CHAPTER 16
METAL OXIDES AS HPLC STATIONARY PHASES

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ABSTRACT

The paper contrasts the main drawbacks of silica based packings such as their relative thermal and chemical instability with excellent stability of metal oxides. The paper concerns mainly ZrO_2, TiO_2 and Al_2O_3. Surface chemistry of the oxides is however very different from that of silica. Ability of the oxides to ion- and ligand-exchange is discussed from a chromatographic point of view.

1 INTRODUCTION

The goal of the present work is to summarize recent achievements in HPLC with metal oxide substrates, that is, non-silica-based inorganic phases. Some years ago we published two reviews on the chemistry of zirconia and its use in chromatography [1,2]. Forgács and Cserháti [3] recently reviewed liquid chromatographic studies of bioactive compounds on non-silica-based supports. However, that paper only showed a few separations on metal oxides. A very recent review of Pesek and Matyska [4] describes alumina supports for HPLC. Several synthetic approaches to modify alumina surfaces were described.. This paper will be mainly devoted to explaining the chromatography of many different classes of compounds, especially organic bases, on modified metal oxides. Just last year Dunlap et al. reviewed some of the more promising aspects of chromatography on zirconia-based stationary phases in a very brief feature article in Analytical Chemistry [5].

We will demonstrate that due to the differences in chromatographic retention and selectivity arising from the surface chemistry these differences in surface chemistry relative to silica can frequently be used to very good advantage to achieve otherwise difficult separations. Additionally, metal oxide supports offer very high efficiency (> 100,000 plates/meter), which in contrast to that of polymeric materials is similar to silica's whereas their, especially zirconia's, chemical and thermal stability are vastly superior to silica's

Zirconia, titania and alumina are oxides which deserve more attention from chromatographers. So far, in comparison to all other types of phases (metal oxides, carbon, and polymers) silica-based reversed phases completely dominate HPLC. Silica surface chemistry and surface modification reactions are well understood. However, the limitations of silicaceous materials are also well known. First, silica's backbone i.e. the Si-O-Si bond hydrolyzes at pH > 8 and does so quite rapidly at elevated temperatures (> 40 ^{o}C) and in the presence of certain very common but highly deleterious species such as phosphate and carbonate. Essentially silica is soluble in aqueous/organic media in even slightly alkaline media at even moderately elevated temperatures [6]. Second, the siloxane bond is unstable at pH < 4-5 and becomes increasingly less stable as the pH is lowered. Although silica does not dissolve at low pH this instability of the Si-O-Si bond leads to the loss of the stationary phase bonded to the silica. In contrast, polymeric reversed phases, are stable from pH 1 to 13 [7]. The materials can be used in RPLC as supports for bonded phases or as stationary phases themselves. However, polymeric phases often suffer excessive swelling and low mechanical stability under common use conditions. Most polymeric phases have poor chromatographic efficiency despite years of study and development [8,9].

In our opinion the design features of the next generation of column technology are now clear [5]:

- The particle synthesis method must be highly reproducible and yield solids with a narrow particle size distribution, that is monodisperse, with a porosity, surface area and pore diameter relevant to the analyte of interest
- The columns must be highly durable under adverse conditions (especially high and low pH and super-ambient temperatures).
- Column-to-column and batch-to-batch reproducibility must be improved.
- The particles should allow for faster analysis.
- The chromatographic performance (peak width and symmetry) towards cationic (basic) analytes should be improved.
- Selectivities differing significantly from those currently available are quite desirable.
- Columns will be designed for specific applications such as LC-MS and for specific purposes such as the minimization of solvent consumption and improved performance in highly aqueous eluents.
- New formats with novel flow characteristics especially monolithic phases for increasing speed at lower pressure drops will be most welcome.

In view of the above requirements we can easily point out the weaknesses of silicaceous phases: they are not sufficiently stable at extreme pHs or elevated temperatures. On the other hand, polymeric supports swell excessively, are not sufficiently mechanically stable and are much too inefficient. Thus there remains considerable interest in development of new, more stable phases. The quest for such phases has encompassed various metal oxides such as zirconia, alumina, titania and to a lesser extent thoria and ceria [10,11]. If we look at ceramic stationary phases we see that when properly formed into particles alumina, zirconia and titania roughly meet all the specifications enumerated above. However, these metal oxides have a “richer” surface chemistry than does silica and thus upon first glance they appear to be more difficult to use. Consequently detailed studies have been conducted on their surface chemistry and its influence on the retention properties of these oxides. A central theme of this review is that this surface chemistry is in fact easy to deal with and in many instances can be used to very great advantage to influence and control chromatographic selectivity.

The silicas used for chromatography are amorphous hence they are termed gels [12-16] but metal oxides are, by and large, crystalline: alumina is used as the α or γ (and also in amorphous form) [17]; zirconia is monoclinic and titania is in the anatase form [18,19]. Another problem, which seems to be at least partially solved, is the reproducibile synthesis of chromatographic grade particles; today nicely spherical, monodisperse, particles with excellent pore morphology comprised of titania, zirconia and to a lesser extent alumina HPLC packings are commercially available. As most HPLC separations are conducted in the reversed phase mode it is of great interest to obtain ceramic packings modified so as to impart a low polarity surface. However, as we pointed out above, the chemistry of the oxides is very different from that of silica. Thus simple silanization chemistry does not provide satisfactory results.

2 THE PH AND THERMAL STABILITY OF MODERN STATIONARY PHASES FOR HPLC.

The applications of HPLC today are unbelievably numerous. Most HPLC separations are done in the reversed phase mode. There exist an altogether intimidating variety of octyl and octadecyl silica based phases. More than 400 octadecyl silica based phases, not including polar embedded phases, are available [20]. Their properties vary substantially from one manufacturer to another. Although column performance, stability and reproducibility have improved greatly over the past 20 years there is still need for more durable columns and concommitantly the resulting more durable analytical methods. Improvements in stationary phase stability have been and remain a significant driving force for research into the development of new stationary phases. Several companies are now marketing much more stable silica-based reversed phase columns with nominal pH stability up to pH 11.5. These achievements are impressive. The rational use of silica columns at high pH has been reviewed [16]. Similarly there have been advances in improving the low pH stability [21,22].

Silica and silica-based HPLC stationary phases have been extensively used in different LC modes; however, reversed phase applications are by far predominant. The most widely used reversed phase silica-based stationary phase is the organosilane-bonded phase. Organosilane bonded phases are the most common because the synthesis is highly reproducible and the mass transfer characteristics of the resulting phases are the best available [23,24-26]. We will limit this discussion to bonded phases, the important factors affecting their chemical stability, and the various chemical approaches taken during phase production to enhance their chemical stability.

Typically conventional bonded phases are chemically stable when the pH of the mobile phase is between 3 and 9. McCalley has stated recently that the long-term stability is limited to pHs between 3 and 7 [21].

When the pH of the mobile phase is less than 4, acid catalyzed hydrolysis of the siloxane bond between the silica surface and the organosilane becomes significant [16,22,27,28]. This results in continuous loss of the bonded phase and concomitant loss of chromatographic retention. At mobile phase pH values greater than 9, the incompletely shielded silica support begins to dissolve and in addition the bonded phase is cleaved from the support [16,28,29,30,31]. The dissolution of the underlying silica leads to a progressive and often rapid loss in column efficiency, development of a void at the top of the column and eventually an intolerably backpressure. Continual exposure to pH $\geq$ 9 mobile phases leads to collapse of the column bed and complete loss of chromatographic efficiency.

The simplest solution of the pH stability problem is to avoid using mobile phases with pH values outside the range of 3 to 9. However, this severely limits the use of mobile phase pH as a variable for optimizing the separations of many classes of solutes. For example, the reversed phase separation of small, basic pharmaceuticals often benefits from the use of either highly acidic and highly basic eluents [28]. Lowering the pH suppresses deleterious interactions between the silanol groups on the silica surface and the solutes leading to significant peak shape and efficiency improvements. Recently Neue has demonstrated the existence of a class of silanols whose pKa is between 3 and 5 on one specific high purity silica [32]. The separation of these solutes also benefits from high pH mobile phases. Raising the mobile phase pH deprotonates the solutes or converts them into the “free base” form. Analyzing basic solutes as free bases often leads to higher chromatographic retention and symmetric, highly efficient peaks [28]. Mobile phase pH is such a powerful variable for optimizing reversed phase separations [16,21] that numerous approaches for enhancing the

chemical stability of reversed phase silica-based stationary phases have been examined. In addition, it should be understood that loss of bonded phase does not altogether cease at pH above 3 nor does dissolution of silica stop at pHs below 8. Fundamentally the lifetime of the column at any pH is ultimately limited by these stability issues.

2.1 Metal oxide phases

Metal oxides such as zirconia, titania and alumina are the most interesting prospects. These metal oxides offer much better chemical stability than do silica. Chromatographers can expect the following benefits from enhanced column packing stability [33]:

- more stable column packing = longer column lifetime = lower cost of analysis,
- more stable packing leads to a larger accessible range in pH and temperature and this gives the analyst more flexibility in developing analytical methods and offer the promise of better, more robust conditions
- more stable column packing = lower bleed from the column, easier development of LC-MS methods or the use of light-scattering detectors,
- the possibility of cleaning dirty, columns by use of extremely harsh conditions (extreme pHs and/or high temperature) without damaging column performance.

TABLE 1
Comparison of silica and metal oxide based packings.

Property	**SiO_2**	**$Ti\ O_2$**	**$Al_2\ O_3$**	**ZrO_2**
Monodispersity	++	++	++	++
Spherical micron sized particles available for all the oxides				
Pore structure	++	?	?	++
Silica and zirconia have well connected pores, not much has been published on the porosity of spherical titania and alumina .				
Surface area/pore diameter	++++	++	+++	++
Wider range available for silica				
Controllable surface chemistry	++++	?	++	++
Silica by silane chemistry, metal oxides by polymer deposition, zirconia - carbon deposition, not much is known on titania's surface chemistry				
Mechanical strength	++	?	?	+++
Silica and zirconia can tolerate >10,000 psi				
Chemical stability	-	++ (?)	+++	++++
Silica: 2<pH<8 (some to pH 11.5 0, no phosphates), alumina 3<pH<13, zirconia 1<pH<14, no stability studies are available for titania				
Thermal stability	-	?	?	+++
Silica:not higher than 60-70 ^oC, zirconia up to 200 ^oC, lack of temperature stability of alumina and titania				
Column efficiency	+++	?	++	+++
Silica and zirconia comparable, alumina slightly lower, no studies on titania				
Energetic homogeneity	++	+	+	+
Silica has better homogeneity of the surface (from an HPLC point of view) than metal oxides, all metal oxides have comparable surface chemistry.				

Table 1 can be summarized as follows: silica is available in the widest variety of pore and particle sizes. However, its thermal and chemical stability are much lower than the metal oxide-based packings.
The practicing chromatographer must choose an HPLC column from those available on the market. Thus the chromatographic practioner needs a real comparison of commercially available products. The Table 2 shows a comparison of eight stable HPLC columns.

TABLE 2
Commercially available high pH "stable" columns for RPLC.

Manufacturer	**ZirChrom**	**ZirChrom**	**Polymer Labs**	**Biotage**
Column name	DiamondBondC 18	ZirChrom-PBD	PLRP-S	Unisphere -PBD
Support	zirconia	Zirconia	polymer	alumina
Particle size [µm]	3	3	5	10
Pore size [Å]	300	300	100	220
Dimensions [mm]	50 x 4.6	150 x 4.6	150 x 4,6	250x4.6
Price [$]	675.00	595.00	480.00	NA
Low pH	1	1	1	2
High pH	14	13	14	13
High temp. limit [^{o}C]	200	150	150	NA
Manufacturer	**ES Industries**	**Phenomenex**	**Waters**	**Zorbax**
Column name	GammaBond RP-1	Luna	XTerra	Extend
Support	alumina	silica	silica	silica
Particle size [µm]	5	3	3.5	3.5
Pore size [Å]	80	100	130	80
Dimensions [mm]	150 x 4.6	150x4.6	150 x 4.6	150 x x4.6
Price [$]	695.00	435.00	425.00	540.00
Low pH	1.3	1.5	1	2
High pH	12	10	12	11.5
High temp. limit [^{o}C]	NA	NA	80	60

Table 2 gives the impression that all the proposed chromatographic packings are very similar (except perhaps for the high temperature limit): even the silica-based supports appear to have chemical stability comparable to polymer and zirconia or alumina . However, the chromatographer must be concerned with the long-term stability and ruggedness of the columns. Comparison of these properties for silica and zirconia columns can be easily assessed from the following studies.

Figures 1-2 compare the stabilities of two popular silica-based reversed phases to that of a zirconia-based phase using a number of basic pharmaceuticals as test compounds at extreme pH. It is important to note that this study was performed in the

presence of phosphate, which seriously destabilizes silica so in practice with the use of less corrosive media the silica columns would last for a longer time.

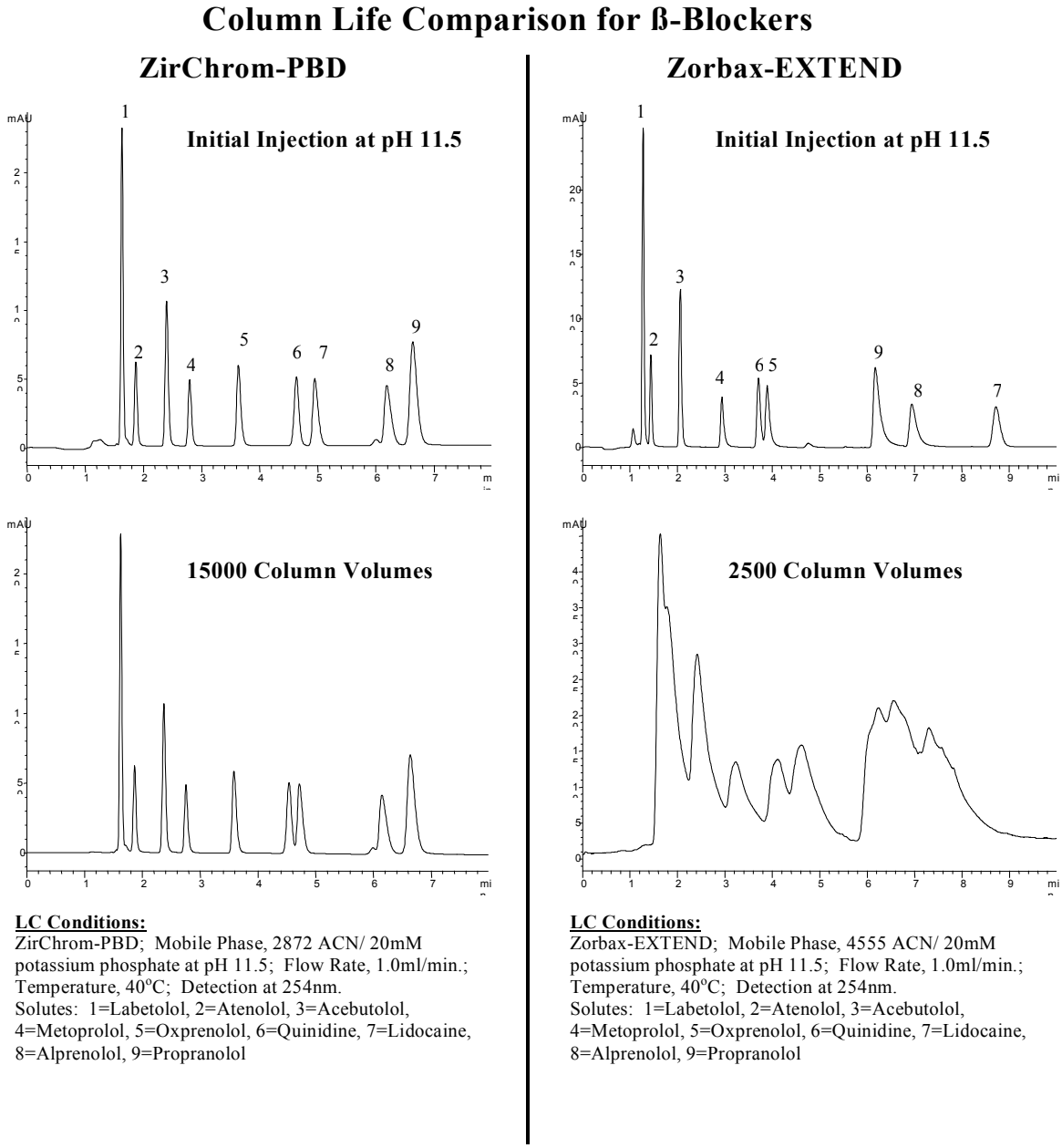
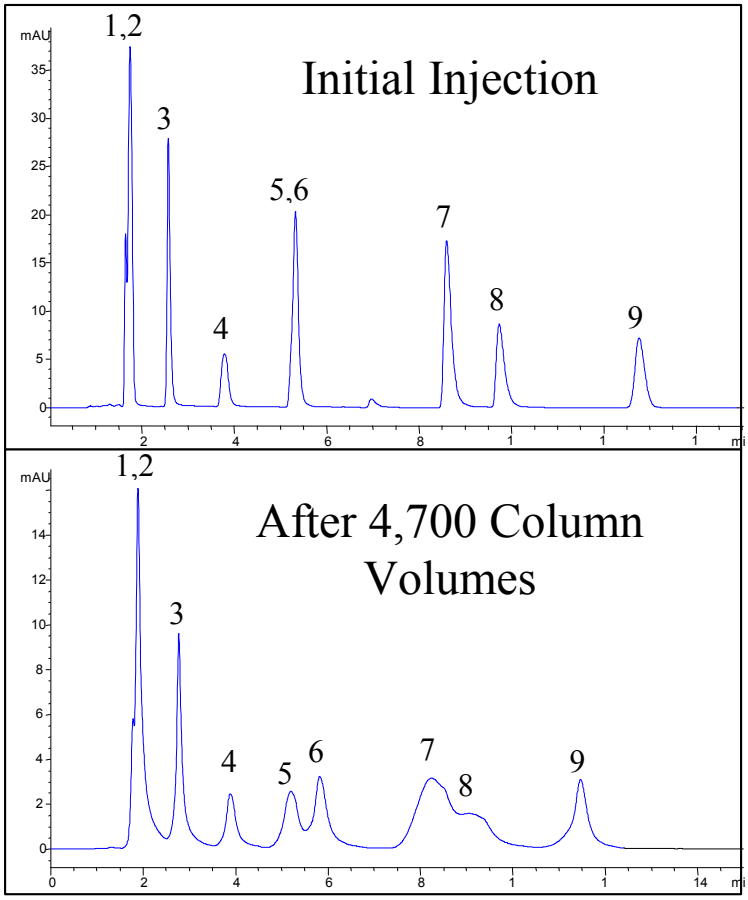


Figure1. High pH stability of PBD-zirconia vs. Zorbax Extend column.
LC Conditions: ZirChrom-PBD; Mobile Phase, 28/72 ACN/ 20mM potassium phosphate at pH 11.5; Zorbax-EXTEND; Mobile Phase, 45/55 ACN/ 20mM potassium phosphate at pH 11.5; Flow Rate, 1.0ml/min.; Temperature, 40oC; Detection at 254nm.
Solutes: 1=Labetolol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Quinidine, 7=Lidocaine, 8=Alprenolol, 9=Propranolol

Figure 2. Stability test of Phenomenex Luna column at pH 10.



LC Conditions: Phenomenex Luna; Mobile Phase, 45/55 acetonitrile/20mM potassium phosphate at pH 10.0; Flow Rate, 1.0ml/min.; Temperature, 30°C; Detection at 254nm. Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

Most silica-based columns are thought to be stable at pH 7 even in the presence of phosphate. The data shown in Figure 3 were obtained in an accelerated ageing study by holding the columns at 80 °C [5]. The test clearly shows the relative instability of silica-based packings compared to zirconia-based materials under these conditions.
Although the silica–based supports can be exposed at high pH, their stability seem to be rather limited when prolonged use of the column is intended.

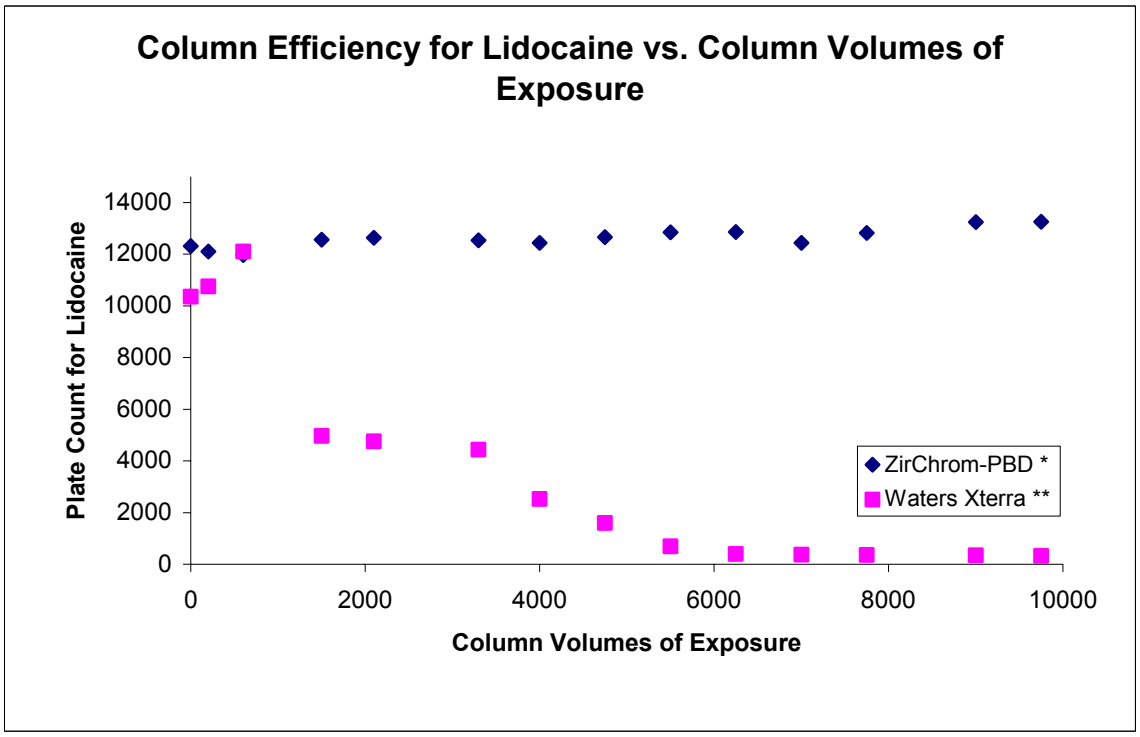


Figure 3. Stability of XTerra column at higher temperature and presence of phosphates. LC Conditions: Column dimension, 150 x 4.6; Mobile phase, ACN/50mM Potassium phosphate, pH 7.0; Temperature, 80 ^{o}C, 5 ul injection; Flow rate, 1.0 ml/min.; Detection at 254nm. *25/75 ACN/Buffer **30/70ACN/Buffer

3 SURFACES PROPERTIES OF ZRO_2, TIO_2 AND AL_2O_3 AND CHEMISTRY OF CHROMATOGRAPHY ON METAL OXIDES.

The chemistry of alumina, titania and zirconia surfaces differ considerably from that of silica. Due to its low pH_{pzc} silica allows only for a cation exchange while metal oxides behave as amphoteric ion exchangers i.e. they can be cation- or anion-exchangers depending on pH. Another difference, which has a great impact on the use of metal oxide phases is the existence of Lewis acid sites on the surface of metal oxides. These sites are responsible for the ligand exchange ability of zirconia, titania and alumina. Much more will be said about this Lewis chemistry below.

In chromatography modified metal oxides are used. That is the desired chromatography is induced by modifying the surface by depositing various substances that have the desired properties in the pores. For example, if one desires to do RPLC a low polarity substance (e.g. polybutadiene, polystyrene, elemental carbon) will be deposited on the surface of the pores and suitably immobilized. There is no modification that is known to block all the Lewis sites on the surface and thus the modified oxides almost always offer mixed mode retention. Which of the retention mechanisms prevails depends on the type of solute, the pH, the type of buffer used, the total ionic strength and the amount of organic modifier present in the eluent. It will be shown below that PBD modified oxides interact with nonelectrolytes exclusively by a reversed phase mechanism, organic bases (cationic amines) may be retained by a mixed mode ion-exchange/reversed phase mechanism depending on pH and other factors while the retention of *hard* Lewis bases (principally organo-phosphates, phosphonates, and

carboxylates) will be mainly governed by a mixed-mode ligand exchange/reversed phase mechanism.

3.1 Ion-exchange

Ion exchange properties are based on the ability of surface hydroxyls to dissociate or to be protonated depending on the eluent's pH:

$$M\text{-}OH + H^+ \leftrightarrow M\text{-}OH^+_2 \qquad K^{int}_1$$

$$M\text{-}OH + OH^- \leftrightarrow M\text{-}O^- + H_2O \qquad K^{int}_2$$

where K^{int}_1 and K^{int}_2 are the intristic ionization constants.

According to the model of Yates [189] H^+ and OH^- are the potential determining ions. When the number of positively ($M\text{-}OH^+_2$) charged species is equal to number of negatively ($M\text{-}O^-$) charged species then the surface has a zero net charge. The pH of the point of zero charge (pH_{pzc}) depends on ionization constants:

$$pH_{pzc} = 0.5\ (pK^{int}_1 + pK^{int}_2)$$

This is shown in Fig. 4. Practically when $pH < pK^{int}_1$ metal oxide will act as an anion exchanger while at $pH > pK^{int}_2$ it will be a cation exchanger. As the Yates' model seems to be too simplistic the reader is referred to more sophisticated models of ion adsorption on metal oxide surfaces [35-37].

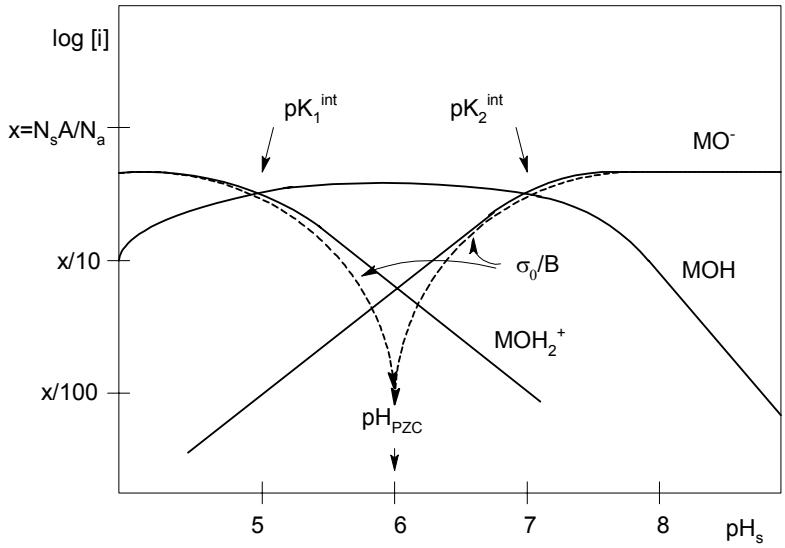


Figure 4. Variation of charged and uncharged species concentration with the surface pH (pH_s)

Isoelectric points (pI) of oxides can be measured by various methods [1] and in simple electrolyte systems, that is those which do not contain ions that specifically adsorb on the surface, then the pI will be equal to pH_{pzc} (point of zero charge).

The trouble with the chromatography of amines on silica-based supports originate in the low pI value of silica. Due to the low pH_{pzc} silanols dissociate at neutral pH (in fact even at pH 2 silanol effects cannot be totally avoided [38,39]) and the surface acquires a negative charge. Aliphatic bases are protonated at neutral pH and thus they interact strongly with silica surface via electrostatic interactions (ion-exchange) [12,16]. The chromatographer can choose to:

- decrease the pH to decrease the dissociation of silanols (but at pH<3 most bonded phase are unstable),
- increase the pH to deprotonate the organic bases (but at pH> 8 the silica backbone dissolves).
- add a strong ion exchange displacer otherwise known as a silanol group blocking agent to the eluent.
- add an anionic ion-pairing agent to form a tight ion pair with the cationic base and inhibit the Coulombic interaction with the surface.

Metal oxides offer much higher value of pH_{pzc}; i.e. at neutral pH the surface of metal oxide does not have a negative charge and thus it will not interact with charged bases via electrostatic interactions. The metal oxides are also stable at high pH [1,33,40,41] thus giving the analyst a chance to deprotonate the charged bases.

There are many examples of HPLC of organic bases on bare metal oxide supports: the slightly basic character of titania surface allows using this oxide for the separation of organic bases in normal phase chromatography [19]. Very similar separations can be done on alumina and zirconia [42,43,44]. Normal phase chromatography for alumina, titania and zirconia was compared by Kurganov et al. [44] and Grün et al. [43]. According to them the retention of the test solutes was mostly governed by the Brönsted acid-base properties of the surfaces. In the above separations of bases ion-exchange forces did not play a dominant role. However, there were attempts to take advantage of cation-exchange silica's ability to separate amines [45,46].

Important fundamental studies on the LC separation of inorganic anions on alumina were carried out by Schmitt and Pietrzyk [47]**.** Analyte anion exchange selectivities on alumina were determined and were found to be different from those observed on PS-DVB based R_4N^+ type anion exchangers. Schmitt and Pietrzyk noted excellent efficiency (40,000-70,000 plates/m), selectivity and resolution of inorganic anions on alumina. Smith and Pietrzyk have shown that selectivity of alumina depends on pH, ionic strenght, counterion, analyte concentration as well as mobile phase solvent composition. Strong affinity of alumina for sulphates and carboxylates was also noted [47]. Similar strong affinity of titania to carboxylates is also reported by Tani and Kubojima [48]. Detailed adsorption studies of halide ions on alumina were recently carried out by Szczepaniak and Kościelna [49]. Ion-exchange properties of alumina were examined and used for the separation of inorganic ions [50,51-54], of heroin samples [55] and a mixture of proteins [56]. The ion-exchange properties of titania [48,57] and zirconia [41] were also studied. It is interesting to note that there are some differences between the ion exchange behavior of the oxides: the halides elute from zirconia in the order: Cl^-, Br^-, I^-, while the sequence of elution for these anions on alumina is reversed [41,47]. The ion exchange properties of titania are similar to those of zirconia. All of the metal oxides strongly interact with fluoride, which is hard Lewis base [1,48,58-61]. Al_2O_3 is even used for removal of excessive concentrations of fluoride from drinking water [60].

3.2 Ligand exchange.

The oxides' ability to ligand exchange originate from:

- the presence of Lewis acid sites on the surface (i.e. coordinatively unsaturated Al^{3+}, Zr^{4+} or Ti^{4+}),
- the presence of water molecules and other easily displaced ligands coordinatively bonded to the sites.

The ligand exchange phenomena have been thoroughly studied by HPLC only for zirconia surfaces. Although the results below were obtained from studies of native (non coated) zirconia it is important to understand that coating zirconia's surface with polymers or elemental carbon does not form a uniform, impervious layer which completely blocks access to the surface. Thus the ligand exchange sites which are clearly evident in studies of native zirconia (and other metal oxides) are very relevant to understanding the chromatography of the coated materials as encountered in reversed phase liquid chromatography. Some of these ligand exchange sites remain available on the coated phase. Coordinatively bonded water molecules play a key role. These molecules can be exchanged for other Lewis base molecules; the harder the base the easier the exchange. Jaroniec et al. [17] have determined the amount of adsorbed water on metal oxides by TGA (for from ambient temperatures ambient to 350 ^{o}C) this water is , at least in part, available for ligand exchange. The amounts ranged from ~ 20 μmol/m^2 for zirconia to about 30 μmol/m^2 for alumina. However, according to earlier findings of Moterra et al. [62] coordinated water is removed from zirconia below 200 ^{o}C. Thus Jaroniec et al. measured both coordinatively bonded water but also water generated by partial dehydroxylation of the metal oxide surfaces. TGA analysis indicates that the surface concentration of zirconia hydroxyls is 20.2 μmol/m^2 [63]. Jaroniec et al. [17] found 22.1 μmol/m^{2}of adsorbed water on titania surface while Rodrigez et al. [64] found only 3.82 μmol/m^2.

The following chemical equilibria have been used to describe the ligand exchange reactions on metal oxide surfaces [1,58]:

$$M(OH)(H_2O) + L_1^- \leftrightarrow M(H_2O)L_1 + OH^- \quad (1)$$

$$M(OH)(H_2O) + L_1^- \leftrightarrow M(OH)L_1^- + H_2O \quad (2)$$

$$M(OH)(H_2O) + L_2^- \leftrightarrow M(H_2O)L_2 + OH^- \quad (3)$$

$$M(OH)(H_2O) + L_2^- \leftrightarrow M(OH)L_2^- + H_2O \quad (4)$$

$$M(OH)L_1^- + L_2^- \leftrightarrow M(OH)L_2^- + L_1^- \quad (5)$$

Where M represents the metal oxide metal, L_1 and L_2 represent a Lewis base present in the eluent and a Lewis base solute respectively.

Processes (1) and (2) describe the modification of the surface sites, which occurs when a Lewis base is present in the eluent. Processes 3-5 show the exchange of the eluent Lewis base (L_1^-) for a solute base (L_2^-). The contribution of H_2O and OH^- to ligand exchange will depend strongly on pH. At lower pH processes 2, 3, 4 and 5 are possible. At high pH the contribution of processes 1 and 5 to the overall ligand exchange process is likely to be minimal as OH^- ion is a very strong, hard Lewis base. On zirconia the OH^- ion is the strongest, monovalent Lewis base known. It will displace every other simple (monovalent) base. An example of a mixed mode retention showing the behavior of some antihistamines on PBD-ZrO_2 is presented in Figure 5. The shortest retention times are observed for acetate containing mobile phases. Acetate is a weaker Lewis base than fluoride or phosphate. Although fluoride and phosphate block Lewis sites more effectively than acetate, they introduce a larger negative charge on a PBD modified zirconia surface than does acetate and the surface becomes a stronger cation-exchanger. The cation-exchange mechanism becomes the dominant interaction and it results in much longer retention times of the bases.

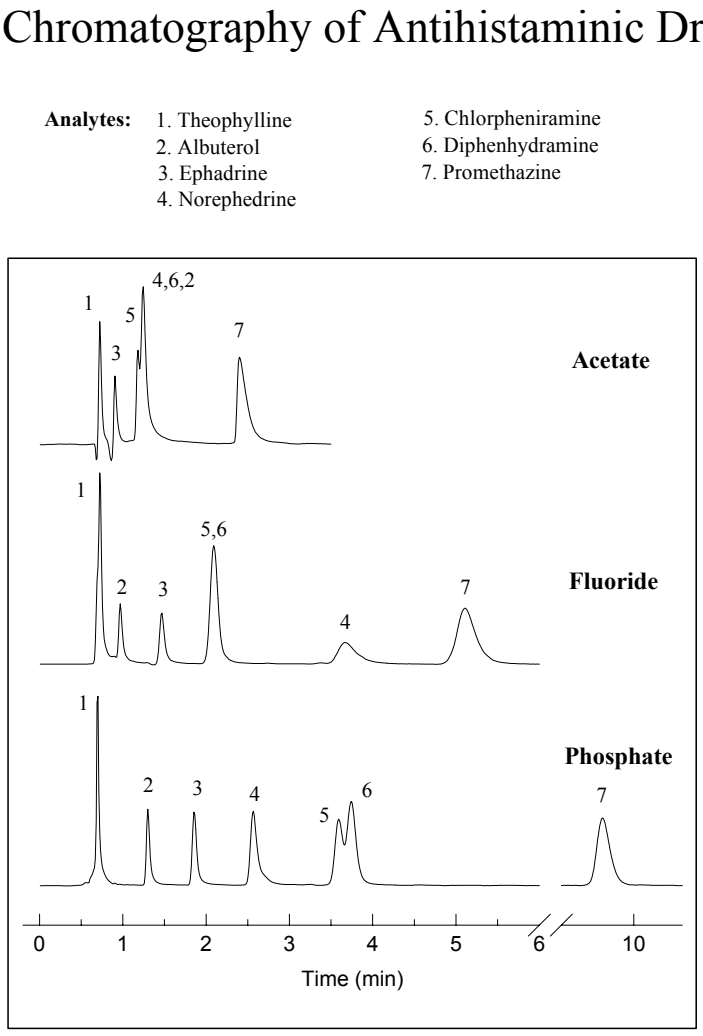


Figure 5. Chromatograms of test basic solute mixture on PBD-ZrO2 in mobile phases containing acetate, fluoride and phosphate, Mobile phase: 30% ACN, 20 mM Lewis base additive (NH_4+), pH 7; 0.8 mL/min; 40 ^oC 1. Ammonia acetate, 2. Ammonia fluoride, 3. Ammonia phosphate dibasic. Solutes: 1. Lidocaine, 2. Norpseudoephedrine, 3. Tryptamine, 4. Quinidine, 5. Amitriptyline, 6. Nortriptyline

Phosphate modified PBD zirconia shows both reversed phase and cation exchange properties even under acidic mobile phase conditions [65]. The choice of Lewis base buffer type and concentration, ionic strength, pH and the nature of the buffer counter-ion can control the interactions. An understanding of the retention mechanisms is necessary to control the chromatography of basic solutes. An example of the influence of ionic strength on retention is shown in Figure 6. An increase in buffer concentration from 20 mM to 100 mM decreased the retention times by more than 50%. [66].

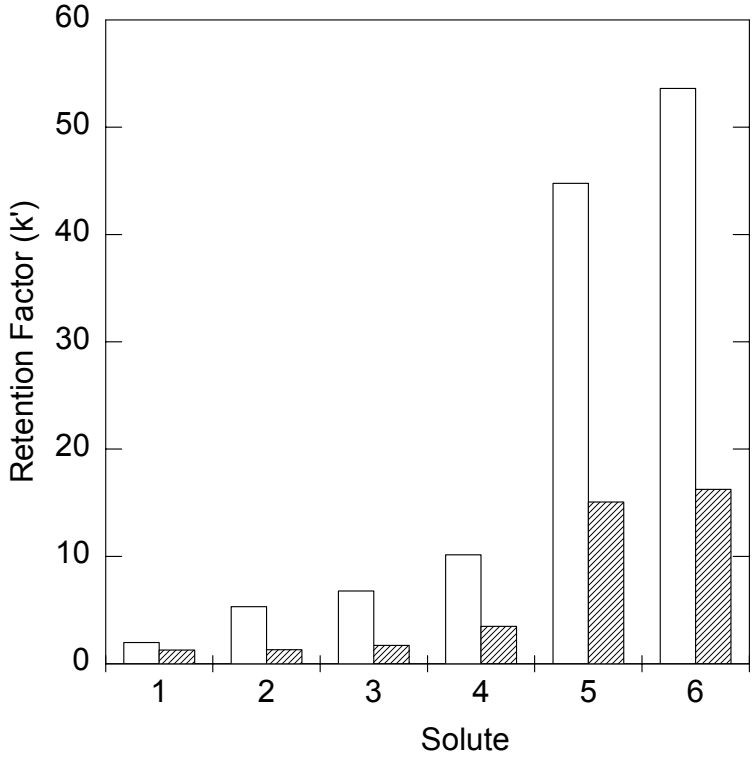


Figure 6. Effect of ionic stregth on the retention factors of test basic solutes on PBD-ZrO_2. Mobile phase: 30%ACN/buffer at pH 7. flow rate: 0.8 ml/min, 40^{o}C, 210 nm, Open bars: 20 mM ammonia phosphate monobasic, Full bars: 100 mM ammonia phosphate monobasic, Solutes: 1. Lidocaine, 2. Norpseudoephedrine, 3. Tryptamine, 4. Quinidine, 5. Amitriptyline, 6. Nortriptyline

pH is another variable that is used to control retention when ion-exchange is the main retention mechanism. This is shown in Figure 7. Amitriptyline and nortriptiline are strongly retained by ion exchange. Increasing the pH to 12 decreased the ion-exchange contribution to retention of the bases. Reversed phase retention becomes dominant and amitriptyline and nortriptyline change elution order.

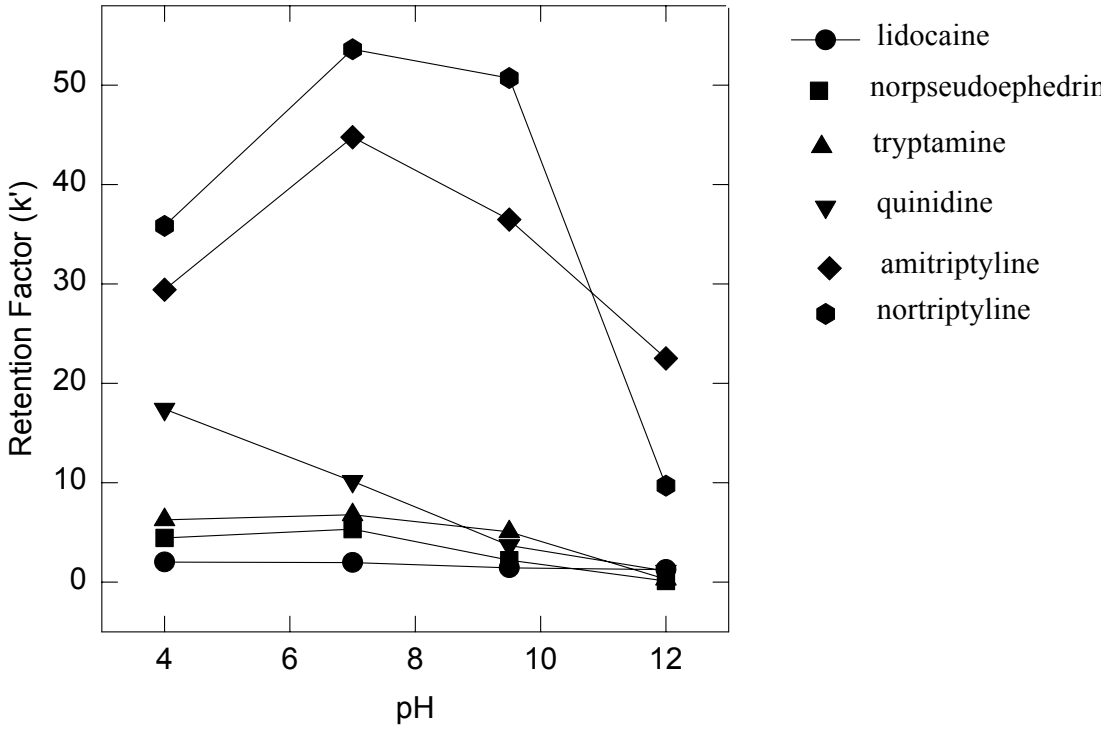


Figure 7. Effect of mobile phase pH on the retention factors of basic solutes on PBD-ZrO_2. Mobile phase: 30%CAN + 20 mM ammonia phosphate buffer adjusted to different pH, except for pH 12 that was obtained with 20mM of sodium phosphate buffer. Flow rate: 0.8 ml/min, 40^{o}C, 210 nm,

4 CONCLUSIONS

The basic thesis of this review is that metal oxide-based stationary phases are not only viable alternatives to silica-based stationary phases but in many instances and for some purposes are superior to them. In this review, we focused on the properties of metal oxides, which are most relevant to their chromatographic performance. In contrast, in our earlier review [1] we centered our interest on the general physical and chemical properties of zirconium dioxide. Since that time a great deal of additional research has appeared. It is now evident that metal oxides especially zirconia allows one to do a number of forms of HPLC at both extremely low and extremely high pH under conditions where silica-based phases are utterly unstable. In addition, metal oxide-based phases can be used at temperatures well above those which can be tolerated by silica-based phases due to the dissolution of the silica in aqueous media or to the loss of silane bonded phases. In contradistinction to stationary phases based on synthetic organic

polymers these significant improvements in chromatographically relevant properties are not obtained at the price of decreased efficiency (plate count) or increases in column operating back pressure or slow response to changes in solvent due to local or global shrinking or swelling.

REFERENCES

[1] J. Nawrocki, M.P.Rigney, A.McCormick, P.W.Carr, J.Chromatogr. **657,** 229 (1993).
[2] J. Nawrocki, C.J.Dunlap, P.W.Carr, J. A.Blackwell, Biotechnology Progress **10,** 561 (1994).
[3] E. Forgács, T. Cserháti, Adv. Chromatogr. **40**, 359 (2000).
[4] J. J.Pesek, M.T.Matyska, J.Chromatogr. **A 952**, 1 (2002).
[5] C.J.Dunlap, C.V.McNeff, D.Stoll, P.W.Carr, Anal. Chem. **73(21)**, A598 (2001).
[6] A.Wehrli, J.C.Hildebrand, H.P.Keller, R.Stampfli, R.Frei, J.Chromatogr. **149**, 199 (1987).
[7] L.R.Snyder, J.L.Glajch, J.J.Kirkland, "Practical HPLC Method Development", Wiley, New York, 1997.
[8] J. Li and F. F. Cantwell, J Chromatogr. **A 726**, 37-44 (1996).
[9] B. Ells, Y. Wang and F.F. Cantwell, J Chromatogr. **A 835**, 3-18 (1999).
[10] Y. Akama, H. Kanno, Anal. Chim. Acta **309**, 153 (1995).
[11] Y. Akama, Talanta **42**, 1943 (1995).
[12] K.K.Unger, Porous Silica, Elsevier, 1979.
[13] R.P.W.Scott, *Silica Gel and Bonded Phases*, John Wiley & Sons Ltd, New York 1993.
[14] J.Nawrocki, Chromatographia **31,** 177 (1991).
[15] J.Nawrocki, Chromatographia **31,** 193 (1991).
[16] J.Nawrocki J.Chromatogr. **A 779,** 29 (1997).
[17] C.P.Jaroniec, M.Jaroniec, M.Kruk, J.Chromatogr. **A 797,** 93 (1998).
[18] M.Abboud, M.Turner, E.Duguet, M.Fontanille, J.Mater. Chem. **7(8),** 1527 (1997).
[19] J.Winkler and S.Marmé, J.Chromatogr. **A 888**, 51 (2000).
[20] K.Miyabe, G.Guiochon, J.Chromatogr. **A 903,** 1 (2000).
[21] D.V. McCalley**,** J. Chromatogr. **A**, **902**, 311 (2000).
[22] J. J. Kirkland, J.L. Glajch, J. Kohler; J. Chromatogr. **384,** 81 (1987).
[23] H. Engelhardt,; G. Ahr, Chromatographia, **14,** 227 (1981).
[24] U. D.Neue, In *Encyclopedia of Analytical Chemistry*; R. A.Meyers, Ed.; John Wiley and Sons: New York, 2001.
[25] L. C.Sander, S. A.Wise, *Critical Reviews in Analytical Chemistry*, **18**, 299 (1987).
[26] K. K.Unger, *Packings and Stationary Phases in Chromatographic Techniques*; M. Dekker: New York, 1989.
[27] J. J. Kirkland, J.L. Glajch, R. D.Farlee, Anal. Chem. **61**, 2 **(**1989).
[28] L. R.Snyder, J. L. Glajch, J. J. Kirkland, *Practical HPLC Method Development*, 2nd ed.; Wiley-Interscience: New York, 1996.
[29] A. Berthod,.J. Chromatogr., ***549***, 1 (1991)
[30] J. J.Kirkland, M. A. Straten, H. A. Claessens, J. Chromatog. **691**, 3 (1995).
[31] H. A. Claessens, M. A. Straten, J. J.Kirkland, J. Chromatog. **A 728**, 259 (1996).
[32] C.McNeff, L.Zigan, K.Johnson, P.W.Carr, A.M.Weber-Main, LC-GC **18(5)**, 515 (2000).
[33] A. Méndez, E. Bosch, M. Rosés, U.D. Neue, J Chromatogr. **A 986**, 33 (2003).
[34] D.E.Yates, S.Levine, T.W.Healey, J.Chem.Soc. Faraday Trans.I **70**, 1807 (1974).

[35] T.Hiemstra, W.H.Van Riemsdijk, G.H.Bolt, J.Colloid Interface Sci. **133(1)**, 91 (1989).
[36] T.Hiemstra, J.C.M. De Wit, W.H.Van Riemsdijk, J.Colloid Interface Sci. **133(1)**, 105 (1989).
[37] N.J.Barrow,J.W.Bowden, J.Colloid Interface Sci. **119(1)**, 236 (1987).
[38] C.Stella, S.Rudaz, J.-L.Veuthey, A.Tchapla, Chromatographia **53** (Supl.) S-113 (2001).
[39] C.Stella, S.Rudaz, J.-L.Veuthey, A.Tchapla, Chromatographia **53** (Supl.) S-132 (2001).
[40] M.P.Rigney PhD Thesis, University of Minneapolis, 1988.
[41] M.P.Rigney, E.F.Funkenbush, P.W.Carr, J.Chromatogr. **499**, 291 (1990).
[42] U.Trüdinger, G.Müller, K.K.Unger, J.Chromatogr. **535,** 111 (1990).
[43] M.Grün, A.A. Kurganov, S.Schacht, F.Schüth, K.K.Unger, J.Chromatogr. **A 740,** 1 (1996).
[44] A.Kurganov, U.Trüdinger, T.Isaeva, K.K.Unger, Chromatographia **42(3/4),** 217 (1996).
[45] H. Richardson, B. A. Bidlingmeyer, J. Pharm. Sci. **73(10)**, 1480, (1984)
[46] B. A Bidlingmeyer. J.K Del Rios, J.Korpi, Anal. Chem. **54(3)**, 442-7 (1982).
[47] G.L.Schmitt, D.J.Pietrzyk, Anal. Chem. **57**, 2247 (1985).
[48] K.Tani, H.Kubojima, Chromatographia **47(11/12),** 655 (1998).
[49] W.Szczepaniak, H.Kościelna, Anal. Chim. Acta **470**, 263 (2002).
[50] C.Laurent, H.A.H.Billiet, L. de Galan, Chromatographia **17(5)**, 253 (1983).
[51] C.Laurent, H.A.H.Billiet, L. de Galan, Chromatographia **17(7)**, 394 (1983).
[52] S.C.Churms, J.Sud.Afr. Chem.Inst. **19**, 98 (1966).
[53] S.-Y.Shio, R.E.Meyer, J.Inorg.Nucl. Chem. **43(12)**, 3301 (1981).
[54] M.Lederer, C.Polcaro, J.Chromatogr. **84**, 379 (1973).
[55] C.J.C.M.Laurent, H.A.H.Billiet, L. De Galan, J.Chromatogr. **285**, 161 (1984).
[56] C.J.C.M.Laurent, H.A.H.Billiet, L. De Galan, J.Chromatogr. **287**, 45 (1984).
[57] J.C.Yu, F.Qu, J.Lin, HL. Lam, ZL.Chen, J.Liq.Chromatogr. Relat. Technol. **24(3)**, 367 (2001).
[58] J.Blackwell, P.W.Carr, J.Chromatogr. **549**, 59 (1991).
[59] J.J.Pesek, M.T.Matyska, J.Ramakrishnan, Chromatographia **44(9/10)** (1997) 538.
[60] Guo-Xun Xu, Aqua (London) **43(2)**, 58 (1994).
[61] Surendra K.V. Nath, S.N.Tandon, J.Liq.Chromatogr. **11(7)**, 1433 (1988).
[62] V.Bolis, C.Morterra, M.Volante, L.Orio, B.Fubini, Langmuir **6**, 695 (1990).
[63] J.Nawrocki, P.W.Carr, M.J.Annen, S.Froelicher, Anal. Chim. Acta **327**, 261 (1996).
[64] R.Rodrígez, M.A.Blesa, A.E.Regazzoni, J.Colloid Interface Sci. **177**, 122 (1996).
[65] L.Sun, P.W.Carr, Anal. Chem. **67** (1995) 2517.
[66] Y. Hu, X. Yang and P.W. Carr, J. Chromatogr. **A 968,** 17-29 (2002).