 to 0. Considering hold-up times larger than $t_{0,exp}$ would not make physical sense because this would assume that thiourea is excluded from part of the pores to which the other solutes would have access. The most striking result in this figure is the considerable change in the behavior of the adsorption isotherm at high concentra-

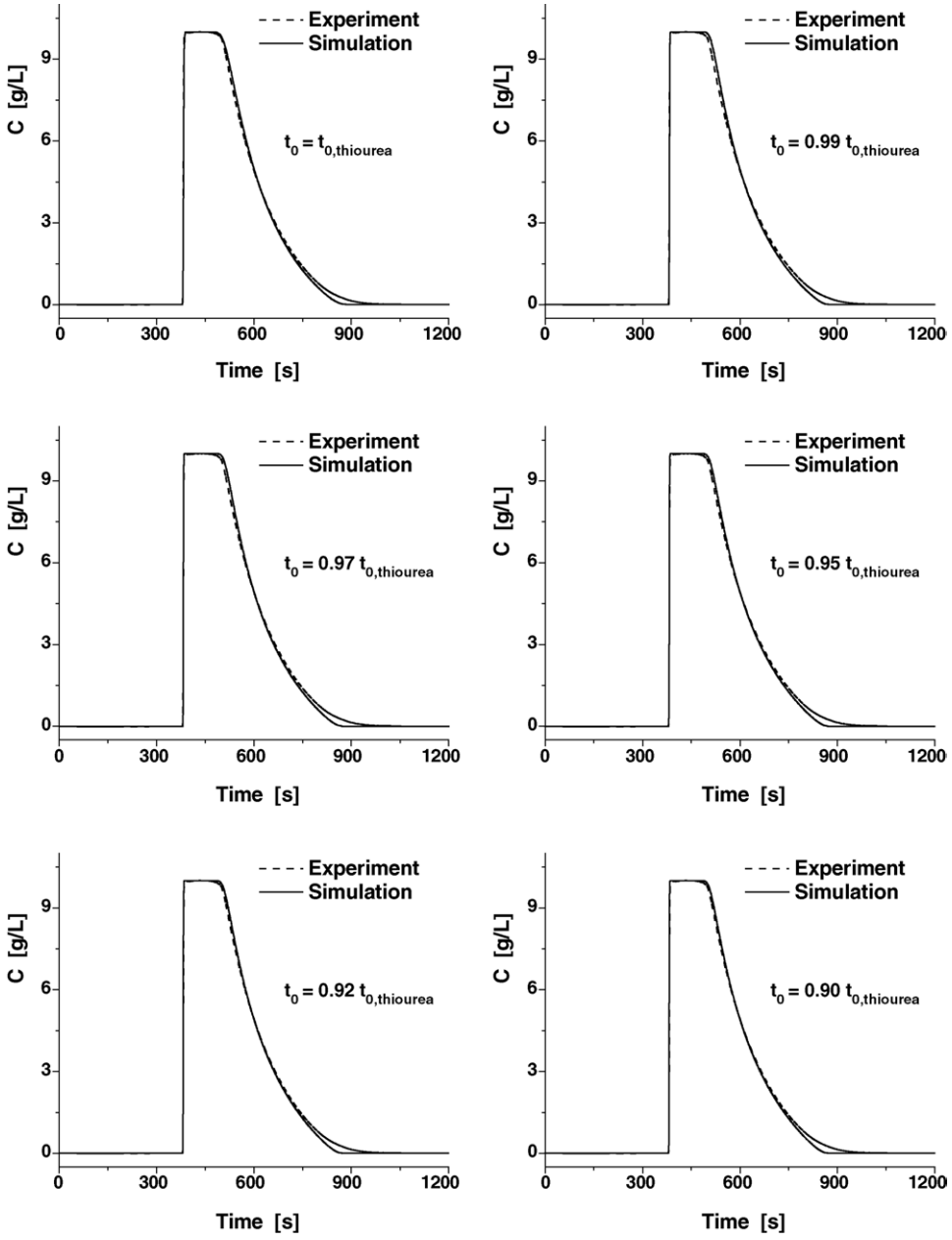

Fig. 16. Comparison between the experimental and simulated breakthrough curves of phenol for low and high concentrations (10 and 160 g/L injected, respectively). The six plots correspond to six different simulations using the best isotherm parameters that fit the data containing all the data points for different values of the hold-up times. Note the systematic and excellent agreement.

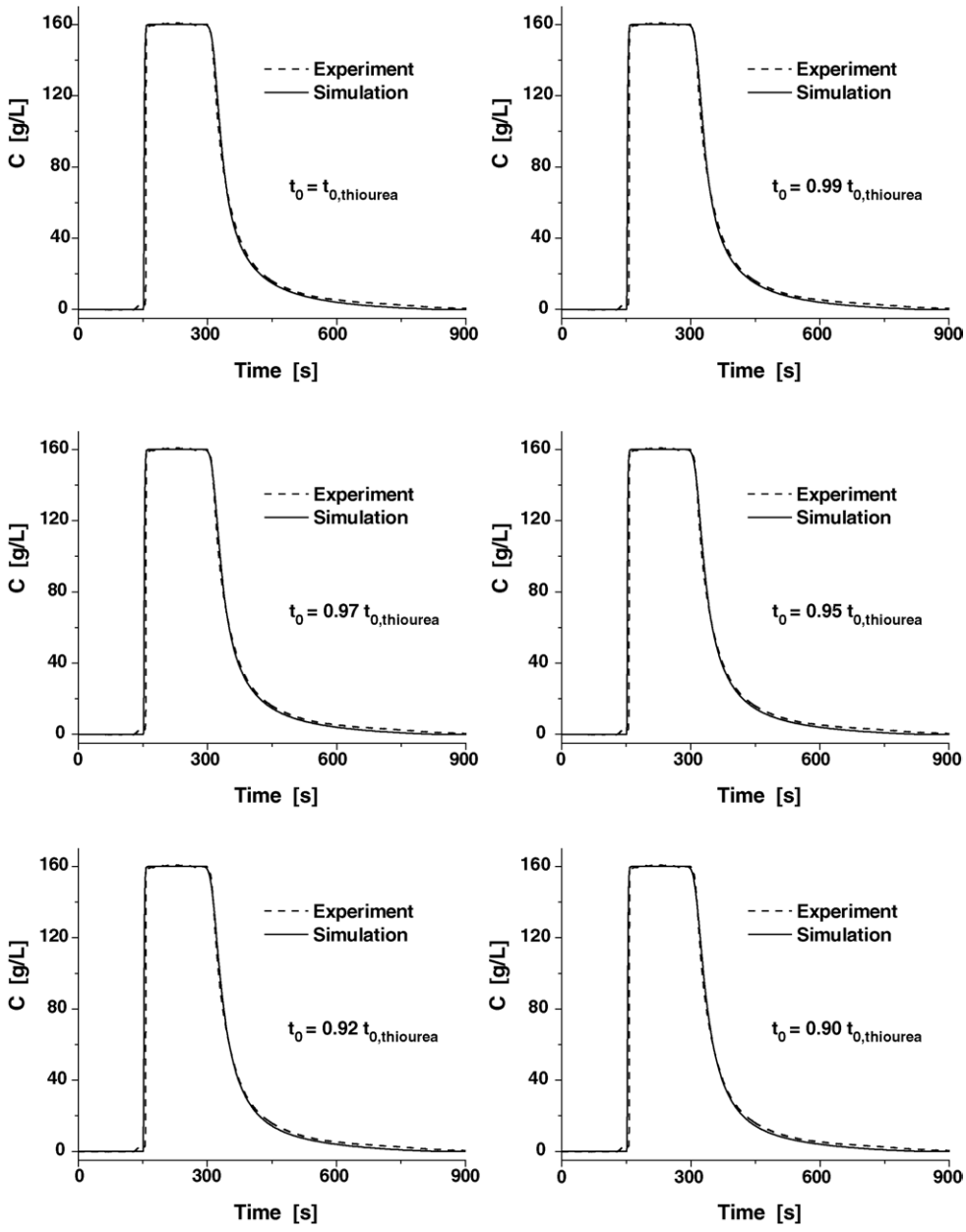

Fig. 16. (*Continued*).

tions. The amount of phenol adsorbed at equilibrium in the concentration range between 160 and 200 g/L changes dramatically. When $t_0 = t_{0,thiourea}$, it is lower for $C = 200$ g/L than for $C = 160$ g/L. This isotherm behavior was predicted by Sajonz [8]. This amount progressively increases and the isotherm has no longer a maximum. This suggests that the choice made earlier for t_0 was an overestimation of the true hold-up volume of the Gemini column, as expected from the weak retention of thiourea.

The different sets of adsorption data were used to calculate the AED (Fig. 14B) and were fitted to the bi-Langmuir model. The equilibrium constants b_1 and b_2 vary considerably (Fig. 15A), by factors of about 3 and 1/3, respectively, when the hold-up time decreases by 15%. Because the curvature of the adsorption data change mostly at high concentrations, the parameters of the sites of type 1 are more sensitive to the change in the hold-up time. This is expected since the relative change in $t_{shock} - t_0$ is most important at high concentrations, when the sites of type 1 begin to fill. This result is confirmed by the evolution of the adsorption energy distribution that shows a progressive shift of the bands towards the low energies (Fig. 14B), with a more important shift for the band of type 1. The saturation capacity $q_{s,2}$ increases by 50% but remains almost unchanged as long as the hold-up time is less than 92% of $t_{0,thiourea}$. The change in $q_{s,1}$ is similar to but less important than that of $q_{s,2}$, about 15% at most.

These results show how sensitive is the determination of the isotherm parameters to the choice of the value of the hold-up volume. However, the agreement between the calculated and the experimental profiles of overloaded bands remains excellent, whatever the hold-up column volume (Fig. 16). Errors made

on the measurement of the hold-up volume cannot be revealed by the degree of agreement between calculated and experimental band profiles. Sajonz [8] has showed much larger variations between the overloaded band profiles calculated assuming different values of the error made on t_0. However, he considered much larger errors, almost unrealistic errors, between ±20% and ±40%, to prove his point. From our data, it is most likely that the error made on t_0 is less than 15%. Lindholm et al. [26] demonstrated the consequences of using different hold-up volumes measured in different ways and the error on the simulated peak profiles.

5. Conclusion

This work demonstrates how the FA method, albeit it is considered as the most accurate chromatography method of adsorption isotherm data measurements for solid–liquid systems, gives results that are very sensitive to the way in which the adsorption data are collected and interpreted. First, whatever model best fits the whole set of data acquired, the best numerical values of its parameters depend on the range of concentrations probed. This range should be as wide as possible, given the finite solubility of the analyte in the liquid phase. Accordingly, a poor solubility may drastically limit the accuracy of the isotherm model and particularly the amount of information obtained regarding the low energy adsorption sites.

Second it is important that data should also be acquired at such low concentrations that the isotherm behavior becomes practically linear. Significant differences between the initial slope of the isotherm and the ratio of the retention factor and the column phase ratio (i.e., k'/F) indicate often that the lowest data point was obtained at too high a concentration and that more data at lower concentrations are needed. A frequent error in chromatography consists in assuming that the tailing observed for peaks of small-size samples is always due to a slow mass transfer kinetics (e.g., a slow kinetics of desorption from the adsorbent surface, which happens to be rare, or rather a slow rate of internal diffusion). As a matter of facts, and particularly with the modern, high-performance, endcapped RPLC columns, peak tailing at low sample sizes arises from a nonlinear behavior of the isotherm. The surface of RPLC columns is heterogeneous. Some adsorption sites may behave as high-energy sites for certain, polar or basic compounds, not for more neutral ones with the result that the range of sample sizes within which a column behaves linearly depends on the nature of the compound studied. A simple method to ensure that FA data were collected at low enough concentrations consists in recording successive breakthrough curves with decreasing feed solution concentration and comparing the degree of asymmetry of their front and rear. This can be done quantitatively by comparing the derivatives of these two parts of the breakthrough curve. However, we have previously reported cases in which the detector sensitivity was insufficient to reach the linear range [27].

Third, the numerical values of the coefficients of the adsorption isotherm depend strongly on the method selected to estimate the column hold-up volume. Therefore, all reports providing values of isotherm coefficients should indicate clearly which method was used to estimate the column hold-up volume.

Interestingly, in the case under study, the accuracy of the results of calculations of overloaded band profiles was little affected by the significant variations in the numerical values of the isotherm parameters that arose from the use of a very low density of isotherm data points, by a relative lack of high-concentrations or of low concentrations data, or by an important error on the value of the hold-up time. This observation explains the reputation of accuracy of the FA method. It makes it most attractive for the acquisition of the data necessary for computer-assisted modeling of preparative chromatography. Of great importance, however, is the achievement of a high degree of reproducibility of the measurements of the elution times of the breakthrough curves. This requires the use of a very precise HPLC instrument that provides a highly constant flow rate (fluctuations less than a few parts per thousand over a time equal to the retention time of the compound studied under analytical conditions), a highly stable column temperature (fluctuations less than 0.5 K), and a high precision and accuracy in the ratio of the flow rates of the two solutions used to prepare the feed solution for the breakthrough curves. These requirements are met by the HP 1090 and 1100 used in our work.

Doubtlessly, the dynamic FA method is accurate and very precise. The errors discussed here arise not during the measurement process itself but in connection with the interpretation of the data and their use to determine the equilibrium isotherm of the compounds studied. If the equilibrium isotherm is needed to permit computer optimization of preparative separation, these errors have little consequences since we have shown that the differences between the calculated and the experimental band profiles are practically negligible. If the equilibrium isotherms are to be used for a study of the retention mechanism, we must remain cautious in their interpretation as the actual systematic errors made in the measurement of these isotherms are probably far greater than the random error.

One might prefer to use a static method. The use of this method does not require an estimate of the phase ratio in the chromatographic system. However, similar problems arise in the selection of the number of data points and of the concentration range that they should encompass. The static method is much less precise than FA because the analyte concentration in the solution must be measured before and after its equilibrium with the solid adsorbent. Concentrations are more difficult to measure than times. From a fundamental point of view, an agreement between the results of the static and the FA method is expected. A comparison between these results would provide an estimate of the actual importance of the selection of a truly unretained marker for the FA measurements.

Finally, we must keep in mind that conventional isotherm models used in HPLC imply that the mobile and the adsorbed phases follow ideal thermodynamic behavior, even at high concentrations. Actually, the concentration dependence of the activity coefficients should be taken into account in the expression of the adsorption models, using for instance the UNIFAC method [28] for the mobile phase and the Flory-Huggins [29,30] expression for the adsorbed phase. This advanced procedure will affect

the numerical values of the fitted thermodynamic parameters (saturation capacities, equilibrium constants), hopefully leading to values less dependent on the high concentration range of the measurement. On the other hand, it will cause an increase of the number of fitting parameters in the adsorption model, especially in the case of heterogeneous adsorption. As a consequence, the estimates of these parameters will be less precise.

Acknowledgments

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