

The combined effect of silanols and the reversed-phase ligand on the retention of positively charged analytes

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Received 17 August 2004; received in revised form 29 September 2004; accepted 29 November 2004

Abstract

The nature of the interaction of positively charged analytes with the surface of reversed-phase bonded phases has been investigated as a function of both pH and volume fraction of organic modifier. Studies of the combined effect of both the parameters have been previously reported by us, and the data presented here further demonstrate a multiplicative interaction between pH and the concentration of organic modifier in the mobile phase. Fitting of the data as functions of pH and eluent composition clearly shows that the hydrophobically assisted ion-exchange process dominates over a purely reversed-phase or a pure ion-exchange retention mechanism. The underlying theory is developed in detail, and the mechanism is elucidated using several reversed-phase packings of substantially different character.

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Keywords: Silanols; Silanol activity; Retention theory; Reversed-phase chromatography

1. Introduction

It has been understood since the beginnings of HPLC that the interaction of an analyte with the reversed-phase sites of a packing is not the only factor responsible for retention. In addition, silanols interact with analytes containing basic functional groups. This can create excessive retention [1] or tailing [2,3]. As early as the late 1970s and early 1980s, attempts were made to understand the combined effect of reversed-phase and “silanophilic” interaction [1,4–9].

Recently, there has been renewed interest in the interaction of charged analytes with reversed-phase packings, specifically with surface silanols on silica or hybrid-based bonded phases [10–17]. Some of the previous work reflects a continued concern about the effects of silanols on the peak shape obtained with different packings ([13], and references therein), the selectivity of the stationary phase [18–23] or the repro-

ducibility of packings towards positively charged analytes [17]. Other studies are driven by the desire to understand the loadability of basic compounds in preparative chromatography [14,15,24].

In the classical model of the interaction of a charged analyte with the surface of a silica-based reversed-phase packing, it was assumed that two separate mechanisms take place [8,9]. One of these is the reversed-phase mechanism encountered otherwise with purely hydrophobic molecules. In addition, an interaction of suitable analytes with the surface silanols is occurring as well. If the second mechanism is ion-exchange, we can summarize the classical dual mechanism as an additive mechanism:

$$k = k_{rp} + k_{ex} \quad (1)$$

where k is the retention factor, k_{rp} is the contribution of the reversed-phase process and k_{ex} the contribution of the ion-exchange with surface silanols. This formalism is equivalent to the two types of sites being on separate particles or even in tandem columns.

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Neue et al. [10] formulated a model in which the total free energy of transfer of the solute is taken as the sum of independent contributions from hydrophobic and Coulombic effects which is equivalent to a multiplicative formulation of Eq. (1). Essentially, the purely hydrophobic retention process combines with the ion-exchange process as follows:

$$k = k_{\text{rp}}k_{\text{ex}} \quad (2)$$

where k_{rp} is the contribution of the reversed-phase process and k_{ex} the contribution of the ion-exchange with surface silanols. This ad-hoc model explains the increase in ionic interaction with increased hydrophobic interaction, but it fails in two important points. Firstly, it cannot account for the change in retention with a change in the hydrophobicity of the packing. Secondly, the retention will disappear as *either* the ionic interaction or the hydrophobic interaction vanishes. Neither can be the case (e.g. it has been shown that retention remains when all ionic interactions are eliminated), and a more thorough model is required.

Such an improved model was proposed by Yang et al. [11]. The model combines our model of multiplicative interaction (called a one-site hydrophobically assisted ion-exchange in [11]) with a purely hydrophobic retention. Thus, there are two mechanisms that act in parallel: pure hydrophobic interaction and the multiplicative mechanism of combined hydrophobic and ion-exchange interaction. The following equation shows this model:

$$k = k_{\text{rp}}(1 + k_{\text{ex}}) = k_{\text{rp}} + k_{\text{rp}}k_{\text{ex}} \quad (3)$$

As the ionic interaction increases, the multiplicative interaction between reversed-phase and ion-exchange dominates. As the ion-exchange interaction vanishes, the purely hydrophobic interaction dominates. One may call the second term either an ion-exchange-assisted hydrophobic interaction or a hydrophobically assisted ion-exchange process.

From the form of Eq. (3), it is clear that as the hydrophobic interaction decreases, the retention disappears. Yet this cannot be the case either, since now the ion-exchange process should take over. We therefore need to formulate a third case, where the interaction with silanols is the dominant step, and the combined hydrophobic and ion-exchange interaction is a possible step that accompanies the ionic interaction. Although Yang et al. [11] did not find such a mechanism on the silica-based reversed-phase packings that they studied, they did encounter it on a polybutadiene-coated zirconia when used in phosphate media. Mixed-mode interactions of this nature also emerge on the surface of a mixed-mode divinylbenzene-based ion-exchanger, where the longer-range ion-exchange interaction combines with the hydrophobic interaction with the DVB backbone [25]. Both phenomena, the augmentation of hydrophobic retention with ion-exchange and the augmentation of ion-exchange with hydrophobic interaction have been reported for ion-exchange materials by Lee and O'Gara [25]. The mixed-mode interaction of trialkylammonium ions on a styrene-divinylbenzene-based ion-

exchanger has also been reported by Rahman and Hoffman, but no quantitative description was given [26].

On the surface of a silica-based reversed-phase packing, we find hydrophobic bonded-phase ligands and silanols. In principle for the interaction between a hydrophobic and positively charged analyte with such packings, we may find pure hydrophobic interactions, pure silanophilic interactions or combinations of both with the multiplicative interaction, as shown in Eq. (4):

$$k = k_{\text{rp}} + k_{\text{rp}}^*k_{\text{ex}}^* + k_{\text{ex}} \quad (4)$$

It is possible that the retention factors underlying the combined effect, which are marked here with an asterisk, and the retention factors for the individual interactions are different. Such events could be caused, if the sites responsible for the multiplicative interaction were substantially different than for example the sites responsible for the purely hydrophobic interaction. Due to the substantially different nature of the packings used in this study, it is unlikely that a special site distinct from those sites responsible for the individual interactions is causing the multiplicative interaction. For the sake of simplicity, we will therefore assume in this paper that the single retention factors and the retention factors in the multiplicative term are identical. On a series of packings with variable ratios of reversed-phase ligands and silanols, the mixture of the three mechanisms varies with the packing. The particular mixture of properties is a function of the details of the design of the packing. In this paper, we will study several packings of substantially different character to examine the nature of the interaction between a hydrophobic quaternary amine and these packings.

As will become clear in the discussion of the underlying theory and the results in this paper, the complex combined mechanism cannot be uncovered with measurements in a single mobile phase composition, in addition not even under conditions where only the pH is altered without varying the concentration of organic solvent. This has been the drawback of previous investigations. It also answers the question why such a fundamental effect, i.e. the multiplicative combination of hydrophobic and silanophilic interaction, has remained hidden in 30 years of HPLC, and was unearthed only recently [10,11].

An interesting aspect of the inorganic-organic hybrid packing underlying the XTerra packings is the fact that the $\text{p}K_{\text{a}}$ -values of the surface silanols shift into the alkaline pH compared to a silica-based packing [10]. This is the expected shift based on the differential effect of an oxygen versus an alkyl substituent on the acidity of the SiOH group. This phenomenon has been confirmed by Méndez et al. [12]. The consequence of this is a much lower silanol activity compared to silica-based packings. This needs to be taken into account in the interpretation of the chromatographic retention data of ionic and ionizable analytes. Méndez et al. [12] have also corroborated the difference in the ionization between a classical silica and a high-purity silica, as well as for the respective

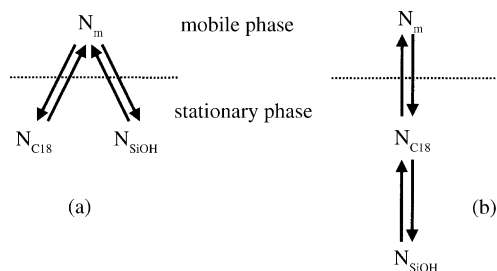


Fig. 1. Interaction mechanisms for the reversed-phase retention of positively charged analytes. (a) Parallel hydrophobic interaction and ion-exchange. (b) Sequential hydrophobic interaction and ion-exchange.

C_{18} -bonded phases. The use of significantly different packings in the current study will increase the knowledge of the dependence of ionic interactions on the make-up of the stationary phase.

Another complication in the interaction of a packing with analytes arises from dewetting phenomena in highly aqueous mobile phases [27–31]. Since we are varying the concentration of organic solvent from purely water to 50% acetonitrile, we will need to take this phenomenon into account as well.

2. Theory

In Section 1, we have outlined the existing view of the multiple interaction mechanism between a positively charged hydrophobic analyte and the surface of a silica-based packing covered with a hydrophobic layer. In this section, we will briefly describe a mechanism that leads to the identical equations, and is indistinguishable from the thermodynamic model published in reference [11]. As a matter of fact, there is no possibility to distinguish between the model shown here and the previous model in terms of the mathematical form of the dependence of k on the experimental variables, but we believe that the model shown here nicely rationalizes the multiple interaction model postulated above and may lead other researchers to uncover more details of the multiple interaction process. In any case, it is a thermodynamically legitimate approach to deriving the form of the relationships.

The classical model of the interaction of a positively charged hydrophobic analyte with the C_{18} chains and the silanols of a packing assumes a simple additivity of the interactions of the analyte (Fig. 1a).

In this case, both sites are independent, and the retention factor can be calculated as follows. The number of molecules in the stationary phase N_s consists of molecules that are retained by hydrophobic retention N_{C18} and those that are retained by silanophilic interaction N_{SiOH} :

$$N_s = N_{C18} + N_{SiOH} \quad (5)$$

With the number of molecules in the mobile phase N_m , the retention factor is defined as:

$$k = \frac{N_s}{N_m} = \frac{N_{C18}}{N_m} + \frac{N_{SiOH}}{N_m} \quad (6)$$

The concentrations in the different phases are:

$$c_m = \frac{N_m}{V_m} \quad (7a)$$

$$c_{C18} = \frac{N_{C18}}{V_{C18}} \quad (7b)$$

$$c_{SiOH} = \frac{N_{SiOH}}{A_{SiOH}} \quad (7c)$$

V are the volumes of the respective phases. For the sorption on silica, the concentration is defined as a two-dimensional concentration on the surface of the silica A_{SiOH} , i.e. as the number or molar quantity of silanols per unit surface area. This is only a formalism that does not affect the remainder of the treatment. Substituting these definitions into Eq. (6) gives the following expression for the retention factor:

$$k = \frac{V_{C18}}{V_m} \frac{c_{C18}}{c_m} + \frac{A_{SiOH}}{V_m} \frac{c_{SiOH}}{c_m} \quad (8)$$

With the phase ratio for the C_{18} layer and the silica:

$$\phi_{C18} = \frac{V_{C18}}{V_m} \quad (9a)$$

$$\phi_{SiOH} = \frac{A_{SiOH}}{V_m} \quad (9b)$$

and the distribution or sorption coefficients:

$$K_{C18} = \frac{c_{C18}}{c_m} \quad (10a)$$

$$K_{SiOH} = \frac{c_{SiOH}}{c_m} \quad (10b)$$

we obtain the final equation for the combined retention according to the classical model:

$$k = \phi_{C18} K_{C18} + \phi_{SiOH} K_{SiOH} \quad (11)$$

The key feature of this model is that both contributions to retention are independent and additive.

In reference [10], we had postulated a third type of interaction, the multiplicative combination of hydrophobic and silanophilic interaction. Adding this mechanism to the other possible mechanisms, we obtain:

$$k = \phi_{C18} K_{C18} + \phi_m K_{C18} K_{SiOH} + \phi_{SiOH} K_{SiOH} \quad (12)$$

where ϕ_m is the phase ratio for this mixed-mode interaction. In the following, we will briefly develop an alternative model that arrives at the identical result. Thus, within the studies shown here, this second model is indistinguishable from the first model. The second model assumes that the sorption to the silica surface takes place subsequent to solute partitioning into the C_{18} layer. We caution the reader that thermodynamic

measurements cannot reveal the order of steps in a process and we do not insist on this sequence as precisely the same result can be obtained by assuming that first the analyte adsorbs on an ion exchange site and then partitions into a hydrophobic layer. As in Eq. (5), some of the analyte molecules in the stationary phase are bound to the C₁₈ ligand, and some to the silanols. However, for this second process, shown as a diagram in Fig. 1b, we can define a secondary distribution coefficient K_2 of the analytes between the C₁₈ layer and the silica layer as follows:

$$\frac{N_{\text{SiOH}}}{N_{\text{C18}}} = \frac{A_{\text{SiOH}}}{V_{\text{C18}}} \frac{c_{\text{SiOH}}}{c_{\text{C18}}} = \phi_2 K_2 \quad (13)$$

Substituting this into Eq. (5), we obtain:

$$N_s = N_{\text{C18}}(1 + \phi_2 K_2) \quad (14)$$

This means that the interaction with the silanols is not independent of the interaction with the C₁₈ layer, but is a secondary process that is preceded by a partitioning of the analyte into the C₁₈ layer. The retention factor is now:

$$k = \frac{N_s}{N_m} = \frac{N_{\text{C18}}(1 + \phi_2 K_2)}{N_m} \quad (15)$$

$$k = \frac{V_{\text{C18}}}{V_m} \frac{c_{\text{C18}}}{c_m} (1 + \phi_2 K_2) \quad (16)$$

$$k = \phi_{\text{C18}} K_{\text{C18}} (1 + \phi_2 K_2) \quad (17)$$

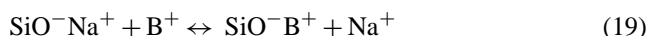
This equation is identical in form to the one derived in reference [11], but its interpretation is different. While the authors in reference [11] interpreted this form of the relationship as a simultaneous interaction with C₁₈ and silanols, this new model is based on a sequential interaction, first with the C₁₈ layer and then in a second step with the underlying silica layer. Both models result in exactly the same equation. They are therefore thermodynamically indistinguishable.

We will now expand Eq. (12) to derive the details of the dependence of the retention factor on the mobile phase composition. The hydrophobic interaction depends on the solvent composition. Usually, curved relationships are observed, especially if the measurements are carried out over a broad range of solvent composition. We have used the following equation to account for this interaction (10):

$$k_{\text{C18}} = \phi_{\text{C18}} K_{\text{C18}} = k_{\text{H}_2\text{O}} \times 10^{-Ar/(1+Br)} \quad (18)$$

$k_{\text{H}_2\text{O}}$ is the purely hydrophobic retention factor of the compound in water, i.e. without silanophilic interaction. A and B are constants, and r is the (volumetric) ratio of organic solvent to the aqueous component of the mobile phase.

The interaction of the positively charged quaternary amine with the negatively charged silanols is an ion-exchange process:



With the equilibrium constant:

$$K_{\text{ieX}} = \frac{[\text{SiO}^- \text{Na}^+][\text{B}^+]}{[\text{Na}^+][\text{SiO}^- \text{B}^+]} \quad (20)$$

The distribution coefficients K_2 and K_{SiOH} are defined as the partitioning ratios:

$$\frac{[\text{SiO}^- \text{B}^+]}{[\text{B}^+]} = \frac{1}{K_{\text{ieX}}} \frac{[\text{SiO}^- \text{Na}^+]}{[\text{Na}^+]} \quad (21)$$

Since the relationships for K_2 (the secondary interaction with silanols) and K_{SiOH} (the direct interaction with silanols) are identical, we will use the general terms from now until Eq. (25). The total silanol concentration on the surface is the sum of the ionized silanols and the undissociated silanols:

$$[\text{SiOH}]_{\text{T}} = [\text{SiO}^- \text{Na}^+] + [\text{SiOH}] \quad (22)$$

At the same time, the concentration of the silanol groups available for ion-exchange is a function of the hydrogen-ion concentration:



With the following ratio of undissociated silanols to total silanols:

$$\frac{[\text{SiOH}]}{[\text{SiOH}]_{\text{T}}} = \frac{1}{1 + (K_{\text{D}}/[\text{H}^+])} \quad (24)$$

$[\text{H}^+]$ is the hydrogen ion concentration, and K_{D} is the dissociation constant for the silanols. Combining Eqs. (21)–(24), we obtain for the partitioning ratios of a positively charged ion K_2 and K_{SiOH}

$$\frac{[\text{SiO}^- \text{B}^+]}{[\text{B}^+]} = \frac{1}{K_{\text{ieX}}} \frac{[\text{SiOH}]_{\text{T}}}{[\text{Na}^+]} \frac{1}{1 + ([\text{H}^+]/K_{\text{D}})} \quad (25)$$

Substituting these results together with Eq. (18) into Eq. (12), we obtain:

$$k = k_{\text{H}_2\text{O}} \times 10^{-Ar/(1+Br)} + k_{\text{mix}} \times 10^{-Ar/(1+Br)} \frac{1}{1 + 10^{\text{p}K_{\text{SiOH}} - \text{pH}}} + k_{\text{SiOH}} \frac{1}{1 + 10^{\text{p}K_{\text{SiOH}} - \text{pH}}} \quad (26)$$

k_{mix} is the retention factor in water for the mixed-mode interaction, and $\text{p}K_{\text{SiOH}}$ is the negative logarithm of the dissociation constant of the silanols. The abbreviation k_{SiOH} stands for the following:

$$k_{\text{SiOH}} = \phi_{\text{SiOH}} \frac{1}{K_{\text{ieX}}} \frac{[\text{SiOH}]_{\text{T}}}{[\text{Na}^+]} \quad (27)$$

Eq. (26) will be used for curvefitting of the experimental data from weakly acidic to alkaline pH.

It is not impossible that $\text{p}K_{\text{SiOH}}$ is a function of the solvent composition as well. It may however be a different and potentially weaker dependence on solvent composition than the change in $\text{p}K_{\text{a}}$ of a mobile phase buffer or an analyte in

the mobile phase. The reason for this is that the primary environment of the silanol group is the hydrophobic C₁₈ layer to which the organic modifier of the mobile phase is adsorbed. We will deal with this subject further in the discussion of the results of the data analysis. Similarly, the ion-exchange with the free silanols as described by the constant in Eq. (27) may also depend on the solvent composition. However, this problem is outside the current study.

3. Experimental

The HPLC system used in this study was an Alliance 2690 Solvent Manager with a 2487 Detector set at 254 nm equipped with a microbore cell with a 2.6 μ L cell volume. The time constant was set to 0.1 s, the data rate was 10 points/s. The system operated under the control of Millennium 32 version 3.2. The columns were Sentry Guard Columns packed with the appropriate packings. The columns were held in a Sentry Universal Guard Holder that was contained in a Neslab refrigerated recirculating water bath set to 23 °C. The dimensions of the guard columns are 3.9 mm \times 20 mm. The packings used in this study were Symmetry C₁₈ and XTerra RP₁₈, both with a particle size of 5 μ m, and Nova-Pak C₁₈, particle size 4 μ m as a representative of an older packing not based on a high-purity silica. The characteristics of these packings are shown in Table 1. Two additional packings were tested as well, but were excluded from this report. One was Resolve C₁₈, an unencapped packing with a very high silanol content. In this case, the retention time of the bretylium ion was for the most part outside the measurement window ($k \gg 100$). The other was XTerra MS C₁₈, a hybrid packing with a very low silanol activity. In this case, the change in retention with pH was too low to discern an interpretable pattern. The extra-column volume contained in the connection tubing was measured and the value subtracted from the retention volumes measured under the different conditions. The flow rate used was 2 mL/min. This setup permits the measurement of retention factors approaching 100 within a reasonable time. The sample was a solution of 1 mg/mL bretylium tosylate in water, of which 5 μ L were injected onto the column.

All mobile phases were prepared by mixing 20% of the buffer solution with 80% of appropriate mixtures of acetonitrile and water. The acetonitrile content of the mobile phase was varied between 0 and 50%.

All buffers were based on a constant concentration of sodium of 30 mM, resulting in a total mobile-phase sodium concentration of 6 mM. Buffers for pH 11, 8, 7, 6, 3 and 2 were prepared by adding phosphoric acid to a solution of 30 mM

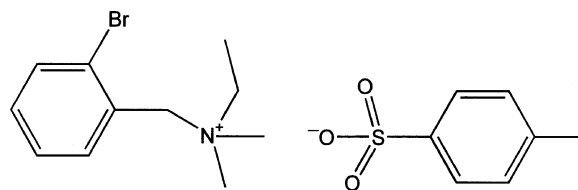


Fig. 2. Bretylium tosylate, the compound used in this study.

sodium hydroxide. Buffers for pH 5 and 4 were prepared from the 30 mM sodium hydroxide solution via the addition of acetic acid. Buffers for pH 10 and 9 were prepared by mixing the 30 mM sodium hydroxide solution with a 30 mM sodium hydrogen carbonate solution. The exact procedure for pH adjustment is described below.

All experiments were carried out at each pH-value from high organic content to low organic content, and then from low pH to high pH. This experimental protocol minimizes artifacts from the dewetting of the stationary phase to dissolution of the silica in basic mobile phases. Only Nova-Pak C₁₈ with its smaller pore size and pore volume exhibited dewetting in 100% aqueous mobile phases. In addition, fresh columns were used under alkaline conditions if there was concern about the stability of the stationary phase. If inconsistent results were obtained, the data were not included in the analysis.

During instrument operation, the mixtures of the buffers with the different concentrations of organic modifier were prepared automatically. We also prepared the same solutions off-line to be able to measure the actual pH of all solutions. The pH meter, an Orion Research digital ionalyzer/501 equipped with a ThermoOne PerpHect Triode, was calibrated with standard buffer solutions in water. The pH measurement was carried out in the presence of the organic solvent. This procedure is therefore in agreement with the requirements published in the literature [32].

The curvefitting procedures were carried out using ProStat Version 3.5 for Windows from Polysoftware International, Pearl River, USA. The data were fitted to the equations described using the Levenburg algorithm refined by Marquardt.

4. Results and discussion

In the following, we will demonstrate the complexity of the interaction of silanols with a positively charged analyte. We have chosen the bretylium ion for this purpose. Bretylium is a rather simple quaternary ammonium compound with a single aromatic ring (Fig. 2). Its charge is independent of

Table 1
Properties of the packings used in this study

Packing	Type	Pore size (nm)	Specific pore volume (mL/g)	Specific surface area (m ² /g)
Nova-Pak C ₁₈	Classical silica	6	0.30	120
Symmetry C ₁₈	High-purity silica	10	0.90	335
XTerra RP ₁₈	Hybrid packing with embedded polar group	12.5	0.70	175

Table 2

s_w pH values measured for the buffers shown on the right for acetonitrile–water mixtures of the concentrations indicated

	0%	10%	20%	30%	40%	50%
Phosphoric	1.99	2.11	2.23	2.37	2.51	2.65
Phosphate	2.98	3.13	3.33	3.49	3.75	3.84
Acetic	4.00	4.19	4.43	4.67	4.92	5.16
Acetate	5.00	5.18	5.43	5.68	5.93	6.18
Phosphoric	6.00	6.24	6.45	6.64	6.78	6.89
Phosphate	6.98	7.22	7.43	7.60	7.75	7.88
Phosphate	8.00	8.22	8.40	8.55	8.68	8.79
Bicarbonate	9.00	9.30	9.57	9.82	9.98	10.16
Bicarbonate	10.00	10.27	10.55	10.78	10.97	11.13
Phosphate	11.01	11.04	11.19	11.24	11.30	11.53

pH. It interacts with a reversed-phase bonded phase via hydrophobic interaction, as well as ionic interaction with ionized silanols.

We have studied the retention behavior of this compound using Symmetry C₁₈, a modern monofunctional C₁₈ bonded phase based on a high-purity silica [3,17], XTerra RP₁₈, a reversed-phase packing with an embedded carbamate group [33] based on an inorganic–organic hybrid packing with incorporated methyl groups [34] and Nova-Pak C₁₈, a C₁₈ packing based on a high-strength classical silica with a low pore volume and a small pore size. We varied the organic solvent concentration in the mobile phase from 0 to 50% acetonitrile. The pH was varied from pH 2 to 11 in steps of one pH unit, as measured in water. Afterwards, the pH was measured in the presence of the organic solvent according to the procedure recommended by Rosés and Bosch [32]. As described in Section 3, the sodium concentration of the buffer was held constant during the entire study. The details of the buffer preparation are described in Section 3.

The s_w pH-values measured at the different acetonitrile concentrations are shown in Table 2. All the buffers used in this study were anionic buffers. Therefore, the pH values shift towards larger values as the concentration of the organic solvent is increased. The measured s_w pH-values are the correct values to be used in correlating the retention of a cationic analyte with the solvent composition of reversed-phase mobile phases. Values used in a solvent system where the calibration of the measurement is also carried out in the presence of the organic solvent correlate with the s_w pH-values via a constant function. The experimental procedure allowed us to investigate simultaneously the influence of mobile phase composition and pH on the retention behavior of the bretylium ion.

In order to understand the retention behavior of the quaternary amine on reversed-phase packings, we will first show the results expected from theory, and then the actual measurements on the various packings. The oldest interpretation of the combined interaction of silanols and hydrophobic chains with positively charged analytes assumed that both interactions are additive (see Eq. (1) [1,8]). If this were the dominant form of the interaction, the retention pattern shown in Fig. 3

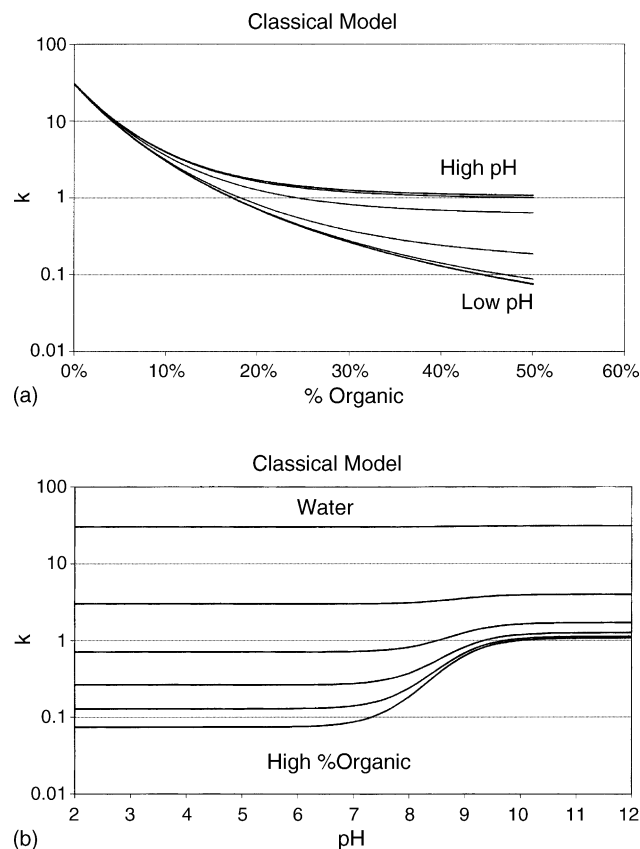


Fig. 3. Retention pattern for a quaternary amine after the classical model. (a) As a function of the solvent composition. (b) As a function of pH. The solvent concentration varies from 0 to 50%, and the pH varies from pH 2 to 12.

would result. For ease of comparison, Fig. 3 and the following figures are organized in the same way, i.e. Fig. 3a shows the retention factor as a function of the concentration of acetonitrile, and Fig. 3b depicts the dependence of the retention factor on the pH of the mobile phase. The numerical values chosen for the parameters in the theoretical figures were similar to the actual values found for the packings used in this study. For the reversed-phase interaction, the model uses the following equation:

$$k_{rp} = 30e^{-30x/(1+3x)} \quad (28)$$

x is the volume fraction of the organic solvent used as the x -axis in Figs. 3–5. For the ion-exchange interaction, the following model was used:

$$k_{ex} = \frac{1}{1 + 10^{-pH+8.9}} \quad (29)$$

For Fig. 3, both the parameters are added to each other, as shown in Eq. (1). In all figures, the vertical axis is on a logarithmic scale. We can see in Fig. 3 that the retention depends strongly on pH at a low hydrophobic retention, i.e. at a high concentration of the organic modifiers. The retention increases with increasing pH due to the increased influence of the ion-exchange with silanols at high pH. On the other

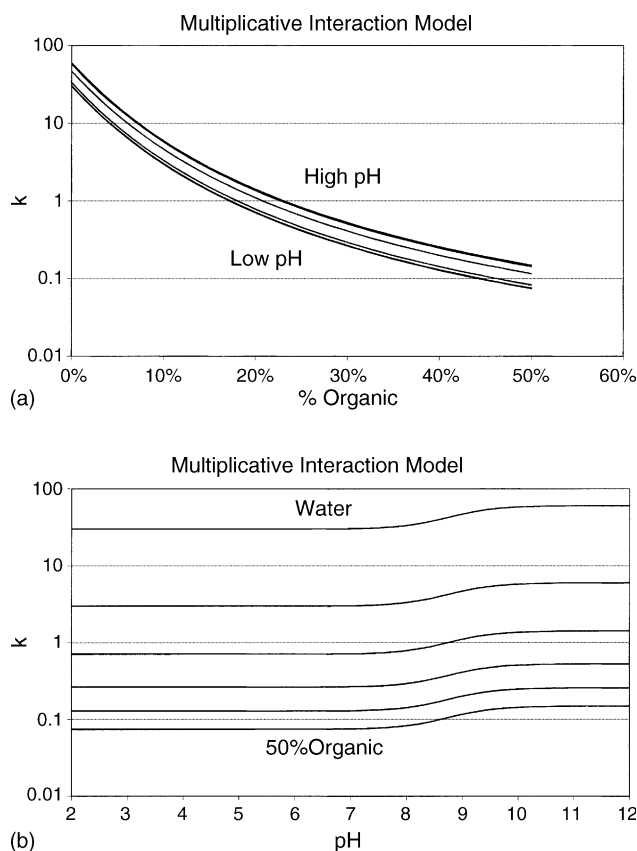


Fig. 4. Retention pattern for a quaternary amine after the multiplicative interaction model. (a) As a function of the solvent composition. (b) As a function of pH. The solvent concentration varies from 0 to 50%, and the pH varies from pH 2 to 12.

hand, at a low concentration of acetonitrile or even in water, the additive retention due to ion-exchange vanishes compared to the increased retention caused by the reversed-phase process. Thus, the retention changes only by a nearly negligible amount with pH, as the reversed-phase retention process dominates.

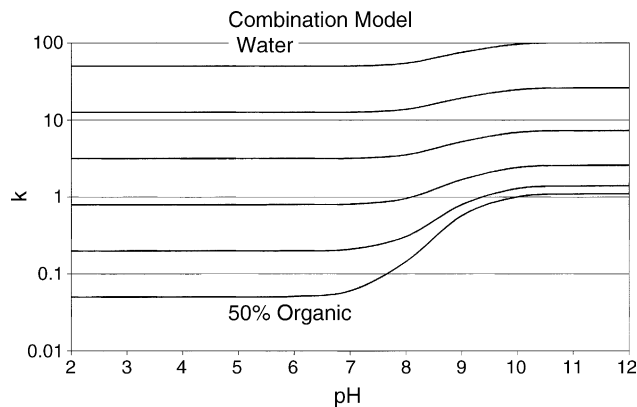


Fig. 5. Retention pattern for a quaternary amine after the model combining sequential hydrophobic interaction and ion-exchange with pure ion-exchange as a function of the pH.

The previously proposed multiplicative mechanism (see Eq. (17)) that combines the hydrophobic retention mechanism and the silanol activity [10,11] is shown in Fig. 4. The model follows Eq. (3), with the values of Eqs. (28) and (29) for k_{rp} and k_{ex} . As one can see, on both plots the different lines are parallel to each other. This means that the ratio of the retention factor at high pH to the retention factor at low pH is a constant, independent of the concentration of organic modifier in the mobile phase. At the same time, the ratio of the retention factors at any organic concentrations do not depend on the pH. Both observations can easily be seen from the underlying equation describing the multiplicative interaction, i.e. they are the essence of the multiplicative interaction model. This model accounts for the still large retention difference measured between low and high pH when the hydrophobic interaction dominates, while the classical model shown in Fig. 3 fails to explain such an observation. From this standpoint, the multiplicative model is superior to the classical model. On the other hand, the multiplicative model does not show any difference in the logarithm of the retention factor between low and high pH for a low hydrophobic interaction and a high hydrophobic interaction, as can be seen in the fact that all curves in Fig. 4a and b are parallel to each other.

The two separate models elucidate different parts of the experimental results. The classical model explains the larger difference in retention with increasing pH observed when the hydrophobic interaction is small. The multiplicative model furnishes an explanation for the still large retention difference between low and high pH when the hydrophobic retention is large. Thus, both models rationalize separate elements of the observed data. As a consequence, a combination of both models will deliver a more complete description of the retention process of cationic hydrophobic solutes on reversed-phase packings. Eq. (4) describes such a model. The results are shown in Fig. 5. For this plot, we assumed—as pointed out earlier—that the retention factors contributing to the mixed mode interaction are identical to the retention factors for the independent interactions. It can be seen that the increase in retention of the brenylion ion from ion-exchange with the silanols at high pH decreases, as the influence of the hydrophobic retention increases, but it does not disappear, as the classical model would indicate. For some of our data, such a combined interaction will be needed to explain the measured data.

In order to understand the interaction with silanols of a positively charged analyte on different surfaces, we examined three different packings. We will first discuss Nova-Pak C₁₈, then later Symmetry C₁₈, and finally XTerra RP₁₈.

The experimental results for Nova-Pak C₁₈ are shown in Fig. 6. Fig. 6a shows the dependence of retention on the solvent composition. In Fig. 6b, the dependence of retention on the pH is presented. In Fig. 6a, the solvent composition is displayed as the ratio r of the volume fraction of the organic solvent to the volume fraction of water. As can be seen on the left side of the graph, the retention decreased under

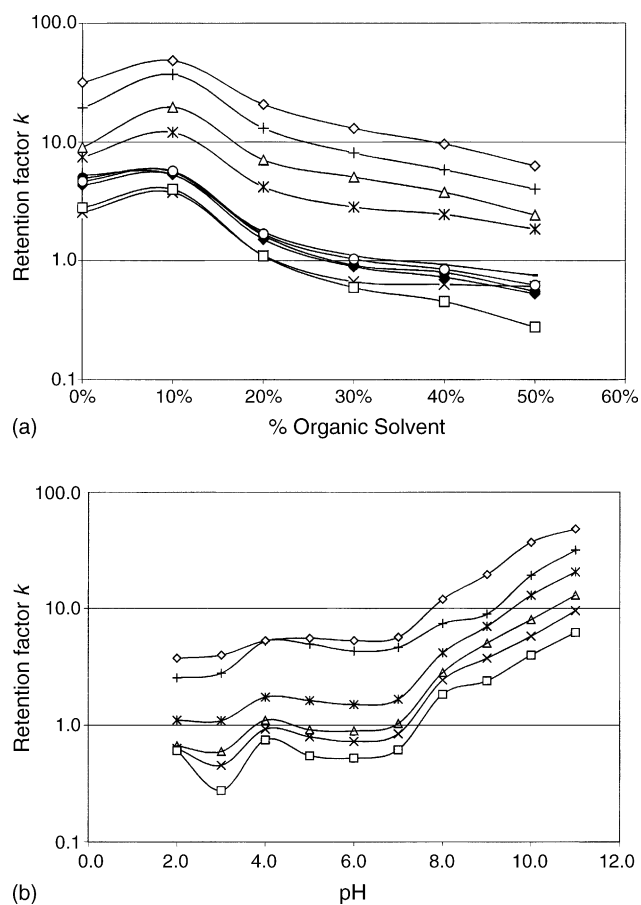


Fig. 6. Retention pattern of the bretylium ion on Nova-Pak C₁₈. (a) As a function of the acetonitrile concentration: (\diamond) pH 11, (+) pH 10, (Δ) pH 9, (\times) pH 8, (\circ) pH 7, (\blacklozenge) pH 6, (\bullet) pH 5, (–) pH 4, (\square) pH 3, (\times) pH 2. (b) As a function of the pH: (+) 0% acetonitrile, (\diamond) 10% acetonitrile, (\times) 20% acetonitrile, (Δ) 30% acetonitrile, (\times) 40% acetonitrile, (\square) 50% acetonitrile.

fully aqueous mobile phase conditions. This phenomenon is known and reasonably well understood [27–31].

In the following, we will briefly review the current understanding of the phenomenon that causes the loss of retention in highly aqueous mobile phases. Under these mobile phase conditions, the stationary phase “dewets” and drives the mobile phase out of the pores. The underlying mechanism is described by the equation by Laplace and Young [35], commonly used to describe pore penetration in mercury porosimetry:

$$\Delta P = -\frac{4\gamma \cos(\theta)}{d} \quad (30)$$

γ is the surface tension, θ the wetting angle, and d the pore diameter. As the mobile phase approaches a high water content, the wetting angle between the hydrophobic stationary phase and the mobile phase exceeds 90° , which creates a positive pressure that forces the mobile phase out of the pores. Since the pore diameter is in the denominator, this phenomenon is more severe with packings with a small pore size, and in a given packing with a given pore-size distribution, it occurs

first in the smallest pores. Furthermore, it happens at the downstream or low-pressure end of the column before it happens at the upstream end of the column. Counter to anticipation, the retention on Nova-Pak C₁₈ is therefore lower in a mobile phase containing 100% water than in a mobile phase containing 90% water. However, an analysis of the details of the retention pattern for bretylium shows also that some retention is lost in a mobile phase as weak as 90% water. For the purpose of our analytical goals, it was not possible to describe the retention pattern as a function of the mobile phase composition with the same equation as will be used later for Symmetry C₁₈ and XTerra RP₁₈.

In Fig. 6b, we observe a strong increase retention in the alkaline pH range. This is in agreement with expectation. Interestingly, the retention gap between low and high pH is roughly the same for mobile phases with a high and a low water content. This observation agrees with the prediction of the combined mixed-mode retention mechanism and thus suggests that for Nova-Pak C₁₈ the sole interaction with the free silanols plays an entirely subordinate role and adds only an insignificant element to the retention of the bretylium ion. This observation is in complete agreement with the pattern expected following Eq. (3) (or (15)), and the example shown in Fig. 4, especially Fig. 4b. The continued increase in retention and the lack of flattening of the curves at high pH are most likely due to the complexity of the silanol acidity on a classical, i.e. low-purity silica.

In order to eliminate the influence of “dewetting” on retention, we examined the retention changes as a function of pH at each solvent composition individually. For that purpose, the data were normalized by the average retention factors obtained at pH 5–7 that is the flat region in the plot. The results are shown in Fig. 7. In general, the retention pattern does not vary to a significant degree at a high concentration of organic solvent. This is again in good agreement with expectations based on the multiplicative model (see Fig. 4). In other words, it demonstrates that for Nova-Pak C₁₈ the retention pattern of the bretylium ion is dominated by the dual

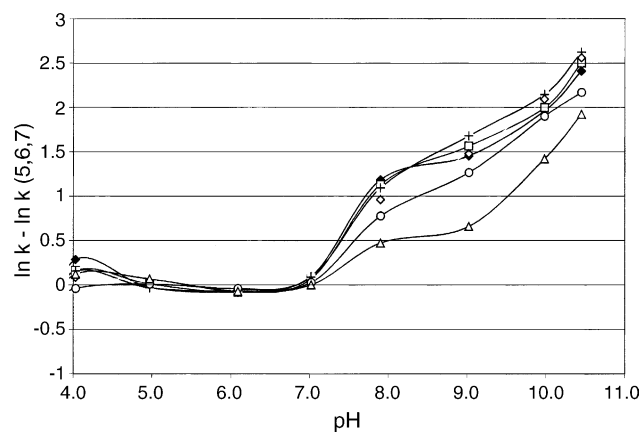


Fig. 7. pH influence on normalized retention on Nova-Pak C₁₈: (Δ) 0% acetonitrile, (\circ) 10% acetonitrile, (\diamond) 20% acetonitrile, (+) 30% acetonitrile, (\times) 40% acetonitrile, (\blacklozenge) 50% acetonitrile.

retention mechanism, and that the influence of free silanols (or pure hydrophobic interaction) is negligible. As some of the surface becomes unavailable for interaction in a mobile phase composition containing 10% or less acetonitrile, the interaction with the silanols decreases as well. The change is more pronounced for pH 9 than for the higher pH values, but the reason for this behavior is unclear at this time. One can speculate that the more acidic silanols are located in the smaller pores, which would be in agreement with the expectation that the silanol pK_a depends on the hydrophobicity of its environment.

For Nova-Pak C₁₈, the multiplicative interaction mechanism is dominant and it is potentially the sole mechanism. However, due to the broad pH range of increasing silanol activity, fitting procedures designed to determine a single pK_a of the silanols failed. This is most likely due to the fact that the simple fitting procedure used here together with the limited data set could not accommodate the possibility of a wide range of silanol pK_a values, as is expected for a classical silica based on the result of Méndez et al. [12]. At the same time, the limited data set could not accommodate more complex equations with multiple silanol pK_a values.

However, in order to gain further insight into the pattern observed for Nova-Pak C₁₈, the data were curve fit only over a narrower range in solvent composition. Using this procedure, it became clear that the curve fitting procedure inverted the dependence of the pH on solvent composition as shown in Table 2. The reason for this finding is simply that it is not the pH in the mobile phase that determines the retention pattern of the quaternary amine via the dual-step reversed-phase retention mechanism, but that it is the pH in the stationary phase that causes the ionization of the silanols. This value is different from the pH in the mobile phase, since the solvent environment inside the stationary phase layer is different from the mobile phase, i.e. this environment is much more hydrophobic. This phenomenon is the fundamental cause of the shift in pK_a observed in our data on reversed-phase packings compared with pK_a values measured on unmodified silica (e.g. reference [10]). However, the pH in the stationary phase cannot be measured by the means used here. In the plots shown above for Nova-Pak C₁₈, we used therefore the aqueous mobile phase pH.

The “dewetting” difficulties found with Nova-Pak C₁₈ were not encountered with the Symmetry C₁₈ and XTerra RP₁₈ phases. For XTerra RP₁₈, a dewetting is not expected, since the embedded polar group allows wetting in a fully aqueous mobile phase. Symmetry C₁₈ has a larger pore size, thus the phenomenon is not expected to be as pronounced as for Nova-Pak C₁₈, and the pressure in the post-column tubing was sufficient to prevent dewetting. The results for Symmetry are shown in Fig. 8. Fig. 8a shows the change in retention as a function of the solvent composition from 50 to 0% acetonitrile. One can see immediately that the different lines obtained at different pH values are not parallel to each other, as was the case for Nova-Pak C₁₈ and as the theory of an exclusively multiplicative retention mechanism as shown

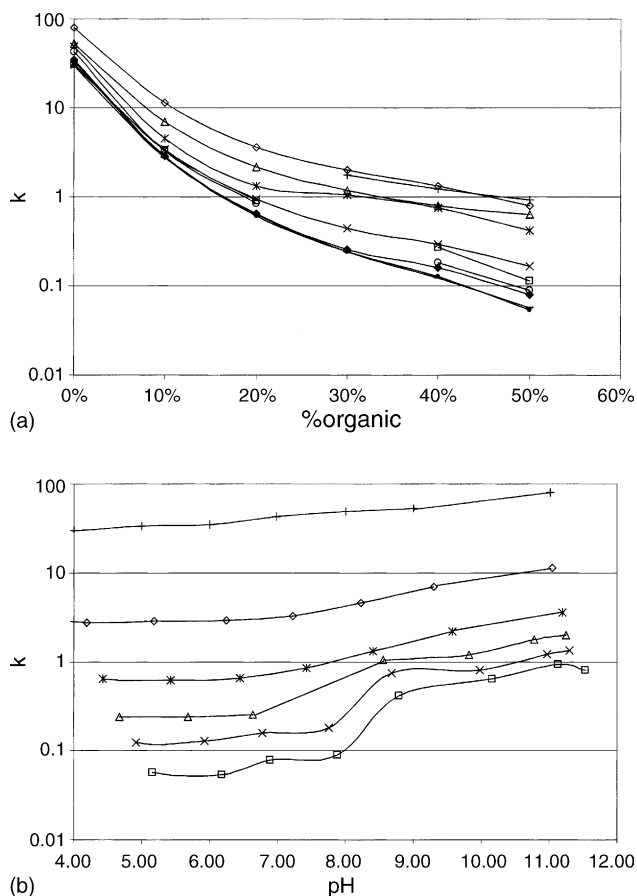


Fig. 8. Retention pattern of the bretylium ion on Symmetry C₁₈. (a) As a function of the acetonitrile concentration: (x) pH 2, (□) pH 3, (-) pH 4, (●) pH 5, (◆) pH 6, (○) pH 7, (✕) pH 8, (△) pH 9, (+) pH 10, (◇) pH 11. (b) As a function of the pH: (+) 0% acetonitrile, (◇) 10% acetonitrile, (✕) 20% acetonitrile, (△) 30% acetonitrile, (x) 40% acetonitrile, (□) 50% acetonitrile.

in Fig. 4 would predict. The data obtained for the pH range 4–12 are displayed in Fig. 8b. One can see a clear transition range in the activity of the silanols, including a leveling off at higher pH. The behavior in Fig. 8b suggests that there are multiple interactions, as shown in the example pattern of Fig. 5. Two mechanisms occur simultaneously: (1) a multiplicative (that is a reversed-phase assisted) ion-exchange mechanism and (2) a pure ion-exchange mechanism. Based on this assessment, we will attempt to do curve fitting to Eq. (26).

In a first curvefitting attempt, we assigned two different pK_a values to the silanols that show direct interaction compared to those involved in the multiplicative retention mechanism. The curvefit results were good, but the error windows of the two pK_a values for the postulated two types of silanol groups overlapped. This means that there are not two statistically distinguishable types of silanols, but only a single type that should be considered for further fitting procedures.

In Table 3a and b, the curvefit results for Symmetry C₁₈ to a single type of silanol with a single pK_a are shown. In Table 3a, the curvefit is done assuming that the pH value

Table 3
Results for Symmetry C₁₈

Parameter	(a) Curvefit 1 with pH correction		(b) Curvefit 2 without pH correction	
	Result	Standard deviation	Result	Standard deviation
$k_{\text{H}_2\text{O}}$	31.0	2.7	29.5	2.6
A	-29.1	1.8	-29.3	1.7
B	3.68	0.3	3.78	0.28
K_{mix}	65.2	11.0	51.2	8.0
$\text{p}K_{\text{SiOH}}$	8.80	0.10	8.11	0.09
k_{SiOH}	0.73	0.09	0.82	0.09
r^2	0.9948		0.9955	

Table 4
Results for XTerra RP₁₈

Parameter	(a) Curvefit 1 with pH correction		(b) Curvefit 2 without pH correction	
	Result	Standard deviation	Result	Standard deviation
$k_{\text{H}_2\text{O}}$	4.90	0.14	4.79	0.14
A	-14.5	0.4	-14.3	0.41
B	2.25	0.09	2.22	0.09
k_{mix}	4.6	0.8	2.7	0.4
$\text{p}K_{\text{SiOH}}$	10.33	0.12	9.44	0.11
k_{SiOH}	0.095	0.015	0.135	0.016
r^2	0.9982		0.9983	

responsible for the interaction of the bretylium ion with the silanol groups in the C₁₈ layer is a function of the organic solvent concentration, in Table 3b we assume that this is not the case. The latter curvefit was triggered by the finding for Nova-Pak C₁₈ that a correction of the pH of the mobile phase with the organic solvent composition was not in agreement with the curvefitting results. In agreement with the findings for Nova-Pak C₁₈, better results are obtained, if we use the aqueous pH for curvefitting instead of the pH measured in the mobile phase (results Table 3b). As for Nova-Pak C₁₈, this finding for Symmetry C₁₈ indicates that the silanol ionization is either not or a very weak function of the mobile phase composition, due to the fact that the local environment of the silanols does not vary significantly with the organic content of the mobile phase.

Both fitting methods result in a silanol $\text{p}K_{\text{a}}$ above 8. The higher $\text{p}K_{\text{a}}$ of the silanols on a C₁₈ (between 8 and 9) compared to the free silanols on a silica (around 7) is in agreement with our previous results [10], as well as the results of others [36] and in agreement with the reasonable expectation of a lower dielectric constant in the less polar surface region as compared to the mobile phase.

Next, the same two-dimensional curvefitting procedure was used for XTerra RP₁₈, a well-deactivated packing with an embedded polar group. Table 4 and Fig. 9 show the results of this procedure. As above, the data in Table 4a show the figures for the fit to the pH measured in the presence of the organic solvent, while the data in Table 4b reflect the results of the fit using the aqueous pH. The quality of the data is even better than the quality of the Symmetry C₁₈ data, with an overall correlation coefficient of 0.9983 compared to 0.9955

for the same curvefit for Symmetry. There are several important differences between the results obtained for Symmetry C₁₈ and XTerra RP₁₈. The retention factor for the bretylium ion is much lower on XTerra RP₁₈. In addition, the values of k_{mix} and k_{SiOH} , which reflect the influence of the silanol groups on retention, are also much smaller on XTerra RP₁₈. Especially the value for the direct interaction with silanols k_{SiOH} is nearly negligible. Most importantly, the $\text{p}K_{\text{a}}$ of the silanols on this packing has shifted still further into the alkaline pH range compared to Symmetry C₁₈, to a value of 10.33 with pH correction or 9.44 without pH correction. This is in agreement with previous results and with expectation. The shift in $\text{p}K_{\text{a}}$ for the hybrid packings has been measured

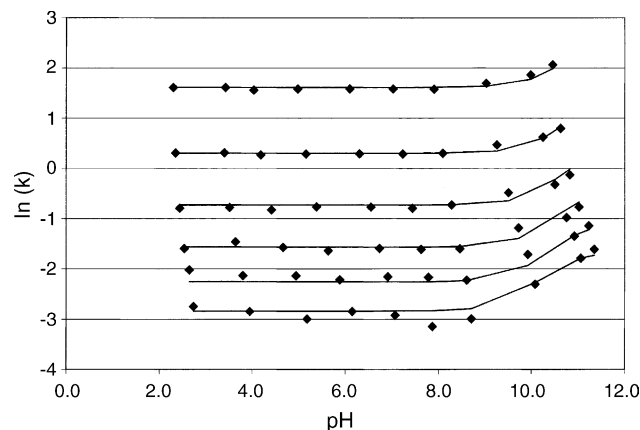


Fig. 9. Retention pattern of the bretylium ion on XTerra RP₁₈ as a function of pH and solvent composition. The curvefit (lines) is overlaid to the measured data.

before for the underivatized material in reference [10]. For the C₁₈-bonded hybrid, a shift of the pK_a towards the alkaline pH has also been established in reference [12], but the pK_a of the surface silanols was not determined by these authors.

We have therefore observed the combined reversed-phase ion-exchange mechanism on three substantially different reversed-phase packings. We conclude that the multiplicative mechanism is of major significance in the interaction of basic analytes with silica-based reversed-phase packings.

A brief comment on the residual scatter in the data is also necessary. Repetitive experiments showed, that only a small part of the scatter is related to experimental errors. We believe that there is a more fundamental reason. It is of course impossible to carry out a study over such a broad pH range with a single buffer. The consequence of this is the fact that the negatively charged buffer ion needed to change, and with this, counterion effects on retention [37] could not be avoided. Even the adsorption of the acetate ion on the surface in a fully aqueous mobile phase cannot be excluded. At the same time, these effects were small compared to the pH effects that were the primary purpose of our investigation.

5. Conclusions

The influence of silanols on the retention pattern of positively charged analytes is different from what has been described in the older literature. It is dominated by an interaction mechanism that combines reversed-phase interaction of the analyte with the hydrophobic C₁₈ layer with ionic binding to charged silanols in a multiplicative manner. The mechanism of pure ion exchange with silanols can also be observed, but it plays a smaller role. The multiplicative reversed-phase ion-exchange interaction mechanism has been observed on three different packings with substantially different character: Nova-Pak C₁₈, a bonded phase based on a classical silica, Symmetry C₁₈ based on a high-purity silica, and XTerra RP₁₈, a bonded phase with an embedded polar group based on an inorganic–organic hybrid packing. The multiplicative interaction is the sole factor determining the retention of the bretylium ion for Nova-Pak C₁₈. As the multiplicative reversed-phase ion-exchange mechanism becomes less dominant on modern, highly deactivated packings, a contribution to retention by pure ion-exchange can be observed as well. A similar multiplicative interaction has been observed previously on zirconia-based packings [11]. Based on these observations by two different groups on a broad range of packings, there remains little doubt that the multiplicative reversed-phase ion-exchange mechanism is rather universal and dominant in the interaction of charged bases with reversed-phase packings.

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