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Determination of the thermodynamic contribution to peak asymmetry of basic solutes in reversed-phase liquid chromatography

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Abstract

To this day packing materials manufacturers are still trying to develop reversed-phase stationary phases that have silica more completely reacted with bonding ligands to afford more homogeneous particle surfaces. Incomplete bonding causes inhomogeneous effects that are readily observed when separating basic solutes because of the acidic silanols that are unreacted. However, it is still not understood exactly what types of silanol sites are unreacted or if metal impurities are contributing to the resulting peak asymmetry observed. A method is presented which utilizes (1) the frontal analysis method of chromatography to obtain adsorption/partition isotherms, (2) a heterogeneous Langmuir distribution model for the resulting isotherm, (3) an expectation-maximization numerical procedure to solve the mathematical problem to yield the most probable distribution of adsorption parameters, and (4) the equilibrium–dispersive model of chromatography incorporating the fitted isotherm model to check the validity of the sorption model with experimental observations. Correlation of packing materials characterization parameters with results obtained by this procedure will indicate what type of silanols or other sites are responsible for observed tailing behavior. Developers and manufacturers will then be able to more efficiently target their synthetic designs for ''base-deactivated'' reversed-phase silicas.

Keywords: Thermodynamic parameters; Peak shape; Adsorption isotherms; Stationary phases, LC

1. Introduction

The chemical modification of silica to afford reversed-phase stationary phases in high-performance liquid chromatography (HPLC) has proceeded well since the 1970s, especially by the leading manufacturers of these products. However, it is well known and accepted that the chemical derivatization reactions are not performed at 100% yield, and that a significant portion of silanol sites are left unreacted due to problems such as steric hindrance in the case of octadecylsilanated silica, the most popular of the reversed phases.

It is also well known and accepted in HPLC that the band profiles recorded in an elution chromatogram are not perfectly Gaussian in shape as predicted by theory. Possible reasons for nonideality can be hydrodynamic or chemical in nature. Hydrodynamic asymmetry can result from poorly packed columns yielding non-uniform flow distributions of mobile phase across the packed bed [1–4]. Chemical reasons can result from mass transfer limitations (kinetic effects) and from the inhomogeneous surface chemistry that results from incomplete derivatization reactions.

An inhomogeneous surface chemistry may be more dispersed than a simple binary model of reacted and unreacted (e.g., nonpolar and polar) sites because it is well documented in the literature that silanol sites may exist in different forms: isolated,

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vicinal and geminal [5]. In addition to these silanol sites are siloxane sites and a myriad of possible metal contamination sites that may be ''active'' due to complexation reactions with various solutes. This distribution of stationary phase interaction sites (see Fig. 1) can be accompanied by a distribution of thermodynamic interaction energies or partition coefficients, dependent on the specific system studied. The result of band tailing is often attributed to these reasons, however the specific types and quantity of these sites that give rise to the observed behavior has proven to be information fairly difficult to acquire and validate.

The separation of basic solutes by reversed-phase HPLC is often plagued by poor peak asymmetry [6,7]. This is presumed to be due to an ionic interaction between the deprotonated, negatively-charged acidic silanol sites and the protonated, positively-charged basic compounds at issue. Although experimental techniques exist to deal with this problem and reduce asymmetry [8], it would be of interest to packing manufacturers to know what chemical types of active sites are causing the observed peak tailing, and how many of these sites exist.

Deconvoluting the effects of parallel, active sites in reversed-phase HPLC has proceeded with ^{29}Si cross polarized magic angle spinning nuclear magnetic resonance (NMR), Fourier transform (FT) IR, and other experiments [7], although verification of the resulting distribution of silanol sites giving rise to the observed peak asymmetry is typically incomplete.

In this contribution, a chromatographic method of determining the reversed-phase thermodynamic sorption heterogeneity is presented. It is based on determining the sorption isotherm of the basic solute over several orders of magnitude in concentration using the frontal analysis method. The isotherm is modeled by an extension of the Langmuir equation, in which the ''*b*'' parameter is allowed to simultaneously exist across a range of values. Solution of the resultant integral equation yields a plot of the saturation capacity vs. the *b* parameter. Thus the quantity of sites at each central *b* value is determined as well as the range of existing values. This model is the main contribution of the report, because it allows examination of the different sorption partition coefficients and their relative strengths and populations.

To validate this model, it is incorporated into the equilibrium–dispersive model of chromatography.

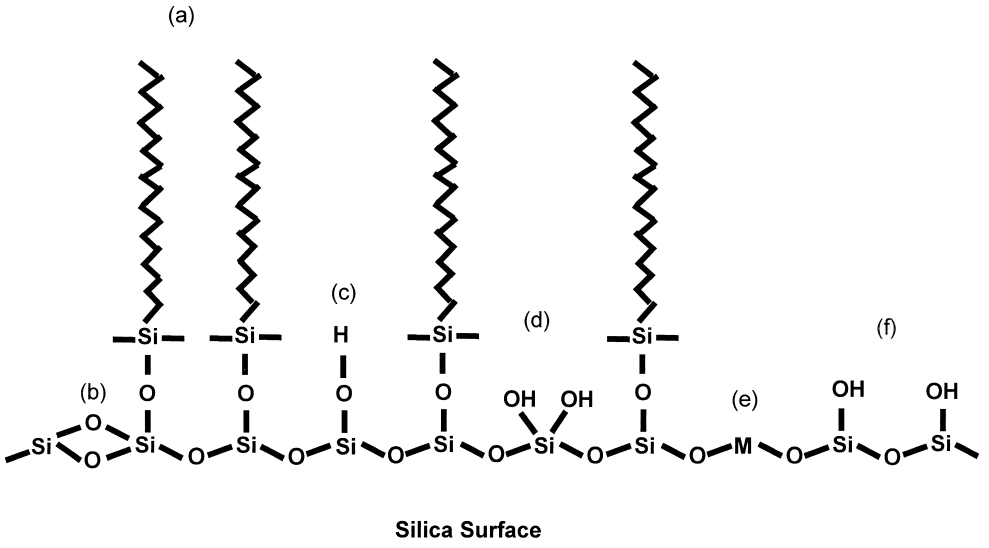

Fig. 1. Types of active sites on a C_{18}-modified silica surface: (a) dimethyloctadecylsilane ligands, (b) siloxane bridges, (c) isolated silanols, (d) geminal silanols, (e) metal impurities, and (f) vicinal silanols. Representation is exaggerated for clarity. The silanol sites for most reversed phases have been at least 70% deactivated by alkane ligands. Obtaining the distribution of remaining active sites is the goal of the method presented in this work.

With this model, the sorption isotherm dictates the shape of the resulting band profile. Kinetic effects are assumed to be negligible. Validation occurs if the predicted chromatogram agrees with the experimentally observed one, using this Langmuir distribution isotherm model.

Future contributions will show the correlation of the Langmuir distributions introduced here with silica characterization figures such as bonded-phase coverage and metal content, mobile phase parameters such as methanol–water ratio and pH, and of course the chemical properties of the solute. In this paper, the methodology is described, and is supported with the results from one system to illustrate the potential of the method. A few remarks concerning the reproducibility of the results obtained from similar chromatographic systems is also offered.

2. Theory

2.1. Isotherm determination

The frontal analysis (FA) method of isotherm determination was used in this study. It has been extensively described in the literature and will not be reviewed here [9]. It is based on the elution volume of increasing steps of solute concentration, and the amount sorbed on the stationary phase at step i is calculated from the amount adsorbed at the previous step and the difference in equilibrium mobile phase concentration between those two steps:

$$q_i = q_{i-1} + (C_i - C_{i-1})(V_{F,i} - V_0)/V_a \quad (1)$$

where q is the equilibrium concentration of solute in the stationary phase, C is the equilibrium concentration of solute in the mobile phase, V_F is the breakthrough volume of the solute, V_0 is the breakthrough volume of the mobile phase, and V_a is the volume of the adsorbent or stationary phase.

2.2. Isotherm model

The isotherm model used in this study is the ubiquitously successful Langmuir model:

$$q = \frac{aC}{1 + bC} \quad (2)$$

where a is a numerical coefficient that describes the initial slope of the isotherm. b is a numerical coefficient that serves as an empirical thermodynamic binding constant [9], exponentially related to the sorption energy.

The Langmuir model has been very successful in describing the sorption of numerous solutes on reversed-phase chromatographic stationary phases [10]. However, it only describes sorption onto one type of binding ''site'', and is thus not suitable for a study of sorption heterogeneity. Different sites are accommodated by summing additional terms, for example the bi-Langmuir model has enjoyed much success in the literature [10,11].

A true study of heterogeneity, however, cannot assume one, two, or any integer number of discrete sites, because a limit and bias is placed on the solution of the problem by imposing a degree of heterogeneity beforehand [12]. To circumvent this problem, the Langmuir model may be expressed as an integral sum over an appropriate range of b values. In this way, the data itself determines the degree of heterogeneity. To express this Langmuir distribution model, the saturation capacity, q_s, for each b is used [10]:

$$q_s = a/b \quad (3)$$

The saturation capacity is valid in the Langmuir model, because this model assumes that only monolayer coverage occurs and that there are no solute–solute interactions. This results in a convex upwards shaped isotherm. At high solute concentrations this assumption often breaks down, and concavely-shaped isotherms can result, at which point the model is no longer valid, and the saturation capacity can no longer be calculated.

The Langmuir distribution model calculates the saturation capacity at each possible b value in a distributed b parameter space with a Fredholm integral of the first kind:

$$q = \int_{b_{min}}^{b_{max}} \frac{q_s(b)bC}{1 + bC} db \quad (4)$$

This model is solved for $q_s(b)$, and the resulting plot of q_s vs. b is a graphical description of the heterogeneity of the system in terms of the numerical

binding constant, b. The ordinate q_s values are valuable in describing the concentration of binding sites at each b. The distribution of b is valuable in describing the relative strengths of sorption of the different sites on the heterogeneous surface; however, the theoretical exponential relationship between b and the sorption energy has not been rigorously developed yet. A correlation of b distributions with system parameters such as bonded phase coverage, metal content, pore size distribution, and solute parameters such as pK_a, polarity and molecular size would reveal important chemical variables that b is functionally dependent on. This is the first report of a Langmuir distribution model describing a wide concentration range of sorption for a reversed-phase liquid chromatographic system.

2.3. Solution of isotherm model

The solution of the classical, single-site Langmuir model (Eq. (2)) is easily accomplished by nonlinear least-squares regression. The Langmuir distribution model (Eq. (4)) must be solved by special techniques due to its ill-posed nature. Linear Fredholm integrals of the first kind are ill-conditioned, in that many solutions exist which satisfy the data to within the experimental error in the least-squared sense. Several techniques have been proposed in the literature for numerically solving this equation [13]. We have found that the iterative method of expectation-maximization (EM) results in the smoothest solution, which is desirable because ill-conditioned problems easily result in solutions with spurious artifactual information (amplified noise) that is physically meaningless [14,15]. There are other arguments for the smoothest solution that have been discussed in the literature and will not be reviewed here [13–15].

The EM method calculates the isotherm data, $q_{cal}(C)$, from the sorption model $\theta(C,b)$ by replacing the integral with a sum across a grid of b values:

$$q_{cal}(C_j) = \sum_{b_{min}}^{b_{max}} q_s(b_i)\theta(C_j, b_i)\Delta b \tag{5}$$

where

$$\theta(C_j, b_i) = \frac{b_i C_j}{1 + b_i C_j} \tag{6}$$

The b values span a grid indexed by i with spacing Δb, and the isotherm is calculated at the solute concentrations indexed by j. The solution, $q_s(b)$, is updated iteratively

$$q_s^{k+1}(b_i) = q_s^k(b_i) \sum_{C_{min}}^{C_{max}} \theta(C_j, b_i)\Delta b \cdot \frac{q_{exp}(C_j)}{q_{cal}(C_j)} \tag{7}$$

where $q_{exp}(C)$ is the experimental isotherm data and k is the iteration number. Thus, the EM algorithm consists of calculating the isotherm with Eq. (5), starting with an initial estimate of the sorption distribution, and then correcting this distribution with Eq. (7), repeating until no further, significant change in the solution occurs.

2.4. Chromatography model and solution

To validate the Langmuir sorption distribution model for chromatographic purposes, it must be able to adequately describe experimentally observed chromatograms. The chromatographic model used in this study is the equilibrium–dispersive (E–D) model for single-component systems [16]:

$$\frac{\partial C}{\partial t} + F\frac{\partial q}{\partial t} + u\frac{\partial C}{\partial z} = D_a \cdot \frac{\partial^2 C}{\partial z^2} \tag{8}$$

where F is the phase ratio, u is the linear flow velocity, D_a is the apparent axial dispersion coefficient, t is time, and z is the axial position along the column. The solution of this model requires knowledge of the sorption isotherm, $q(C)$, F, u, an estimate of D_a obtainable from the column efficiency, and an appropriate set of boundary conditions. Solution yields $C(t)$, the chromatogram at $z=L$, the end-length of the column.

Numerical solutions of this model have been discussed in the literature, and the Rouchon algorithm, popularized by the Guiochon group, is used here [17]. The model assumes equilibrium and fast kinetic mass transfer processes. It has been shown to be highly successful for reversed-phase stationary phases when using the Langmuir sorption model for $q(C)$ [17]. We report here the first use of the Langmuir distribution model with the E–D model for a liquid chromatographic system.

3. Experimental

3.1. Equipment and materials

A Shimadzu HPLC system (Cole-Scientific, Moorpark, CA, USA) equipped with two LC-10AS pumps, a SPD-10AV UV–visible detector, an Eldex heater, and the Axxiom chromatography data acquisition system was used to measure all elution and frontal chromatograms. The flow-rate was set at 1.0 ml/min and the temperature was kept constant at 30°C. A 10×0.46 cm column was packed in the laboratory with Zorbax Pro 10-150 stationary phase (10 μm particle diameter, 150 Å pore diameter) with a packing apparatus (YMC, Wilmington, NC, USA) constructed with a Haskel (Model MS-110) amplifier pump. The silica was prepared in an isopropanol–methlylene chloride (70:30) slurry, sonicated for 5–10 min. The column was then packed for 5–10 min at a pressure of 6000 p.s.i. using the same solvent used to prepare the slurry (1 p.s.i. = 6894.76 Pa).

The mobile phase was methanol–water (60:40) and the solute was *N*,*N*-dimethylaniline (Spectrum, Gardena, CA, USA). The pH was set to 5.0 with HCl. HCl of high enough concentration was used so that only a few drops were required to adjust the pH and thus the methanol–water mobile phase composition ratio was not altered significantly. The same mobile phase stock was used to prepare the dimethylaniline (DMA) solutions, although the pH of those solutions was set separately.

3.2. Isotherm determination

The FA experiments were performed across five-orders of magnitude in DMA concentration, in staircase mode, from 0.83 μ*M* to 83 m*M*. This was done in five separate stages as detailed in Table 1. The pH of each solution was set to 5.0 with HCl, as described above. Each stage corresponded to 10 steps, each step increasing in concentration by 10% increments of the total feed concentration placed with one of the pumps (pure mobile phase was present in the other pump). The first step in each range was deleted from the overall isotherm plots due to overlap with the previous range, although necessary for the isotherm calculation in each stage. The individual stages were then spliced together to obtain the overall, total sorption isotherm.

3.3. Isotherm modeling

The classical Langmuir model (Eq. (2)) was modeled by nonlinear least-squares using the JMP program (SAS Institute, Cary, NC, USA). The Langmuir distribution model was calculated with the EM algorithm (Eqs. (5–7)) programmed in the laboratory in Fortran code. One hundred grid points in *b* space were used from 0.01 to 5000. One hundred thousands iterations were performed. On a 350 MHz Pentium II computer this calculation takes about 1.5 min.

3.4. Elution experiments

The experimental chromatograms were obtained with 10-μl injections of 8.3 m*M* DMA, or 83 nmol (Rheodyne Model 7725 injector). The mobile phase conditions were supplied by the same stock as used for the FA experiments. Detection was monitored at 247 nm. Injections of uracil and benzene were performed to obtain the hold-up time and column efficiency, respectively. The hold-up time for injection-mode is only used to check the veracity of the phase ratio measurements as used in the solution of the E–D model. The number of theoretical plates, *N*, is needed in the E–D model to account for the axial band dispersion. A calculation of *N* from the

Table 1
Experimental parameters for FA experiments

Stage	Concentration (*M*)	Step time (min)	Detection wavelength (nm)
1	0.83 to $8.30 \cdot 10^{-6}$	20	247
2	0.83 to $8.30 \cdot 10^{-5}$	10	282
3	0.83 to $8.30 \cdot 10^{-4}$	5	320
4	0.83 to $8.30 \cdot 10^{-3}$	5	330
5	0.83 to $8.30 \cdot 10^{-2}$	5	340

DMA peaks is not an accurate assessment of the column efficiency, because the majority of the total dispersion of the DMA peaks is asymmetric and due to the nonlinear nature of the sorption isotherm.

The experimental band profiles were converted to concentration units on the ordinate axis from the inherent calibration data present in the FA runs performed at 247 nm (lowest concentration range). The E–D model solution provides $C(t)$ as discussed previously. Further details and verification of our procedures comparing the experimental and E–D model band profiles can be found in Ref. [4].

4. Results and discussion

pH is an important variable in these studies. It was our goal to deliberately probe the active adsorption sites to the maximum extent possible, so that they could easily be detected in the Langmuir distribution (q_s vs. b) plots. This requires that the observed tailing be substantial and that the UV detection of the probe possess adequate sensitivity at the lower concentrations that tail badly. A pH of 5 was found to maximize the tailing. At higher and lower pH values, retention times and asymmetry factors were observed to decrease, especially at the lower pH values (see Fig. 2). A pH of 5 is close to the pK_a of DMA. At lower pH, DMA becomes completely protonated, but the increased competition with H^+ for silanol sites causes reduced retention and does not allow the DMA to probe these sites with an ionic-type interaction. At higher pH, the probe deprotonates, and the lack of any ionic interaction with the active sites again reduces retention.

It is necessary to perform FA at the lowest possible concentrations to detect the strongest adsorption in the isotherm plots. At concentrations less than measured here, at least one, higher b, adsorption interaction may be quantified, as shown below.

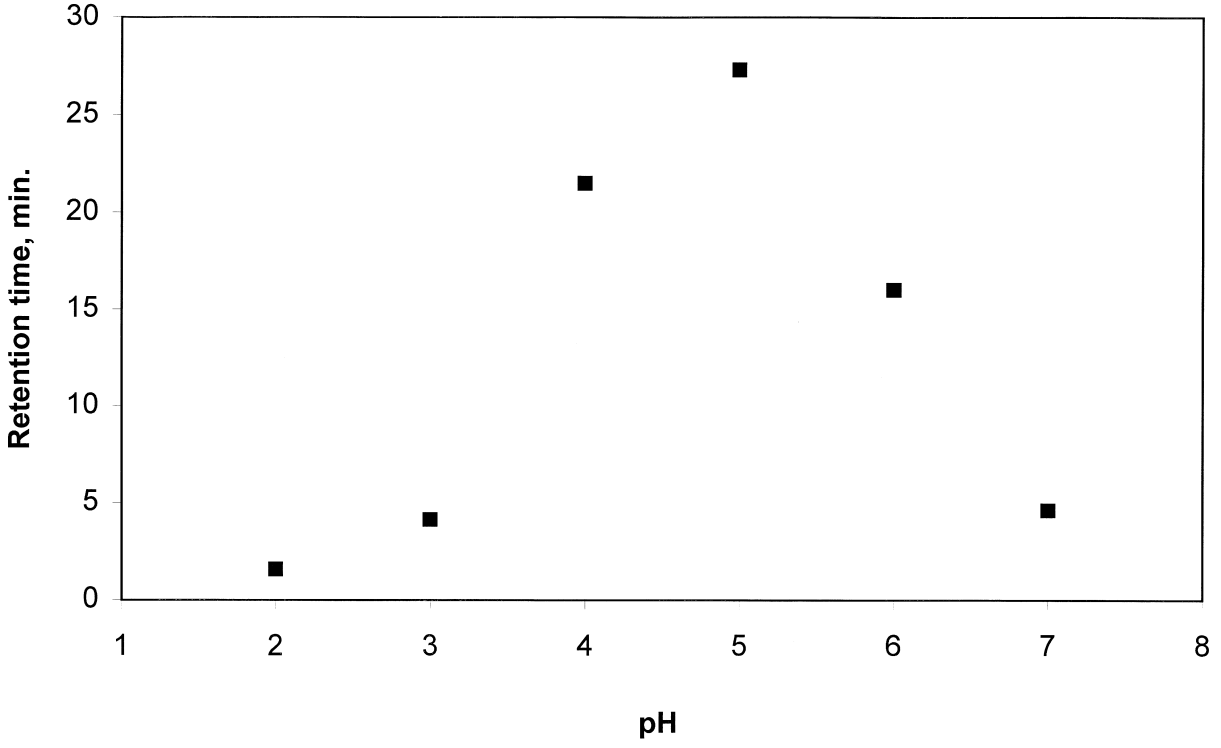

Fig. 2. Retention of 17 nmol *N,N*-dimethylaniline injections as a function of mobile phase pH. Stationary phase: Zorbax Pro 10-150, 10×0.46 cm column. Mobile phase: methanol–water (60:40), pH set with HCl or NaOH. Flow-rate: 1.0 ml/min.

However the detection limit for DMA existed in the next lowest order of magnitude in concentration (0.083 to 0.83 μ*M*).

It is prudent to perform FA measurements to the highest concentrations possible that conforms to two conditions: firstly and obviously the solubility of the solute in the mobile phase can not be exceeded and this occurs over 100 m*M* for DMA in methanol–water (60:40); secondly, the isotherm must retain its convex-upwards shape to conform to the Langmuir model. We found that this condition was violated and the slope of the isotherm began to increase over 10 m*M* in DMA mobile phase concentration; therefore, the highest concentration range in Table 1 was deleted from the isotherm modeling step (see data in Table 2). High concentrations should be measured when modeling sorption distributions to deconvolute different sorption behavior from each other to the greatest extent possible. This has been shown to increase the accuracy of the modeled parameters [18,19]. The corrected breakthrough volumes, $(V_{F,i} - V_0)$, for all the concentration steps are given in Table 2. In this example, we were unable to detect monolayer saturated sorption into the C_{18} chains, which would have been indicated by monotonically decreasing retention volumes at the highest concentrations sampled.

The sorption isotherm across the entire concentration range, minus the concave-upwards region at the highest concentration range, is shown in Fig. 3a. In Fig. 3b the lowest two concentration ranges are shown. Although the data looks linear at high concentrations, this is presumably due to solute partitioning into the C_{18} chains of the stationary phase. At lower concentrations, the nonlinearity becomes noticeable, and is due to the increased retention volumes recorded at these concentrations.

The entire isotherm of Fig. 3a was modeled by the classical, one-site Langmuir model and the proposed Langmuir distribution model. The results are given in Fig. 4, with the statistical results given in Table 3. It is clearly observed that the Langmuir distribution model fits the data better. More dramatically, the difference in sorption models is demonstrated by calculating the E–D chromatograms with the fitted models, as shown in Fig. 5. The Langmuir model can not predict band tailing at ''infinite dilution'', i.e., the analytical concentrations where the isotherm is assumed to be linear. The prediction of the apical retention time is actually better for the single-site Langmuir model in this example with the Langmuir distribution model exhibiting an approximately 10% error. However, the general shape and tailing of the band profiles is simulated well with the distributed model. If smaller amounts are injected, observed retention times increase considerably. The Langmuir

Table 2
Breakthrough volumes for *N*,*N*-dimethylaniline (DMA) on Zorbax Pro 10-150 in methanol–water (60:40) at pH 5 for entire, measured concentration range[a]

Stage	Equilibrium concentration of DMA in mobile phase, C_i in Eq. (1)									
	Corrected retention volume on 10×0.46 cm column, $(V_{F,i} - V_0)$ in Eq. (1)									
	Step									
	1	2	3	4	5	6	7	8	9	10
	0.83 μ*M*	1.65 μ*M*	2.48 μ*M*	3.31 μ*M*	4.13 μ*M*	4.96 μ*M*	5.79 μ*M*	6.61 μ*M*	7.44 μ*M*	8.26 μ*M*
1	21.1 ml	15.0 ml	9.9 ml	8.6 ml	7.8 ml	7.4 ml	6.7 ml	6.5 ml	6.2 ml	5.9 ml
	8.3 μ*M*	16.5 μ*M*	24.8 μ*M*	33.1 μ*M*	41.3 μ*M*	49.6 μ*M*	57.9 μ*M*	66.1 μ*M*	74.4 μ*M*	82.6 μ*M*
2	11.5 ml	5.9 ml	5.1 ml	4.8 ml	4.7 ml	4.6 ml	4.5 ml	4.4 ml	4.4 ml	4.4 ml
	83 μ*M*	167 μ*M*	248 μ*M*	331 μ*M*	413 μ*M*	496 μ*M*	579 μ*M*	661 μ*M*	744 μ*M*	826 μ*M*
3	9.0 ml	4.2 ml	4.1 ml	4.0 ml	4.0 ml	4.0 ml	4.0 ml	4.0 ml	4.0 ml	3.9 ml
	0.83 μ*M*	1.67 m*M*	2.48 m*M*	3.31 m*M*	4.13 m*M*	4.96 m*M*	5.79 m*M*	6.61 m*M*	7.44 m*M*	8.26 m*M*
4	4.7 ml	4.0 ml	3.9 ml	3.9 ml	3.9 ml	3.9 ml	3.8 ml	3.9 ml	3.9 ml	3.9 ml
	8.3 μ*M*	16.7 m*M*	24.8 m*M*	33.1 m*M*	41.3 m*M*	49.6 m*M*	57.9 m*M*	66.1 m*M*	74.4 m*M*	82.6 m*M*
5	4.0 ml	3.9 ml	4.0 ml	4.0 ml	4.0 ml	4.2 ml	4.3 ml	4.5 ml	4.7 ml	4.7 ml

[a] Breakthrough volumes are listed for concentration steps above the previous concentration listed in the columns to the left (staircase FA). First column breakthrough volumes' previous concentration was 0.0 *M* (first step of each range).

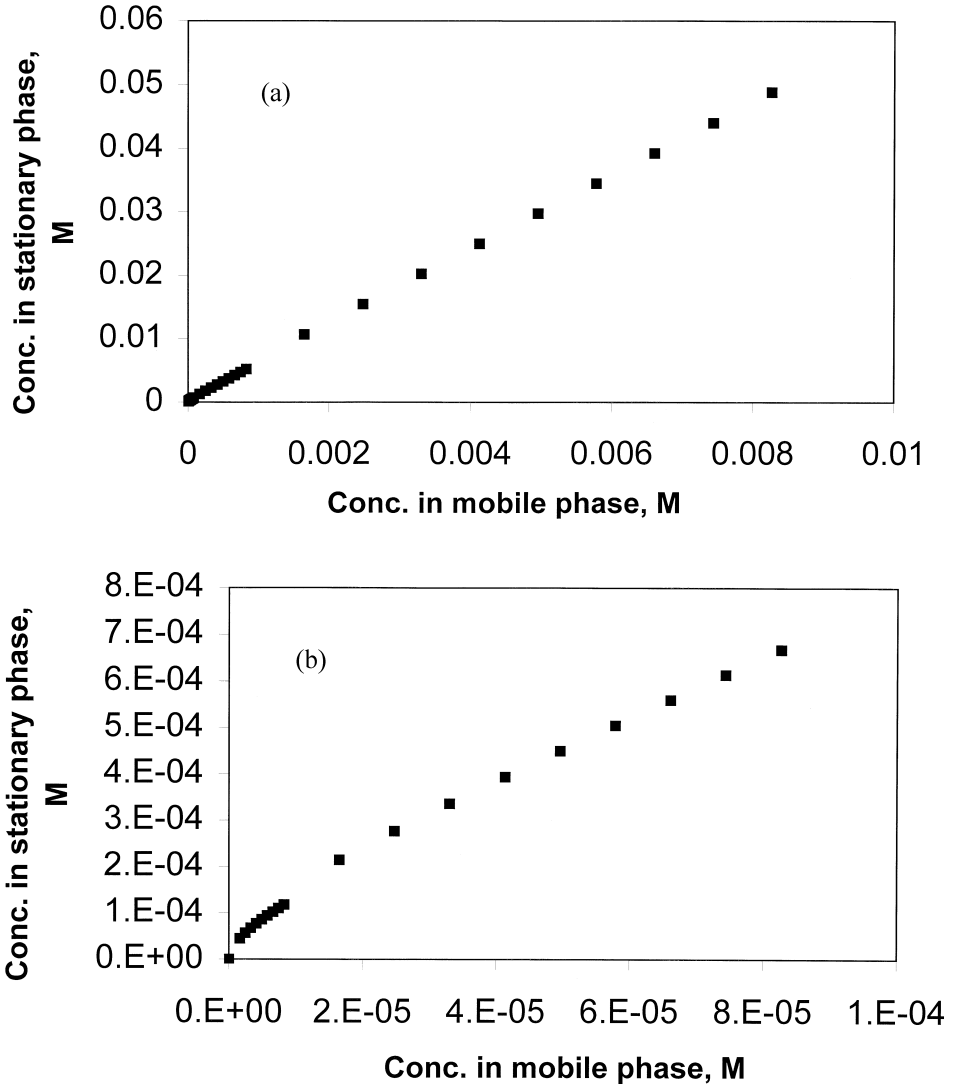

Fig. 3. (a) Total isotherm of *N*,*N*-dimethylaniline on Zorbax Pro 10-150 in methanol–water (60:40) at pH 5.0. (b) Isotherm in (a) at lowest two concentration ranges measured.

distribution model always predicts the retention better relative to the one-site model for these cases. The inaccuracy of the Langmuir distribution model is due to an inadequately sampled isotherm and poor reproducibility.

The Langmuir distribution is shown in Fig. 6. The majority of the sorption isotherm is modeled by low *b* values, or a low sorption energy ''site''. The sorption in this region is interpreted as due to a hydrophobic partitioning mechanism between the DMA molecules and the C_{18} chains. It completely dominates the total sorption signal, as should be the case by design. The active adsorption is revealed by changing the scale of the plot at higher *b* values (see Fig. 6b). A peak of *b* values centered at $b=250$ is required to fit the data. The width of sorption peaks is only a moderate indication of the dispersion in sorption strength around the central value, because numerical dispersion is incurred in the EM method. The peak width at half-height is approximately $b\pm60$

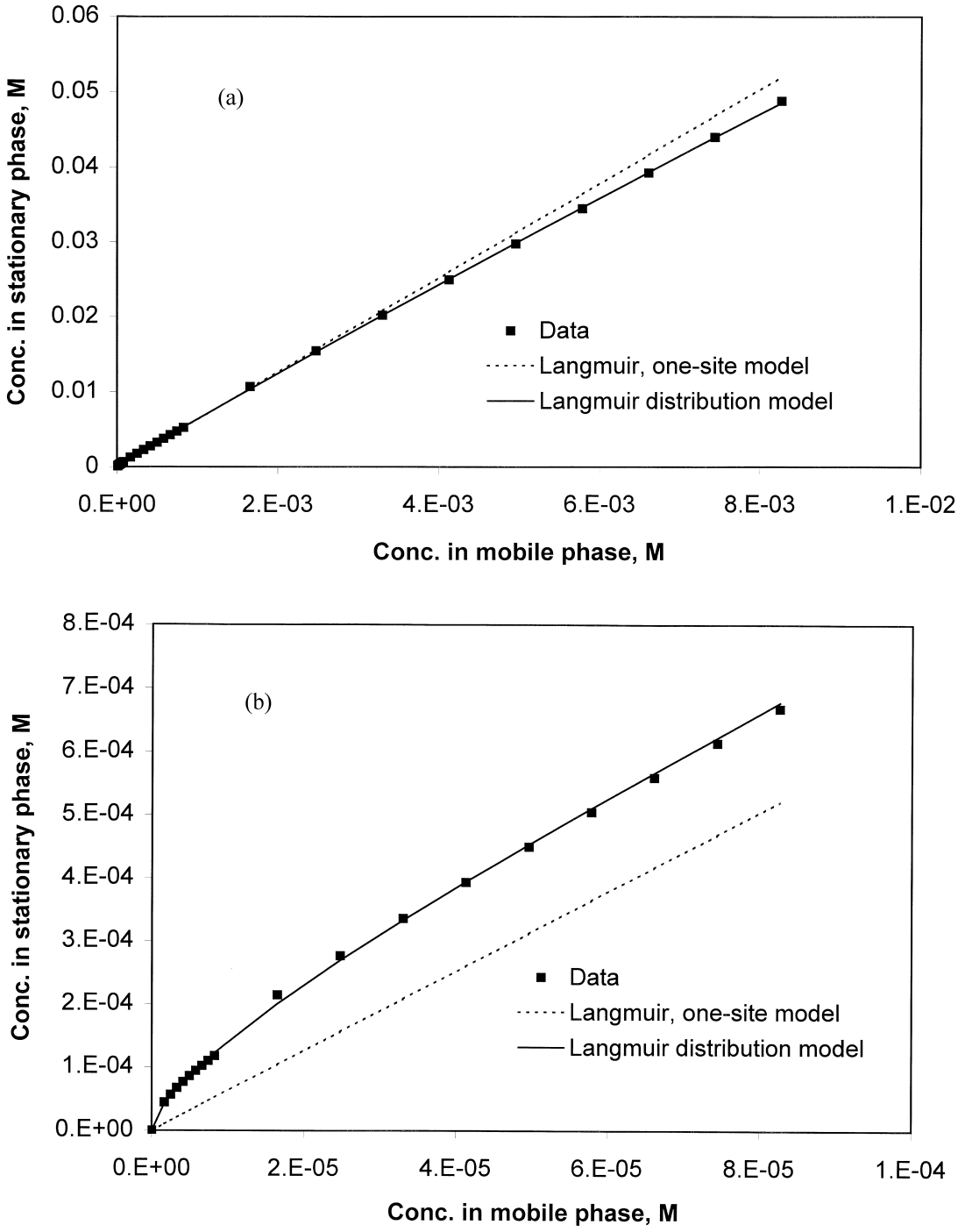

Fig. 4. (a) Fit of isotherm data to the single-site Langmuir model and the Langmuir distribution model. (b) Same fit as in (a) at lowest two concentration ranges measured.

Table 3
Results of Langmuir one-site and distribution fits of isotherm data for *N,N*-dimethylaniline (DMA) on Zorbax Pro 10-150 in methanol–water (60:40) at pH 5 for entire, measured concentration range

Model	*b* Value(s) (M^{-1})	q_s (M)	SSE[a] (M^2)
Langmuir	0.073	0.71	$7.5 \cdot 10^{-7}$
Langmuir distribution	<0.1, 250, >5000	1.6, $1.49 \cdot 10^{-4}$, $4.69 \cdot 10^{-5}$	$2.7 \cdot 10^{-7}$

[a] Summed squared error of solute concentration in the stationary phase: $\Sigma(q_{exp} - q_{cal})^2$.

for this data. A better indication of the thermodynamic dispersion can be achieved by numerical simulation of the data with discrete *b* values and deconvolution back to the Langmuir distribution [18].

The central, high sorption strength *b* constant is approximately 3500-times greater for adsorption onto these sites than that observed for hydrophobic solute partitioning, by comparison to the best fit *b* value taken from the one-site Langmuir model (see Table 3). If the *b* distribution is integrated, the concentration of active sites in the stationary phase that was successively probed in this experiment is obtained. The result is 170 μM for the peak centered at $b = 250$; the total active site concentration probed may be greater due to the detection of higher energy adsorption (discussed below). Using the stationary phase volume, we can calculate that 113 nmol of DMA were adsorbed onto this site. In the elution experiments, considering the injection volume of 83 nmol, it is not surprising that the band profiles tailed as badly as shown in Fig. 5. However, this active site concentration value is much too small to account for all the unreacted silanol sites. Using the surface area of this silica, we calculate only 0.6 nmol/m^2. The bonded-phase coverage for this silica is reported by the manufacturer as 3.2 μmol/m^2. Even if only a small fraction of this latter number corresponded to the unreacted silanol concentration, we can conclude that only a small fraction of the silanol sites are detected in this method. However, this apparently is the fraction that is ''active'' in nature, either due to their chemical nature or to whether they were exposed in the experiment or not.

It is tempting to label the active adsorption peak revealed in this work as due to silanol adsorption, but this can not be confirmed until similar results are correlated with independent measures of silanol

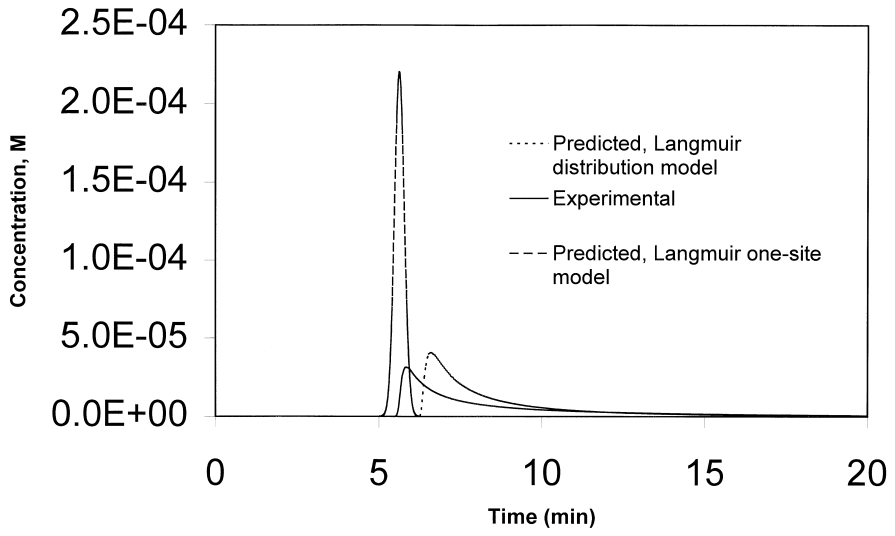

Fig. 5. Experimental vs. predicted chromatograms based on the Langmuir and Langmuir distribution models.

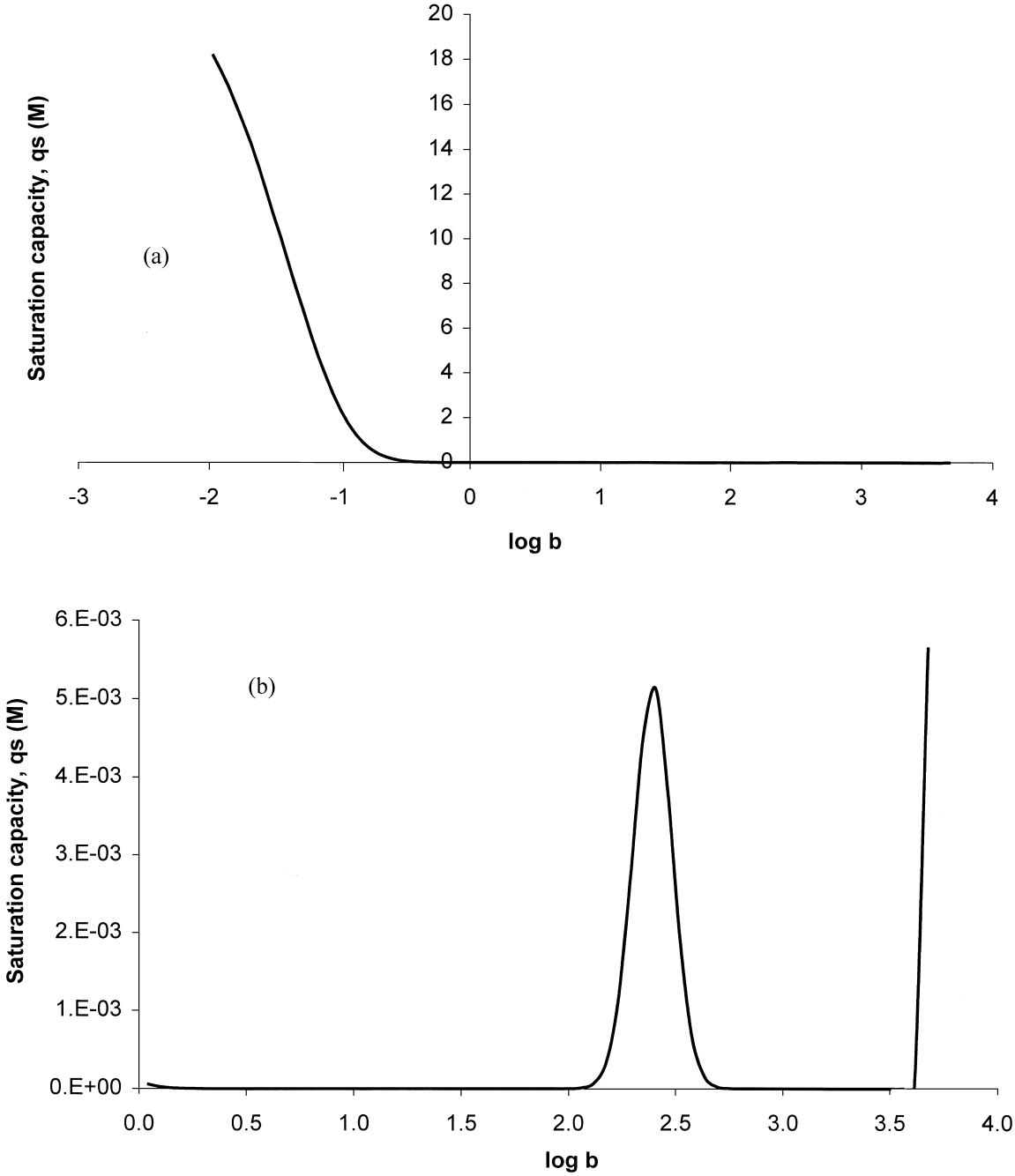

Fig. 6. (a) Langmuir *b*-distribution of sorption isotherm recorded in Fig. 3a and modeled in Fig. 4a. (b) Langmuir *b*-distribution at higher binding strengths and lower saturation capacities, blown up from (a).

concentration. Another significant possibility is metal impurities. Furthermore, the type of silanol site is not known unless correlation studies with independent measures of silanol type are performed. These studies are currently in progress in our laboratory. The results suggested here are that only a tiny fraction of the probable number of silanol sites are active and cause band tailing. According to Kirkland et al. [7], these sites may be the isolated or geminal sites. The vicinal sites, or patches of silanol sites, may not contribute significantly to peak asymmetry.

Note that at each end of the *b*-distribution is information suggesting sorption off the scale of the plot. It was not possible to obtain peaks in these regions because they are undersampled in our experiments. Higher concentrations and a continuous decrease in the slope of the sorption isotherm is required to quantitate the low-*b*, C_{18}-partitioning peak. The experimental limitations of this were discussed previously. Due to this experimental limitation and the ramifications on the data analysis, there is little confidence in the saturation capacity for the low energy sorption, using either the one-site or Langmuir distribution model. To obtain a peak at higher *b* values requires adsorption data at concentrations near or below the detection limit. It is apparent, however, that adsorption is occurring at greater strength than the above-discussed ''silanol'' peak, due to the information revealed at the high-*b* end of the distribution. This could be due to a more energetic type of silanol site or a metal complexation reaction. In conclusion, one should not quantitate the results obtained from the Langmuir distributions unless a peak is contained in the results. Divergent signals at the endpoints help model the data, may be due to real chemical phenomena, and make more accurate the peak(s) that are contained, but are not sufficient to accurately model the data in these regions. Further data in the regions past the endpoints are required (lower concentrations for higher *b* values, higher concentrations for lower *b* values).

4.1. Reproducibility

Although run-to-run reproducibility is better than 2% for these studies, dozens of runs are performed for an entire experiment such as described here, encompassing 3–4 l of mobile phase, and one week of data collection. Retention times recorded at the beginning of this period can differ from those obtained at the end of this period rather significantly, approaching as high as 10%. With this observation, the difference in the Langmuir distribution model band profile and the experimental one shown in Fig. 5 may be due to poor reproducibility.

To obtain reproducible sites, extensive conditioning with the mobile phase is required between runs to completely rinse off the active sites. Reproducibility was achieved simply by monitoring the effluent signal with high amplification and waiting for a true baseline only affected by noise or random drift. Approximately 120 ml of mobile phase were required to reproduce the data presented here. This added cost reveals the main disadvantage of obtaining the type of information reported here. Approximately 4 l of mobile phase and one week of data collection is required to make up all the FA solutions and obtain all of the data required for a complete analysis at one set of conditions.

We have also observed that the reproducibility decreases as the asymmetry increases. This is a burden in the chromatography of basic solutes. When probing active sites for study, more time and solvent must be expended to adequately sample the sites. We are currently determining conditioning and equilibration conditions to improve reproducibility and lower solvent costs. However, it should be stressed that reproducibility for its own stake is meaningless in this type of study. In order to study the active sites, they must all be ''activated'' before each chromatographic experiment is performed. Moreover, conditions must be chosen such that the probe adequately senses the activated sites. This increased sampling of active sites is encumbered by an increased sensitivity to the cleanliness of the stationary phase packing at these sites.

5. Conclusions

Frontal analysis isotherm determination coupled with a thermodynamically heterogeneous Langmuir distribution model is able to model peak tailing of basic solute elution in C_{18} reversed-phase HPLC. The nature of the sorption model is such that hydrophobic solute partitioning can be differentiated

from higher energy silanol adsorption or possibly other types of interactions. Different types of active sites may be distinguished if the retention behavior corresponding to these sites differs accordingly and sufficient data is acquired in these regions.

The distributed Langmuir model works with the equilibrium–dispersive model of chromatography in predicting band tailing. A kinetic model allowing for slow adsorption–desorption processes is not required to produce this type of asymmetry.

Reproducibility, solvent and time costs are the disadvantages of this method. Poor reproducibility results in the attempt to establish the same silanol site distribution before each experiment. Several liters of mobile phase are required to obtain results over several orders of magnitude in concentration range. This range must be obtained in order to successively probe several orders of magnitude in the binding parameter, *b*.

For dimethylaniline sorption on Zorbax octadecylsilanated silica in methanol–water (60:40) at pH 5, one complete ''silanol peak'' was determined, and a second was detected at higher adsorption strength, although not quantifiable. The results indicate a very small active silanol concentration relative to the surface as a whole ($<1\%$). However, this concentration can cause severe peak asymmetry for analytical work. Future studies must be directed at the correlation of the sorption peaks with physicochemical characteristics of the silica. Such correlations will uniquely identify and quantify which characteristics are responsible for the peak tailing phenomena observed for each application.

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