ELSEVIER

Journal of Chromatography A, 968 (2002) 17–29

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Mixed-mode reversed-phase and ion-exchange separations of cationic analytes on polybutadiene-coated zirconia

Yue Hu[1], Xiqin Yang, Peter W. Carr*

Department of Chemistry, University of Minnesota, Kolthoff and Smith Halls, 207 Pleasant Street, S.E., Minneapolis, MN 55455, USA

Received 6 February 2002; received in revised form 3 June 2002; accepted 3 June 2002

Abstract

The retention and selectivity of the chromatographic separation of basic (cationic) analytes on a polybutadiene-coated zirconia (PBD–ZrO_2) stationary phase have been studied in greater detail than in previous studies. These separations are strongly influenced by the chemistry of the accessible surface of zirconia. In the presence of buffers which contain *hard* Lewis bases (e.g., phosphate, fluoride, carboxylic acids) zirconia's surface becomes negatively charged due to adsorption of the buffer anion at the hard Lewis acid sites. Consequently, under most conditions (e.g., neutral pH), cationic analytes undergo both hydrophobic and cation-exchange interactions. This mixed-mode retention process generally leads to greater retention factors for cations relative to those on silica-based reversed phases despite the lower surface areas of the zirconia phase, but, more importantly, adsorption of hard Lewis bases can be used to control the chromatographic selectivity for cationic analytes on these zirconia-based stationary phases. In contrast to our prior work, here we show that when mixed-mode retention takes place, both retention and selectivity are easily adjusted by changing the *type* of hard Lewis base buffer anion, the type of buffer counter-ion (e.g., sodium, potassium, ammonium), the pH, and the ionic strength of the eluent as well as the type and amount of organic modifier.

Keywords: Mixed-mode retention; Silanophilic interactions; Lewis acid–base interactions; Stationary phases, LC; Mobile phase composition; Cationic drugs; Polybutadiene; Zirconia

1. Introduction

Zirconia-based phases are very useful alternatives to silica-based supports due to their high chemical and thermal stability [1–11]. Zirconia is insoluble in water from pH 1 to 14. Thus far, polybutadiene-coated zirconia (PBD–ZrO_2) has been the most studied zirconia-based reversed-phase material [4] and has proven to be very useful for the separation of nonpolar and polar solutes over a wide range in pH and at temperatures up to 200 °C [12–14]. It is more chemically and thermally stable [1,14,15] than are the sterically protected [16,17], bidentate [18,19], and/or hybrid silane polymer-silica phases [20] that have been introduced over the past several years. The major advantage of using silica for HPLC is its chemical flexibility. Silanol groups can be easily modified to provide many different kinds of station-

*Corresponding author. Tel.: +1-612-624-0253; fax: +1-612-626-7541.

E-mail address: carr@chem.umn.edu (P.W. Carr).

[1]Current address: Bristol-Myers Squibb Co., One Squibb Drive, P.O. Box 191, New Brunswick, NJ 08903-0191, USA.

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00754-9

ary phases, while the silane bonding chemistry is not stable on zirconia. Si–O– bonds to alumina or zirconia are highly ionic and thus quite unstable. This led Schomburg and Koehler to use PBD-coated alumina instead of silanization chemistry to make their RPLC phase [21].

One of the most chemically interesting aspects of using zirconia supports is understanding their surface chemistry, which is radically different from that of silica supports [22]. In particular, there are many *hard* Lewis acid sites on zirconia's surface [23,24]. In aqueous media the surface of zirconia is populated with hydroxylated and aquated Zr(IV) sites which can undergo ligand exchange with hard Lewis base adsorbates. This type of true coordination chemistry does not take place on silica as it is not a metal [25]. The chemistry of silica is dominated by the population of the various types of silanol groups, many of which are moderately strong ($pK_a > 4$) Bronsted acids [25]. Thus where silica tends to adsorb amines, zirconia and alumina adsorb carboxylates and other hard Lewis bases, but they do not specifically chemically interact with amines by Lewis acid–base chemistry. In our previous work, Blackwell studied and used the ligand-exchange chemistry of the Lewis acid sites on zirconia to separate carboxylic acids, amino acids, peptides and proteins [26–29]. Further he determined the adsorption capacity and eluotropic strength of a wide variety of Lewis bases (e.g., hydroxide > phosphate > fluoride ≫ carboxylate, etc.) [24]. The effect of pH and ionic strength on the adsorption of fluoride on uncoated zirconia were also studied in detail [30]. Rigney et al. and Schafer et al. quantified the adsorption of organic and inorganic phosphate on PBD-coated surfaces [31,32]. It is quite clear that even particles which are heavily loaded [>4% (w/w)] with PBD have a high population of accessible Lewis acid sites [33]. Interaction of hard Lewis base analytes with these sites generally involves rather slow desorption kinetics, which, in turn, can cause severe peak broadening and tailing or even irreversible analyte adsorption [12,34]. Even with very high loads of polymer [>4–5% (w/w) polymer/zirconia] current polymer coating techniques cover no more than 70% of zirconia's surface with PBD [12]. The commercial zirconia phases are loaded to only about 3% (w/w) to provide very fast mass transport in the pores, thus issues related to the surface chemistry are inevitable whenever a PBD–ZrO_2 phase is used to separate ionic or ionogenic analytes.

It is most important to understand that unless a prohibitively thick [>7–10% (w/w)] coating of polymer is applied it is virtually impossible to completely eliminate analyte access to the zirconia surface. At coating levels of greater than 3–4% (w/w) mass transfer in the pores is significantly impeded [35] and the chromatographic performance is unacceptable. Various theories and experimental studies of polymer adsorption indicate the importance of chain conformational entropy during polymer adsorption. The entropic price of forming a thin, complete, protective film by deposition of a polymer prevents complete coverage of the surface [36]. Thus, ionized carboxylic acid analytes show strong ligand-exchange interactions with the accessible hard Lewis acid sites on PBD-ZrO_2. This leads to long retention and very poor peak shape. We have demonstrated that addition of hard Lewis base additives such as phosphate and fluoride to the eluent suppresses such deleterious effects and good separations are achieved [12]. Recent work has shown that the adsorption of phosphate on PBD–ZrO_2 can be used in the novel thermally tuned tandem column technique to achieve some very interesting separations, although the adsorption process was not studied in detail and only phosphate buffers were used in that study [37].

The addition of a hard Lewis base to the eluent is roughly analogous to the use of amines [38–41] and, more recently, group II metals to block adsorption of amines on ionized silanol groups [42]. However, the population of active hard Lewis base sites on zirconia is quite high (about 4–6 $\mu mol/m^2$) [22] and the interaction involves some degree of covalency and is thus slow. In contrast, the active sites on RPLC silica responsible for the poor peak shape of amines are fewer in number (quite likely much less than 1 $\mu mol/m^2$) [43,44] and the desorption of amines, albeit not instantaneous, is faster than that of hard Lewis bases from zirconia [45–47].

The surface chemistry of PBD–ZrO_2 phases can also affect the retention of basic (cationic) species. Although a few studies have touched on the fact that good separations of cationic analytes can be obtained on PBD–ZrO_2 [13,34,37], no *systematic* studies of

the retention mechanism of amine bases (i.e., cations) on zirconia have been conducted. Based on subsidiary observations in our earlier study [37] we strongly believe that, in addition to hydrophobic interactions, other retention modes, most especially cation-exchange interactions, also take place. There are at least two sources of such secondary interactions. First, accessible zirconia sites may be dynamically modified by adsorption of the hard Lewis base component of the buffer used to control the mobile phase pH. Hard Lewis base buffer species (phosphates, carboxylates, etc.), but not soft Lewis bases (e.g., amines), strongly interact with hard Lewis acid surface sites and strongly adsorb. This adsorption imparts negative charges to the sites. These sites can, and do, interact with cationic solutes. As pointed out above, native zirconia, when dynamically modified by adsorption of hard Lewis bases such as phosphate, phosphonates, fluoride and carboxylates, can be used as a stationary phase for protein analysis [6,48,49]. Second, there are a significant number and variety of hydroxyl groups on zirconia's surface which, upon loss of a proton, become negatively charged. Consequently, when cationic analytes are separated on PBD–ZrO_2 phases using eluents containing hard Lewis bases, they can undergo both hydrophobic interactions with the PBD coating and cation-exchange interactions with both adsorbed buffer anions and ionized zirconols. These two cation-exchange processes can be described as:

$$Zr\text{–}L^-{:}X_S^+ + B_M^+ = Zr\text{–}L^-{:}B_S^+ + X_M^+ \quad (1)$$

$$Zr\text{–}O^-{:}X_S^+ + B_M^+ = Zr\text{–}O^-{:}B_S^+ + X_M^+ \quad (2)$$

where B^+, X^+, $Zr\text{–}O^-$, and $Zr\text{–}L^-$ denote the positively charged analyte, the eluent counter-ion, the ionized zirconol, and the adsorbed hard Lewis base sites, respectively. The subscripts M and S indicate that the species are present in the mobile and stationary phase, respectively.

Secondary retention modes can lead to peak broadening and peak tailing when the retention sites are overloaded or if the process is kinetically slow [50]. They are not necessarily deleterious per se. The best-known example of such behavior in RPLC is the silanophilic interaction between basic solutes and residual silanol groups on silica-based alkylsilane-bonded phases. Interaction between amines and silanols often causes severe peak broadening and tailing [25,51,52]. In order to obtain good separations, such secondary interactions can be suppressed by using amine modifiers such as triethylamine and dimethyloctylamine in the mobile phase to compete with basic solutes for the silanol groups [25,38–42], or by working at high pH to suppress the charge on the cationic analyte [25]. It is extremely doubtful that such interactions are entirely absent in the separation of cationic analytes on silica-based RPLC media.

However, one should not consider such secondary interactions as being entirely deleterious. They are responsible for important selectivity effects. For example, most chiral separations involve two or more types of interactions such as hydrophobic interactions, hydrogen bonding, π–π interaction, and dipole–dipole interaction [53–55]. Proteins and peptides are often separated by mixed-mode retention processes [56,57]. In fact, zirconia-based mixed-mode stationary phases have been used to separate both peptides and proteins. In one example, fluoride-modified zirconia was used to separate cationic proteins by both cation-exchange and ligand-exchange interactions [6]. In another example, a PBD–ZrO_2 phase was used to analyze peptides by reversed-phase and cation-exchange interactions [10]. Recently, a mixed-mode column was deliberately prepared by combining strong cation-exchange and reversed-phase packing materials; the resulting column provided unusually high retention for strongly basic solutes [58].

The present work is aimed at providing additional evidence for simultaneous cation-exchange and reversed-phase chromatography on PBD–ZrO_2 [37], showing its utility in achieving selectivity in the separation of cationic analytes and demonstrating the effect of different buffer types on selectivity and retention. We hope to answer the following questions: How does the surface chemistry of zirconia affect the retention behavior of basic solutes? Can the effect be controlled by changing the chromatographic conditions? And, can the effect be used to advantage for some separations? To do so, we studied the effect of changing the type and the concentration of Lewis base buffer, mobile phase pH, and the type of buffer counter-ion on the

retention behavior of a test set of basic solutes. The results clearly demonstrate that basic solutes almost always undergo chromatographically significant cation-exchange interactions with zirconia's surface and the interaction strength can be controlled by manipulating the above chromatographic variables. Finally, we show that the mixed-mode retention mechanism exhibited by PBD–ZrO_2 phases can provide unique selectivities for analyte mixtures of very different properties.

2. Experimental

2.1. Reagents

All reagents were obtained from commercial sources and, unless noted otherwise, were reagent grade or better. Ammonium acetate, ammonium fluoride, ammonium phosphate monobasic, ammonium phosphate dibasic, sodium phosphate dibasic, and potassium phosphate dibasic were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Isopropanol (Mallinckrodt, Paris, KY, USA), acetonitrile (Burdick and Jackson, Muskegon, MI, USA), and acetone (Mallinckrodt) were all HPLC grade.

Antidepressant drugs, antihistamine drugs, and anti-arrhythmic drugs were purchased from Sigma (St. Louis, MO, USA). Other test solutes were purchased from Aldrich (Milwaukee, WI, USA).

House deionized water was further treated by a Barnsted Nanopure II deionizing system with an organic-free cartridge and a 0.2 μm filter, and finally was boiled to remove carbon dioxide before use.

2.2. Preparation of PBD-coated zirconia columns

The PBD–ZrO_2 (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 m^2/g (by BET) and the average pore diameter is 500 Å.

The zirconia particles were packed by the downward slurry method at 5000 p.s.i. Stainless-steel (316) column blanks with dimensions of 50 mm × 4.6 mm I.D. and 0.5 μm stainless-steel frits were obtained from Isolation Technologies (Hopedale, MA, USA).

2.3. Chromatographic conditions

Chromatographic studies were carried out on a Hewlett-Packard 1090 liquid chromatograph with 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.

Scouting experiments were done by using a binary pumping system. However, for the final results reported here the mobile phases were prepared by first dissolving buffers in water, adjusting the pH with dilute NaOH or HNO_3 solutions, then filtering the solution through a Millipore (type HA) 0.45 μm membrane filter prior to use, and finally mixing the aqueous solution with pre-filtered acetonitrile. The separation temperature was controlled within ±0.2 °C. Sample concentrations were about 1–2 mg/mL and the injection volume was 1–2 μL. The column void volumes were determined using acetone as a marker.

In this work, the plate count (N) was computed with the following equation:

$$N = 2\pi\left(\frac{t_R}{A_p/H_p}\right)^2 \quad (3)$$

where t_R, A_p and H_p are the retention time, peak area and peak height, respectively, as reported by the HP Chemstation software. This approach gives a rather conservative estimate of the plate count since the entire peak is used and any tailing will have a significant impact on the plate count estimate in contrast to methods based on the peak half-width.

3. Results and discussion

A set of six basic compounds including two primary amines (norpseudoephedrine and tryptamine), one secondary amine (nortriptyline), and three tertiary amines (lidocaine, quinidine, and amitriptyline) were chosen for this study. Their structures and the pK_a values of the Bronsted conjugate acids are shown in Fig. 1. We intentionally picked

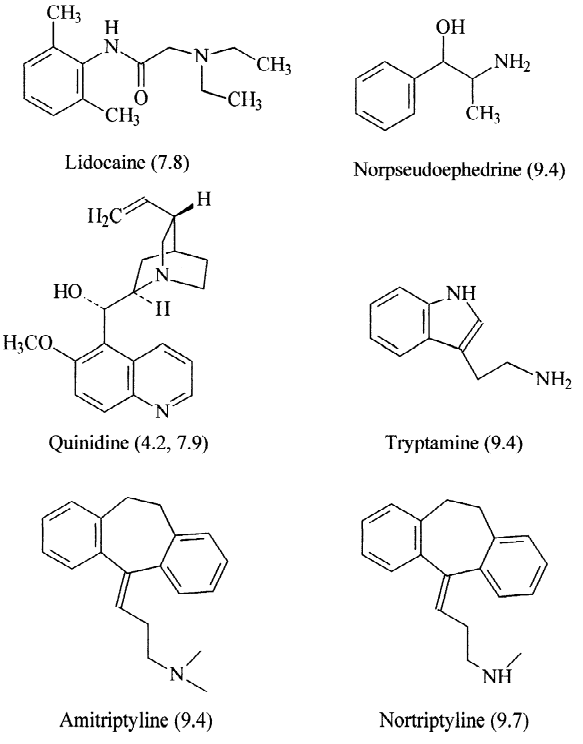

Fig. 1. Structures and pK_as of the basic test solutes for the retention behavior study on the PBD–ZrO_2 phase.

some high-pK_a species because they are more sensitive probes for cation-exchange interactions than low-pK_a species [59,60].

As stated, we believe that the type and amount of Lewis base buffer is one of the most important factors in controlling the surface chemistry of the PBD–ZrO_2 phase. Thus their effect on the retention behavior of basic solutes was studied first.

3.1. Effect of the type of Lewis base buffer

Three buffers (acetate, fluoride and phosphate) were chosen in this experiment because acetate and phosphate are very common buffers for RPLC, and fluoride, although not a common buffer for use with silica, is used as a hard Lewis base eluent additive with PBD–ZrO_2 phases [12,30]. The strength of the Lewis acid–base interaction between these buffers and hard Lewis acid sites on unmodified (i.e. not polymer coated) zirconia increases in the order acetate $\ll$ fluoride $<$ phosphate [24].

Fig. 2 shows chromatograms of a mixture of six basic solutes on a PBD–ZrO_2 phase in eluents containing these three buffers. In each chromatogram the peaks are numbered in their order of elution with phosphate buffer as the eluent. Clearly, the buffer type, at fixed pH, has a dramatic effect on the retention of basic solutes [especially quinidine (3) and the band spacing (see peaks 2 and 4)] as is evident from the very different retention times and significant changes in elution orders among the three chromatograms. In order to better understand the retention behavior, single-solute injections were made and the retention factors and plate counts so obtained are given in Table 1. For all solutes, retention was lower in acetate than either other buffer and in four of six cases retention was lower in fluoride than in phosphate. The elution orders of the test solutes are different from those predicted by the usual reversed-phase considerations. For instance, amitriptyline has one more methyl group than nor-

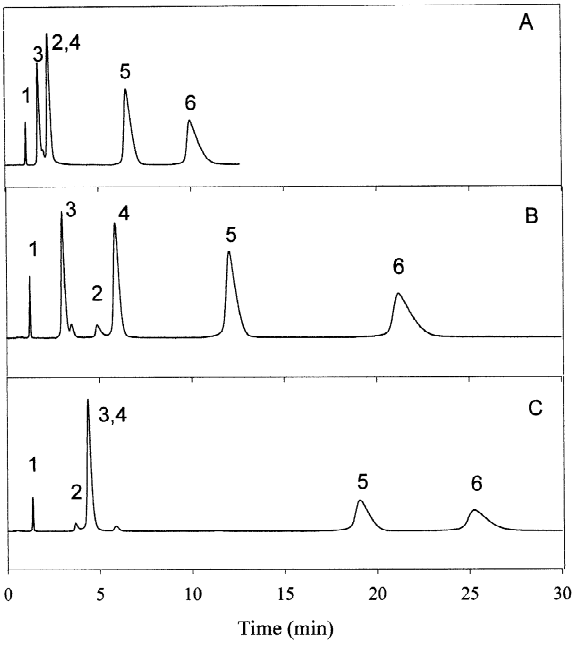

Fig. 2. Chromatograms of the test basic solute mixture on PBD–ZrO_2 in mobile phases containing acetate, fluoride and phosphate, respectively. Mobile phase: 30% ACN+20 m*M* buffer (pH 7.0). (A) ammonium acetate; (B) ammonium fluoride; (C) ammonium phosphate monobasic. Other experimental conditions: 1 mL/min; 40 °C; 254 nm. Samples: 1, lidocaine; 2, norpseudoephedrine; 3, quinidine; 4, tryptamine; 5, amitriptyline; and 6, nortriptyline.

Table 1
Effect of the type of Lewis base buffer on the retention factors and plate counts of the basic test solutes on the PBD–ZrO_2 phase

Solute	k' [a]			N [a]		
	Acetate	Fluoride	Phosphate	Acetate	Fluoride	Phosphate
Lidocaine	0.87 (1)[b]	1.13 (1)	1.29 (1)	2400	1450	2600
Norpseudoephedrine	2.61 (3)	6.85 (3)	5.23 (2)	200	660	1200
Quinidine	1.69 (2)	3.76 (2)	5.76 (3)	500	900	1600
Tryptamine	2.66 (4)	8.39 (4)	6.43 (4)	400	550	700
Amitriptyline	8.98 (5)	18.98 (5)	29.75 (5)	450	850	1200
Nortriptyline	13.97 (6)	32.70 (6)	39.49 (6)	300	800	1000

[a] The values were obtained from individual-solute injections. The mobile phases are 30% ACN containing 20 m*M* ammonia acetate, ammonia fluoride or ammonia phosphate monobasic, respectively, adjusted to pH 7. Other experimental conditions: 1 mL/min; 40 °C; 254 nm.

[b] The number in parentheses is the elution order of the corresponding solutes in each medium.

triptyline and thus should be more hydrophobic. If both solutes were retained by purely reversed-phase mode, then amitriptyline should be more retained than nortriptyline. Instead, we observed that amitriptyline always elutes before nortriptyline. Similarly, in both acetate and fluoride media, norpseudoephedrine, a relatively hydrophilic solute, had longer retention times than quinidine, a more hydrophobic solute.

Since all the other chromatographic conditions except the buffer type were held constant, the difference in retention time can only be attributed to the difference in the buffer type. Although the 20 m*M* buffer used in this experiment is not likely to change the properties of the mobile phase (surface tension, dielectric constant) significantly, it can cause a substantial change in the surface chemistry of the PBD–ZrO_2 stationary phase and introduce retention modes in addition to the reversed-phase mode provided by the PBD coating. As described above, adsorption of buffer anions imparts negative charges on the surface for ion-exchange interactions with cationic analytes. The fact that the elution order of nortriptyline and amitriptyline deviates from that predicted by the reversed-phase retention model can be easily explained by a secondary cation-exchange interaction. At pH 7, nortriptyline and amitriptyline are both positively charged and thus undergo cation-exchange interactions besides hydrophobic interactions. This results in longer retention for both of these solutes. We note that, at higher pH, their retention decreases (see below). Furthermore, amitriptyline has an additional methyl substitute on the positively charged nitrogen atom compared to nortriptyline. An increase in the size of the charged center will weaken the cation-exchange interaction [61] and thus make amitriptyline less retained by Coulombic (ion-exchange) interactions than nortriptyline. The fact that amitriptyline elutes before nortriptyline in all three buffer systems tested indicates that cation-exchange interactions play a significant role in the retention of basic solutes on PBD–ZrO_2 phases.

The strength of the cation-exchange interaction also depends on the type of buffer used. The data in Table 1 and Fig. 2 suggest that the strength of the cation-exchange interaction on the PBD–ZrO_2 phase follows the order acetate < fluoride < phosphate based on the assumption that a stronger cation-exchange property of the PBD–ZrO_2 phase leads to longer retention times. This order is in good agreement with our previous findings for adsorption of these species on *bare* zirconia [24]. We attribute the increase in retention to the increase in the number of negatively charged sites available for cation-exchange interactions brought about by changing the type of buffer from acetate to fluoride to phosphate. At pH 7, about 50% of the solution phosphate ions are doubly negatively charged and phosphate binds to the surface more strongly than acetate and fluoride. Thus, in the presence of phosphate, we expect that there are more negative charges on the surface.

In Table 2, the selectivities of the peak pairs in order of their elution in each medium are compared. We can see that even though the elution orders of the six solutes in the three media are very similar, the

Table 2
Effect of the type of Lewis base buffer on the selectivity of the separations of the basic test solutes on the PBD–ZrO_2 phase

Pair	Selectivity (α)		
	Acetate	Fluoride	Phosphate
2/1[a]	1.94	3.31	4.05
3/2	1.54	1.82	1.10
4/3	1.02	1.23	1.12
5/4	3.38	2.26	4.62
6/5	1.56	1.72	1.33

[a] Peak pair in order of elution order in each medium.

selectivities are dramatically different. This indicates that we can use the type of buffer to adjust selectivity.

The plate counts for these basic solutes are shown in Table 1. They generally follow the trend acetate < fluoride < phosphate, which means that the separation efficiencies of the basic solutes actually improve as cation-exchange interactions become stronger. One may argue that this is because the experiment was conducted at pH 7, which is within the buffer range of phosphate but is out of the buffer range of acetate and fluoride. However, this explanation contradicts some of our observations. First, we see the same trend when the same experiment was conducted at pH 4, which is within the buffer range of acetate and fluoride but out of the buffer range of phosphate. Second, the same trend was also observed when we separated some basic drugs at pH 7.5 where each of the above three buffers was used in conjunction with a second non-Lewis base species which had good buffer capacity at around 7.5 (see below). There are many possible reasons why these trends might be as they are, but we did not investigate them further.

3.2. Effect of buffer concentration

The eluent's buffer concentration has a big impact on solute retention in ion-exchange chromatography (IEC) but only a minor effect in RPLC. Thus it is important to examine how changes in buffer concentration can affect retention on PBD–ZrO_2. 5, 10, and 20 m*M* ammonium phosphate buffers were chosen to ensure that the effect is large enough to be detected and the experiment was carried out under chromatographic conditions similar to those discussed above. Since the set of the six basic solutes have very long retention even in 20 m*M* phosphate, another set of five solutes were used in this experiment. The retention factors of the solutes at these concentrations are given in Table 3.

For ion-exchange chromatography, the relationship between the retention factor of an ionic analyte and the salt concentration in the mobile phase is generally estimated by using the following equation [62]:

$$\log k' = -s \log [C] + \text{constant} \quad (4)$$

where s is a constant dependent on the charge of the analyte and the displacer (counter-ion) and C is the counter-ion concentration. Eq. (4) indicates that a plot of $\log k'$ vs. the logarithm of the buffer concentration should be linear with a negative slope. The $\log k'$ vs. log[phosphate] is plotted in Fig. 3.

Table 3
Retention factors of basic solutes as a function of phosphate concentration

	$\log k'$ [a]			R^2 [c]	Slope[d]	S.D.[e]
Buffer conc. (m*M*):	5[b]	10[b]	20[b]			
Lidocaine	0.50	0.35	0.22	0.9989	0.46±0.02	0.007
Procainamide	0.71	0.46	0.20	1.0000	0.85±0.01	0.000
Atenolol	0.80	0.54	0.28	1.0000	0.86±0.01	0.001
Nadolol	1.13	0.89	0.64	0.9999	0.82±0.01	0.003
Doxylamine	1.15	0.93	0.69	0.9999	0.76±0.01	0.004

[a] Measured in 30% ACN + phosphate buffer at pH 7.0, 40 °C.
[b] Buffer concentration in m*M*.
[c] R^2 for the regression of $\log k'$ versus log[phosphate] in Fig. 3.
[d] Slope of Eq. (4) with ±standard deviation.
[e] Standard deviation of the overall linear regression.

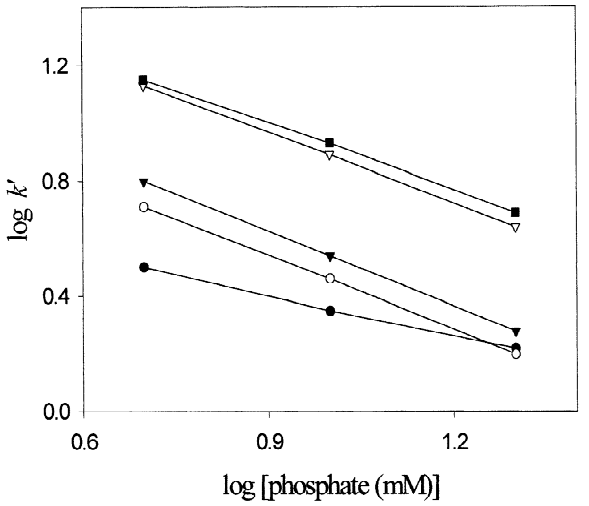

Fig. 3. Plot of $\log k'$ for basic solutes versus logarithm of phosphate buffer concentration in m*M* on PBD–ZrO_2. Mobile phase: 30% ACN + buffer adjusted to pH 7.0. Other experimental conditions: 1 mL/min; 40 °C; 254 nm. Solutes: (●) lidocaine; (○) procainamide; (▼) atenolol; (▽) nadolol; (■) doxylamine.

The slope, correlation coefficient, and also the retention factors are listed in Table 3. For all the solutes, retention decreases as the buffer concentration is increased. This confirms that ion-exchange interactions do contribute substantially to retention on PBD–ZrO_2 phases. Obviously, there are many negatively charged sites on the surface of zirconia when there is a hard Lewis base in the mobile phase. An increase in the ionic strength of the mobile phase significantly weakens the electrostatic interaction between the basic solutes and zirconia's surface, thereby decreasing the retention. While it is possible that the surface concentration of phosphate might vary with the eluent concentration of phosphate, studies have shown that the surface is saturated when the concentration of phosphate in the mobile phase is greater than about 1–3 m*M* [63]. So when the 5, 10, and 20 m*M* phosphate buffers were used, the adsorbed phosphate concentration is almost unchanging.

We also noticed that the slopes for the different solutes are different. This means that when we change the concentration of the eluent additive, the change of retention of different solutes will be different. Thus, the selectivity (band spacing) will vary as the buffer concentration is changed.

3.3. Effects of the type of buffer cation

We studied the effect of sodium, potassium, ammonium, and triethylammonium (TEA^+) phosphate as the buffer cation. The data are given in Table 4. In general, sodium, potassium, and ammonium show similar effects on the retention of all basic solutes except for quinidine, which elutes slightly earlier in sodium, and amitriptyline and nortriptyline, which elute a bit later in potassium buffers. This observation is not consistent with the observed cation-exchange elution series for a conventional IEC system: $Na^+ < NH_4^+ < K^+$ [64,65]. At this time, we do not have an explanation for these results except to point out that the aqueous–organic eluents used here are not the typical mobile phases used for IEC. Table 4 also shows that there is a

Table 4
Effect of buffer cation on the retention factors and plate counts of the basic test solutes on the PBD–ZrO_2 phase

Solute	k' [a]				N [a]			
	Na^+	K^+	NH_4^+	TEA^+	Na^+	K^+	NH_4^+	TEA^+
Lidocaine	0.88 (1)[b]	0.95 (1)	0.98 (1)	0.92 (1)	3800	3900	4000	3900
Norpseudoephedrine	2.84 (2)	2.99 (2)	2.99 (2)	64.0 (5)	1300	1600	2200	500
Quinidine	3.83 (3)	4.90 (4)	5.03 (4)	7.70 (2)	1900	2700	2900	2300
Tryptamine	3.86 (4)	4.08 (3)	3.71 (3)	150 (6)	2700	3300	3800	1800
Amitriptyline	18.5 (5)	22.4 (5)	18.8 (5)	33.6 (3)	3300	3400	3400	3600
Nortriptyline	24.2 (6)	29.7 (6)	23.8 (6)	94.6 (4)	3000	3400	3900	2000

[a] The values were obtained from individual-solute injections. The mobile phases are 35% ACN solutions containing 30 m*M* sodium phosphate, potassium phosphate, ammonia phosphate, and triethylamine phosphate, respectively, adjusted to pH 7.5. Other experimental conditions: 1.0 mL/min; 40 °C; 254 nm.

[b] The number in parentheses is the elution order of the corresponding solutes in each medium.

minor improvement in separation efficiency in the order $Na^+ < K^+ < NH_4^+$. This trend coincides with the relative solubility of these cations in acetonitrile.

In comparison to sodium, potassium, and ammonium ions, triethylammonium is very different and much less effective in eluting the basic solutes, especially the primary and secondary amines. This is reflected not only by the extremely long retention times of most test solutes (other than lidocaine and quinidine), but also by the markedly lower separation efficiency in the TEA-phosphate buffer. A possible explanation is that the triethylammonium cation is much bigger than the other three cations, which makes it a weaker eluent for IEC. Alternatively, adsorption of TEA with its high amount of carbon might increase the hydrophobicity of the stationary phase. However, the difference in retention data of a series of alkylbenzenes in the ammonium and TEA buffer is less than 5% (data not shown). We conclude that our first explanation is the more reasonable one. The ineffectiveness of triethylammonium in suppressing cation-exchange interactions is also evident in our observation that when a water–acetonitrile solution containing 20 m*M* of triethylamine was used for the separation of antidepressant drugs on a PBD–ZrO_2 phase, all secondary amines showed very long retention times and poor peak shapes (data not shown).

The results observed here are rather surprising considering that triethylamine is much more effective in suppressing silanophilic interactions on silica-based reversed phases than are sodium, potassium or ammonium [39,41,64] and that one generally sees lower retention in TEA buffers than in ammonium buffers when silica-based RPLC media are used. Our data suggest that the interactions between the basic solutes and the anionic sites on the PBD–ZrO_2 phase are fundamentally different from ''silanophilic'' interactions. This difference will be the subject of additional study.

3.4. Mobile phase pH

Ammonium phosphate was used as the buffer in our study of the effect of mobile phase pH (see Fig. 4). Based on the changes in retention factor with pH, the test solutes can be divided into two groups:

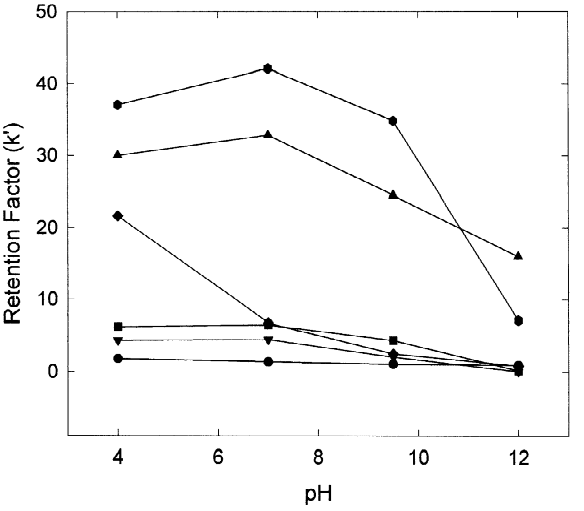

Fig. 4. Effect of mobile phase pH on the retention factors of basic solutes on PBD–ZrO_2. Mobile phase: 30% ACN + 20 m*M* ammonia phosphate buffer adjusted to different pH except that pH 12 was obtained with 20 m*M* of sodium phosphate buffer. Other experimental conditions: 1 mL/min; 40 °C; 254 nm. Samples: (●) lidocaine; (▼) norpseudoephedrine; (■) tryptamine; (♦) quinidine; (▲) amitriptyline; and (⬢), nortriptyline.

amitriptyline and nortriptyline in one group and all others in the second.

Amitriptyline and nortriptyline (the two tricyclic antidepressant drugs) were much more retained than the other solutes under all conditions. This is likely due to the strong hydrophobic interaction between their aromatic rings and the PBD coating. As the mobile phase pH was increased, their retention first increased and then decreased, reaching a maximum at about pH 7. This results because changing the mobile phase pH affects the charge state of both the basic analyte and zirconia's surface. This in turn affects both the hydrophobic and cation-exchange interactions. At low pH (two units below their pK_a), the cation-exchange interaction is likely dominant; and at high pH the hydrophobic interaction is likely dominant. At intermediate pH values, both interactions contribute significantly to the retention, leading to a maximum in retention vs. pH. In direct contrast to the above observation on PBD–ZrO_2, it is well known that the retention of amines on silica-based RPLC media increases as the pH is raised up to and above the pK_a, reflecting an increase in the hydrophobicity of the analytes upon deprotonation [18,66].

Most interestingly, we also observed a switch in elution order of amitriptyline and nortriptyline as the pH was increased. Below pH 12, nortriptyline always eluted after amitriptyline, which is ''anti-reversed-phase'' retention behavior. In contrast, at pH 12, nortriptyline eluted before amitriptyline, indicating that the hydrophobic interaction became the dominant retention factor. This result shows that amines can be separated by a predominantly reversed-phase interaction on PBD–ZrO_2 phases at extremely high pH. Such separations are made possible by the excellent chemical stability of the PBD–ZrO_2 phase.

Among the less-retained solutes, retention of norpseudoephedrine and tryptamine first increased slightly then decreased significantly as the pH was increased. In contrast, retention of lidocaine and quinidine simply decreased monotonically. These solutes are much more hydrophilic than those in the former group; thus they have much weaker hydrophobic interactions. Consequently, their retention times are controlled principally by the cation-exchange interaction. As the pH was increased, the effect incurred by an increase in hydrophobic interaction is likely to be overwhelmed by that of a significant decrease in the cation-exchange interaction.

The mobile phase pH also affects the separation efficiency (see Fig. 5). Nearly all the solutes show their highest plate count at pH 12, which is reasonable because all the solutes are uncharged. In this case, the reversed-phase mechanism dominates and makes the peak shape much better. Thus, in order to get the best separation efficiency, amines should be separated at high pH at least two units above their pK_a values.

3.5. Application of mixed-mode retention selectivity

So far, we have found that, under most chromatographic conditions, amine solutes are retained by both hydrophobic and cation-exchange interactions on PBD–ZrO_2 phases. Although a predominantly reversed-phase retention mode can be obtained at extremely high pH (around pH 12), this high pH may not be useful for the separation of hydrophilic basic solutes which have minimal hydrophobic interactions with the stationary phase. Instead, a reversed-phase and cation-exchange mixed-mode retention can provide longer retention and unique selectivity for such analytes. In this section, two sample separations were studied to demonstrate the utility of the mixed-mode retention feature of PBD–ZrO_2 phases.

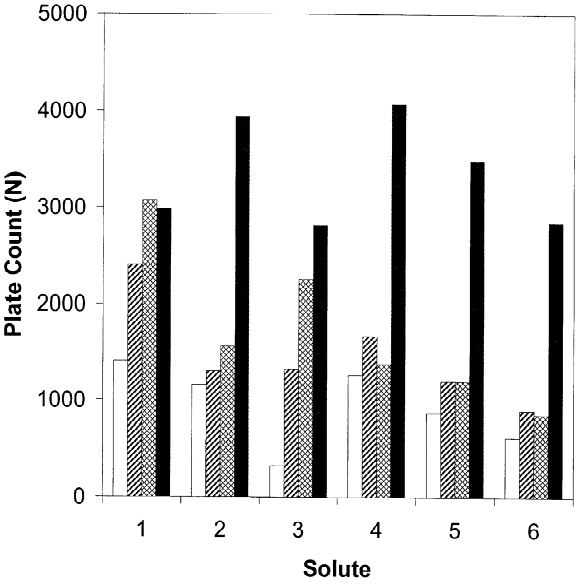

Fig. 5. Effect of mobile phase pH on the separation efficiency of basic solutes on PBD–ZrO_2. The experimental conditions were the same as those described in Fig. 4. Bars: (□) pH 4.0, (▨) pH 7.0, (▩) pH 9.5, (■) pH 12. Samples: 1, lidocaine; 2, norpseudoephedrine; 3, quinidine; 4, tryptamine; 5 amitriptyline; and 6, nortriptyline.

The first example is the separation of seven antihistamine and antidepressant drugs. Fig. 6 shows three separations of a mixture under otherwise similar conditions except the eluent buffer system was varied slightly. Each buffer system was comprised of a common buffer, Tris, and one of the three Lewis bases studied (acetate, fluoride, and phosphate). Tris was used to control the mobile phase pH at 7.5. Since Tris does not have a hard Lewis base functionality, we do not expect it to interfere with the Lewis acid/base interaction between acetate, fluoride, and phosphate and zirconia's surface. Thus, these Lewis base buffers will impart different cation-exchange properties to the PBD–ZrO_2 phase.

As shown in Fig. 6, the mixture was well separated in all mobile phases. When acetate was used, the seven compounds were separated in less than 4 min. Replacing acetate with fluoride or phosphate increased the retention for most solutes due to

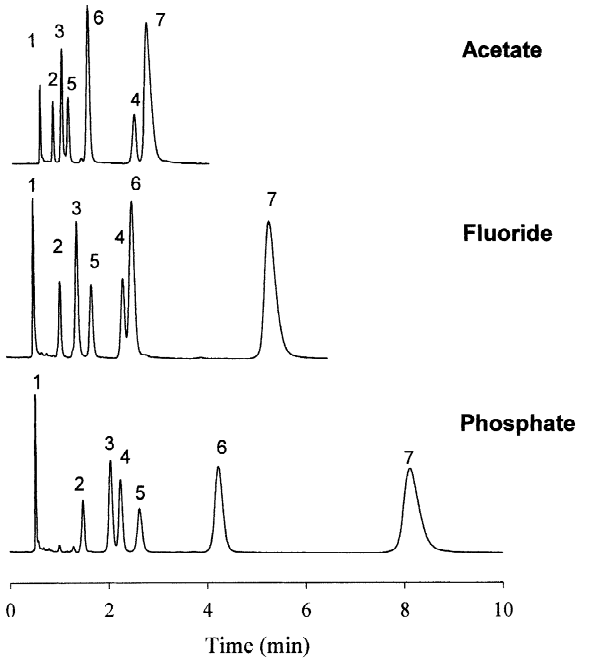

Fig. 6. Separation of drug mixture on the PBD–ZrO_2 phase. Mobile phase: 37.5% ACN containing 15 m*M* Tris and 30 m*M* ammonium acetate; ammonium fluoride; or ammonium phosphate monobasic adjusted to pH 7.5. Other experimental conditions: 1 mL/min; 40 °C; 254 nm. Samples: 1, dimenhydrinate; 2, doxylamine; 3, pyrilamine; 4, meclizine; 5, chlorpheniramine; 6, doxepin; and 7, desipramine.

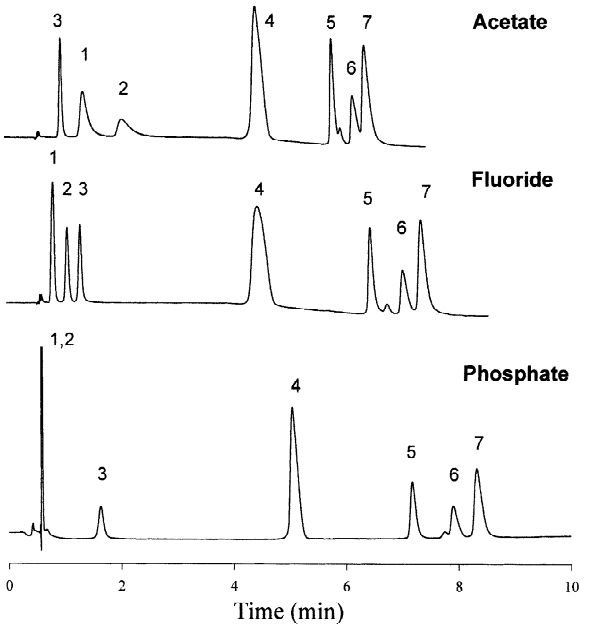

Fig. 7. Separation of anti-arrhythmic drugs on the PBD–ZrO_2 phase. Mobile phase: A, aqueous solution containing 15 m*M* Tris and 30 m*M* of a Lewis base buffer adjusted to pH 7.5; B, 50% ACN solution containing 15 m*M* Tris and 30 m*M* of a Lewis base buffer adjusted to pH 7.5. Gradient elution: 15% B for 3 min, 15–70% B in 2 min, 70–100% B in 5 min. Other experimental conditions: 1 mL/min; 40 °C; 254 nm. Samples: 1, chlorpropamide; 2, tolbutamide; 3, procainamide; 4, lidocaine; 5, quinidine; 6, alprenolol; and 7, propranolol.

enhancement of the cation-exchange interaction. The mixture is still well separated under these two conditions. However, the important point is the significant change in elution order and selectivity.

The second example is the separation of a set of anti-arrhythmic drugs. This solute set is more complex than the previous one because it includes the acidic solutes chlorpropamide and tolbutamide. This mixture was separated under gradient conditions (see Fig. 7). When acetate was included in the mobile phase, the peaks were reasonably well separated. However, the two acidic solutes, chlorpropamide and tolbutamide, showed very broad, tailing peaks. These solutes have a sulfonamide group whose pK_a is around 4.9 [67,68]. At pH 7.5, the sulfonamide groups are deprotonated making these solutes Lewis bases which can undergo Lewis acid–base interactions with zirconia's surface just as do the hard Lewis base buffers. This led to long retention, peak broadening and tailing. Apparently, acetate is not strong enough to suppress such interactions. When fluoride was used, chlorpropamide and tolbutamide eluted much earlier and the peaks were much sharper. This is because the Lewis acid–base interactions were effectively suppressed by the much stronger hard Lewis base present in the eluent. Their retention times were further reduced due to the electrostatic repulsion effect from the negatively charged surface. In contrast, basic solutes were more retained due to the increased cation-exchange interaction in fluoride media. This actually created some ''space'' in the separation window to accommodate the early eluted acidic solutes. Consequently, all peaks were well separated (see Fig. 7). When phosphate was used in the eluent, the retention times of basic solutes increased further, but the two acidic solutes were unretained. In fact, the acidic solutes eluted before the system peak, indicating that these

solutes were strongly repelled from the stationary phase by the adsorbed phosphate. Therefore, considering the overall performance, PBD–ZrO_2 modified with fluoride provided the best separation in this case.

4. Conclusions

This work shows that, under most chromatographic conditions, amine solutes experience both hydrophobic and cation-exchange interactions when separated using PBD–ZrO_2 stationary phases that are operated in eluents containing hard Lewis bases at pH values below the pK_a of the Bronsted conjugate acid form of the analyte. The cation-exchange sites are due to both the inherent zirconol groups, but more importantly they result from the adsorbed Lewis base anionic buffer constituents on accessible zirconia surface sites. The properties of these cation-exchange sites depend on the type of Lewis base buffer, the type of counter-ion, ionic strength, and the eluent pH. These secondary cation-exchange interactions can increase retention and provide unique, ''tunable'' selectivities for basic analytes on these types of RPLC supports. When the number of cation-exchange sites is relatively small, amine solutes apparently easily overload these sites thereby causing peak tailing. Increasing the number of these sites can greatly improve the peak shape.

Very interestingly, when the pH of the eluent is increased from neutral (pH 7) to alkaline (pH 12), the retention of all the basic solutes decreased, which is totally different from the observation on silica-based phases [18,66]. Also, the ineffectiveness of TEA in comparison to ammonium ion in eluting basic solutes from PBD–ZrO_2 demonstrates that some fundamental differences exist between the ion-exchange interaction on PBD–ZrO_2 and silica-based phases.

Although a predominantly reversed-phase retention mode can be obtained at extremely high pH (around pH 12), we have shown that the mixed-mode retention mechanism in which IEC interactions are more important than hydrophobic interactions is often preferable. Finally, a few sample separations of pharmaceuticals were used to test the utility of the mixed-mode retention feature on this phase.

Acknowledgements

The authors acknowledge the financial support by grant from the National Institutes of Health and thank ZirChrom Separations for their donation of the PBD–ZrO_2 particles used in this work.

References

[1] C.J. Dunlap, C.V. McNeff, D. Stoll, P.W. Carr, Anal. Chem. 73 (2001) 598A.
[2] M. Kawahara, H. Nakamura, T. Nakajima, J. Chromatogr. 515 (1990) 149.
[3] U. Trudinger, G. Müller, K.K. Unger, J. Chromatogr. 535 (1990) 111.
[4] M.P. Rigney, T.P. Weber, P.W. Carr, J. Chromatogr. 484 (1989) 273.
[5] T.P. Weber, P.W. Carr, E.F. Funkenbusch, J. Chromatogr. 519 (1990) 31.
[6] J.A. Blackwell, P.W. Carr, J. Chromatogr. 549 (1991) 59.
[7] J. Yu, Z.E. Rassi, J. Chromatogr. 631 (1993) 91.
[8] H.J. Wirth, M.T.W. Hearn, J. Chromatogr. 646 (1993) 143.
[9] C. McNeff, Q.H. Zhao, P.W. Carr, J. Chromatogr. A 684 (1994) 201.
[10] L. Sun, P.W. Carr, Anal. Chem. 67 (1995) 2517.
[11] Y. Hu, P.W. Carr, Anal. Chem. 70 (1998) 1934.
[12] Y. Hu, Ph.D. Thesis, University of Minnesota, Minneapolis, MN, 1998.
[13] J. Li, P.W. Carr, Anal. Chem. 69 (1997) 2202.
[14] J. Li, Y. Hu, P.W. Carr, Anal. Chem. 69 (1997) 3884.
[15] J. Li, P.W. Carr, Anal. Chem. 69 (1997) 837.
[16] J.L. Glajch, J.J. Kirkland, LC·GC 8 (1990) 140.
[17] J.J. Kirkland, J.W. Henderson, J. Chromatogr. Sci. 32 (1994) 473.
[18] J.J. Kirkland, J.D. Martosella, J.W. Henderson, C.H. Dilks, J.B. Adams, Am. Lab. 31 (1999) 22.
[19] J.J. Kirkland, J.B. Adams Jr., M.A. Van Straten, H.A. Claessens, Anal. Chem. 70 (1998) 4344.
[20] Y.-F. Cheng, T.H. Walter, Z. Lu, P. Iraneta, B.A. Alden, C. Gendreau, U.D. Neue, J.M. Grassi, J.L. Carmody, J.E. O'Gara, R.P. Fisk, LC·GC 18 (2000) 1162.
[21] U. Bien-Vogelsang, A. Deege, H. Figge, J. Koehler, G. Schomburg, Chromatographia 19 (1984) 170.
[22] J. Nawrocki, M.P. Rigney, A. McCormick, P.W. Carr, J. Chromatogr. A 657 (1993) 229.
[23] J.A. Blackwell, P.W. Carr, Anal. Chem. 64 (1992) 853.
[24] J.A. Blackwell, P.W. Carr, Anal. Chem. 64 (1992) 863.
[25] J. Nawrocki, J. Chromatogr. A 779 (1997) 29.
[26] J.A. Blackwell, P.W. Carr, J. Chromatogr. 549 (1991) 59.
[27] J.A. Blackwell, P.W. Carr, J. Liq. Chromatogr. 15 (1992) 727.
[28] J.A. Blackwell, P.W. Carr, J. Liq. Chromatogr. 15 (1992) 1487.
[29] J.A. Blackwell, P.W. Carr, J. Chromatogr. 596 (1992) 27.

[30] J.A. Blackwell, P.W. Carr, J. Chromatogr. 549 (1991) 43.
[31] M.P. Rigney, E.F. Funkenbusch, P.W. Carr, J. Chromatogr. 499 (1990) 291.
[32] W.A. Schafer, P.W. Carr, E.F. Funkenbusch, K.A. Parson, J. Chromatogr. 587 (1991) 137.
[33] J. Li, P.W. Carr, Anal. Chem. 68 (1996) 2857.
[34] M.P. Rigney, Ph.D. Thesis, University of Minnesota, Minneapolis, MN, 1989.
[35] J. Li, P.W. Carr, Anal. Chem. 69 (1997) 2193.
[36] G.J. Fleer, J. Lyklema, Makromol. Chem., Macromol. Symp. 17 (1988) 39.
[37] Y. Mao, P.W. Carr, Anal. Chem. 73 (2001) 4478.
[38] K.E. Bij, C. Horváth, W.R. Melander, A. Nahum, J. Chromatogr. 203 (1981) 65.
[39] J.S. Kiel, S.L. Morgan, R.K. Abramson, J. Chromatogr. 320 (1985) 313.
[40] R.J. Vervoort, M.W. Derksen, A.J. Debets, J. Chromatogr. A 765 (1997) 157.
[41] D.W. Hill, J. Liq. Chromatogr. 13 (1990) 3147.
[42] M. Reta, P.W. Carr, J. Chromatogr. 855 (1999) 121.
[43] D.V. McCalley, J. Chromatogr. A 793 (1998) 31.
[44] M.J. Wirth, M.D. Ludes, D.J. Swinton, Anal. Chem. 71 (1999) 3911.
[45] D.B. Marshall, C.L. Cole, D.E. Connolly, J. Chromatogr. 361 (1986) 71.
[46] D.B. Marshall, K.A. Stutter, C.H. Lochmuller, J. Chromatogr. Sci. 22 (1984) 217.
[47] P. Van Der Voort, I. Gillis-D'Hamers, E.F. Vansant, J. Chem. Soc., Faraday Trans. 86 (1990) 3751.
[48] W.A. Schafer, P.W. Carr, J. Chromatogr. 587 (1991) 149.
[49] A.M. Clausen, P.W. Carr, Anal. Chem. 70 (1998) 378.
[50] T. Fornstedt, G. Zhong, G. Guiochon, J. Chromatogr. A 741 (1996) 1.
[51] D.V. McCalley, R.G. Brereton, J. Chromatogr. A 828 (1998) 407.
[52] D.V. McCalley, LC·GC 17 (1999) 440.
[53] A.M. Krstulovic, Chiral Separations by HPLC: Applications to Pharmaceutical Compounds, Ellis Horwood, Chichester, 1989.
[54] S.G. Allenmark, Chromatographic Enantioseparation: Methods and Applications, Wiley, New York, 1988.
[55] W.H. Pirkle, T.C. Pochapsky, Chem. Rev. 89 (1989) 347.
[56] D.R. Nau, in: M.T.W. Hearn (Ed.), HPLC of Proteins, Peptides and Polynucleotides, VCH, New York, 1991, p. 331.
[57] B.Y. Zhu, C.T. Mant, R.S. Hodges, J. Chromatogr. 594 (1992) 75.
[58] M. Walshe, M.T. Kelly, M.R. Smyth, H. Ritchie, J. Chromatogr. A 708 (1995) 31.
[59] D.V. McCalley, J. Chromatogr. A 738 (1995) 169.
[60] D. Chan Leach, M.A. Stadalius, J.S. Berus, L.R. Snyder, LC·GC 1 (1988) 22.
[61] F. Helfferich, Ion Exchange, McGraw-Hill, New York, 1962.
[62] H.F. Walton, R.D. Rocklin, Ion Exchange in Analytical Chemistry, CRC Press, Boca Raton, FL, 1990.
[63] I. Tsukerman, M.S. Thesis, University of Minnesota, Minneapolis, MN, 2002.
[64] L.R. Snyder, J.J. Kirkland, Introduction to Modern Liquid Chromatography, Wiley–Interscience, New York, 1979.
[65] H. Small, Modern Analytical Chemistry, Plenum Press, London, 1989.
[66] U.D. Neue, T.H. Walter, B.A. Alden, Z. Jiang, R.P. Fisk, J.T. Cook, K.H. Glose, J.L. Carmody, J.M. Grassi, Y. Cheng, Z. Lu, R.J. Crowley, Am. Lab. (1999) 36.
[67] R.C. Weast, CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 1981.
[68] J.A. Dean, Lange's Handbook of Chemistry, McGraw-Hill, New York, 1979.