

Analysis and critical comparison of the reversed-phase and ion-exchange contributions to retention on polybutadiene coated zirconia and octadecyl silane bonded silica phases

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Abstract

The two major modes of retention of basic compounds in reversed-phase liquid chromatography on both octadecyl silane bonded silica-based (ODS) and polybutadiene coated zirconia (PBD-ZrO₂) materials are hydrophobic and ion-exchange (Coulombic) interactions. Although the influence of reversed-phase and Coulombic interactions on the chromatography of organic cations is qualitatively well recognized, the quantitative relationship between hydrophobic and ion-exchange interactions remains unclear. In this work, the retention mechanisms on both of the above types of phases were probed by studying the retention of a homologous series of *p*-alkylbenzylamines as a function of the ammonium concentration in the eluent. The various columns tested were studied in terms of plots of retention factor vs. the inverse of the displacing cation concentration. The analysis of such plots as well as plots of log *k'* vs. number of methylene groups in the solutes and plots of log *k'* vs. log[NH₄⁺] clearly shows that at least two types of sites—a pure reversed-phase site and a “hydrophobically-assisted ion-exchange site” similar to the type of site described by Neue [J. Chromatogr. A 925 (2001) 49] are needed to explain the observations. In addition, we have found a quantitative measure of the relative amount of reversed-phase and ion-exchange interaction on a given solute has on a given stationary phase which allows unambiguous classification of columns. It is now clear that ion-exchange contributions to retention on PBD-ZrO₂, sometimes exceeding 90%, are even more important than previously thought and relative to hydrophobic interaction much more significant on PBD-ZrO₂ than on ODS type-B silicas.

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1. Introduction

The analysis of basic compounds by RPLC continues to be of great interest and importance to

chromatographers [1–3]. Most such analyses are performed on alkyl silane bonded silica-based stationary phases [4]. Solutes are retained predominantly by hydrophobic (reversed-phase) interactions with the bonded phase. However, the presence of residual ionized silanol groups on silica's surface greatly complicates the retention processes. There is wide agreement that basic solutes undergo a mixed-

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mode retention mechanism on silica-based bonded phases which involves both reversed-phase and ion-exchange interactions [1,2,5]. Ion-exchange interactions between basic solutes and the surface negative charges often lead to tailed peaks, poor efficiency, and irreproducible retention [6]. Adsorption data for organic bases are well fit by a bi-Langmuir [7,8] isotherm clearly suggesting the existence of two classes of independent retention sites but certainly ruling out a single site mechanism. Hydrophobic interactions take place on the weaker but more numerous type of site, whereas the ion-exchange interactions take place on the stronger but less populous silanol site. According to the data fits, the number of the weaker site is 20–60 times larger than the number of stronger site [7,8]. The site-type ratio is not in agreement with the relative number of bonded alkyl silane chains and residual silanol groups. This suggests that only a small fraction of the silanol sites are involved in the ion-exchange retention process.

The high chemical and thermal stability of zirconia-based phases [9–11] and the radically different selectivities for basic molecules [12] make them very useful complements to silica-based supports. To date, polybutadiene-coated zirconia (PBD-ZrO₂) has been the most studied zirconia-based reversed-phase material [13]. It has proven to be very useful for the separation of nonpolar and polar solutes over a wide range in pH (1–14) and at temperatures up to 200 °C [14,15]. The main distinction between zirconia and silica phases is their surface chemistry [16]. While the various kinds of silanol groups are the biggest complication on silica-based phases, the large number of *hard* Lewis acid sites on zirconia's surface, which can be identified with coordinatively unsaturated Zr(IV) sites, present a significantly different challenge [17,18].

The chemistry of chemically bonded silica is controlled in part, especially for cationic analytes, by the various types of silanol groups. Some of these silanol groups are moderately strong ($pK_a < 4$) Bronsted acids [5]. The fact that some sets of basic analytes exhibit considerably different elution orders on different silica based phase at pHs as low as 3 combined with the significant improvement in the peak shape of certain solutes (e.g. meclizine [19]) when the pH is decreased from 1 to 0.5 by increasing

the amount of trifluoroacetic acid in the eluent, leads us to believe that even on type-B silica there must exist some very acidic silanols despite the average silanol pK_a is about 7 [1,2,5]. Very recent work of Rosés et al. [20] indicates a class of silanols on certain silicas with a pK_a as low as 3. Early work by Weber [21] and others [3,22–24] showed that older type-A (i.e. low purity, non-metal free) silicas were able to act as ion-exchangers at pHs as low as pH 2.

In contradistinction to the behavior of silanol groups, Zr(IV) sites (these are *hard* Lewis acids) on zirconia cause *hard* Lewis base analytes (R-SO₃⁻, R-PO₃⁻, R-COO⁻, etc.) to adsorb quite tenaciously [15,17,18,25]. Such Lewis acid–base interactions are characterized by especially slow desorption kinetics, which cause broad and tailed peaks. However, when a strongly competing Lewis base (PO₄³⁻, F⁻, carboxylates) is deliberately added to the eluent at sufficiently high concentration, the accessible Zr(IV) sites are dynamically blocked by adsorption of the eluent [26]. Addition of a hard Lewis base to the eluent thereby greatly improves the peak shape of the above mentioned analytes [26]. Concomitantly the chemisorption of these anionic Lewis base eluent additives (PO₄³⁻, F⁻, etc.) generates a surface with a high negative charge. Under these conditions, PBD-ZrO₂ phases acquire cation-exchange characteristics in addition to their inherent reversed-phase sorption potential (see Fig. 1) [26]. It has been argued that the very significant differences in selectivity of ODS-silica and PBD-ZrO₂ in phosphate media at pH 7 [27] is due to the existence of a larger contribution to retention of ion-exchange relative to reversed-phase interactions on the zirconia-type phase compared to a silica type phase.

Evidently, on both silica and zirconia-based phases, basic solutes can undergo mixed-mode retention involving reversed-phase and cation-exchange interactions. We felt it would be interesting to study and compare the retention characteristics of cationic solutes on reversed-phases which are as radically different as ODS and PBD-ZrO₂ in the hope that the comparison would be mutually revealing. Additionally, a number of retention models for mixed-mode retention mechanism [28–31] for silica-based phases, which have distinctly different physical interpretations, have been published. However, little systematic work has been done to evaluate

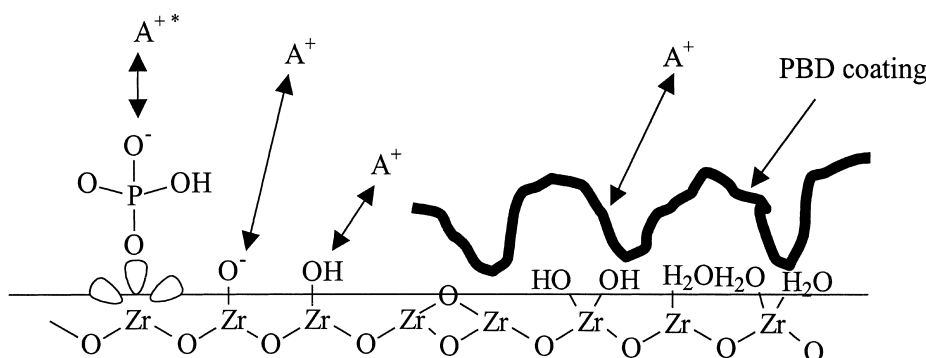


Fig. 1. Multi interactions between analytes and the surface of phosphate dynamically modified PBD-ZrO₂. * A⁺: analyte cation.

these models. In this work, experiments were designed to compare and evaluate the existing models. We hoped to gain more understanding of the surface chemistry on both ODS and PBD-ZrO₂ phases.

2. Theory

There are two distinct, quantitative models of *mixed-mode* retention in RPLC: the one-site and two-site models. We will begin with relevant background on “pure” (not mixed-mode) ion-exchange and “pure” reversed-phase chromatography. For simplicity, the discussion is couched in terms of ODS phases, but we believe that the same *principles* are applicable to PBD-ZrO₂ phases. The purpose of this section is to present the fundamental equations and relationships which will be used to differentiate between different types of one- and two-site models of mixed-mode retention.

2.1. A “pure” ion-exchange (IEX) retention process

Ion-exchange retention of a cationic analyte A⁺ is most commonly described as a stoichiometric displacement reaction:



where SiO⁻ and C⁺ denote the anionic surface site and its counterion, respectively, and the subscript m and s represent the species in the mobile and stationary phase, respectively. The equilibrium constant for this process is:

$$K_A = \frac{[\text{SiO}^-:\text{A}^+]_s \cdot [\text{C}^+]_m}{[\text{SiO}^-:\text{C}^+]_s \cdot [\text{A}^+]_m} \quad (2)$$

Upon rearrangement, Eq. (2) leads to the ion-exchange retention factor for A⁺:

$$\frac{[\text{A}^+]_s}{[\text{A}^+]_m} \equiv \frac{[\text{SiO}^-:\text{A}^+]_s}{[\text{A}^+]_m} = \frac{K_A \cdot [\text{SiO}^-:\text{C}^+]_s}{[\text{C}^+]_m} \quad (3)$$

$$k'_{\text{IEX}} = \phi_{\text{IEX}} \cdot K_{\text{IEX}} = \phi_{\text{IEX}} \cdot \frac{K_A \cdot [\text{SiO}^-:\text{C}^+]_s}{[\text{C}^+]_m} \quad (4)$$

ϕ_{IEX} and K_{IEX} are the appropriate phase ratio and distribution ratio for the ion-exchange process, and $[\text{SiO}^-:\text{C}^+]_s$ is the *total* number of available silanol sites per unit area. For low buffer concentrations (below 0.1 M), it equals the total ion-exchange capacity of the column divided by the volume of the stationary phase [32]. For a given column all of the terms in Eq. (4) except for $[\text{C}^+]_m$ can be combined into a single constant B_{IEX} :

$$k'_{\text{IEX}} = \frac{B_{\text{IEX}}}{[\text{C}^+]_m} \quad (5)$$

We note in passing that for 1:1 displacement processes Eq. (5) leads to the conclusion that there is no retention ($k'_{\text{IEX}} = 0$) upon extrapolation to $\frac{1}{[\text{C}^+]_m} = 0$.

From Eq. (5), the well-known log–log relationship between the ion-exchange retention factor for a singly charged analyte ion and the concentration of a univalent counterion can be derived:

$$\log k'_{\text{IEX}} = -\log[\text{C}^+]_m + \log B_{\text{IEX}} \quad (6)$$

In this work we have used only univalent displacing cations and univalent cationic analytes thus the slope of plots of $\log k'_{\text{IEX}}$ vs. $\log[\text{C}^+]_{\text{m}}$ should be -1 .

Ståhlberg [33,34] and others [35] have used an electrostatic (non-stoichiometric) model of Coulombic contributions to retention, which does not assume the existence of specific sites but rather treats the surface as having a uniform electric potential that attracts oppositely charged analytes. We use the simpler stoichiometric model in this work. It is almost impossible to distinguish between these two approaches at the low concentrations of salt in the eluent used here.

2.2. A “pure” reversed-phase (RP) retention process

A very fundamental characteristic of RPLC which we will use later to differentiate between models is the linear additivity of the free energy of retention of methylene groups which leads to a linear relationship between $\log k'_{\text{RP}}$ and the number of methylene groups (n_{CH_2}) for a homolog series of probe solutes [36,37]. This relationship is called the Martin equation:

$$\log k'_{\text{RP}} = \log(\phi_{\text{RP}} \cdot K_{\text{RP}}) = A + Bn_{\text{CH}_2} \quad (7)$$

where ϕ_{RP} and K_{RP} are the phase ratio and distribution ratio for a pure reversed-phase retention process, A is the intercept of a plot of $\log k'$ vs. homolog number and varies with the terminal functional group of the homolog series, while the slope B is related to the free energy of transfer of a methylene group from the mobile to the stationary phase. To a good approximation B is independent of the homolog series used [38].

2.3. The one-site model of mixed-mode retention of cations

We turn now to a one-site model of *mixed-mode* retention of basic solutes in RPLC. One such model was explicated in a recent paper by Neue and his coworkers [28]. Among the many important issues addressed in that paper, they attempted to explain the *simultaneous* involvement of reversed-phase and ion-exchange contributions to net solute retention within

the context of a one-site model. For a cationic solute, they assumed that both reversed-phase and ion-exchange interactions take place simultaneously at a *single type of site*. They combined the retention effects of cation-exchange and reversed-phase for the analyte cation by summing up the free energies of both types of interactions:

$$\ln k' = \ln(\phi) + \left(\frac{\Delta G_{\text{RP}}^\circ}{RT} + \frac{\Delta G_{\text{IEX}}^\circ}{RT} \right) \quad (8)$$

where ϕ is the phase ratio for the single type of site; $\Delta G_{\text{RP}}^\circ$ and $\Delta G_{\text{IEX}}^\circ$ are the free energy contributions from the reversed-phase and ion-exchange interaction processes, respectively. While a number of models of free energies of solvation represent the *total* free energy of solvation as resulting from independent contributions attributed to different types of interactions (e.g. the LSER [39–41] and solubility parameter [42–45] models) or independent contribution from different functional groups (e.g. the Martin equation [36,37] and the UNIFAC model [46,47]), nonetheless it is evident that such independent additivity is a simplification and approximation. We now make explicit the dependence of k' on the counterion concentration which is implicit in Neue’s work by substituting Eq. (4) into Eq. (8):

$$k' = k'_{\text{RP}} \cdot \frac{K_{\text{A}} \cdot [\text{SiO}^-:\text{C}^+]_{\text{s}}}{[\text{C}^+]_{\text{m}}} \quad (9)$$

The physical meaning of this specific equation is that there is only *one type of site*, which is an ion-exchange type site, and that the *only* interaction that a basic solute undergoes is a *simultaneously* interaction with both the hydrocarbon chain and the ionized silanol group (see Fig. 2A). We believe that Eq. (9) is consistent with Neue’s model and equations. It is evident that as $1/[\text{C}^+]_{\text{m}}$ approaches zero the retention factor of the basic solute *must* become zero. The fact that k' must become zero means that there is no contribution to retention from a “pure” hydrophobic site or any other non ion-exchange site. Thus this site in which solute hydrophobicity augments retention on an inherently ion-exchange like site is what we call a “*hydrophobically assisted ion-exchange one-site model*”. It is our contention that retention in RPLC of bases cannot be due solely to one type of site be it a hydrophobically-assisted

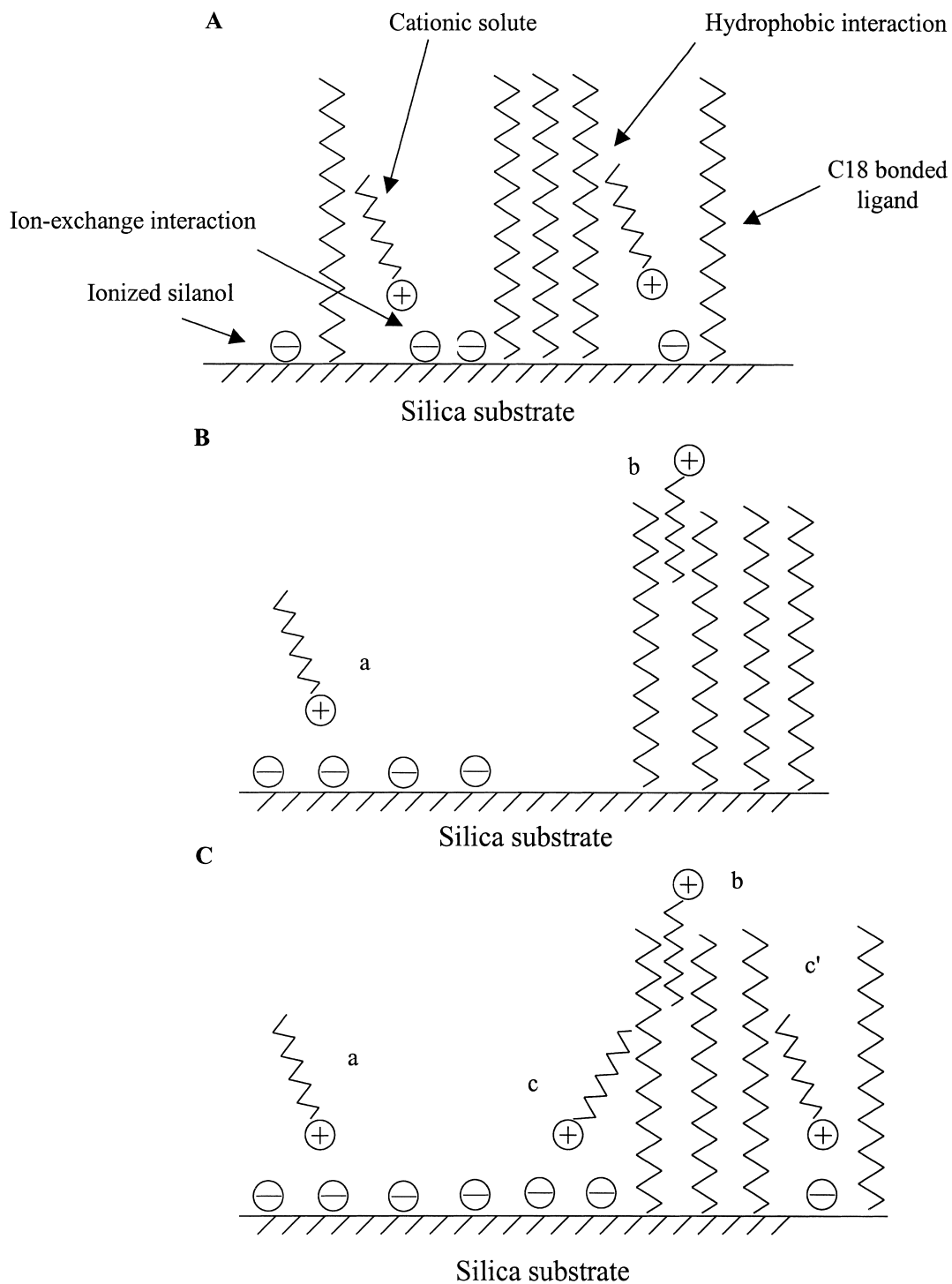


Fig. 2. Cartoon illustrating interactions between a cationic solute and an ODS phase. (A) One-site model; (B) two-site model; (C) three-site model. (a) Pure ion-exchange site; (b) pure reversed-phase site; (c) hydrophobically assisted ion-exchange site; (c') intimate hydrophobically assisted ion-exchange site.

site, a simple reversed-phase site or a pure (non-hydrophobically assisted) type site. As will evolve we believe that one of the sites involved in retention of organic bases in RPLC must be a hydrophobically-assisted site of a type consistent with Eq. (8) and (9). Furthermore we point out that all single-site models are inconsistent with the bi-Langmuir adsorption isotherm studies mentioned above [7,8].

Taking the logarithm of Eq. (9) gives:

$$\log k' = A' + \log k'_{\text{RP}} - \log[\text{C}^+]_{\text{m}} \quad (10)$$

where A' is a constant equal to $\log(K_{\text{A}} \cdot [\text{SiO}^- \cdot \text{C}^+]_{\text{s}})$. Because the two logarithmic terms on the right-hand side are independently additive, combination of Eqs. (7) and (10) leads to an equation that makes explicit the dependence of $\log k'$ on n_{CH_2} and $\log[\text{C}^+]_{\text{m}}$:

$$\log k' = A'' + Bn_{\text{CH}_2} - \log[\text{C}^+]_{\text{m}} \quad (11)$$

where A'' is a constant related to A and A' . This equation, based on the one-site hydrophobically assisted ion-exchange model of mixed-mode retention, predicts that the slope of $\log k'$ vs. $\log[\text{C}^+]_{\text{m}}$ for a univalent cation *must* be -1 and that for a homolog series of such species the slope of $\log k'$ vs. n_{CH_2} *must* be the same as for a nonpolar, non-ionic homolog series.

2.4. The two-site model of mixed-mode retention of cations

A quite different kind of model for basic solute binding in RPLC, a two-site model, was originally proposed by Sokolowski and Wahlund [29] and more completely elaborated by Horvath and coworkers [30,31]. Solutes are assumed to bind to the surface at distinct reversed-phase and silanophilic sites. A fraction of the population of the stationary phase solute molecules are bound to the hydrophobic sites (e.g. alkyl chains) and the remainder to the independent, chemically distinct silanol sites. The key assumption of this model is that the two different binding sites, i.e. the hydrocarbon chain and the silanol groups, are separate and independent (see Fig. 2B). Therefore, the ensemble of solute molecules *independently* interact with the two types of sites; and retention is the sum of two independent processes:

$$k' = \phi_{\text{RP}}K_{\text{RP}} + \phi_{\text{IEX}}K_{\text{IEX}} = k'_{\text{RP}} + k'_{\text{IEX}} \quad (12)$$

The observed retention factor of the solute, k' , is equal to the sum of the reversed-phase retention, k'_{RP} , and the ion-exchange (silanophilic) retention, k'_{IEX} . We note in passing that in Horvath's work [30,31] no assumption is made concerning the nature of the silanophilic interaction which is here assumed to be an ion-exchange process as suggested by Neue. Thus retention is treated as if it were identical to the situation that would exist if there were two different columns coupled in series, retention by one of the columns being pure reversed-phase, while the other allows for ion-exchange interactions between the solute and the stationary phase. It is quite possible that the ion-exchange site could be of the type described by Neue, i.e. it could be a "hydrophobically-assisted" ion-exchange site wherein a more hydrophobic solute would bind more tightly to the surface charged site than a less hydrophobic solute.

To examine how the logarithm of k' for the two-site model varies with conditions (i.e. eluent displacer concentration and solute methylene number) we first sum the two retention factors and then take the logarithm. Because Eq. (12) is very different in form than Eq. (9) it follows that Eq. (13) will be very different than Eq. (10):

$$\log k' = \log(k'_{\text{RP}} + k'_{\text{IEX}}) \quad (13)$$

Note that K_{RP} will vary with homolog number according to Eq. (7) and K_{IEX} is defined implicitly in Eq. (4) and thus Eq. (12) can be rewritten as:

$$k' = \phi_{\text{RP}}K_{\text{RP}} + B_{\text{IEX}}/[\text{C}^+]_{\text{m}} \quad (14)$$

For convenience, we will call this the "two-site mixed-mode model". Note that the combination of two independent sites, with their individual phase ratios and binding constants, is quite consistent with a bi-Langmuir isotherm for analyte adsorption [7,8]. The dependence of k' on $[\text{C}^+]_{\text{m}}$ that which Horvath et al. [31] derived is slightly different from that shown in Eq. (14) but when the equilibrium constant for binding of the silanol blocker agent are large their equation becomes indistinguishable from Eq. (14). Differences would only become apparent at very low values of $[\text{C}^+]_{\text{m}}$, where k' values would be quite large on most phases.

It is important to point out that Eqs. (6) and (7)

are still valid for the individual contributions to retention in Eq. (13). However, it is impossible to combine them into separable and independent terms as is done in Eq. (11). We now combine Eqs. (13) and (14):

$$\log k' = \log(k'_{\text{RP}} + B_{\text{IEX}}/[C^+]_m) \quad (15)$$

Because k'_{RP} is only very weakly dependent on salt concentration we see that the first term inside parenthesis of the logarithm on the right hand side of Eq. (15) is more or less a constant whereas the second varies inversely with the eluent counterion concentration. The relationship between $\log k'$ and $\log[C^+]_m$ will be quasi-linear and its apparent slope must depend on which term is dominant. When k'_{RP} greatly exceeds the second term then the slope, predicted by Eq. (15), will be much closer to zero than to the value of -1 as predicted by Eq. (11) based on the one-site model. On the other hand when k'_{IEX} is much larger than k'_{RP} the two-site model leads to a slope of -1 for $\log k'$ vs. $\log[C^+]_m$.

One of the most glaring differences between Eqs. (9) and (14) is that for the one-site model all retention must disappear when the eluent concentration of salt is made very large whereas Eq. (14), based on the two-site model, allows the existence of a finite value of k' at very high salt concentration. The difference in dependence of retention on eluent concentration of counterion for the one and two-site mixed-mode retention models are summarized and compared in Table 1.

We turn now to a discussion of the effect of n_{CH_2} on k' for the two-site model. To do so we must consider two distinct situations assuming that the addition of methylene group on the solutes of the homolog series does not affect the size of the charge center on the solutes (see below):

Table 1
Dependence of the slope of $\log k'$ vs. $\log[C^+]_m$ on retention mechanism and retention model

Retention mechanism	One-site model	Two-site model
Reversed-phase (RP)	0	0
Ion-exchange (IEX)	-1	-1
Mixed-mode (RP dominates)	-1	~0
Mixed-mode (IEX dominates)	-1	~-1

Case A: The net ion-exchange equilibrium constant is assumed to be independent of solute hydrophobicity. We do not believe this case applies to any ODS type phase but is possible on a PBD-ZrO₂ phase given the non-uniform distribution of polymer in the pores.

Case B: The net ion-exchange equilibrium constant is assumed to vary with solute hydrophobicity (hydrophobically assisted ion-exchange).

In case A, we assume that only the reversed-phase contribution to retention varies with the solute's hydrophobicity and for a homolog series of solutes it does so in accord with Eq. (7). If we combine Eqs. (7) and (13) and plot $\log k'$ vs. n_{CH_2} we will only get a good straight line with the same slope as for a series of nonpolar solutes when the reversed-phase contribution to retention is much larger than the ion-exchange contribution ($k'_{\text{RP}} \gg k'_{\text{IEX}}$).

As pointed out above in case B, we assume the dependence of the strength of the ion-exchange process on solute hydrophobicity. In this case, in the context of the present two-site mixed-mode model, it is possible to conceive of an ion-exchange site (see Fig. 2C, site c and c') whose strength is augmented by the solute's hydrophobicity. Neue's one-site model constitutes such a site. In this case one cannot use the dependence of $\log k'$ on n_{CH_2} to differentiate between the one and two-site mixed-mode models.

2.5. Analysis of k' vs. $1/[C^+]_m$ plots

Cox and Stout [48] first used the intercept of a plot of k' vs. inverse counterion concentration to search for a second non-ion-exchange process at very high buffer concentrations. Recently, McCalley and co-workers [49,50] have made extensive use of such plots to test for the presence of ion-exchange contributions to retention. Eq. (4) (for a pure ion-exchange model), Eq. (9) (for the one-site mixed-mode model) and Eq. (14) (for a two-site mixed-mode model, both case A and case B) indicate that a plot of k'_{IEX} against the reciprocal of the eluent counterion concentration will be a straight line with a slope, B_{IEX} , proportional to the ion-exchange equilibrium constant and the number of accessible silanol sites. If there is only one type of site, specifically an ion-exchange site, *the intercept must be zero*. If there are

two types of sites, one of which is not due to ion-exchange, then *the intercept must be finite*. A large slope relative to the intercept indicates that ion-exchange interactions predominate.

3. Experimental

3.1. Instruments

All chromatographic experiments were carried out with a Hewlett-Packard 1090 chromatographic system, equipped with a binary pump, an autosampler, a temperature controller, and a diode array detector (Hewlett-Packard, Wilmington, DE, USA). Data were collected and processed using Hewlett-Packard Chemstation software.

3.2. Analytical columns

The PBD-ZrO₂ (Batch No. 24-124) particles used in this work were obtained from ZirChrom Separations (Anoka, MN, USA). The average particle size is 4.1 μm. The surface area of the packing is 11.2 m²/g (by BET) and the average pore diameter is 500 Å. The carbon content is 2.5% (w/w). The zirconia particles were packed by the downward slurry method at 5000 p.s.i. Stainless-steel (316) column blanks with dimensions of 50×4.6 mm I.D. and 0.5-μm stainless-steel frits were obtained from Isolation Technologies (Hopdale, MA, USA).

The ODS columns used (5 μm particle size, 15×0.46 cm I.D.) were Ace 5 C₁₈ and Alltima C₁₈. Ace was obtained from Advanced Chromatography Technologies (Aberdeen, Scotland, UK). The surface area is 300 m²/g and the pore size is 100 Å with a carbon loading of 15.5% (w/w). Alltima columns were purchased from Alltech (Alltech Associates, Deerfield, IL, USA). The surface area of this column is 350 m²/g and the pore size is 100 Å with a carbon loading of 16% (w/w).

3.3. Reagents

All chemicals were reagent grade or better. HPLC-grade methanol (MeOH) was from Pharmco (Brookfield, CT, USA). HPLC water was obtained from a Barnsted Nanopure deionizing system (Dubuque, IA,

USA) and run through an “organic-free” cartridge followed by a 0.2-μm particle filter. The water was boiled to remove carbon dioxide. All solvents were filtered through a 0.45-μm filter (Lida Manufacturing, Kenosha, WI, USA) before use. Unless otherwise mentioned, all solutes were purchased from Aldrich (Milwaukee, WI, USA).

3.4. Preparation of *p*-alkylbenzylamines

Approximately 15 ml of ammonia were added to a 50-ml round bottom flask, which was put in an ice bath. Next, 0.34 g (2 mmol) of *p*-ethylbenzoyl chloride was added dropwise. The reaction solution was then stirred for 10 min after adding the *p*-ethylbenzoyl chloride. White solid precipitated from the solution, which was filtered and washed with water and hexane. The solid was then dried under vacuum and the structure was determined by NMR to be *p*-ethylbenzamide. The solid and 10 ml tetrahydrofuran was added to a 25-ml round flask equipped with a septum and nitrogen inlet, and 2 ml of 1.0 M LiAlH₄ was added dropwisely by using a syringe. The resulting solution was stirred and refluxed at 65 °C overnight under a nitrogen atmosphere. To separate the final product, *p*-ethylbenzylamine, from the reactant, *p*-ethylbenzamide, the solution was first acidified with 1 M hydrochloric acid solution. The aqueous phase was then separated from the organic layer by using a separation funnel and neutralized by 1 M sodium hydroxide to free the amine, which was then extracted with ethyl ether. The ether extract was dried with anhydrous sodium sulfate and the ether was removed by distillation. Roughly 0.22 g (yield: 80%) of *p*-ethylbenzylamine was obtained and NMR confirmed the compound identity. Other *p*-alkylbenzylamines used (about 0.2 g each) were synthesized by the above procedure from *p*-alkylbenzoyl chloride (C₂–C₆). The final product was dissolved in acetonitrile for use as a solute in the HPLC studies.

3.5. Chromatographic conditions

All measurements were made at a flow-rate of 1 ml/min with detection at 254 nm. The injection

volume was 1 μl with analyte concentrations of 1–2 mg/ml. The column temperature was controlled to 35 $^{\circ}\text{C}$ with a precision of ± 0.2 $^{\circ}\text{C}$. The dead time was determined by injecting uracil and acetone for the ODS and PBD-ZrO₂ column, respectively. The mobile phases were prepared by first dissolving appropriate amounts of phosphoric acid and ammonium hydroxide in water for the desired pH and concentration. These solutions were then filtered through a Millipore (type HA) 0.45- μm membrane filter prior to use, and finally mixed with pre-filtered MeOH.

4. Results and discussions

Three homolog series (alkylbenzenes, alkylphenones, and *p*-alkylbenzylamines) were used as the solutes in this study. Under our experimental conditions (pH 6), the amines are fully protonated and bear a single positive charge [51]. This was confirmed by ¹H NMR studies not reported here. Because of the structure of the *p*-alkylbenzylamines homolog (*p*-CH₃(CH₂)_{*n*}-C₆H₄-CH₂NH₃⁺), the chain length of the alkyl group ought to have only a minimal effect on the bulkiness of the charge center (nitrogen atom) and the ion-exchange characteristics of the solutes.

Two ODS columns, Ace and Alltima, were deliberately chosen for this work. Previously [12], we studied a group of seven ODS phases selected according to their silanol activity from a study [52], in which a set of 60 phases were ranked by the retention and peak shape for amitriptyline. These seven phases included all but the most silanophilic phases from the set of 60 phases and included phases with very low to moderate silanol activity. To obtain reasonable peak shapes for the basic solutes, we did not study columns with the highest silanol activities. All seven ODS phases were tested with the same set of 17 strong bases (a selection of antidepressants and antihistamines, see Ref. [12]). The relative silanol activities of these seven phases agreed with the commercial literature [52], based specifically on the behavior we observed towards amitriptyline and the other strong bases. We showed that the Ace column was the least acidic (it has the lowest silanol

activity) ODS phase, while the Alltima column had the highest silanol activity among the seven phases.

4.1. Effect of buffer concentration

From Eq. (5), it is evident that a plot of $\log k'_{\text{IEX}}$ against the logarithm of the eluent counterion concentration should be a straight line with a slope equal to -1 , when both the analyte ion and the counterion are singly charged [32]. However, as discussed above the one- and two-site models lead to different results. Here, we varied the concentration of the buffer and measured the solute retention factors (see Table 2). The results for *p*-butylbenzylamine are shown in Fig. 3. They are quite typical of the behavior of the entire homolog series.

It is clear that the slopes on the Ace and Alltima columns are not -1 . *This is strong evidence per se that the one-site model is wrong.* Based on Eq. (10), $\log k'_{\text{RP}}$ can only affect the intercept of the plot. According to the one-site mixed-mode model, the slope of a plot of $\log k'$ for a univalent cation versus $\log[\text{NH}_4^+]$ should always be -1 regardless of the k'_{RP} value; this is not consistent with the data. Thus, the one-site model must be wrong.

The two-site model very easily explains the low slope observed above. According to this model the apparent slope of $\log k'$ vs. $\log[\text{NH}_4^+]$ will depend on the contributions of both k'_{RP} and k'_{IEX} to k' (see Eq. (13)). When ion-exchange dominates retention, the slope will be close to -1 (see Table 1). On the other hand, if reversed-phase processes govern retention, the slope will be close to zero. Clearly, on the two ODS phases, reversed-phase dominates. On the PBD-ZrO₂ phase the ion-exchange interaction is predominant because the slope is very close to -1 . This agrees well with our previous conclusion that ion-exchange dominates the retention of cations on PBD-ZrO₂ [26,27].

The fact that the slope of $\log k'$ vs. $\log[\text{NH}_4^+]$ on PBD-ZrO₂ is close to -1 also invalidates any argument that the slope of the same plot on ODS is not -1 due to partial protonation of the analytes since identical eluents were used in both studies. The retention on the Ace column is virtually independent of the eluent concentration of ammonium ion. Clearly ion-exchange contributes almost negligibly to the retention of the *p*-alkylbenzylamines on this phase.

Table 2
Effect of ammonium concentration on retention of amine homologs

Column	Solute	log k' at $[\text{NH}_4^+]$ (mM)				r	SD	Slope \pm SD
		15	25	35	50			
Ace	<i>p</i> -Propylbenzylamine	0.091	0.091	0.079	0.067	0.901	0.006	-0.046 ± 0.018
	<i>p</i> -Butylbenzylamine	0.377	0.384	0.375	0.369	0.591	0.006	-0.017 ± 0.019
	<i>p</i> -Pentylbenzylamine	0.647	0.656	0.656	0.655	0.796	0.003	0.015 ± 0.010
	<i>p</i> -Hexylbenzylamine	0.994	0.998	0.991	0.985	0.715	0.005	-0.018 ± 0.014
Alltima	<i>p</i> -Propylbenzylamine	0.442	0.382	0.364	0.344	0.979	0.010	-0.186 ± 0.031
	<i>p</i> -Butylbenzylamine	0.722	0.670	0.656	0.638	0.975	0.010	-0.159 ± 0.030
	<i>p</i> -Pentylbenzylamine	0.985	0.941	0.934	0.923	0.949	0.011	-0.117 ± 0.032
	<i>p</i> -Hexylbenzylamine	1.356	1.297	1.290	1.272	0.953	0.014	-0.155 ± 0.040
PBD-ZrO ₂	Benzylamine	0.974	0.786	0.639	0.477	0.999	0.014	-0.949 ± 0.042
	<i>p</i> -Methylbenzylamine	1.107	0.916	0.771	0.610	0.999	0.012	-0.950 ± 0.037
	<i>p</i> -Ethylbenzylamine	1.289	1.093	0.952	0.790	0.999	0.011	-0.952 ± 0.032
	<i>p</i> -Propylbenzylamine	1.504	1.313	1.168	1.007	0.999	0.012	-0.951 ± 0.036
	<i>p</i> -Butylbenzylamine	1.735	1.552	1.400	1.244	0.998	0.015	-0.943 ± 0.044

All chromatographic conditions are given in Fig. 3.

r = correlation coefficient of regression of log k' vs. log $[\text{NH}_4^+]$.

SD = standard deviation of regression of log k' vs. log $[\text{NH}_4^+]$.

Slope \pm SD = slope and its standard error for regression of log k' vs. log $[\text{NH}_4^+]$.

It is also quite clear from Fig. 3 that the amines have much higher retention on PBD-ZrO₂ than on the two ODS phases. We know that both ODS phases have much larger surface areas ($>300 \text{ m}^2/\text{g}$) than does the PBD-ZrO₂ phase ($11.2 \text{ m}^2/\text{g}$), so the high retention on PBD-ZrO₂ cannot be due to reversed-

phase retention. We attribute the stronger retention on PBD-ZrO₂ to very strong ion-exchange interactions on this material.

4.2. Effect of the number of methylene units on log k'

In Fig. 4, the log k' values for the three homolog series are plotted versus n_{CH_2} . On both ODS columns, the slopes of the three series are statistically the same. Given this, we cannot tell which model is correct because the contribution of ion-exchange retention is small and reversed-phase effects apparently dominate on both ODS columns. However, there are additional issues (see below) that make these slopes the same.

In contrast, on the PBD-ZrO₂ phase, the slope for the amine series is *considerably smaller* than that for the alkylbenzenes and alkylphenones. This result is additional confirmation that the one-site model is *not correct* at least on the PBD-ZrO₂ column because the one-site model of mixed-mode retention requires the slope of log k' vs. n_{CH_2} to be the same regardless of the strength of the ion-exchange relative to the reversed-phase interaction. Furthermore the slopes on the PBD-ZrO₂ column for the nonionic homologs

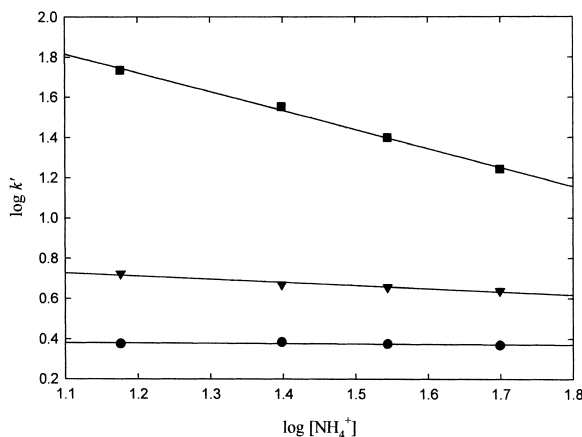


Fig. 3. Dependence of log k' on log $[\text{NH}_4^+]$ for *p*-butylbenzylamine. (■) PBD-ZrO₂, log $k' = 2.854 - 0.954 \times \log[\text{NH}_4^+]$; (▼) Alltima, log $k' = 0.902 - 0.159 \times \log[\text{NH}_4^+]$; (●) Ace, log $k' = 0.401 - 0.017 \times \log[\text{NH}_4^+]$. Mobile phase: 55% MeOH + ammonium phosphate buffer adjusted to pH 6.0. Other experimental conditions: 1 ml/min; 35 °C; 254 nm.

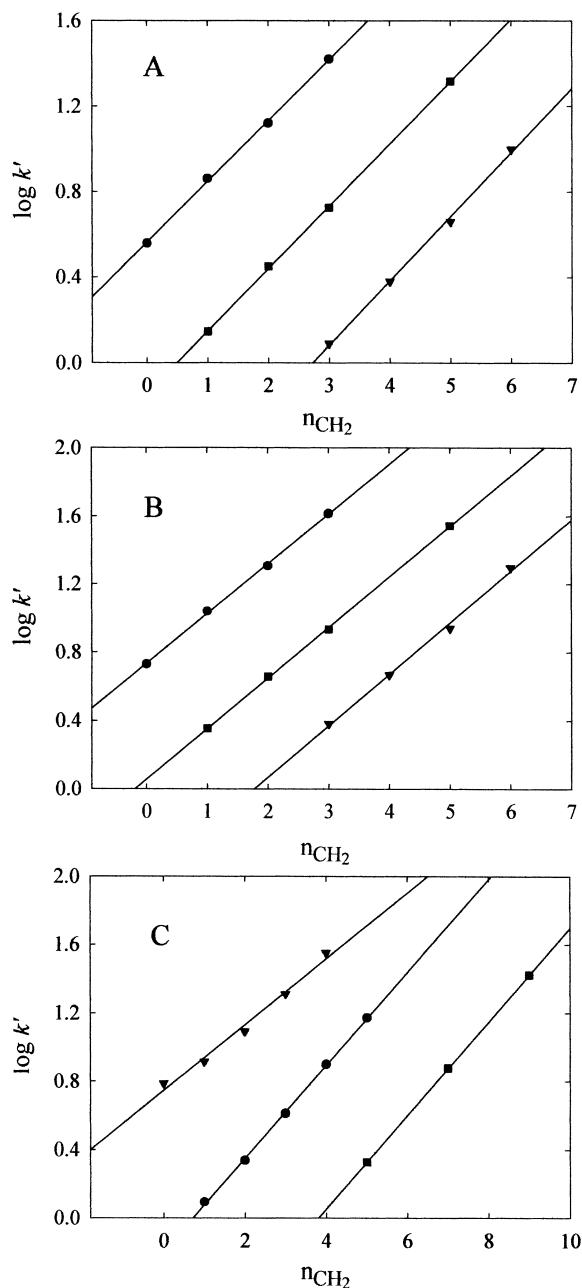


Fig. 4. Plots of $\log k'$ vs. n_{CH_2} of homologs. Mobile phase: 55% MeOH + 25 mM ammonium phosphate buffer adjusted to pH 6.0. Other conditions are the same as in Fig. 3. (A) Ace; (B) Alltima; (C) PBD-ZrO₂. (●) Alkylbenzenes; (■) alkylphenones; (▼) *p*-alkylbenzylamines.

are nearly identical and only slightly smaller than the slopes on the two ODS phases. Clearly the slopes for these two nonionic homolog series are a good measure of the “hydrophobicity” of these two classes (i.e. ODS and PBD-ZrO₂) of reversed-phases.

We also note that the elution orders for these three homolog series on the three phases are radically different. On the ODS phases, the alkylbenzenes and alkylphenones have longer retention than do the amines. However, the amines are much more retained on PBD-ZrO₂ than are the neutral compounds. The difference in strengths of the reversed-phase and ion-exchange interactions is so strong that to keep the retention times reasonable, solutes with different chain lengths had to be used on the ODS and PBD-ZrO₂ phases. We know that alkylbenzenes and alkylphenones are retained only by reversed-phase interaction while the amines are also retained by ion-exchange interactions, thus, this again confirms that on the two ODS phases, the reversed-phase interaction governs but on PBD-ZrO₂ the ion-exchange interaction is dominant.

Ion-exchange theory indicates that ion-exchange contributions to k' must decrease as the eluent concentration of counterion is increased. Thus as we increase the buffer concentration and thereby decrease k'_{IEX} , the slopes of $\log k'$ vs. n_{CH_2} for the *p*-alkylbenzylamines should become closer to the slopes observed for the other two homolog series at least on the PBD-ZrO₂ column, where ion-exchange interactions are clearly dominant. However, most surprisingly, the results show that the slopes of $\log k'$ vs. n_{CH_2} for the three homolog series are independent of the concentration of the buffer on all three columns (see Table 3). We will discuss this in more detail below.

4.3. Analysis of mixed-mode retention in terms of k' vs. $1/[\text{NH}_4^+]$ plots

As discussed above, for pure ion-exchange chromatography with univalent analytes and displacers, a plot of k' against the reciprocal of the counterion concentration should be a straight-line. If no other retention mechanism exists, the line should pass through the origin with a slope proportional to the

Table 3
Study of retention of homolog series as function of n_{CH_2} and ammonium concentration^a

Column	Homolog	r	SD	[NH ₄ ⁺] (mM)							
				15 mM		25 mM		35 mM		50 mM	
				Slope±SD	Intercept±SD	Slope±SD	Intercept±SD	Slope±SD	Intercept±SD	Slope±SD	Intercept±SD
Ace	Alkylbenzenes	1.000	0.013	0.285±0.006	0.557±0.011	0.285±0.006	0.563±0.011	0.285±0.006	0.559±0.011	0.285±0.006	0.553±0.011
	Alkylphenones	1.000	0.010	0.293±0.003	-0.146±0.010	0.292±0.003	-0.143±0.010	0.292±0.003	-0.143±0.010	0.293±0.003	-0.151±0.010
	<i>p</i> -Alkylbenzylamines	0.998	0.026	0.298±0.012	-0.814±0.054	0.299±0.010	-0.815±0.047	0.302±0.008	-0.833±0.036	0.304±0.006	-0.850±0.029
Alltima	Alkylbenzenes	1.000	0.013	0.294±0.006	0.742±0.011	0.292±0.006	0.734±0.011	0.294±0.006	0.739±0.011	0.294±0.006	0.745±0.011
	Alkylphenones	1.000	0.011	0.298±0.004	0.066±0.012	0.296±0.004	0.058±0.011	0.298±0.004	0.060±0.012	0.298±0.004	0.066±0.012
	<i>p</i> -Alkylbenzylamines	0.997	0.037	0.301±0.017	-0.476±0.077	0.302±0.013	-0.534±0.061	0.306±0.012	-0.564±0.055	0.307±0.010	-0.587±0.047
PBD-ZrO ₂	Alkylbenzenes	1.000	0.014	0.273±0.004	-0.195±0.015	0.272±0.004	-0.194±0.015	0.272±0.004	-0.197±0.015	0.272±0.004	-0.190±0.015
	Alkylphenones	1.000	0.003	0.274±0.001	-1.044±0.006	0.274±0.001	-1.041±0.004	0.274±0.001	-1.041±0.004	0.274±0.001	-1.039±0.004
	<i>p</i> -Alkylbenzylamines	0.995	0.036	0.192±0.011	0.938±0.028	0.193±0.013	0.746±0.031	0.193±0.013	0.602±0.029	0.193±0.013	0.439±0.030

^a $\log k'$ is regressed against n_{CH_2} for each homolog series at each of the indicated concentrations of ammonium ion.

r =correlation coefficient of the regression described in footnote a at [NH₄⁺]=15 mM. Data of other concentrations are not shown.

SD=overall standard deviation of the least-square regression at [NH₄⁺]=15 mM. Data of other concentrations are not shown.

Slope±SD=slope and its standard error of least square regression.

Intercept±SD=intercept and its standard error of least-square regression.

ion-exchange equilibrium constant and to the number of negatively charged sites on the stationary phase surface (see Eq. (4)). The existence of other retention mechanisms will result in an intercept (on the k' axis at $1/[\text{NH}_4^+] = 0$) which corresponds to retention under conditions where retention by ion-exchange has been abolished [48].

Fig. 5 shows that the retention factors of the *p*-alkylbenzylamines are inversely proportional to the ammonium ion concentration. We show only the data on the Alltima and PBD-ZrO₂ columns, because

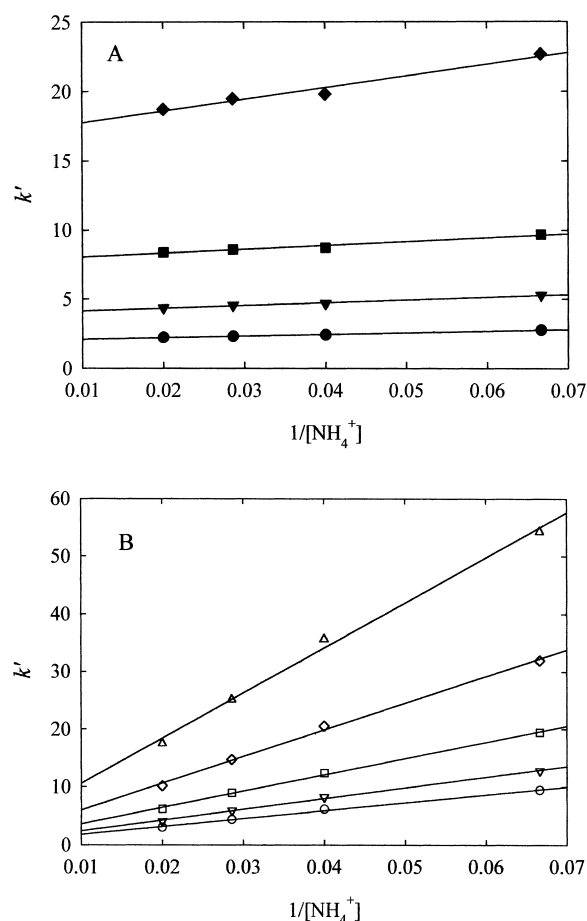


Fig. 5. Dependence of k' on $1/[\text{NH}_4^+]$ for *p*-alkylbenzylamines. Conditions are the same as in Fig. 3. (A) Alltima and (B) PBD-ZrO₂. (\bullet) *p*-Propylbenzylamine; (\blacktriangledown) *p*-butylbenzylamine; (\blacksquare) *p*-pentylbenzylamine; (\blacklozenge) *p*-hexylbenzylamine; (\circ) benzylamine; (∇) *p*-methylbenzylamine; (\square) *p*-ethylbenzylamine; (\diamond) *p*-propylbenzylamine; (\triangle) *p*-butylbenzylamine.

there is virtually no ion-exchange interaction on the Ace column (see above) and thus virtually no change with buffer concentration. It is evident that the both intercepts are finite. Furthermore, the intercepts increase as the solute alkyl chain length increases; this is the expected result for a reversed-phase interaction (see Eq. (7)).

The finite values of these intercepts unambiguously invalidate the one-site model of mixed-mode retention of cations. The intercepts are inconsistent with Eqs. (5) (for pure ion-exchange model) and (9) (for a hydrophobically assisted ion-exchange model).

Note that the slopes of these plots, which are a measure of the strength of the ion-exchange interaction (see Eqs. (5) and (14)) also increase as the solute chain length is increased. The results are summarized in Table 4. The increase in slope with solute hydrophobicity is predicted (see Eq. (9)) by Neue's model of hydrophobically assisted ion-exchange. Furthermore, Rahman and Hoffman [53] observed a similar phenomenon with a polymeric ion-exchange phase. They saw that both the intercepts and slopes of plots of k' vs. $1/[\text{C}^+]_m$ increased with an increase in solute homolog number for a series of phenylalkylamines [53]. They concluded that reversed-phase and ion-exchange interactions aided each other in a mixed-mode type retention process even though they were using a pure polymer type ion-exchange phase.

Based on the two-site model, we know that k'_{RP} , the intercept in Fig. 5, must be an ion-exchange-free contribution to retention since it corresponds to k' at infinite counterion concentration, and B_{IEX} , the slope is proportional to the strength of the ion-exchange interaction. In case A of the two-site model the parameters k'_{RP} and B_{IEX} were assumed to be from independent sites. Nonetheless, we find for both the Alltima and the PBD-ZrO₂ phases (see Fig. 6) a quite strong linear relationship between these two parameters as the number of methylene units in the amines is increased. Thus, we find that as the solutes become more hydrophobic, both the ion-exchange and non ion-exchange interaction increase in strength.

The existence of such a relationship between the propensity of a solute to bind more strongly in both reversed-phase and ion-exchange mode to an ODS phase as the hydrocarbon content of the solute is

Table 4
Dependence of k' on reciprocal of ammonium concentration^a

Column	Solute	Slope \pm SD	Intercept \pm SD	r	SD
Alltima	<i>p</i> -Propylbenzylamine	11.9 \pm 0.6	1.96 \pm 0.03	0.997	0.021
	<i>p</i> -Butylbenzylamine	19.8 \pm 1.4	3.94 \pm 0.06	0.995	0.048
	<i>p</i> -Pentylbenzylamine	27.8 \pm 3.5	7.76 \pm 0.15	0.984	0.123
	<i>p</i> -Hexylbenzylamine	84.2 \pm 10.8	16.91 \pm 0.46	0.984	0.380
PBD-ZrO ₂	Benzylamine	136.3 \pm 5.9	0.43 \pm 0.25	0.998	0.209
	<i>p</i> -Methylbenzylamine	185.4 \pm 7.2	0.56 \pm 0.31	0.998	0.253
	<i>p</i> -Ethylbenzylamine	282.6 \pm 9.1	0.78 \pm 0.39	0.999	0.318
	<i>p</i> -Propylbenzylamine	463.0 \pm 17.4	1.37 \pm 0.74	0.999	0.612
	<i>p</i> -Butylbenzylamine	784.2 \pm 38.8	2.72 \pm 1.65	0.998	1.362

^a k' is regressed against $1/[\text{NH}_4^+]$ (mM).

Slope \pm SD=slope and its standard error of the regression described in footnote a.

Intercept \pm SD=intercept and its standard error of the regression described in footnote a.

r =correlation coefficient of the regression described in footnote a.

SD=overall standard deviation of the regression described in footnote a.

increased is not unheralded. It is well known, for example, that triethylamine and dimethyloctylamine are stronger silanol blockers than are less hydrophobic organic amines and univalent inorganic cations [2,31,54,55]. Thus, it is not unprecedented that more hydrophobic solutes should also bind more strongly by ion-exchange processes.

4.4. The three-site model

Based on analysis of plots of $\log k'$ vs. $\log[\text{C}^+]_m$ and $\log k'$ vs. n_{CH_2} we have proven that two independent sites one which is purely reversed-phase and the other which is inherently ion-exchange are required. The fact that the slope of a plot of k' vs. $1/[\text{C}^+]_m$ which is a measure of the strength of ion-exchange equilibrium constant increases with solute hydrophobicity indicates that a hydrophobically assisted ion-exchange mechanism similar to that suggested by Neue must be involved in the ion-exchange retention process. The augmentation of the ion-exchange interaction by an increase in analyte hydrophobicity is certainly consistent with the schematic models of Fig. 2A and substructure c and c' in Fig. 2C.

A control experiment (see Table 5) using “bare” zirconia, i.e. zirconia not coated with PBD, but otherwise identical conditions clearly indicates that *p*-alkylbenzylamines are strongly retained by pure ion-exchange interactions. It therefore seems likely given the physics of the PBD deposition process on

zirconia that some “pure” ion-exchange (that is not hydrophobically assisted) type sites exist on at least the PBD-coated zirconia. This suggests the need for a three-site model on the hydrophobic zirconia phase, which includes a “pure” reversed-phase, a “pure” ion-exchange, and a hydrophobically assisted ion-exchange type of site.

What was most interesting to us is the fact that the linear plot (see Fig. 6) between the intercepts (k'_{RP}) and slopes (B_{IEX}) passes through the origin. The regression lines are:

Alltima phase:

$$B_{\text{IEX}} = -1.3(\pm 6.0) + 4.87(\pm 0.62)k'_{\text{RP}}; \\ r = 0.984, \text{SD} = 7.1, n = 4 \quad (16)$$

PBD-ZrO₂ phase:

$$B_{\text{IEX}} = 45(\pm 25) + 278(\pm 18)k'_{\text{RP}}; \\ r = 0.994, \text{SD} = 33, n = 5 \quad (17)$$

It is evident that the intercepts of both lines are very close to zero. Thus for either phase the k' can be written as:

$$k' = k'_{\text{RP}} \left(1 + \text{const.} / [\text{NH}_4^+] \right) \quad (18)$$

where k'_{RP} varies from solute to solute on each phase but the same const. applies to each solute on a given phase. The first term (k'_{RP}) on the right in Eq. (18) clearly indicates the existence of a pure hydrophobic site whereas the second term ($\text{const.}k'_{\text{RP}}/[\text{NH}_4^+]$)

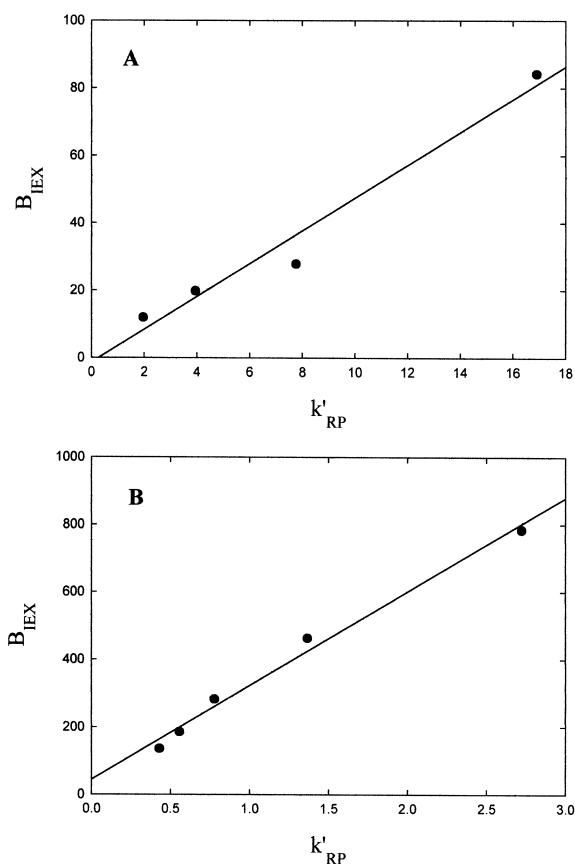


Fig. 6. Plots of k'_{RP} vs. B_{IEX} for different *p*-alkylbenzylamines. k'_{RP} and B_{IEX} are the intercept and slope of the linear relationships shown in Fig. 5 for the different analytes. (A) Alltima; (B) PBD-ZrO₂.

represents the hydrophobically assisted ion-exchange term.

The implications of this plot are that a totally nonhydrophobic primary amine (i.e. a hypothetical

Table 5

Retention factors of *p*-alkylbenzylamine on PBD-ZrO₂ and Bare-ZrO₂^a

Solute	k'	
	PBD-ZrO ₂	Bare-ZrO ₂
Benzylamine	5.87	3.69
<i>p</i> -Methylbenzylamine	7.82	3.20
<i>p</i> -Ethylbenzylamine	11.83	2.97
<i>p</i> -Propylbenzylamine	19.29	2.81
<i>p</i> -Butylbenzylamine	33.11	2.67

^a All chromatographic conditions are the same as in Fig. 4.

amine whose k'_{RP} is zero) cannot displace ammonium ions (the eluent ion) from the ion-exchange sites (thus $B_{IEX} = 0$) and vice versa that is, a nonionic molecule (i.e. a hypothetical amine whose B_{IEX} is zero) has no propensity to bind to the hydrophobic sites. This result is surprising at least for PBD-ZrO₂ since based on our results on bare zirconia one would expect ion-exchange interactions to take place even for a totally nonhydrophobic analyte on totally nonhydrophobic sites such as shown in Fig. 2C, site a, if such existed.

Another possibility is that sites like site a in Fig. 2C do exist but the ion-exchange interaction are so weak that they are unimportant. This leads us to conclude that on PBD-ZrO₂ sites such as those in Fig. 2A and sites c and c' in Fig. 2C dominate over all other types of sites. What this means is that the free energy of a solute ion is much lower when it interacts simultaneously with both a negatively charged site and a hydrophobic site than with just an isolated non-hydrophobic negatively charged site.

In fact, based on the above conclusion, we can explain the above surprising result that the slopes of $\log k'$ vs. n_{CH_2} on PBD-ZrO₂ have no dependence on $[NH_4^+]$. Since the hydrophobically assisted ion-exchange sites dominate on PBD-ZrO₂, both k'_{IEX} and k'_{RP} will decrease as $[NH_4^+]$ increases. Thus based on Eq. (13), it is possible that the slopes of such plots will be the same over a certain range of $[NH_4^+]$.

Another result that is explainable through the above conclusion is why the slope of the $\log k'$ vs. n_{CH_2} for the alkylbenzylamines is not equal to that of the nonpolars on PBD-ZrO₂, but is equal on the ODS phases. On PBD-ZrO₂ we believe that the hydrophobically assisted ion-exchange type site (that is, Neue's type site see Fig. 2C sites c and c') is the most significant site. In our opinion while the cationic moiety of the amine is interacting with the anionic surface site on PBD-ZrO₂, the hydrocarbon chain of the *p*-alkylbenzylamine cannot interact as intimately with the hydrocarbon supplied by PBD. In contrast the ODS chains on silica are much denser than is the PBD on zirconia and on average closer to the ion-exchange sites. Thus, the hydrocarbon portion of a cationic analyte experiences an environment which is as hydrophobic as that of a non-ionic analyte. As shown in Fig. 2C, the degree of the

contact of the solute with the hydrophobic chain on the stationary phase is different on sites c and c'. We believe that on PBD-ZrO₂ site c, the hydrophobically assisted ion-exchange site, which is hydrophobically weaker than a pure hydrophobic site, is dominant. Consequently, the slope of log *k'* vs. *n*_{CH₂} is lower for the PBD-ZrO₂ phase than for the ODS silica phases.

We hypothesize that a given phase begins to overload when the ion-exchange sites in a hydrophobic “neighborhood” become full. The next stronger set of sites, which for a highly hydrophobic species are probably sites such as those in site b in Fig. 2B or C, then fill-up. However, for a species of very low hydrophobicity the next type of sites to fill are likely the pure ion-exchange type of sites (see site a in Fig. 2B or C).

4.5. The relative contributions of ion-exchange and reversed-phase retentions to *k'*

We separated the reversed-phase from the ion-exchange retention by plotting *k'* versus 1/[NH₄⁺] (see Eq. (13)) [30]. The ion-exchange contribution to

retention, *k'*_{IEX}, can be obtained by subtracting *k'*_{RP} from the total retention, *k'*. Table 6 gives the fractional contribution of *k'*_{IEX} to the total retention at different ammonium ion concentrations. On PBD-ZrO₂ most solutes are more than 90% retained by *k'*_{IEX}. However, in view of the previous section one ought not too lightly dismiss the importance of the solute and stationary phase hydrophobicity on the Alltima and PBD-ZrO₂ phases. Nonetheless it is evident that on the Ace column the contribution of the ion-exchange interaction to the total retention is almost nil. On the Alltima column, it is intermediate. These results agree very well with the discussions in prior sections. We have obtained a quantitative estimate of the relative contribution coming from the two different retention mechanisms. *This is to date the first study to evaluate the relative amount of IEX vs. RP for different phases by a rigorous thermodynamically founded method and not by inference based on model compounds.* It is evident that ion-exchange interactions nearly completely control retention on PBD-ZrO₂ whereas reversed-phase interactions by far predominate on modern type-B ODS silica columns.

Table 6
Analysis of slope and intercept (*k'*_{RP}) of plots of *k'* vs. 1/[NH₄⁺]

Column	Solute	<i>k'</i> at [NH ₄ ⁺] (mM)				Intercept (<i>k'</i> _{RP})	% <i>k'</i> _{IEX} / <i>k'</i> at [NH ₄ ⁺] (mM)			
		15	25	35	50		15	25	35	50
Ace	<i>p</i> -Propylbenzylamine	1.23	1.23	1.20	1.17	1.16	6	6	3	1
	<i>p</i> -Butylbenzylamine	2.38	2.42	2.37	2.34	2.35	1	3	1	-1
	<i>p</i> -Pentylbenzylamine	4.44	4.53	4.53	4.52	4.58	-3	-1	-1	-1
	<i>p</i> -Hexylbenzylamine	9.86	9.96	9.80	9.67	9.69	2	3	1	0
Alltima	<i>p</i> -Propylbenzylamine	2.77	2.41	2.31	2.21	1.96	29	19	15	11
	<i>p</i> -Butylbenzylamine	5.28	4.67	4.53	4.35	3.94	25	16	13	9
	<i>p</i> -Pentylbenzylamine	9.67	8.72	8.59	8.37	7.76	20	11	10	7
	<i>p</i> -Hexylbenzylamine	22.69	19.81	19.48	18.72	16.91	25	15	13	10
PBD-ZrO ₂	Benzylamine	9.41	6.11	4.36	3.00	0.43	95	93	90	86
	<i>p</i> -Methylbenzylamine	12.79	8.24	5.90	4.07	0.56	96	93	91	86
	<i>p</i> -Ethylbenzylamine	19.46	12.40	8.95	6.17	0.78	96	94	91	87
	<i>p</i> -Propylbenzylamine	31.93	20.54	14.72	10.16	1.37	96	93	91	87
	<i>p</i> -Butylbenzylamine	54.33	35.67	25.10	17.53	2.72	95	92	89	84

Intercept (*k'*_{RP}) = intercept of *k'* vs. 1/[NH₄⁺] (mM).

$$\% k'_{\text{IEX}}/k' \equiv 100 \cdot \frac{k' - k'_{\text{RP}}}{k'}$$

4.6. Study of the individual contributions to retention

Since we can now estimate the individual contributions of ion-exchange and reversed-phase process to retention we can assess the dependence of $\log k'_{\text{RP}}$ on n_{CH_2} and $\log k'_{\text{IEX}}$ on $\log[\text{NH}_4^+]$ to test whether the two-site model and the evaluation method of the relative amount of k'_{IEX} vs. k'_{RP} are accurate. If the two-site model were completely accurate, we expect to see that the slope of a plot of $\log k'_{\text{RP}}$ vs. n_{CH_2} for

p-alkylbenzylamines would be the same as the slope for the alkylbenzenes and alkylphenones on PBD-ZrO₂. The results show that the slope of the $\log k'_{\text{RP}}$ vs. n_{CH_2} plot (0.199 ± 0.013) is still the same as the slope of the original, i.e. “uncorrected”, $\log k'$ vs. n_{CH_2} plot (0.192 ± 0.013) for the alkylbenzylamines; it is not even close to that for the alkylbenzenes (0.272 ± 0.004).

This clearly proves that the low slope of $\log k'$ on PBD-ZrO₂ vs. n_{CH_2} for the alkylbenzylamines is not due to the high strength of ion-exchange relative to the reversed-phase interactions on this material. As discussed above, it is our opinion that this is due to the lack of intimate interaction of ionic solutes with the PBD coating.

Fig. 7 shows the linear relationship between $\log k'_{\text{IEX}}$ for the *p*-alkylbenzylamines and $\log[\text{NH}_4^+]$ on the Alltima and PBD-ZrO₂ columns. Again, the data for the Ace column are not given since the ion-exchange contribution to retention on that phase is very small. It is clear that the slopes of the plots are now very close to -1 , the theoretical value. This is strong evidence showing that the two-site model is superior to the one-site model even if it is not as yet a complete description of the complex set of interactions in mixed-mode RPLC.

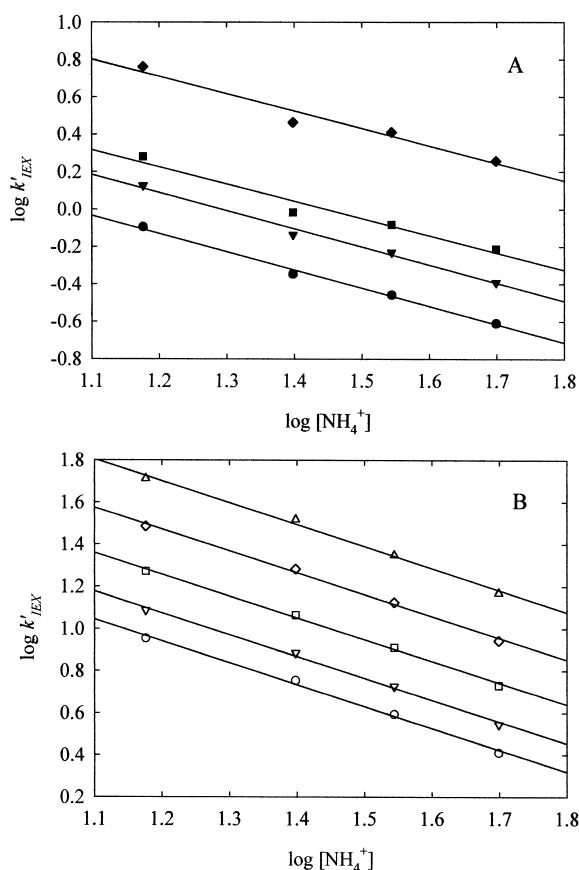


Fig. 7. Dependence of $\log k'_{\text{IEX}}$ vs. $\log[\text{NH}_4^+]$ for *p*-alkylbenzylamines. Conditions are the same as in Fig. 3. Symbols represent the same compounds as in Fig. 5. (A) Alltima; for *p*-hexylbenzylamine (\blacklozenge), regression line is: $\log k'_{\text{IEX}} = 1.8(\pm 0.2) - 0.93(\pm 0.14) \log[\text{NH}_4^+]$; $r = 0.979$, $\text{SD} = 0.05$, $n = 4$. (B) PBD-ZrO₂; for *p*-butylbenzylamine (\triangle), regression line is: $\log k'_{\text{IEX}} = 2.95(\pm 0.08) - 1.04(\pm 0.05) \log[\text{NH}_4^+]$; $r = 0.997$, $\text{SD} = 0.02$, $n = 4$.

5. Conclusions

The main conclusions of this study are:

(1) A one-site hydrophobically assisted ion-exchange model of mixed-mode RP-IEX is incomplete for ODS silica and for PBD-ZrO₂; a two-site model is more correct. A three-site model may be required in some cases and seems unavoidable on PBD-ZrO₂ in view of the strong ion-exchange effect on bare ZrO₂ and the unusually weak dependence of $\log k'$ on n_{CH_2} for the homolog series of benzylamines. We have no evidence for three types of sites on Type-B ODS phases.

(2) Pure reversed-phase sites exist but there are substantial parts of the stationary phase environment wherein cationic solutes *simultaneously* interact with both the hydrocarbon phase and the surface charge.

(3) Increases in solute hydrophobicity increase interactions with the pure reversed-phase sites but

they also act to strengthen the ion-exchange interaction between ionic solutes and the surface charge in agreement with Neue's hydrophobically assisted ion-exchange model.

(4) For cationic solutes, ion-exchange interactions largely govern, in some cases they provide more than 90% of the retention on PBD-ZrO₂, whereas reversed-phase interactions dominate retention on high quality (type-B) ODS columns; furthermore there are significant differences in the ion-exchange contributions on different ODS silicas.

(5) A plot of k' vs. the reciprocal of the counterion concentration ($[C^+]_m$) is a very useful tool for quantifying the relative contributions of reversed-phase and ion-exchange interactions to the retention of basic solutes. They can be used for quantitatively classifying different reversed-phase columns. Plots of $\log k'$ vs. $\log[C^+]_m$ are not nearly as informative.

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References

- [1] D.V. McCalley, LC–GC 17 (1999) 440.
- [2] M.A. Stadalius, J.S. Berus, L.R. Snyder, LC–GC 6 (1988) 494.
- [3] R.J.M. Vervoort, F.A. Maris, H. Hindriks, J. Chromatogr. 623 (1992) 207.
- [4] R.J.M. Vervoort, A.J.J. Debets, H.A. Claessens, C.A. Cramers, G.I. de Jong, J. Chromatogr. A 897 (2000) 1.
- [5] J. Nawrocki, J. Chromatogr. A 779 (1997) 29.
- [6] G.B. Cox, J. Chromatogr. A 656 (1993) 353.
- [7] J.E. Eble, R.L. Grob, P.E. Antle, L.R. Snyder, J. Chromatogr. 384 (1987) 45.
- [8] I. Quinones, A. Cavazzini, G. Guiochon, J. Chromatogr. A 877 (2000) 1.
- [9] C.J. Dunlap, C.V. McNeff, D. Stoll, P.W. Carr, Anal. Chem. 73 (2001) 598A.
- [10] M. Kawahara, H. Nakamura, T. Nakajima, J. Chromatogr. 515 (1990) 149.
- [11] U. Trudinger, G. Müller, K.K. Unger, J. Chromatogr. 535 (1990) 111.
- [12] J. Dai, X. Yang, P.W. Carr, J. Chromatogr. A (2003) in press.
- [13] M.P. Rigney, T.P. Weber, P.W. Carr, J. Chromatogr. 484 (1989) 273.
- [14] J. Li, P.W. Carr, Anal. Chem. 69 (1997) 2202.
- [15] Y. Hu, PhD thesis, University of Minnesota, Minneapolis, 1998.
- [16] J. Nawrocki, M.P. Rigney, A. McCormick, P.W. Carr, J. Chromatogr. A 657 (1993) 229.
- [17] J.A. Blackwell, P.W. Carr, Anal. Chem. 64 (1992) 863.
- [18] J.A. Blackwell, P.W. Carr, Anal. Chem. 64 (1992) 853.
- [19] B. Trammell, PhD thesis, University of Minnesota, Minneapolis, 2002.
- [20] A. Mendez, E. Bosch, M. Roses, U.D. Neue, J. Chromatogr. A 986 (2003) 33.
- [21] S.G. Weber, W.G. Trampusch, Anal. Chem. 55 (1983) 1771.
- [22] J. Nawrocki, B. Buszewski, J. Chromatogr. 449 (1988) 1.
- [23] J. Nawrocki, Chromatographia 31 (1991) 193.
- [24] J. Nawrocki, Chromatographia 31 (1991) 177.
- [25] J.A. Blackwell, P.W. Carr, J. Chromatogr. 549 (1991) 43.
- [26] Y. Hu, X. Yang, P.W. Carr, J. Chromatogr. A 968 (2002) 17.
- [27] Y. Mao, P.W. Carr, Anal. Chem. 73 (2001) 4478.
- [28] U.D. Neue, C.H. Phoebe, K. Tran, Y.F. Cheng, Z. Lu, J. Chromatogr. A 925 (2001) 49.
- [29] A. Sokolowski, K.G. Wahlund, J. Chromatogr. 189 (1980) 299.
- [30] A. Nahum, C. Horvath, J. Chromatogr. 203 (1981) 53.
- [31] K.E. Bij, C. Horvath, W.R. Melander, A. Nahum, J. Chromatogr. 203 (1981) 65.
- [32] H.F. Walton, R.D. Rocklin, Ion Exchange in Analytical Chemistry, CRC Press, Boca Raton, FL, 1990.
- [33] J. Stahlberg, Anal. Chem. 66 (1994) 440.
- [34] J. Stahlberg, J. Chromatogr. A 855 (1999) 3.
- [35] J.G. Chen, S.G. Weber, L.L. Glavina, F.F. Cantwell, J. Chromatogr. A 656 (1993) 549.
- [36] G. Vigh, Z. Varga-Puchony, J. Chromatogr. 196 (1980) 1.
- [37] B. Karger, J.R. Gant, A. Hartkopf, P.H. Weiner, J. Chromatogr. 128 (1976) 65.
- [38] H. Colin, G. Guiochon, Z. Yun, J.C. Diez Masa, J. Jandera, J. Chromatogr. Sci. 21 (1983) 179.
- [39] P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, M.H. Abraham, Anal. Chem. 57 (1985) 2971.
- [40] M.J. Kamlet, M.H. Abraham, P.W. Carr, R.M. Doherty, R.W. Taft, J. Chem. Soc. Perkin Trans. II (1988) 2087.
- [41] P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, W.M. Melander, C. Horvath, Anal. Chem. 58 (1986) 2674.
- [42] R.P.W. Scott, J. Chromatogr. 122 (1976) 35.
- [43] B.L. Karger, L.R. Snyder, C. Econ, J. Chromatogr. 125 (1976) 71.
- [44] R. Tijssen, H.A.H. Billeiet, P.J. Schoenmakers, J. Chromatogr. 122 (1976) 185.
- [45] C. Horvath, W.R. Melander, J. Molnar, J. Chromatogr. 125 (1976) 129.
- [46] S.M. Petrovic, S. Lomic, I. Sefer, J. Chromatogr. 348 (1985) 49.

- [47] A. Fredenslund, R.L. Jones, J.M. Prausnitz, *AIChE J.* 21 (1975) 1086.
- [48] G.B. Cox, R.W. Stout, *J. Chromatogr.* 384 (1987) 315.
- [49] S.M.C. Buckenmaier, D.V. McCalley, M.R. Euerby, *Anal. Chem.* 74 (2002) 4672.
- [50] D.V. McCalley, *J. Chromatogr. A* 902 (2000) 311.
- [51] E. Bosch, P. Bou, H. Allemann, M. Roses, *Anal. Chem.* 68 (1996) 3651.
- [52] MAC-MOD, Comparison Guide to C₁₈ Reversed Phase HPLC Columns, MAC-MOD Analytical Inc, Chadds Ford, PA, 2002.
- [53] A. Rahman, N.E. Hoffman, *J. Chromatogr. Sci.* 28 (1990) 157.
- [54] D.W. Hill, *J. Liq. Chromatogr.* 13 (1990) 3147.
- [55] J.S. Kiel, S.L. Morgan, R.K. Abramson, *J. Chromatogr.* 320 (1985) 313.