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Adsorption Mechanisms and Effect of Temperature in Reversed-Phase Liquid Chromatography. Meaning of the Classical Van't Hoff Plot in Chromatography

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The effect of temperature on the adsorption and retention behaviors of a low molecular weight compound (phenol) on a C_{18}-bonded silica column (C_{18}-Sunfire, Waters) from aqueous solutions of methanol (20%) or acetonitrile (15%) was investigated. The results of the measurements were interpreted successively on the basis of the linear (i.e., overall retention factors) and the nonlinear (i.e., adsorption isotherms, surface heterogeneity, saturation capacities, and equilibrium constants) chromatographic methods. The confrontation of these two approaches confirmed the impossibility of a sound physical interpretation of the conventional Van't Hoff plot. The classical linear chromatography theory assumes that retention is determined by the equilibrium thermodynamics of analytes between a homogeneous stationary phase and a homogeneous mobile phase (although there may be two or several types of interactions). From values of the experimental retention factors in a temperature interval and estimates of the activity coefficients at infinite dilution in the same temperature interval provided by the UNIFAC group contribution method, evidence is provided that such a retention model cannot hold. The classical Van't Hoff plot appears meaningless and its linear behavior a mere accident. Results from nonlinear chromatography confirm these conclusions and provide explanations. The retention factors seem to fulfill the Van't Hoff equation, not the Henry constants corresponding to the different types of adsorption sites. The saturation capacities and the adsorption energies are clearly temperature dependent. The temperature dependence of these characteristics of the different assorption sites are different in aqueous methanol and acetonitrile solutions.

A better understanding of the mechanism(s) of adsorption in RPLC[1−4] would help to improve the analytical performance of chromatography in environmental, food, biological, and pharmaceutical analyses[5] by leading to the development of better stationary phases allowing faster separations and lower detection limits. We showed recently how the results of measurements that are classical in nonlinear chromatography shed new light on these mechanisms and reveal unexpected phenomena in RPLC.[6] First, the surfaces of adsorbents are heterogeneous.[7−9] This fact explains the peak tailing observed at even moderate sample sizes,[10] a tailing that has a nefarious impact on the separation and the detection of analytes. The classical models used to interpret the dependence of the retention factor at infinite dilution, k', on the mobile-phase composition and the temperature are the linear solvent strength model (LSSM) and the Van't Hoff plot, respectively. The LSSM assumes a homogeneous stationary phase and plots ln k' versus the volumetric fraction, ϕ, of the organic solvent while the classical Van't Hoff plots (ln k' versus $(1/T)$) implies that there is a single retention mechanism. So, both approaches imply that the stationary phase, i.e., the adsorbent surface, is homogeneous, which it is not.[6−9] These models are empirical and do not reflect the true retention model of the compounds in RPLC.

We showed recently that a careful analysis of the adsorption data of neutral and ionizable low-molecular-weight compounds (phenol, caffeine, naphthalene sulfonate, propranololium chloride) on conventional RPLC columns demonstrates the coexistence of at least two and a maximum of four distinct types of adsorption sites.[11,12] We showed also that the mechanisms of adsorption of neutral molecules from aqueous solutions of methanol and acetonitrile are quite different.[13] Finally, we showed that the adsorption models of ionizable compounds in the presence of counterions depend considerably on the valence of this counterion since a convex upward, an S-shaped, and a convex downward

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isotherms were observed when the valence of the counterion was one, two, and three, respectively.[14] In all these cases, linear chromatography was clueless to distinguish between these different behaviors because it measures the mere retention time, which is simply the sum of the contributions of the different retention mechanisms to the Henry's constant.

In our previous work, we focused on the effects of the organic solvent, the supporting salts, and the buffers on the adsorption properties of the stationary phase in RPLC. In this work, we followed a similar approach and performed nonlinear chromatography experiments involving the effects of temperature on the adsorption behavior of neutral compounds on RPLC columns. It is well known that retention factors almost always decrease with increasing temperature. The questions that we want to answer are, Why? What does cause this decrease? How do the saturation capacities and the equilibrium constants of adsorbates change with increasing temperature? A similar study was done earlier on an end-capped C_{18}-bonded column (Symmetry, Waters).[15] It showed that the adsorption of phenol followed a two-site, bi-Langmuir isotherm model behavior and that the number of the low-adsorption energy sites $q_{S,1}$ and the equilibrium constant of the high-adsorption sites b_2 decrease rapidly while the number of high-adsorption energy sites $q_{S,2}$ and the equilibrium constant of the low-adsorption energy sites b_1 remain nearly constant when the temperature increases. So, both contributions to $k' = F\sum q_S b$ decrease with increasing temperature, but for different reasons. These results suggest that the interface structure between the bulk mobile phase and the top of the bonded layer, where the chains have a high mobility, is quite sensitive to temperature while the inner structure of the bonded layer, where the chains have a limited mobility, was less sensitive to temperature change.

In this work, we consider the behavior of the same analyte (phenol) with different mobile phases (both methanol and acetonitrile were used as organic solvents), on a different C_{18}-bonded column (Sunfire, Waters) provided by the same manufacturer. We investigate the details of the retention mechanism of phenol on the different types of adsorption sites with different mobile phases (containing methanol or acetonitrile as organic modifier). The validity of applying the classical Van't Hoff law to chromatographic data is then discussed, based on a comparison between the interpretation of the data measured in linear and in nonlinear chromatography.

THEORY

Determination of the Adsorption Isotherm Data by Frontal Analysis (FA). Frontal analysis[2,16,17] was used to measure the single-component adsorption isotherm data used in this work. The mobile-phase composition was selected so that the retention of the probe was sufficiently large to permit the retention data to be measured with accuracy within a reasonable time. The determination of the probe amount that is adsorbed on the column at equilibrium with a solution of known concentration is explained in detail elsewhere.[18] It requires the precise measurement of both the extracolumn volume and the hold-up column volume V_M. The first one was measured from the retention time of the inflection point of the breakthrough curve recorded without a chromatographic column, the second from pycnometric measurements, in which methanol and dichloromethane were used as the two solvents, with $\rho_{CH_3OH} = 0.791$ g/cm^3 and $\rho_{CH_2Cl_2} = 1.326$ g/cm^3. The column hold-up volume is given by

$$V_M = \frac{m_{CH_2Cl_2} - m_{MeOH}}{\rho_{CH_2Cl_2} - \rho_{CH_3OH}} \quad (1)$$

where $m_{CH_2Cl_2}$ and m_{MeOH} are the masses of the column when filled with dichloromethane and methanol, respectively.

The general equation that gives the amount q^* of sample adsorbed per unit volume of adsorbent is simply derived from the mass balance equation written between the moments when the sample solution at concentration C_0 enters the column and when the adsorbent at the outlet of the column ($x = L$) is at equilibrium with this sample solution:

$$q^*(C_0) = \frac{F_v}{V_C - V_M}\int_{t_M}^{\infty} [C_0 - C(t)]\, dt \quad (2)$$

where V_C is the column tube volume, F_v the mobile-phase flow rate, and $C(t)$ the concentration profile recorded at the column outlet ($x = L$). t_M is defined as the ratio (V_M/F_v).

The precision of the measurements of $q^*(C_0)$ is discussed later (see Precision of the FA Data).

Adsorption Isotherm Models. Two different models of adsorption isotherms were found useful in this study, depending on whether methanol or acetonitrile was used as the organic modifier. The adsorption isotherm models that fit best the adsorption data of phenol on end-capped C_{18}-bonded columns with methanol–water mobile phases are the bi-Langmuir[7] or the tri-Langmuir[8,20] model that both characterize adsorption on a heterogeneous surface. The equation of the latter model is

$$q^* = q_{S,1}\frac{b_1 C}{1 + b_1 C} + q_{S,2}\frac{b_2 C}{1 + b_2 C} + q_{S,3}\frac{b_3 C}{1 + b_3 C} \quad (3\text{-A})$$

where $q_{S,1}$, $q_{S,2}$, $q_{S,3}$, b_1, b_2, and b_3 are the saturation capacities and the adsorption–desorption constants of the three types of sites. For the former model, $q_{S,3} = 0$.

The isotherm model that best accounts for the adsorption data of neutral compounds from acetonitrile–ater mixtures on RPLC columns is the BET–Langmuir isotherm model.[13] The Langmuir term describes adsorption on the high-energy sites (monolayer adsorption) and the BET term describes the multilayer adsorption of the compounds on the low-energy adsorption sites.

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The equation of this model is

$$q^* = q_{S,1}\frac{b_S C}{(1 - b_L C)(1 - b_L C + b_S C)} + q_{S,2}\frac{b_2}{1 + b_2 C} \qquad (3\text{-B})$$

where b_S and b_L are the adsorption−desorption constants of the adsorbate on the surface of the adsorbent and on a layer of adsorbate molecules, respectively.

Fitting the Isotherm Data to the Isotherm Models. The adsorption data derived from the FA method were directly fitted to the adsorption isotherm models listed above, using nonlinear regression analysis. Each squared residual was weighed by the factor $(1/q_{exp}^2)$, to avoid discriminating the data points in favor of the high concentration ones. It was verified that the isotherm parameters obtained were independent of each other.

Calculation of the Adsorption Energy Distribution (AED). The adsorption energy distribution or relationship between the surface area occupied by the adsorption sites of type i, i.e., $q_{S,i}$, and the logarithm of the adsorption−desorption constant b_i was calculated using the program developed by Stanley et al. and implementing the expectation-maximization method.[21] The detail of the procedure is given elsewhere.[6] The program assumes that the local isotherm follows Langmuir isotherm model behavior. The overall isotherm is the convolution of the local Langmuir isotherm and the energy distribution. In the case of adsorption from acetonitrile solutions, however, the overall isotherm is a BET-Langmuir model because of adsorbate−adsorbate interactions. In this case, the overall isotherm cannot be deconvoluted into a distribution of Langmuir isotherms and the program does not apply for the determination of the AED of phenol.

EXPERIMENTAL SECTION

Chemicals. The mobile phases used in this work were aqueous solutions of methanol and acetonitrile with concentrations of 20 and 15% (v/v), respectively. Water, methanol, and acetonitrile were of HPLC grade, purchased from Fisher Scientific (Fair Lawn, NJ). Prior to their use, the solvents were filtered on an SFCA filter membrane, 0.2-μm average pore size (Suwannee, GA). Phenol, the only solute used, was obtained from Aldrich (Milwaukee, WI).

Columns. The column used in this study (Sunfire-C_{18}) was given by the manufacturer (Waters, Milford, MA). The tube dimension is 150 × 4.6 mm. The main characteristics of the packing material are summarized in Table 1. The column porosity was measured by pycnometry.

Apparatus. The perturbation signals and the overloaded band profiles were acquired using a Hewlett-Packard (now Agilent Technologies, Palo Alto, CA) HP 1100 liquid chromatograph. This instrument includes a multisolvent delivery system (volume of each tank, 1 L), an autosampler with a 250-μL sample loop, a UV−visible diode array detector, a column thermostat, and a data station. The extracolumn volumes are 0.044 and 0.845 mL, as measured from the autosampler and from the pump system, respectively, to the column inlet. All the retention data were corrected for these contributions. The flow rate accuracy was controlled by pumping the pure mobile phase at 295 K 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 within ±1 K.

Table 1. Physicochemical Properties of the RP-C_{18} Columns Provided by the Manufacturer (Waters)

	C18-Sunfire (Waters)
column dimension (mm × mm)	150 × 4.6
particle size (μm)	5
mesopore size (Å)	90
specific surface area (m^2/g)	349
bonding process	monomeric
carbon content (%)	17.52
surface coverage (μmol/m^2)	3.85
total porosity[b]	0.615
external porosity[a]	0.371
end-capping	yes

[b] Estimated from pycnometric measurements ($MeOH/CH_2Cl_2$). [a] Estimated from inverse size exclusion chromatography measurements (polystyrene/THF).

Precision of the FA Data. The accuracy of the measurements of the amount adsorbed, (i.e., the difference between the measured and the true values) is limited by the precision of the measurements of (1) the flow rate, F_v, delivered by the HPLC pump system (±0.4% for our HP1090 apparatus), (2) the volume of the column tube, V_C (according to the manufacturer, the relative standard deviation of the internal volume of these stainless steel tubes is ∼0.5% around the value corresponding to their average length, 150 mm, and inner diameter, 4.6 mm), and (3) the hold-up volume, V_M (according to the results of the pycnometic measurements, the error on V_M is ∼0.4%). The error made on the integral term in eq 2 is much less important and can be neglected. It depends on the precisions on the concentration, C_0, of the mother solution of the sample in the mobile phase and on the flow rate ratio of the streams of pure mobile phase and mother solution. The mother solution was prepared by weighing the sample (precision of the balance, ±0.000 05 g, lowest mass weighed, 0.5 g) and measuring the volume of mobile phase with a 100-mL volumetric flask (precision, ±0.05%). The error due to the flow rate mixer is less than 0.1%. Accordingly, an error calculation shows that the relative error made on the experimental values of q^* is less than 2.5%. Most importantly, this error is systematic and remains the same for all values of the concentrations of the streams used in a set of FA measurements. This fact is consistent with the smoothness of the plots of the experimental adsorption data that exhibit no noise over the whole range of concentrations.

Because the error made on the FA measurements is systematic and not random, the precision of the parameters of the adsorption isotherm model is always good. It varies between 1 and 10%. Hence, any variation of an isotherm parameter that is lesser than 10% from one temperature to another cannot be considered as significant. On the other hand, an increasing or a decreasing trend of this parameter with an amplitude that exceeds 10% over the temperature range studied (296−351 K) has definitely a physical meaning.

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The high degree of precision of the FA method was demonstrated recently.[19] It was shown that the random error made during a complete set of FA measurements is so small that selecting only 6 data points out of a set of 26 affects the numerical values of the isotherm parameters by less than a few percent if the selected data points cover the whole range of mobile-phase concentrations. This is due to the implementation of the systematic experimental protocol that we have established during the last four years.[6]

RESULTS AND DISCUSSION

In the following section, we assume that the retention of the analyte is based on its equilibrium distribution between a mobile and a stationary phase having volumes V_M and V_S, respectively. In the section on the Case of a Homogeneous Stationary Phase, the stationary phase is assumed to be a single, homogeneous phase while, in the section on the Case of a Heterogeneous Stationary Phase, it is assumed to consist in two immiscible homogeneous phases. The stationary phases are modeled as pure liquid octadecane in the first case or pure octadecane (phase 1) and an equimolar mixture of methanol and octadecane (phase 2) in the second case. Based on the thermodynamics of phase equilibrium between the mobile phase (modeled as methanol−water and acetonitrile−water mixtures) and the stationary phase, the retention factor k' of phenol is predicted from a theoretical point of view. The derivation of the classical Van't Hoff equation is based on the validity of such a distribution model. The suitability of the two models to account for the experimental results obtained in the case studied was tested by comparing the variations of the measured and the calculated retention factors as a function of the reciprocal temperature.

Finally, the experimental retention data of the analyte acquired by frontal analysis are analyzed in Interpretation of the Adsorption Isotherm Data without making any assumption. The adsorption mechanism of phenol is discussed based on the temperature-induced variations of the isotherm parameters (equilibrium constants and saturation capacities). This approach gives a better understanding of the complexity of the retention mechanism in RPLC than the mere measurement of the retention factors and consideration of their Van't Hoff plot.

Classical Linear Chromatography Approach and the Van't Hoff Plot. (1) Case of a Homogeneous Stationary Phase. The effect of temperature in analytical chromatography has been widely investigated. A popular interpretation of retention mechanisms consists of assuming thermodynamic equilibrium of the analyte i between the two phases of the chromatographic system. In RPLC, which uses mostly C_{18}-bonded silica phases, the stationary phase is a bonded layer similar to liquid octadecane. The polar mobile phase is an aqueous solution of either methanol or acetonitrile. By convention, the standard state of the analyte in either phase is the pure analyte, with a molar fraction equal to unity under normal pressure ($P^0 = 1$ bar). Its activity coefficients are unity. The activity coefficients at infinite dilution will be estimated using the UNIFAC group method calculations described in ref 22. The distribution equilibrium of compound i between the two phases is given by writing the equality of its chemical potentials μ_i in the two phases, hence:

$$\mu_i^S = \mu_i^M \leftrightarrow \mu_i^{0,*} + RT \ln \gamma_i^S x_i^S = \mu_i^{0,*} + RT \ln \gamma_i^M x_i^M \quad (4)$$

where the superscripts S and M denote the stationary and the mobile phases, respectively, T is the temperature, and x_i and γ_i are the molar fraction and the activity coefficient of the analyte i, respectively. Note that the chemical potential of the analyte i in its standard state is the same in both the stationary and the mobile phases (pure state marked by the asterisk $*$). In particular, at infinite dilution (i.e., under linear chromatography conditions), the equilibrium condition is written

$$\left(\frac{x_i^S}{x_i^M}\right)_{eq,\infty} = \frac{\gamma_i^{M,\infty}}{\gamma_i^{S,\infty}} \quad (5)$$

where γ_i^∞ is the activity coefficient of the analyte at infinite dilution. At infinite dilution, the molar concentrations of the analyte, C_i^S and C_i^M, are simply related to the molar fractions x_i^S and x_i^M, respectively, by

$$\left(\frac{x_i^S}{C_i^S}\right)_\infty = V_{S,m} \quad \text{and} \quad \left(\frac{x_i^M}{C_i^M}\right)_\infty = V_{M,m} \quad (6)$$

where $V_{S,m}$ and $V_{M,m}$ are the molar volume of the stationary and the mobile phases, respectively.

The retention factor, k', is the ratio of the amounts of solute in the stationary and the mobile phases[23] at infinite dilution:

$$k' = \frac{V_S}{V_M}\left(\frac{C_i^S}{C_i^M}\right)_{eq,\infty} = FH \quad (7)$$

where F is the phase ratio of the chromatographic system and V_S is the volume of octadecyl chains bonded to the surface of the silica. V_S can be easily calculated from the physicochemical properties of the packing material (Table 1). Note that the volume of the silica support should not be taken into account because it is both impermeable to the analyte and inert from a thermodynamic point of view. The Sunfire silica represents 77.3% of the overall weigh of the packing material. Assuming a density of silica equal to 2.12 g/cm^3 and that of the bonded layer equal to the density of liquid octadecane, 0.795 g/cm^3, 1 g of packing material is made of 0.36 cm^3 of silica and 0.29 cm^3 of hydrophobic layer. The volume of liquid phase V_M was measured by pycnometry and is equal to 1.53 cm^3. The volume of bonded phase V_S in the Sunfire-C_{18} column is then 0.43 cm^3.

In eq 7, H is the Henry's constant or simply the ratio of the analyte concentrations in the stationary and the mobile phases at equilibrium. Hence, the Van't Hoff plot equation

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can be written as

$$k' = F\frac{V_{M,m}}{V_{S,m}}\frac{\gamma_M^\infty}{\gamma_S^\infty} \quad (8)$$

It is now possible to test the thermodynamic consistency of the chromatographic results by plotting the following function as a function of the temperature:

$$\frac{\gamma_S^\infty}{\gamma_M^\infty}k' = \phi \quad (9)$$

with $\phi = (V_S/V_M)(V_{M,m}/V_{S,m}) = (N_S/N_M)$. Note that ϕ is simply the ratio of the number of moles of stationary phase, N_S (octadecane), to the number of moles of water, methanol, or acetonitrile, N_M, present in the column void volume. N_S is independent of the temperature, and N_M slightly decreases with increasing temperature since the molar volume of the liquid phase increases with increasing temperature.

Accordingly, under the assumption that equilibrium in the chromatographic system is viewed as the distribution of the analyte between two homogeneous and immiscible phases, one being the mobile phase and the other a liquid similar to octadecane, the left-hand side term in eq 9 should remain close to a constant over the temperature range investigated. This constant can be easily estimated from the characteristics of the column (surface coverage, specific surface area of the silica, mass of silica inside the column, void volume) and the molar volume of the liquid phases used. In the present case, the mass of silica in the column is 1.12 g, the specific surface of the neat silica is 349 m^2/g, and the surface coverage of the silica in C_{18} chains is 3.85 $\mu mol/m^2$. Then $N_S \approx 1.50$ mmol. The void volume of the column is 1.53 cm^3, and the molar volumes of the liquid mixtures CH_3OH/H_2O and CH_3CN/H_2O at 298 K are 20.4 and 22.8 cm^3/mol. Then, at most, $N_M \approx 75$ mmol. The expected value of ϕ is then ~0.02.

Figure 1 shows plots of the left-hand side term of eq 9 versus the temperature. The experimental retention factors k' were derived from the initial slope (Henry's constant H) of the measured adsorption isotherm (section on Interpretation of the Adsorption Isotherm Data) and the column phase ratio F. The activity coefficients were calculated according to the UNIFAC group method.[22] Although the agreement with the experimental results is not as good as one might wish, the main advantage of using the UNIFAC method is its wide range of application for vapor−liquid equilibria of nonelectrolytes mixtures. The typical difference between experimental and calculated values is 10%. The plots in Figure 1A and B are a test of the validity of the hypothesis of a distribution of the analyte between two immiscible liquids as a possible interpretation of the retention mechanism in RPLC. The results showed that such a model completely fails for two reasons: (1) the plot exhibits an obvious decreasing trend while the model predicts a constant or a slightly increasing trend, and (2) the functions vary between 1 and 25 while the model predicts a value 2−3 orders of magnitude smaller (0.02).

The linear model of retention appears to be too simplistic for the main reason that the adsorbed molecules of analyte may not be completely embedded within the octadecane phase. These molecules have not necessarily access to the whole volume V_S of the stationary phase. The analyte may also be simply adsorbed at the interface between the layer of octadecane chains and the mobile phase. In the next section, we will consider a more complex retention model, in which the analyte can be retained by interactions with two distinct stationary phases.

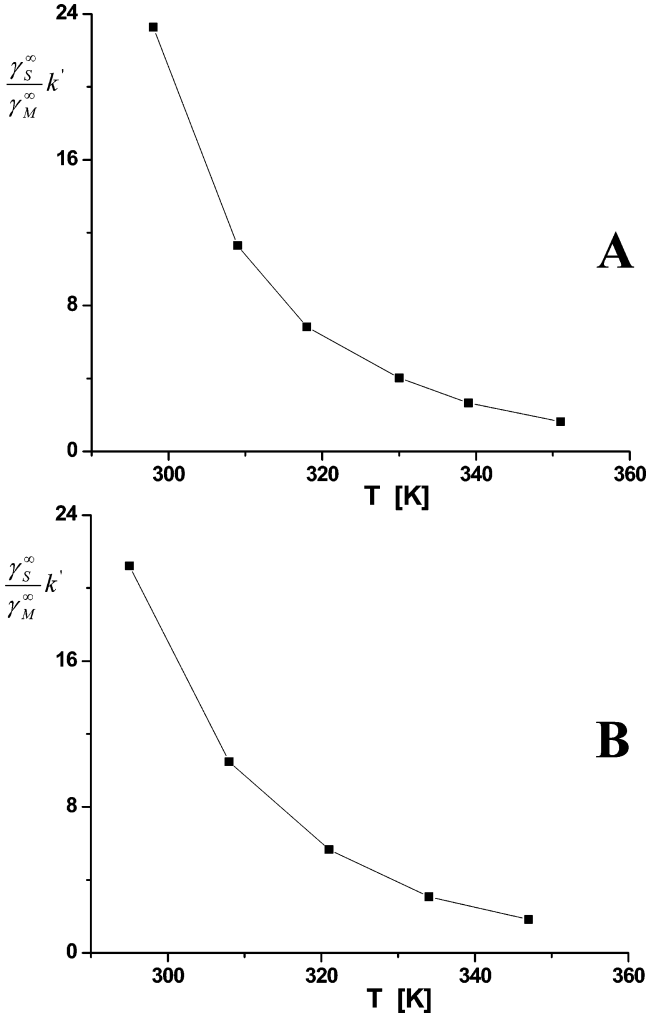

Figure 1. Plot of $(\gamma_i^{M,\infty}/\gamma_i^{S,\infty})K'$ versus the temperature T. The activity coefficients were calculated acording to the UNIFAC group method. Analyte phenol; Sunfire-C_{18} column with mixtures of (A) methanol and water (20:80, v/v) and (B) acetonitrile and water (15:85, v/v) as the mobile phase. Note the inconsistency of the experimental plots with the thermodynamic model given by eq 9.

(b) Case of a Heterogeneous Stationary Phase. Let assume now that the sample compound is distributed not between two but between three homogeneous liquids. One of them is the same mobile phase. Any possible source of mobile-phase heterogeneity will be ignored. However, there are now two distinct stationary phases, j, that act independently but are simultaneously in equilibrium with the mobile phase. Let $V_{S,1}$ and $V_{S,2}$ be the volumes of the stationary phases 1 and 2, respectively.

Consider that stationary phase 1 is similar to pure octadecane (meaning that the compound molecule becomes embedded in the stationary phase) and stationary phase 2 is a hypothetical mixture

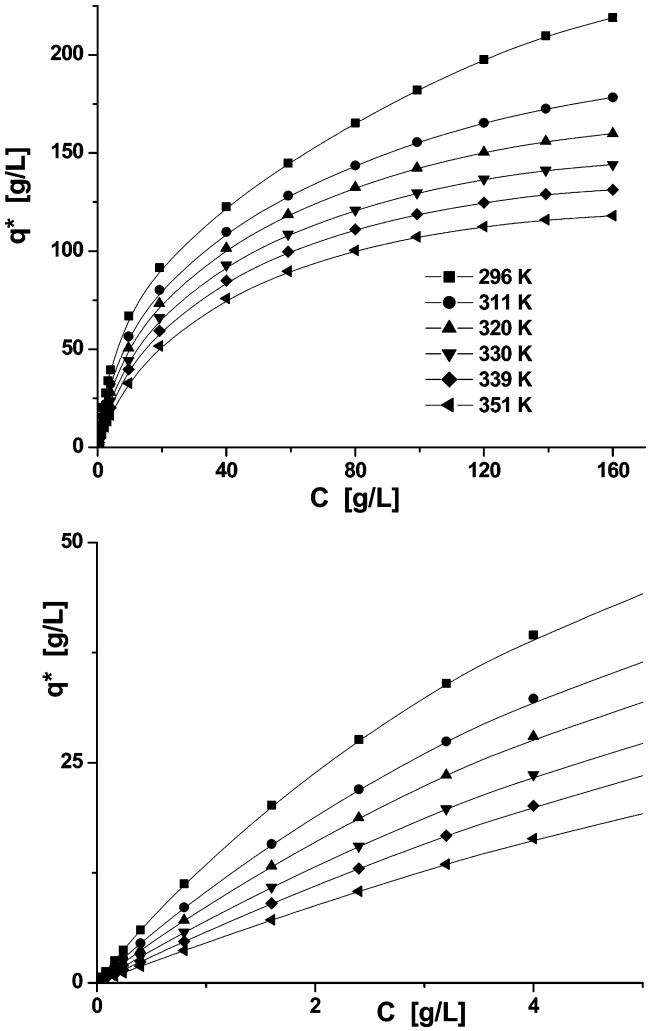

Figure 2. Experimental adsorption isotherms of phenol measured by FA on Sunfire-C_{18} with a mixture of methanol and water (20:80, v/v) as the mobile phase at six different temperatures. Note the important decrease of the saturation capacity of the column at high temperatures.

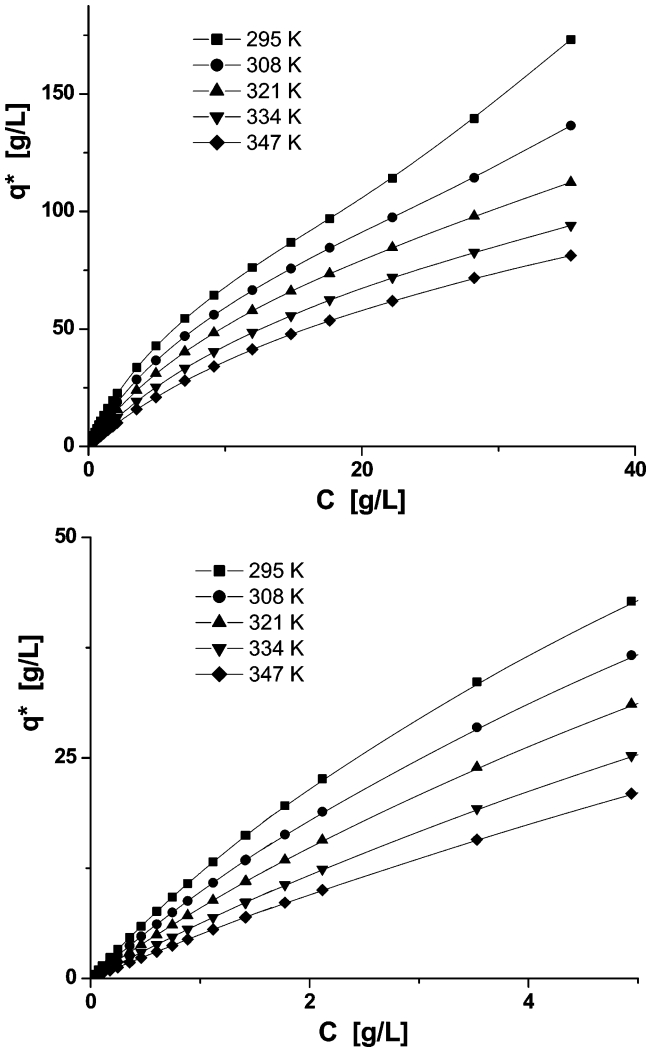

Figure 3. Same as in Figure 2, except the mobile phase is a mixture of acetonitrile and water (15:85, v/v) and the temperatures (five temperatures only). Note the "S" shape of the adsorption isotherms, especially visible at room temperature.

of the octadecane terminal methyl groups and methanol (representing the environment of the analyte molecules adsorbed at the free ends of the bonded alkyl chains, in the adsorbed layer of methanol). The stoechiometry of the mixture methyl groups−methanol is assumed to be 1:1.

The two equilibria are written

$$\left(\frac{x_i^{S,1}}{x_i^{M}}\right)_{eq.,\infty} = \frac{\gamma_i^{M,\infty}}{\gamma_i^{S,1,\infty}} \quad \text{and} \quad \left(\frac{x_i^{S,2}}{x_i^{M}}\right)_{eq,\infty} = \frac{\gamma_i^{M,\infty}}{\gamma_i^{S,2,\infty}} \qquad (10)$$

The retention factor becomes

$$k' = \frac{V_{S,1}\, C_i^{S,1} + V_{S,2}\, C_i^{S,2}}{V_M\, C_i^{M}} = F_1\left(\frac{C_i^{S,1}}{C_i^{M}}\right)_{eq,\infty} + F_2\left(\frac{C_i^{S,2}}{C_i^{M}}\right)_{eq,\infty} = F_1H_1 + F_2H_2 \qquad (11)$$

Introducing the molar fractions instead of the molar concentrations and using eq 10, eq 11 becomes

$$k' = \frac{N_{S,1}}{N_M}\frac{\gamma_i^{M,\infty}}{\gamma_i^{S,1,\infty}} + \frac{N_{S,2}\gamma_i^{M,\infty}}{N_M\gamma_i^{S,2,\infty}} \qquad (12)$$

where $N_{S,1}$ and $N_{S,2}$ are the number of moles of stationary-phase molecules in the phases 1 and 2, respectively. In eq 12, the activity coefficients are fixed and can be easily estimated by applying the UNIFAC method to each one of the three phases at the different temperatures considered. The number of moles of solvent in the liquid phase (an aqueous solution of methanol or acetonitrile) was calculated as mentionned (~75 mmol). k' was measured experimentally.

To fit the experimental data to eq 12, we assumed that the quantities $N_{S,1}$ and $N_{S,2}$ are unknown but remain constant, independently of the temperature. This fit was unsuccessful, the fitting procedure ending up with the best estimate for one parameter being devoid of any physical significance ($N_{S,1} < 0$) and a poor regression coefficient ($R = 0.80$).

Table 2. Isotherm Parameters of Phenol on the Sunfire-C18 Column at Different Temperatures with a Mixture of Methanol and Water (20:80, v/v) as the Liquid Phase

	T (K)					
	295	311	320	330	339	351
$q_{S,1}$ (mol/L)	3.88 (3.92)[a]	2.02 (2.02)	1.63 (1.62)	1.40 (1.39)	1.21 (1.17)	1.11 (1.28)
b_1 (L/moL)	0.339 (0.337)	0.757 (0.750)	1.08 (1.03)	1.29 (1.28)	1.46 (1.33)	1.62 (1.92)
$q_{S,2}$ (mol/L)	0.95 (0.95)	0.81 (0.81)	0.69 (0.71)	0.62 (0.63)	0.58 (0.64)	0.50 (0.30)
b_2 (L/moL)	14.0 (14.5)	12.5 (12.4)	11.4 (11.1)	9.42 (9.38)	7.53 (7.21)	5.98 (7.74)
$q_{S,3}$ (mmol/L)	14.3 (4.4)	0.29 (0.43)	0 (0.06)	0 (0)	0 (0)	0 (0)
b_3 (L/mmoL)	0.109 (0.281)	2.44 (1.14)	/ (?)	/ (/)	/ (/)	/ (/)
Henry's constant	16.2 (16.3)	12.4 (12.1)	9.63 (9.55)	7.65 (7.69)	6.13 (6.17)	4.79 (4.78)
k'	10.2	7.76	6.03	4.79	3.84	3.00

[a] The values in parenthesis are derived from the AED calculation.

The retention model of linear chromatography that is expressed by eq 12 might make better physical sense than the one given by eq 9, but it still does not fit properly to the experimental data. This means that a simple partition model between two or more phases is unrealistic. This challenges the actual meaning of the Van't Hoff plot, which is abundantly applied in liquid chromatography and which assumes a partition equilibrium between two phases characterized by a molar enthalpy and a molar entropy of transfer, $\Delta H°$ and $\Delta S°$, between the two standard states. Despite the fact that linear Van't Hoff plots are often encountered in RPLC, this does not imply that they should necessarily be accounted for a partition mechanism between a polar mobile liquid phase and a hydrophobic stationary liquid phase.

The results reported in this section show that the retention mechanism in RPLC is far more complex than usually believed. This originates from the complexity of the structure of the alkyl bonded stationary phase used and from the nature of the interface of RPLC systems. Any significant variation of the temperature, the mobile-phase composition, and the pressure affects, sometimes drastically, the retention mechanism. The effects of these variations cannot be accounted for based on mere thermodynamics considerations regarding the distribution of the analyte between the mobile and the stationary phases. One way to shed light on the retention mechanisms in RPLC is to accumulate a set of adsorption data and to study empirically the evolution of the adsorption isotherm parameters (q_S and b) with, for example, the temperature, as done in this work. The advantage of this strategy is that no assumption is made and the experimental results are clear, sound, and precise.

The acquisition of retention factors k' in a temperature range is insufficient to reveal the adsorption properties of an analyte in RPLC. Linear chromatography does not provide enough information on the heterogeneity of adsorbent surfaces nor does it afford straightforward conclusions regarding the temperature dependence of the thermodynamic properties of adsorption.

Another limitation of linear chromatography that is encountered even when a truly homogeneous adsorbent is used is that this method does not distinguish between the contributions to the retention factor of the saturation capacity, $q_{S,i}$ (or amount of an analyte forming a monolayer on the surface) and the adsorption−desorption equilibrium constant b_i. In the case of an homogeneous surface (Langmuir adsorption isotherm) we can write

$$k' = FH = Fq_S b \tag{13}$$

and, more generally, for an heterogeneous surface (case of N-Langmuir adsorption isotherms)

$$k' = F \times \sum_{i=1}^{i=N} (q_{S,i} b_i) \tag{14}$$

More experimental data are then needed to provide convincing evidence regarding the degree of heterogeneity of adsorbent surfaces. By measuring retention data in a wide concentration range, we can assess the number of the types of adsorption sites present on the surface, derive their equilibrium constants, and their contributions to the Henry's constant, $H_i = q_{S,i} b_i$.

Interpretation of the Adsorption Isotherm Data. The adsorption isotherms of phenol were measured under the same experimental conditions as those used in linear chromatography. They were measured for phenol concentrations between 0 and 160 g/L and between 0 and 35 g/L with methanol and acetonitrile, respectively (the solubility is much lower in acetonitrile than in methanol). Figures 2 and 3 show the results. The isotherm data in water−methanol were fitted to a tri-Langmuir isotherm model, a model consistent with the shape of the isotherm data and with the adsorption energy distribution derived from the isotherm data. Figure 3 shows that, with acetonitrile, the isotherm is S-shaped (convex upward at low and downward at high concentrations). The data were modeled with a BET-Langmuir isotherm. This result was reported earlier, with other C_{18}-bonded columns.[13] The Langmuir term accounts for adsorption of the compound on the high-energy sites within the C_{18}-bonded layer, the BET term for the accumulation of the analyte in the multilayer of acetonitrile (3−5 monolayers thick[13]) at the top of the C_{18}-bonded chains.

(a) Adsorption of Phenol from Methanol−Water (20:80, v/v). The best tri-Langmuir isotherm parameters are listed in Table 2. These values are compared to those derived from the AEDs, shown in Figure 4. A most interesting result of the AED calculations is to show that there are three types of adsorption sites at ambient temperature but only two at high temperatures. This is confirmed by the results of the regression analysis of the isotherm data. It becomes impossible to calculate a set of parameters for a tri-Langmuir isotherm model at temperatures

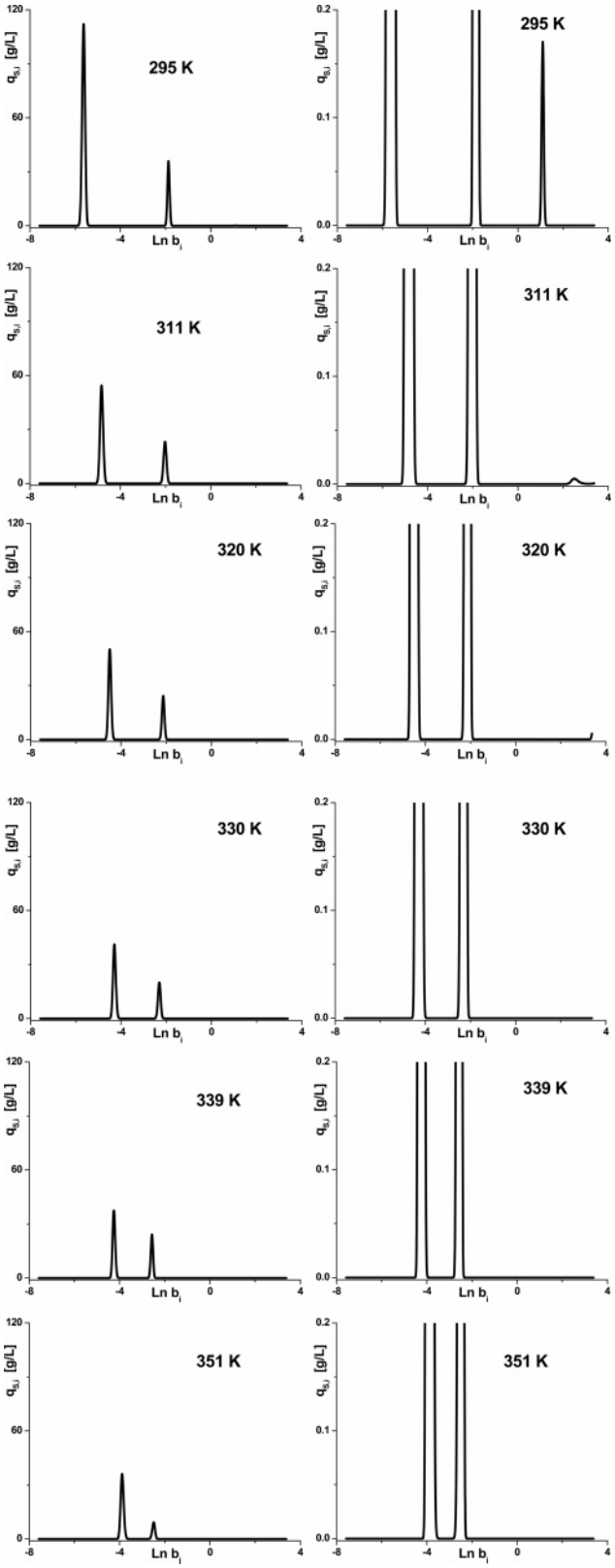

Figure 4. AED showing the logarithm of the adsorption−desorption constant of phenol (mobile phase, methanol−water, 20:80, v/v) on Sunfire-C_{18} versus the number of sites *i*. The results are shown for the six temperatures given in Figure 2. The right-side graphs are enlargements of the left-side ones that illustrate the existence of high-energy sites with very low saturation capacities $q_{S,i}$. Note the decrease of the column heterogeneity as well as the decrease of the difference between the adsorption energies on sites 1 and 2 ($RT \ln(b_2/b_1)$) with increasing temperature.

beyond 311 K. This suggests that access to adsorption sites deep within the bonded layer is affected by the temperature. The sites of type 3 are no longer accessible or disappear (may be morphing into type 2 sites?) when the mobility of the C_{18} chains increases.

The evolutions of the isotherm parameters ($q_{S,1}$, $q_{S,2}$, b_1, b_2) with increasing temperature are shown in Figure 5. Figure 6 compares the contributions of each type of sites to the overall Henry's constant of phenol. It is clear that the variation of the retention of phenol with temperature is controlled by its adsorption on the sites of type 2. The contribution of the sites of type 1 to the retention of phenol is almost independent of the temperature. Most interesting are the differences between the behavior of the sites of types 1 and 2. The saturation capacity $q_{S,1}$ decreases by a factor of nearly 3.5 while the adsorption−desorption constant b_1 increases by a factor of 4.5 when the temperature increases from 295 to 351 K. It is unusual to observe an equilibrium constant that increases with increasing temperature. Actually, the classical equation

$$\ln b_i = \ln b_0 + \frac{\epsilon_{a,i}}{RT} \qquad (15)$$

that describes the dependence of this constant on the temperature[24] no longer applies because the adsorption energy $\epsilon_{a,1}$ is not constant over the temperature range investigated. This may be due to the mobility and the organization of the extremities of the C_{18} chains at the interface with the bulk mobile phase increasing with increasing temperature. It is likely that this provides an increasing surface area of contact for adsorbate molecules. As for the sites of type 2, their saturation capacity decreases by a factor of nearly 2 and their adsorption−desorption constant by a factor of 2.5 in the same temperature range (295−351 K). This explains why these sites control the temperature dependence of the retention of phenol, not the sites of type 1. Because the sites of type 2 are probably buried within the hydrophobic layer, their surface area of contact with the adsorbate and their adsorption energy does not vary much with temperature. Equation 15 applies well to these sites and the adsorption energy derived from it, $\epsilon_{a,2}$, is ~12.6 kJ/mol, a value typical of those that we have found with several other columns for this type of adsorption sites.[7,11,12] However, we must keep in mind that the preexponential factor, b_0, which describes the molecular partition function for the internal degrees of freedom of isolated adsorbate and solute molecules, is assumed to be temperature independent.

It is noteworthy that the higher the temperature, the more homogeneous the Sunfire-C_{18} adsorbent. This effect results from the simultaneous decreases of the number of types of adsorption sites (which drops from 3 to 2) and of the difference between the adsorption energies of the sites of types 2 and 1 (which drops from 9.1 to 3.8 kJ/mol). This result agrees with the AED plots shown in Figure 4, in which the distance between the corresponding two modes diminishes with increasing temperature. However, the two modes are always well resolved, and even at 351 K, the two distinct adsorption modes are clearly observed.

Finally, the total saturation capacity of the Sunfire column decreases by a factor 3 in the temperature range studied,

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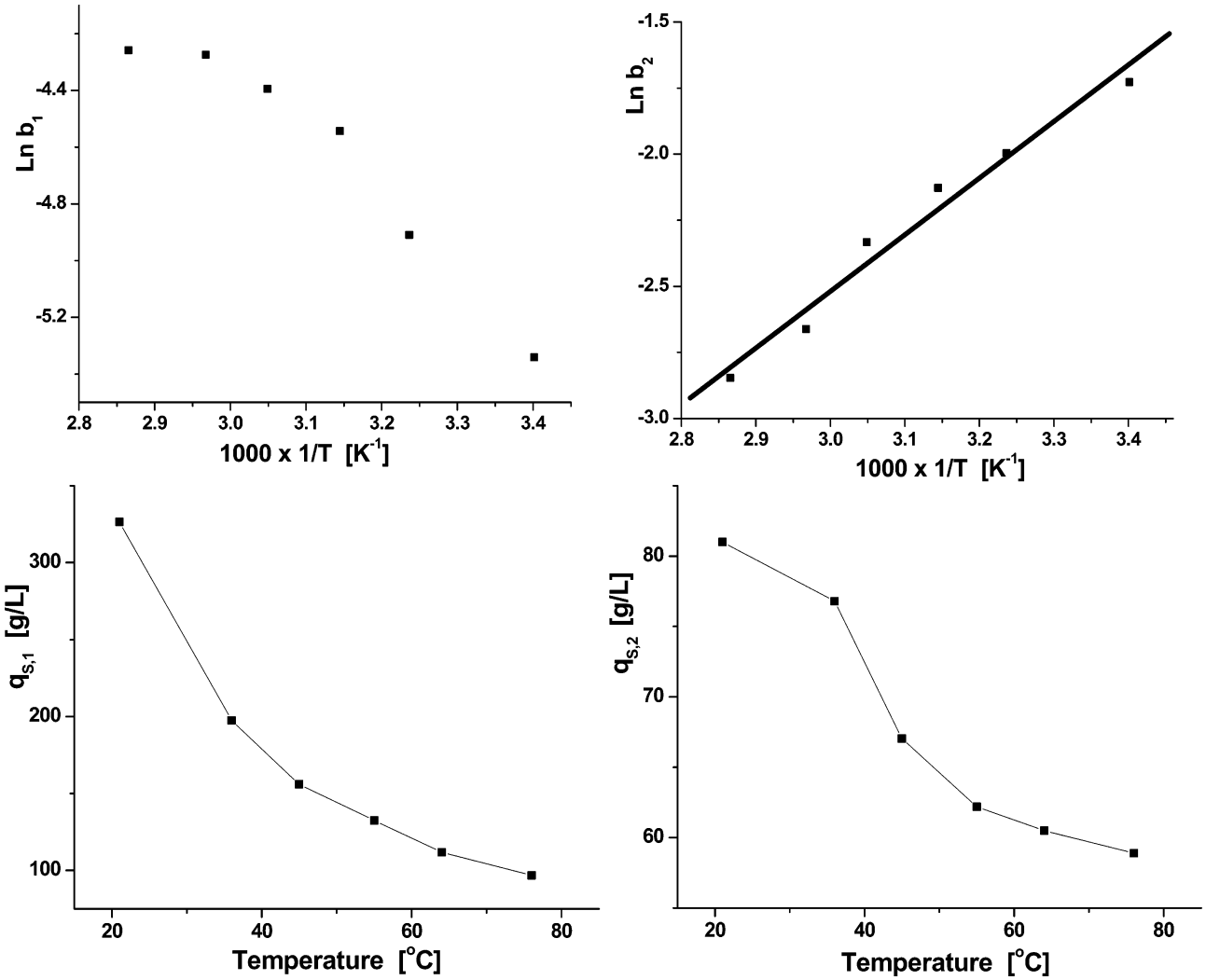

Figure 5. Variation with temperature of the isotherm parameters. Sites 1 and 2 relate to the adsorption of phenol on Sunfire-C_{18}. Mobile phase: methanol−water mixture (20:80, v/v). (Top) Equilibrium constants. (Bottom) Saturation capacities.

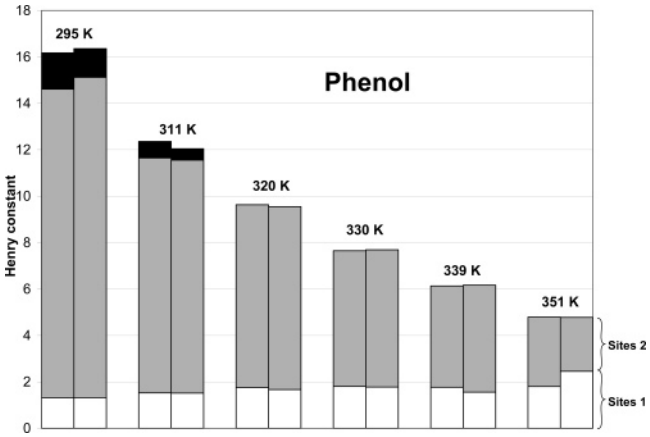

Figure 6. Contributions to the overall Henry's constant of phenol based on the fit of the adsorption data (e.g., $H_i = q_{S,i}b_i$) versus the temperature.

confirming the result observed previously with a Symmetry-C_{18} column.[15] This is an important result for preparative chromatography, a field of application in which a high column capacity is useful. Although a high column temperature would not be helpful from this viewpoint, the negative effect of a loss in saturation capacity is compensated by a decrease in the degree of heterogeneity of the column and a marked increase in the solubility of the feed components in the mobile phase.

Table 3. Isotherm Parameters of Phenol on the Sunfire-C_{18} Column at Different Temperatures with a Mixture of Acetonitrile and Water (15:85, v/v) as the Liquid Phase

	T (K)				
	295	308	321	334	347
$q_{S,1}$ (mol/L)	1.01	1.01	1.00	0.99	0.99
$b_{S,1}$ (L/moL)	12.1	8.58	5.57	2.30	1.03
$b_{L,1}$ (mol/L)	1.34	1.03	0.74	0.46	0.34
$q_{S,2}$ (mol/L)	0.017	0.073	0.194	0.51	0.78
b_2 (L/moL)	101.0	31.2	15.9	8.4	5.4
Henry's constant	13.9	10.9	8.65	6.56	5.23
k'	8.7	6.8	5.42	4.11	3.27

(b) Adsorption of Phenol from Acetonitrile−Water (15:85, v/v). The adsorption isotherm data of phenol from the aqueous solution of acetonitrile are shown in Figure 3 at five different temperatures. The best BET-Langmuir isotherm parameters are listed in Table 3. These isotherms are initially convex downward, exhibiting this tell-tale sign of significant adsorbate−adsorbate interactions. Thus, it was not possible to calculate the

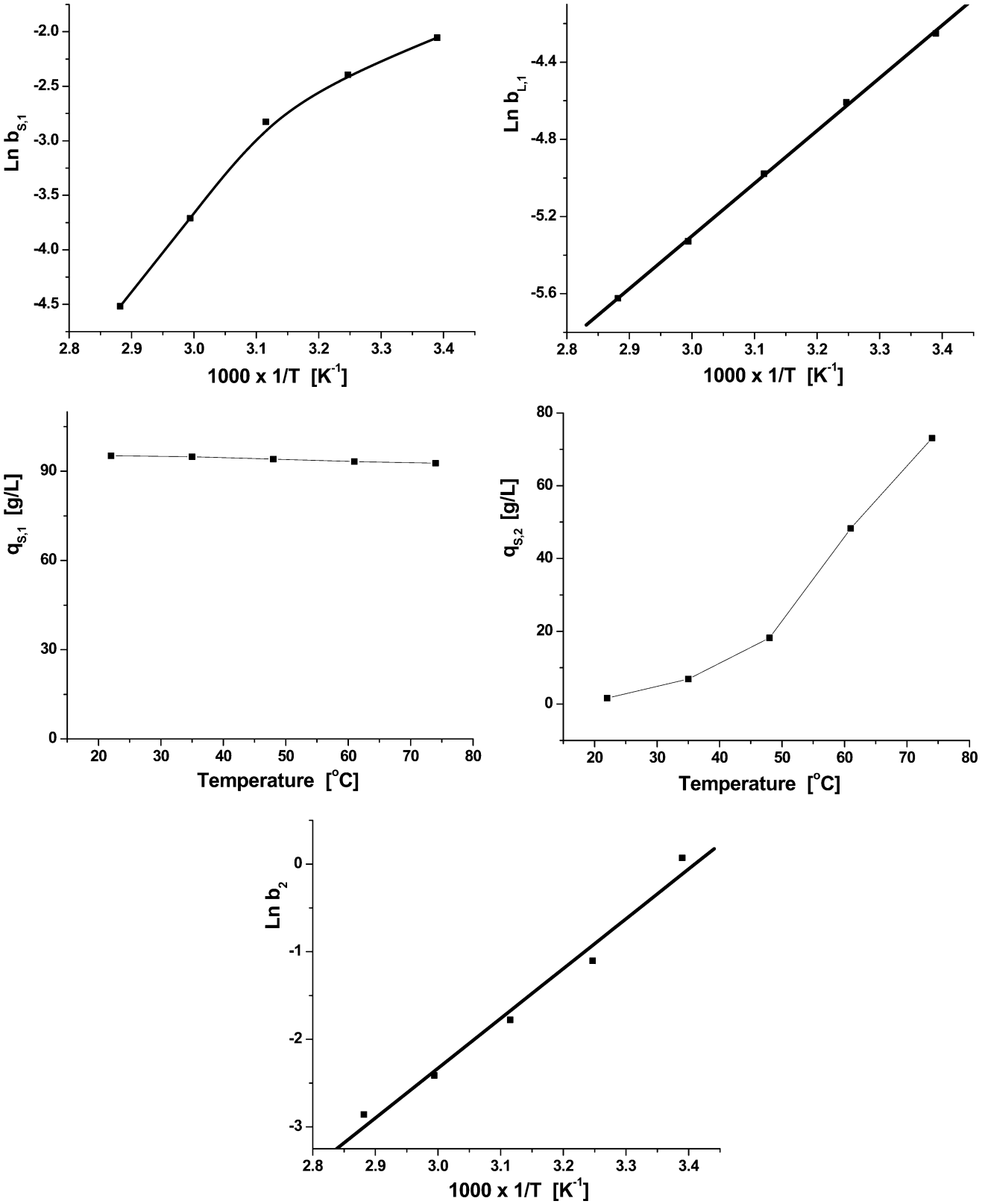

Figure 7. Same as in Figure 5, except the mobile phase, a mixture of acetonitrile and water (15:85, v/v). The description of each parameter of the BET-Langmuir isotherm is given in the text.

AEDs in this case since the EM program assumes local Langmuir isotherm. Any sum of Langmuir isotherms cannot generate an S-shaped isotherm similar to those observed in Figure 3. As a result, the following conclusions are based on the sole regression analysis of the adsorption data, without independent confirmation.

It was previously shown[13] that acetonitrile forms an adsorbed multilayer system on C_{18}-bonded phases. Solutes such as phenol can dissolve and accumulate as a multilayer system forming a "second" stationary phase. Acetonitrile cannot interact strongly with the hydroxyl group of phenol through hydrogen-bonding

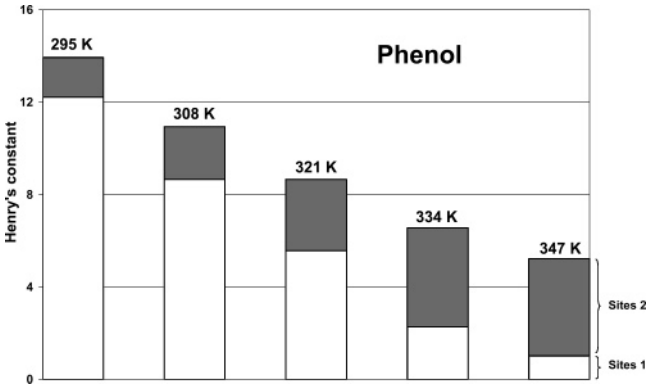

Figure 8. Same as in Figure 6, except the mobile phase, a mixture of acetonitrile and water (15:85, v/v).

interactions, like methanol does. As a result, phenol−phenol hydrogen-bonding interactions take place in the acetonitrile layer, allowing the formation of an adsorbed multilayer of phenol molecules on the surface of the C_{18} adsorbent.

The monolayer saturation capacity on the sites of type 1 is barely affected by the temperature, and in contrast with the methanol case, the adsorption−desorption constant of phenol on the surface of the C_{18}-bonded layer decreases continuously with increasing temperature (Figure 7). However, the plot of ln $b_{S,1}$ versus $1/T$ is not linear and eq 15 does not apply. It seems that the adsorption energy is larger at high than at low temperatures. On the other hand, the adsorption−desorption constant of phenol on a monolayer of adsorbate molecules follows eq 3. Note that this plot is linear if both the adsorption energy and the factor b_0 are considered to be temperature independent, which is theoretically incorrect.[24] The slope of this plot would give an adsorption energy of 22.7 kJ/mol, a value that is surprisingly large.

Figure 3 clearly shows that the concentration of the inflection point of the isotherm increases with increasing temperature. The isotherm tends progressively to become Langmuirian (in this case, it tends toward a bi-Langmuir isotherm), and the role played by the adsorption sites of type 2 becomes more important. The saturation capacity $q_{S,2}$ increases significantly with increasing temperature while the adsorption−desorption constant b_2 decreases.

Figure 8 shows the variations of the Henry's constant of phenol with increasing temperature. Note how the role played by the sites of types 1 and 2 is different whether methanol or acetonitrile is used as the organic modifier. Although the overall retention factor decreases with increasing temperature in both cases, the contribution of the sites of type 1 decreases and that of the sites of type 2 increases when acetonitrile replaces methanol.

Comparison between the Results of Linear and Nonlinear Chromatography. The results derived from linear chromatography and the Van't Hoff equation would suggest that phenol does partition between the mobile phase and a homogeneous liquid stationary phase. Its equilibrium between these two phases as a simple liquid−liquid equilibrium and the associated variations of the enthalpy and the entropy would correspond to the transfer of 1 mol of phenol between the two immiscible phases, the polar mobile phase and the octadecyl layer. As demonstrated in earlier, it is unrealistic, however, to consider the surface of a C_{18} bonded porous silica adsorbent as a homogeneous stationary phase, akin to liquid octadecane, for example. The octadecyl chains are not free but bonded to the silica surface, which reduces considerably their mobility and modifies drastically their organization and structure. Furthermore, the complex nature of the C_{18} bonded layer makes possible for the analyte molecules to adsorb onto some patches of exposed bare silica or to bury themselves in the bonded layer, as well as to adsorb at the interface between these alkyl chains and the mobile phase. These facts render implausible the assumptions underlying the Van't Hoff equation.

In contrast, the results of the measurements carried out at high concentrations demonstrate the complexity of the equilibrium isotherm. This complexity can be explained only by the heterogeneity of the surface of the C_{18}-bonded adsorbent used. The complexity of the temperature dependence of the parameters of the equilibrium isotherm suggests that each one of the retention equilibria identified earlier is not as simple as the analogy with the adsorption at the planar interface between a conventional solid adsorbent and a liquid would suggest. The environment of the adsorbate varies from place to place on the surface, and depending on the solute considered and the brand of RPLC adsorbent investigated, up to four different types of adsorption sites have been found, with adsorption energies ranging from a few to more than 20 kJ/mol.[6] Also, the environment of a given site seems to depend on the temperature. So, the number of adsorption sites of each type seems to depend on the temperature, the sites of certain types morphing into those of another one, due to the reorganization of the bonded C_{18} chains. In the process, the adsorption energy on the sites of certain types also changes and the effect is different for the different sites.

These effects are illustrated in Figures 6 and 8 that show plots versus the temperature of the contributions of the two different types of sites identified for phenol on Sunfire to the Henry constant. These results are not surprising. The number of adsorption sites and the environment between the C_{18} chains, hence the interactions between solute molecules and C_{18} chains, change continuously with increasing temperature. It is impossible to assume that the nature and density of the different patches remain constant when the temperature varies. What is surprising is that the values measured for the overall retention factor fit so well to the van't Hoff equation.

CONCLUSION

This work demonstrates that the problem of deriving thermodynamic parameters related to the transfer of the solute between the liquid and the stationary phase in RPLC has no satisfactory solution. The C_{18}-bonded layer cannot be considered as a conventional adsorbent, defined as a thermodynamic phase. The adsorption behavior of low-molecular-weight compounds is too complex, the adsorbent surface is heterogeneous, the structure of the hydrophobic layer is very flexible, and the arrangement of the C_{18} chains change with the temperature, so retention factors cannot be related to a single distribution constant K between the mobile and the stationary phases. Thus, the classical C_{18}-bonded phase cannot be considered as equivalent to either liquid octadecane or a simple solid surface and retention in RPLC cannot be accounted for by one distribution factor between octadecane and the aqueous solution of an organic modifier.

As a result, it seems that the linear or quasi-linear behavior of the Van't Hoff plot, which is generally observed, should be

considered as accidental. The overall retention factor of solutes measured in linear chromatography is actually the sum of the contributions of different retention mechanisms, i.e., of adsorption on different types of sites. While retention increases on one type of sites with increasing temperature, it decreases more strongly on another type of sites and the compensation between these two effects results in the overall retention factor decreasing with increasing temperature, in agreement with Van't Hoff law (or at least approximately so; see Figure 1). The complexity of RPLC adsorption is further illustrated by the large difference between the temperature effects on the adsorption behavior of phenol that is observed in aqueous solutions of methanol or acetonitrile. With methanol, the contributions of the low- and the high-energy sites to the overall retention increase and decrease, respectively, with increasing temperature. The converse is observed with acetonitrile. This suggests that the temperature effect on the mobility and structure of the C_{18} chains is different in methanol and in acetonitrile. It was already known that these two solvents adsorb very differently on C_{18}-bonded silicas. Our results show that these differences affect the structure of the bonded hydrophobic layer, its dynamic, and the accessibility of the adsorption sites by the analyte molecules.

This work also raises questions on the precision and the accuracy of the adsorption data measured by FA and on their interpretation. The accuracy of FA is well established. We measured and applied the necessary corrections (i.e., for the contribution of the extracolumn volume). The precision of the measurements has been established earlier. It is confirmed by the high degree of self-consistency of the data (see Figures 2 and 3). The modeling of the data leaves a small sum of residuals. Obviously, the precision of the parameters of the two Langmuir sites is less than that of the data, but the validity of the isotherms is confirmed by the agreement (not shown) between the calculated and experimental high-concentration band profiles. The interpretation of the results by the complex structure of the interface between the bulk liquid phase and the RPLC adsorbent and its high sensitivity to changes in the temperature or the mobile-phase composition is highly plausible. Yet, it might also be based on an incorrect extrapolation of an approach that was shown to predict accurately the overloaded band profiles[2]. Thus, independent verifications of the validity of our method and of the present results must be obtained. We plan to apply FA and model adsorption data obtained for more classical solid−liquid systems for which a solid surface is directly in contact with the liquid phase, without the fuzzy layer of the bonded alkyl chains.

Finally, our results show that increasing the column temperature tends to render the surface of C_{18}-bonded silicas more homogeneous. The difference between the adsorption energies on the sites of types 1 and 2 (those that have the lowest two adsorption energy) decreases significantly with increasing temperature. Thus, increasing the column temperature improves the column efficiency by accelerating mass transfers and also by reducing the degree of peak tailing. This conclusion is consistent with recent results obtained by NMR spectroscopy, which have shown the same effect of the temperature on the conformation and mobility of the bonded alkyl chains.[25,26]

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