Review

Part I. Chromatography using ultra-stable metal oxide-based stationary phases for HPLC

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Abstract

The first part of the review 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. Methods of preparation of spherical particles for HPLC are described. 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.
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1. Introduction

Some years ago, we published two reviews on the chemistry of zirconia and its use in chromatography [1,2]. Since quite a number of years have passed and many new applications of zirconia, titania and alumina have appeared, the goal of the present work is to summarize recent achievements in HPLC with metal oxide substrates, that is, non-silica-based inorganic phases. 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].

It must be pointed out that our enthusiasm for the use of zirconia as a stationary phase in HPLC is not shared by all. Buchmeiser [6] stated that chromatography on zirconia-based packings is *only* of academic interest because zirconia's surface chemistry and indeed that of any metal oxide differs from silica's. Among other things, the present review hopes to show that the surface of metal oxides can be very effectively used for HPLC. Furthermore, 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/m), which in contrast to polymeric materials is similar to the efficiency of silica. However, zirconia-based phases share the advantage of polymer based supports in that they are vastly superior to silica in terms of their chemical and thermal stability.

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 °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 [7]. Second, the siloxane bond is unstable at acidic pH and becomes increasingly less stable as the pH is lowered. Increase of temperature also leads to gradual loss of the bonded phase [35,38,226]. 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 [8]. 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 [9,10].

According to Schomburg [11] and Unger and Trüdinger [12] the following characteristics define an ideal HPLC stationary phase:

- Readily variable phase ratio, i.e. retentivity and sample capacity.
- Long-term chemical stability against highly aggressive mobile phases (e.g. with very low or very high pH).
- Mechanical strength to withstand high pressure operation and packing.
- The particles should have a narrow size distribution and high surface area. The pore structure should be open (showing an H1 type hysteresis loop in the BET curve); the pores should have no or few constraints and have a mean pore diameter between 6 and 50 nm [13]. Modern HPLC packings must be spherical to provide low pressure drop and mechanically stable beds.
- The pores must have a diameter appropriate to the analyte's molecular diameter and good connectivity to allow for fast mass transfer.
- The support material should resist thermal and chemical degradation.
- The support material should have a surface that is both energetically homogeneous, yet chemically modifiable.
- Exhibit minimal swelling in case of polymeric stationary phases or polymeric coatings.

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 [14,15]. 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 [16–20] but metal oxides are, by and large, crystalline: alumina is used as the α or γ (and also in amorphous form) [21]; zirconia is monoclinic and titania is in the anatase form [22,23]. 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.

This paper is devoted to the chromatographic properties of new materials based mainly on zirconia, alumina and titania as well as some mixed oxides. This requires a description of the surfaces of the oxides. As the surface chemistry of native zirconia was described in detail in our previous review [1] we will pay less attention to it here. From a chemical point of view, all the oxide surfaces contain hydroxyl groups. However, the amount and properties of the hydroxyls depend very much on the metal oxide. The structure of a hydroxylated silica surface is very different from that of titania, zirconia and alumina. In stark contrast to silica, the common feature of the metal oxide phases is their ability to act as both *ion- and ligand exchangers*. Finally, a major purpose of this review is to describe the actual chromatographic performance of packings based on these oxides.

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 [24]. 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 concomitantly 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 [20]. Similarly there have been advances in improving the low pH stability [36,37].

2.1. Silica-based organosilane-bonded phases—the attempts to increase stability at extreme pHs

Microparticulate silica is, by far, the most widely used substrate for HPLC stationary phases [25]. Silica is an attractive support for many reasons. First, the particles are monodisperse, mechanically strong, and they have high surface area and easily tailored pore size distributions. Second, the availability of a wide variety of silanes allows for covalent surface modification thus offering a diverse suite of chromatographic selectivities enabling reversed, normal, ion exchange and size exclusion. Several excellent books and reviews are available that provide comprehensive treatment

of the chemistry of silica, silica particle synthesis and stationary phase synthesis [6,16,18–20,25–33].

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 [28,32–34]. 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 [35].

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 [20,36–38]. 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 [20,25,38–40]. 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 ≥ 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 3–9. 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 [38]. 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 pK_a is between 3 and 5 on one specific high purity silica [224]. 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 [38]. Mobile phase pH is such a powerful variable for optimizing reversed-phase separations [20,35] 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 pH below

8. Fundamentally the lifetime of the column at any pH is ultimately limited by these stability issues.

2.1.1. Enhancements in chemical stability at low pH

Improvements in the chemical stability of silica-based bonded phases at low pH were first achieved by increasing the size and hydrophobicity of the organosilane. Studies show that a C_{18} bonded phase is significantly more resistant to acid catalyzed hydrolysis compared to C_1, C_4, or C_8 bonded phases [37]. The increase in stability is attributed to better shielding of the siloxane bond by the larger alkyl portion of the organosilane. Thus, the typical trimethysilyl "endcapping" group used to block access to surface silanols is particularly unstable at low pH and phases made for use at lower pHs are not endcapped.

Increasing the number of covalent attachments or strong physical attractive interactions between organosilanes and the silica surface or otherwise increasing the connectivity of a oligomeric silane polymer to the surface also enhances low pH stability. By increasing the number of covalent bonds between the organosilane and the silica surface, a multi-dentate phase is formed. For multi-dentate phases to loose the stationary phase acid catalyzed hydrolysis and subsequent loss of the stationary phase would require multiple covalent bonds to break simultaneously. Multiple bonding decreases the probability of phase loss compared to singly bonded phases. Three approaches for synthesizing multi-dentate phases are discussed below.

Both bifunctional (CH_3RSiX_2) and trifunctional ($RSiX_3$) organosilanes (X = Cl, OR) have been used to bond stationary phases to silica [32,38]. Both reagents bond the organosilane to the silica through two covalent bonds. It is likely that some polymer forms in solution which then attaches to the surface. Although this approach enhances resistance to acid catalyzed phase loss, it is not as reproducible as the bonding of monofunctional organosilanes and residual silanols are generated when the reactive organosilane polymerizes on the surface [32]. Polymeric bonded phases used to show inferior mass transfer kinetics compared to an equivalent, monomerically bonded stationary phase [28]. Today, the efficiency of polymeric phases is actually similar to that of monomeric phases [38]. It is, however, necessary to add here that modern bonded phases based on B class silica are exclusively monomeric (see e.g. [225]).

Kirkland et al. developed a different multi-dentate synthetic approach for improving the low pH stability of bonded phases [37]. A doubly attached or bidentate C_{18}/C_{18} silane-bonded phase shows dramatically increased resistance to acid catalyzed phase loss compared to a conventional C_{18} bonded phase [37]. Unlike polymeric bonded phases, this type of bonding is highly reproducible and the resulting phase has mass transfer very similar to that of a conventional C_{18} bonded phase [41].

Another type of multi-dentate approach for enhancing the chemical stability of bonded phases at low pH was developed by Wirth and co-workers [42,43–45]. Under scrupulously

dry solvent conditions, an octadecyltrichlorosilane and typically a methyltricholorsilane are jointly "self-assembled" on a carefully humidified silica surface [46]. This approach gives an exceedingly dense bonded layer of alkyl chains (nearly $8\,\mu mol/m^2$ as compared to $2.5–3.5\,\mu mol/m^2$ for dimethyl octadecyl silanes) on the silica. Solid state ^{29}Si cross polarization magic angle spinning NMR shows siloxane bonding between the self-assembled moieties in the horizontally polymerized layer and the formation of siloxane bonds between the horizontally polymerized layer and the silica surface [47]. It is claimed that C_{18}/C_1 mixed horizontally polymerized stationary phase have excellent chemical stability at low pH [42]; however, the details of the column aging and comparisons with conventional-bonded phases have not been published.

To date the most successful synthetic approach for enhancing the low pH stability of silica-based bonded phases uses "sterically protected" silanes to derivatize the silica surface. This approach was developed and patented by Glajch and co-workers [37,48,49]. The sterically protected silanes consist of an alkyl (C_3, C_8 or C_{18}) or an aromatic moiety, two, bulky isopropyl or isobutyl groups, and a reactive moiety (chlorine) bonded to a silicon atom. Sterically protected silane (C_3, C_8, C_{18}, propylphenyl) phases are very stable under aggressive, low pH (0.1% trifluoroacetic acid (TFA), $T = 90\,°C$, pH 0.9) mobile phase conditions [37,38,48,49]. Dynamic acid aging tests indicate that the two sterically bulky isopropyl or isobutyl groups are the most effective side groups for shielding the siloxane bond from acid catalyzed hydrolysis [37]. The commercially available sterically protected phases are routinely operable and stable at pH 1 for thousands of column volumes at room temperature and even above ambient temperature [50].

2.1.2. Enhancements in chemical stability at high pH

Synthetic efforts for enhancing the chemical stability of silica-based bonded phases at high pH focus primarily on increasing the coverage or shielding of the underlying silica. Steric hindrance limits the surface density of bonded organosilanes to approximately $2–4\,\mu mol/m^2$ [25,32,38]. This leaves at least 50% of the silanol groups on the surface unreacted and thus a significant fraction of the silica surface is insufficiently shielded. Simply increasing the length of the alkyl chain on the organosilane leads to an increase in high pH stability presumably by improving the shielding of the underlying silica [38]. However, this approach alone still leaves a significant portion of the silica surface underivatized or lightly derivatized; therefore, further shielding of these areas is needed to improve the overall chemical stability at high pH.

Many procedures have been developed to chemically treat the residual silanols on organosilane-bonded phases and improve the shielding of silica surfaces. All of these procedures are described as "endcapping" reactions. Trimethylchlorosilane and hexamethyldisilazane have been used extensively to endcap organosilane-bonded phases [32,33,38]. The smaller size of these silanes allows them to react with silanols that are sterically inaccessible to the larger bonded organosilanes. These procedures give noticeable decreases in silanol activity and dramatic improvements in base stability. "Double endcapping" procedures have also been developed to further reduce the population of residual silanols on organosilane-bonded phases and improve the shielding of the underlying silica [39–41,51,52]. Due to the proprietary nature of these methods, the chemical details of the more effective endcapping procedures are not known. The benefit of double endcapping is discussed below. Additionally, Sudo and co-workers developed a unique endcapping method. They have shown that endcapping of a polymeric C_{18} phase with hexamethylcyclotrisiloxane at temperatures above $350\,°C$ gives a very inert, stable stationary phase [53–55]. Although many endcapping reactions provide a measurable decrease in silanol activity and an increase in base stability, steric hindrance prevents complete shielding of the silica surface.

Several bonded phase synthesis methods combine a unique initial silanization step with the benefits of double endcapping to improve the chemical stability of silica-based phases at high pH. Kirkland et al. synthesized an "extra densely" C_{18}-bonded phase that was double endcapped to help further maximize the shielding of the silica surface [52]. The stability of this phase was superior to a conventional C_{18} bonded phase at pH 11 [52]. Once again, the exact synthetic details of these reactions are not known. Kirkland and co-workers also combined bidentate C_{18}/C_{18} silane bonding with a double endcapping procedure [41,51,52,56,57]. The C_{18}/C_{18} bidentate silane, doubly encapped stationary phase shows greatly enhanced stability compared to a conventional RPLC stationary phase under aggressive high pH conditions (20 mM K_3PO_4, $T = 25\,°C$, pH 11) [41]. This phase shows less silica dissolution and greatly enhanced plate height stability compared to the extra densely bonded, doubly endcapped C_{18} stationary phase [41]. The commercially available bidentate C_{18}/C_{18} bonded phase is given an operating pH range of 2–12 in "non-aggressive" buffers (specifically organic buffers are recommended for use at high pH but phosphate and carbonate buffers should be avoided) by the manufacturer [50]. Independent comparisons of this phase with several commercially available competitors shows that the combination of the bidentate silane bonding to the surface and a double endcapping procedure provides far superior shielding of the underlying silica compared to conventional silica-based bonded phases [58].

A different synthetic approach for enhancing the chemical stability of silica-based bonded phases at high pH was first described by Unger et al. in 1976 [59]. This method reacts an inorganic alkoxysilane with an organic alkoxysilane to form an inorganic/organic hybrid particle [60]. The organic moiety is present in the internal structure and at the surface of the particle. The organic groups on the surface lower the amount of underivatized, unshielded silica before

any silanization chemistry is performed, thus lowering the rate of silica dissolution [60]. The particles contain approximately 66% of the silanol groups present on traditional silica; therefore, typical silanization methods can be used to covalently bond a C_8 or C_{18} chain. A commercial embodiment of this type of phase uses a trifunctional alkylsilane to derivatize the inorganic/organic hybrid. Technical literature shows that the C_{18} derivatized inorganic/organic hybrid stationary phases are more stable at pH 10 compared to several competitor C_{18} bonded phases [60]. The manufacturer states that the phase is stable from pH 2 to 12 [60].

However, the ability of a column to withstand exposure to high pH is not the same as being stable over a long time at high pH. Chromatographic columns are expensive and users are interested in columns, which are stable for months not for just a few days. Frequent system suitablity testing and recalibration of systems when failed columns are replaced adds to the base cost of column mortality. As shown below even silica-based columns specifically designed for use at high pH can only withstand high pH over a limited time typically only 3–6 days of continuous use. There are also other conditions which must be met for the long-term use of silica-based phases at high pH: inorganic buffers (particularly phosphate and carbonate) and temperatures higher than 60–80 °C must be specifically eschewed [36,51]. According to a very recent review [61,62], silica bonded phases are more stable at high pH when organic-rich phases are used and not pure water; this is probably due to the lower solubility of silica in the less polar media.

2.2. 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 [63]:

- 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.

The advantages are summarized in Fig. 1.

The thermal and chemical stability of the base oxide must be considered first. Silica is generally stable up to ~200 °C. At higher temperatures the surface silanols start to condense and the chemical properties of the surface change. Below 400 °C the changes are reversible and it is relatively easy to recover the initial properties of the support. The thermal stability of zirconia is very high. Zirconia has a very high melting point (2750 °C). The post-synthetic treatment of the porous zirconia particles developed in this lab involves sintering at 950 °C for several hours. This treatment makes the
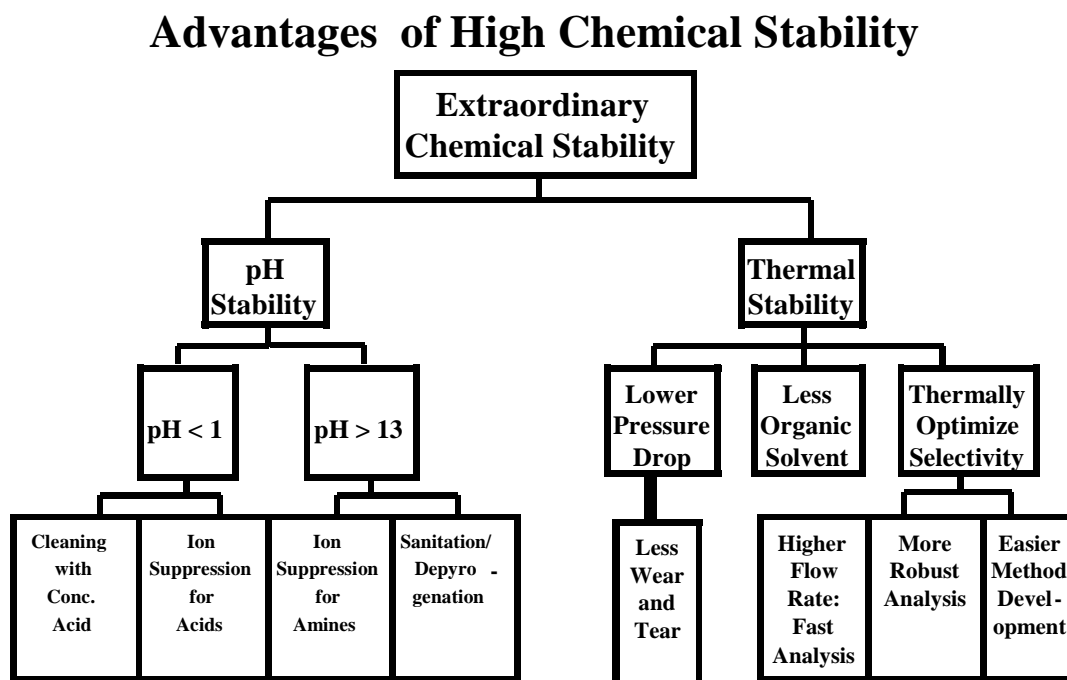

Fig. 1. Advantages of high chemical stability of chromatographic supports.

particles mechanically stable. Zirconia can be coated with a thin film of elemental carbon by heating it to 700–800 °C [64] which in the presence of hydrocarbon vapors leads to their cracking and coke formation [65]. Due to the high sintering temperature of zirconia the pore structure is virtually unchanged by subsequent heating and deposition of the thin carbon film.

It has been shown that metal oxides have much higher thermal stability and can easily be used at temperatures of ~200 °C [66–68]. Thermal stability is highly advantageous since chromatography at higher temperature means that the pressure drop will be lower, or higher flow rates can be used to achieve three to five-fold faster analysis [69–71] and one can adjust the selectivity (i.e. the band spacing) by simultaneously tuning temperature and composition or gradient parameters [72,73].

Alumina, titania and zirconia have been used in chromatography for many years. However, the literature shows that zirconia has been the most systematically studied oxide. Zirconia appears to be thermally and chemically the most stable metal oxide. It is used extensively for capturing and storing radiochemical wastes. Alumina is definitely more soluble in acid especially hydrochloric acid while the stability of chromatographic grades of titania has not been examined experimentally. There are papers on the synthesis of chromatographically useful zirconia particles, numerous attempts to modify its surface and chromatographic applications. The stability of zirconia and alumina have been compared under extreme conditions [1,74,75]. Zirconia is definitely more stable than alumina at both low and high pH. Zirconia is absolutely stable in the pH ranging from 1 to 14 as demonstrated previously [1]. Grün et al. [76] stated that titania is stable from pH 1 to 14 but the statement is not supported by hard data on chromatographic grade titania.

The mechanisms of retention on zirconia packings are relatively well understood. Alumina has been used for chromatography for decades especially for normal phase work but mainly in the form of poorly defined particles having various degrees of crystallinity, surface area and porosity. Alumina has been used extensively for purification of samples and for thin layer chromatography. The chemistry of alumina has been extensively examined. Alumina is available in a form suitable for HPLC. Despite alumina's decades long use in chromatography there has been little in the way of systematic study especially of reversed-phase aluminas that could enable their future use. Titania has been investigated for about 10 years but it has only recently become commercially available in the form of spherical particles for HPLC [23,77].

2.2.1. Stability of metal oxide phases versus silica-based phases

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.

Table 1
Comparison of silica and metal oxide-based packings

Property	SiO_2	TiO_2	Al_2O_3	ZrO_2
Monodispersity[a]	++	++	++	++
Pore structure[b]	++	?	?	++
Surface area/pore diameter[c]	++++	++	+++	++
Controllable surface chemistry[d]	++++	?	++	++
Mechanical strength[e]	++	?	?	+++
Chemical stability[f]	–	++ (?)	+++	++++
Thermal stability[g]	–	?	?	+++
Column efficiency[h]	+++	?	++	+++
Energetic homogeneity[i]	++	+	+	+

[a] Spherical micron sized particles available for all the oxides.

[b] Silica and zirconia have well connected pores, not much has been published on the porosity of spherical titania and alumina.

[c] Wider range available for silica.

[d] Silica by silane chemistry, metal oxides by polymer deposition, zirconia–carbon deposition, not much is known on titania's surface chemistry.

[e] Silica and zirconia can tolerate >10,000 psi.

[f] 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.

[g] Silica: not higher than 60–70 °C, zirconia up to 200 °C, lack of temperature stability of alumina and titania.

[h] Silica and zirconia comparable, alumina slightly lower, no studies on titania.

[i] Silica has better homogeneity of the surface (from an HPLC point of view) than metal oxides, all metal oxides have comparable surface chemistry.

The practicing chromatographer must choose an HPLC column from those available on the market. Thus, the chromatographic practitioner needs a real comparison of commercially available products. Table 2 shows a comparison of eight stable HPLC columns.

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.

Figs. 2–4 compare the stabilities of three 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. Long-term stabilities of a number of commercial "high pH stable" columns are compared in Fig. 5 at the maximum specified operating pH for a number of silica phases.

Most silica-based columns are thought to be stable at pH 7 even in the presence of phosphate. The data shown in Fig. 6 were obtained in an accelerated aging study by holding the columns at 80 °C [5]. The test clearly shows

Table 2
Commercially available high pH "stable" columns for RPLC

Manufacturer	ZirChrom®	ZirChrom®	Polymer Labs	Biotage	ES Industries	Phenomenex	Waters	Zorbax
Column name	DiamondBondC18	ZirChrom®-PBD	PLRP-S	Unisphere™-PBD	GammaBond RP-1	Luna	Xterra	Extend
Support	zirconia	Zirconia	Polymer	Alumina	Alumina	Silica	Silica	Silica
Particle size (μm)	3	3	5	10	5	3	3.5	3.5
Pore size (Å)	300	300	100	220	80	100	130	80
Dimensions (mm)	50 × 4.6	150 × 4.6	150 × 4.6	250 × 4.6	150 × 4.6	150 × 4.6	150 × 4.6	150 × 4.6
Price (US$)	675.00	595.00	480.00	NA	695.00	435.00	425.00	540.00
Low pH	1	1	1	2	1.3	1.5	1	2
High pH	14	13	14	13	12	10	12	11.5
High temperature limit (°C)	200	150	150	NA	NA	NA	80	60
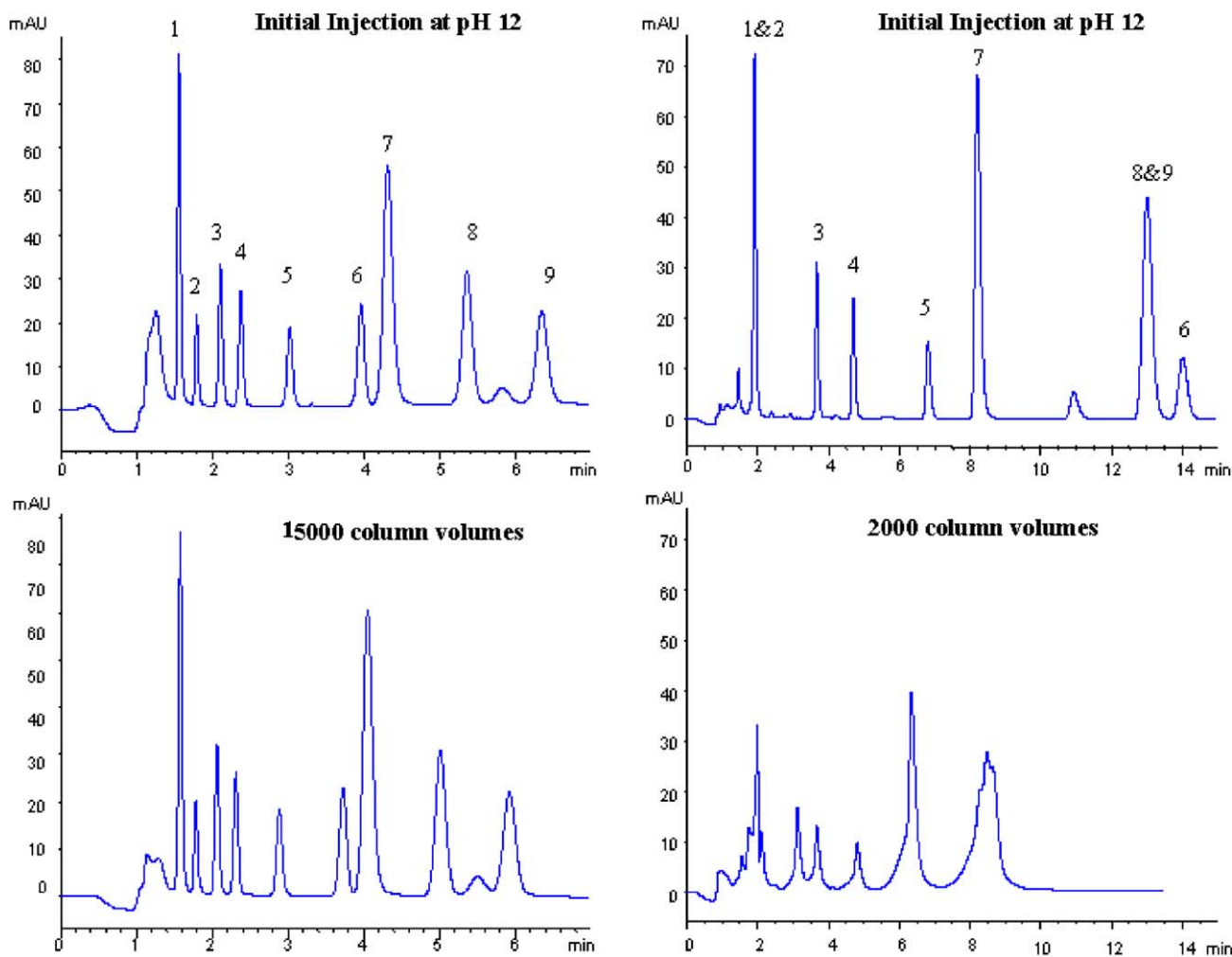
LC Conditions:
ZirChrom®-PBD; Mobile Phase, 28/72 acetonitrile/20 mM potassium phosphate at pH=12.0; Flow Rate, 1.0 mL/min.; Temperature, 30°C; Detection, 254 nm.
Solutes: 1=Labetolol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

LC Conditions:
Waters Xterra™ RP18; Mobile Phase, 35/65 acetonitrile/20 mM potassium phosphate at pH=12.0; Flow Rate, 1.0 mL/min.; Temperature, 30°C; Detection, 254 nm.
Solutes: 1=Labetolol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

Fig. 2. High pH stability of PBD–zirconia vs. Waters XTerra RP 18 column. *LC conditions*: ZirChrom®-PBD; mobile phase, 28/72 ACN/20 mM potassium phosphate at pH 12; Zorbax-EXTEND; mobile phase, 35/65 ACN/20 mM potassium phosphate at pH 12; flow rate, 1.0 ml/min; temperature, 40 °C; detection at 254 nm. *Solutes*: (1) labetolol; (2) atenolol; (3) acebutolol; (4) metoprolol; (5) oxprenolol; (6) quinidine; (7) lidocaine; (8) alprenolol; (9) propranolol.

Column Life Comparison for ß-Blockers

ZirChrom-PBD

Initial Injection at pH 11.5

15000 Column Volumes

Zorbax-EXTEND

Initial Injection at pH 11.5

2500 ColumnVolumes

LC Conditions:
ZirChrom-PBD; Mobile Phase, 2872 ACN/ 20mM
potassium phosphate at pH 11.5; Flow Rate, 1.0ml/min.;
Temperature, 40°C; Detection at 254nm.
Solutes: 1=Labetolol, 2=Atenolol, 3=Acebutolol,
4=Metoprolol, 5=Oxprenolol, 6=Quinidine, 7=Lidocaine,
8=Alprenolol, 9=Propranolol

LC Conditions:
Zorbax-EXTEND; Mobile Phase, 4555 ACN/ 20mM
potassium phosphate at pH 11.5; Flow Rate, 1.0ml/min.;
Temperature, 40°C; Detection at 254nm.
Solutes: 1=Labetolol, 2=Atenolol, 3=Acebutolol,
4=Metoprolol, 5=Oxprenolol, 6=Quinidine, 7=Lidocaine,
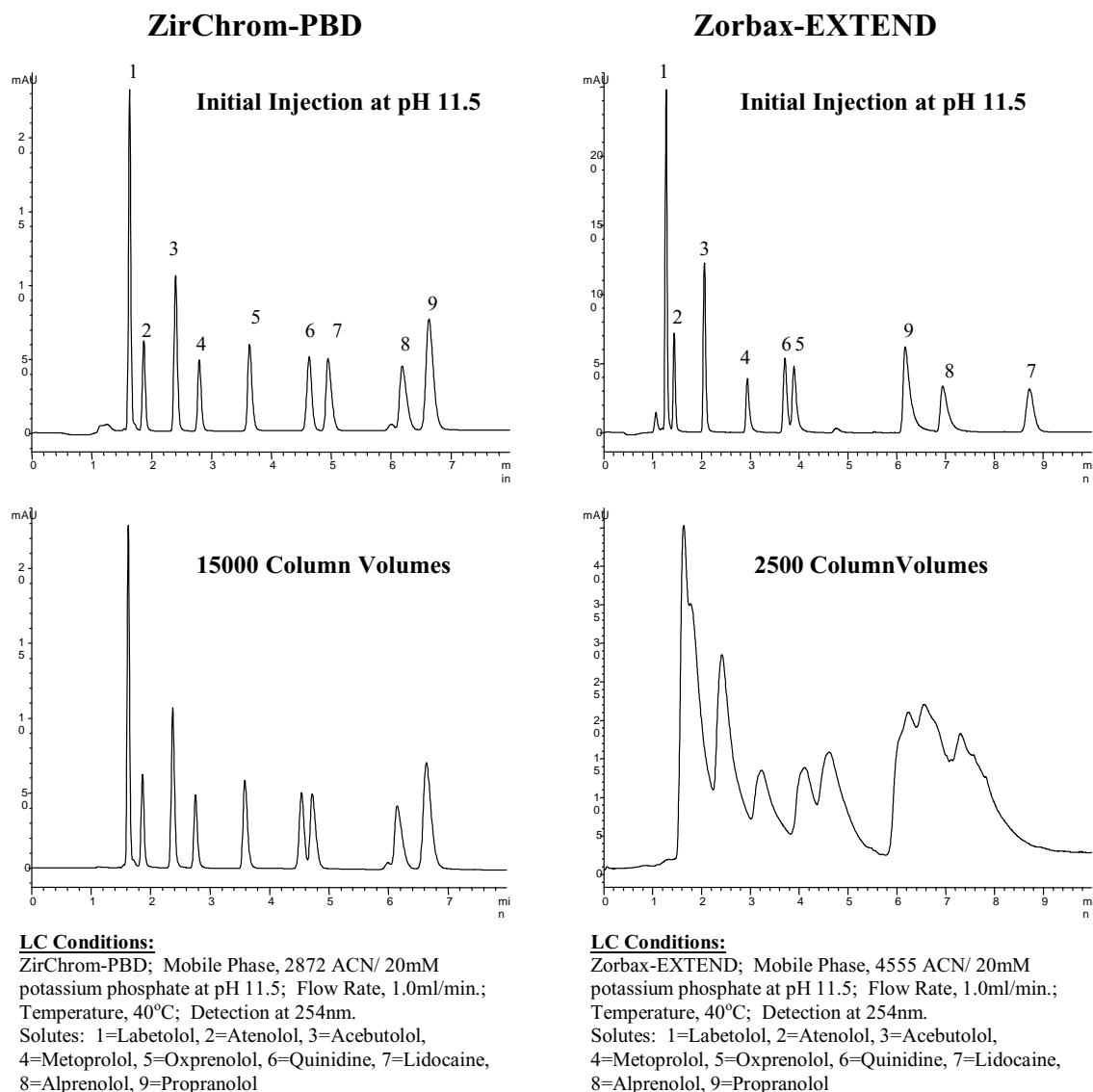8=Alprenolol, 9=Propranolol

Fig. 3. High pH stability of PBD–zirconia vs. Zorbax Extend column. *LC conditions*: ZirChrom®-PBD; mobile phase, 28/72 ACN/20 mM potassium phosphate at pH 11.5; Zorbax-EXTEND; mobile phase, 45/55 ACN/20 mM potassium phosphate at pH 11.5; flow rate, 1.0 ml/min; temperature, 40 °C; detection at 254 nm. *Solutes*: (1) labetolol; (2) atenolol; (3) acebutolol; (4) metoprolol; (5) oxprenolol; (6) quinidine; (7) lidocaine; (8) alprenolol; (9) propranolol.

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.

3. Surfaces properties of ZrO₂, TiO₂ and Al₂O₃

3.1. Zirconia

The surface properties and chromatographic uses of zirconia have been extensively described in two reviews

[1,2]. Most chromatographic applications of zirconia have been done on crystalline (monoclinic) material. However, in one of the earliest papers Trüdinger et al. [13] examined amorphous zirconia. Their material was calcined below 400 °C to avoid the transition from the amorphous to the tetragonal phase. Thus, their zirconia was highly microporous and of relatively high surface area [1,13]. However, the reproducibility of their synthesis method and of the physical properties of the zirconia obtained from it has not been confirmed. Yu and El Rassi [78–80] repeated the procedure of Trüdinger et al. [13] then calcined the particles at 800 °C (6 h) and obtained a nonporous zirconia.
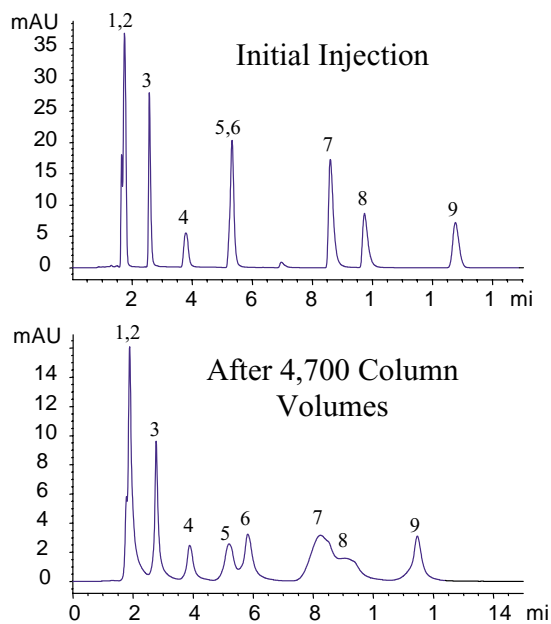
Fig. 4. Stability test of Phenomenex Luna column at pH 10. *LC conditions*: Phenomenex Luna; mobile phase, 45/55 acetonitrile/20 mM potassium phosphate at pH 10.0; flow rate, 1.0 ml/min; temperature, 30 °C; detection at 254 nm. *Solutes*: (1) labetolol; (2) atenolol; (3) acebutolol; (4) metoprolol; (5) oxprenolol; (6) quinidine; (7) lidocaine; (8) alprenolol; (9) propranolol.

Due to lack of commercial chromatographic grade zirconia only laboratory made particles were used in the experiments cited in this review. This is shown in Table 3.

A variety of zirconia-based stationary phases is now available from ZirChrom Separations, Inc. (Anoka, MN, USA). The packings are listed in Table 4.

3.2. Alumina

Alumina is mostly known for its catalytic activity [84]. It can exist in amorphous and a variety of crystalline forms. Several distinct phases have been recognized (γ-, δ-, κ-, η-, θ- and χ-alumina). The phases differ mainly in the amount of water bound to the surface. Anhydrous alumina is designated as α-alumina [85]. Peri [86–88] carried out fundamental studies on surface chemistry of γ-alumina. The studies explained formation of different types of hydroxyls on the surface as well as formation of Lewis acid sites by dehydration at elevated temperatures. Heterogeneity of alumina surfaces was also investigated by gas chromatography [89,90]. Laurent et al. [91] have described the surface chemistry of γ-alumina the most frequently used form in chromatography, in detail. Alumina is hygroscopic and its adsorption properties depend upon the amount of water on its surface. The water is not uniformly bonded on the surface [92]. Despite
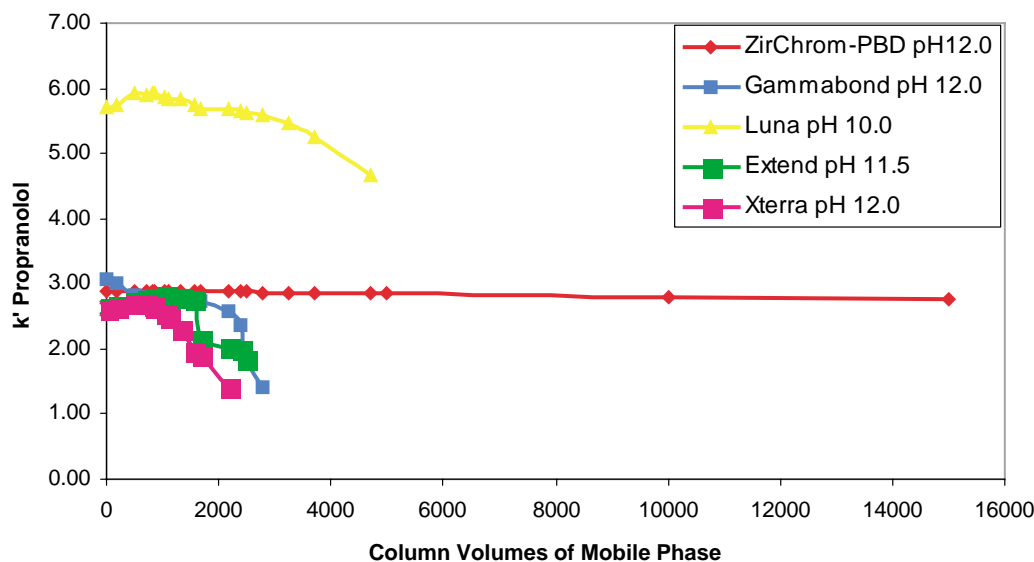

Fig. 5. High pH stability of silica-based columns vs. stability of PBD–zirconia.

Table 3
Nonchromatographic and laboratory made zirconias used for HPLC

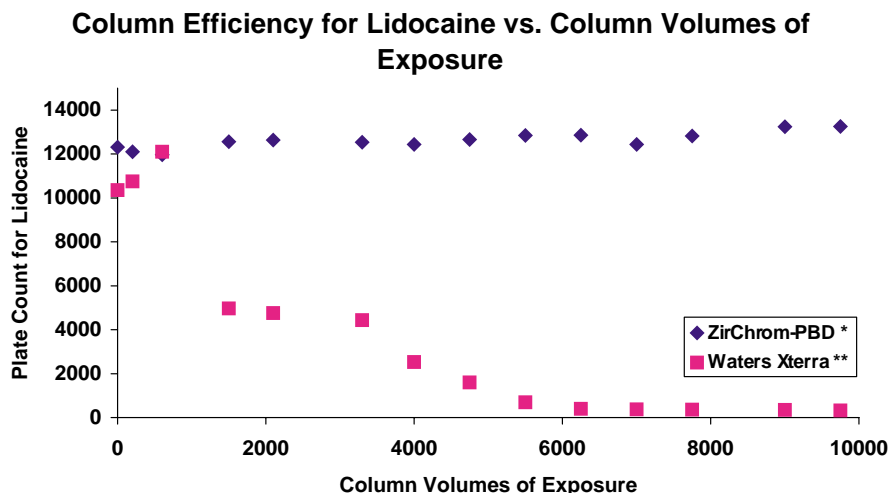| Zirconia	Surface area (m²/g)	Particle size (µm)	Pore size (Å)	Pore volume (cm³/g)	Reference
Magnesium Elektron	9	10 ± 3			[81]
Magnesium Elektron G10	3 ± 1	14			[22]
Degussa	40 ± 10	0.03			[22]
Zirconia PICA-7	33	2.5	25.8	0.15	[21]
Zirconia	52	5	18.1	0.24	[76,82]
Zirconia (Deichi Kigenzo)	22	8.2 (4.6–15.0)			[83]

Fig. 6. Stability of XTerra column at higher temperature and presence of phosphates. *LC conditions*: column dimension, 150 × 4.6; mobile phase, ACN/50 mM potassium phosphate, pH 7.0; temperature, 80 °C, 5 μl injection; flow rate, 1.0 ml/min; detection at 254 nm. *25/75 ACN/buffer; **30/70 ACN/buffer.

the numerous uses of alumina in chromatography its surface properties have not been systematically explored for HPLC.

As is the case for all other metal oxides, alumina's surface properties depend on the partial charge located on the surface hydroxyls. There are many types of hydroxyl groups that differ considerably in their partial charge. The net charge on a particular hydroxyl depends on its location, i.e. on the actual crystal face.

The variety of alumina's adsorption sites and their acid–base characteristics are well described by numerous studies of its catalytic properties at gas–solid interfaces. The presence of both tetragonally and octahedrally coor-

dinated Al atoms leads to the variety of the sites on the surface [84]. Besides the surface hydroxyl groups alumina also contains Lewis acid and Lewis base sites located on the Al^{3+} cation and coordinatively unsaturated oxygen, respectively. It is assumed that surface of alumina contains five types of hydroxyl ion sites [93] (see Fig. 7).

According to the multi site complexation model (MUSIC) [94–96] at liquid–solid interfaces four major hydroxyl sites are active on alumina's surface:
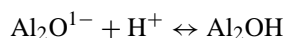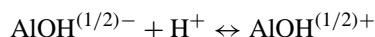
$$AlOH^{(1/2)-} + H^+ \leftrightarrow AlOH^{(1/2)+}$$

$$Al_2O^{1-} + H^+ \leftrightarrow Al_2OH$$

Table 4
Commercially available zirconia-based packings

| Product name	Mode	Chemistry	Pore size (nm)	Particle size (μm)	Surface area (m²/g)	pH range	Temperature limit (°C)
DiamonBond C18	RP	Chemically modified carbon coated zirconia	30	3, 5, 7, 10	30	1–14	200
ZirChrom® Carb	RP	Carbon coated zirconia	30	3, 5, 7, 10	30	1–14	150
ZirChrom® Phase	NP	Bare zirconia	30	3, 5, 7, 10	30	1–14	150
ZirChrom®-PBD	RP	PBD-coated zirconia	30	3, 5, 7, 10	30	1–14	150
ZirChrom® WCX	Weak cation exchange	PEI crosslinked with BUDGE on zirconia	30	3, 5, 7, 10	30	1–10	50
ZirChrom® WAX	Weak anion exchange	PEI coated zirconia	30	3, 5, 7, 10	30	3–9	50
ZirChrom® SAX	Strong anion exchange	PEI crosslinked with BUDGE and quaternized with methyl iodide on zirconia	30	3, 5, 7, 10	30	1–12	50
ZirChrom® SHAX	Strong anion exchange	PEI crosslinked with diiododecane and quaternized with methyl iodide on zirconia	30	3, 5, 7, 10	30	1–12	50
ZirChrom® PEZ	Cation exchange	chemically modified by bonding P-EDTA	30	3, 5, 7, 10	30	1–10	50
ZirChrom® PS	RP	Polystyrene coated zirconia	30	3, 5, 7, 10	30	1–13	150
ZirChrom® EZ	RP	PBD-coated zirconia modified by P-EDTA	30	3, 5, 15, 25	30	1–10	50
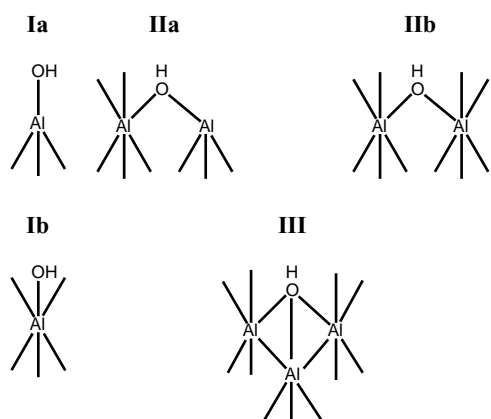
Fig. 7. Alumina surface hydroxyl sites [84].

$$Al_3O^{(1/2)-} + H^+ \leftrightarrow Al_3OH^{(1/2)+}$$

$$Al_2OH + H^+ \leftrightarrow Al_2OH_2{}^+$$
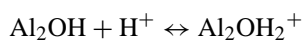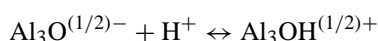
The chemistry of alumina surface is more similar to that of zirconia [1] than to silica. Thus, it is reasonable to expect rather more complex surface properties, It thus should exhibit both ion- and ligand exchange chemistry. Such surface properties are indeed observed and surface heterogeneity is an important feature of alumina [93].

There are several brands of HPLC-grade alumina available on the market. However, not much is known concerning the crystalline structure of these supports. Alcoa's Unisphere^{TM} alumina consists of ~200 nm thick platelets bonded together to form spheroidal particles with inter-platelet macroporosity and intra-platelet microporosity [97,98]. Commercially available HPLC alumina packings are listed in Table 5.

3.3. Titania

Titania exists in three crystallographic forms: anatase, rutile and brookite, The later is unstable thus the discussion is limited to two former crystalline forms [102]. Anatase is thermally stable up to 800 °C and above this temperature a transition to rutile is observed. Using X-ray diffraction, Tani and Miyamoto [103] observed the conversion at temperatures higher than 700 °C. Calcination of titania samples increases titania's density [103]. This is similar to that ob-

served for zirconia [1]. Surface area and pore volume decrease with thermal treatment similarly to zirconia [1,103]. Tani and Miyamoto [103] also observed a decrease of pore diameter with increasing temperature. The pattern of the decrease was similar to that found by Trüdinger et al. [13] for zirconia. In most samples of zirconia however, an increase in pore diameter with temperature was reported [1]. Recently, HPLC grade titania for normal phase chromatography has became available under the trade name Sachtopore (made by Sachtleben, Duisburg, Germany). Particles with 60, 100 and 300 Å pore diameters are available and they are made of pure anatase. The company manufactures also a 2000 Å pore titania but it is made of rutile [23]. The rutile crystalline form of titania is widely used for photocatalytic destruction of water contaminants [104–106]. Recently titania was synthesized by a polymer induced colloid aggregation method (previously developed [1] for zirconia particle synthesis). An extremely narrow particle size distribution of 3.5 ± 0.5 μm was reported and that is better than those obtained for both oil-emulsion and sol–gel methods [77].

3.3.1. Surface hydroxyl groups

Generally speaking the surface chemistry of titania is very complex. As do all metal oxides titania's surface contains hydroxyl (–OH) sites. There are reports on "... at least 12 kinds of –OH groups on titania surface ..." [102]. Only two kinds of residual –OH groups are considered as the most important: those with IR absorption at 3715 and 3675 cm^{-1} [102,107]. Primet et al. [108] found absorption for the same OH groups at 3715 and 3665 cm^{-1}. That is very similar to zirconia's hydroxyl absorption [1], however according to Primet et al. the absorption at 3715 cm^{-1} is due to isolated –OH group while the band at 3675 cm^{-1} is for two H-bonded adjacent –OH groups. Two types of adsorption sites on titania thermally treated above 200 °C were found by Jaroniec et al. [21]. Also Ridrígez et al. [109] confirmed the existence of two kinds of hydroxyls on titania's surface. The titanols differ in partial positive charge and the acid–base character of these sites is represented by the following surface equilibria:

$$\equiv TiOH_2{}^{(2/3)+} \leftrightarrow \equiv TiOH^{(1/3)-} + H^+, \quad K_A^{int}$$

$$\equiv TiO^{(2/3)-} + \equiv H^+ \leftrightarrow \equiv TiOH^{(1/3)+}, \quad K_B^{int}$$
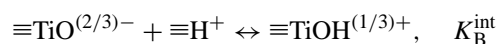
Table 5
Commercially available HPLC alumina packings

Alumina	Surface area (m^2/g)	Particle size (μm)	Pore diameter (nm)	Pore volume (cm^3/g)	Ref.
Biotage	49	8	33.4		[99]
Aluspher AL100	152	5	10.7	0.50	[21]
Alcoa Unisphere^{TM}	103	8	11	0.295	[100]
Aluspher 100	155	5	13	0.5	[76,82,101]
Alox T (Merck)	75	5	13.1	0.24	[101]
Alusorb N 200 (Lachema)	226		5.2	0.29	[101]
Alumina A5Y (Phase Sep., Deeside, UK)	na	na	na	na	[101]

Table 6
Laboratory made and non-chromatographic grades of titania

Titania	Surface area (m²/g)	Pore volume (cm³/g)	Pore diameter (nm)	Particle size (μm)	Ref.
Laboratory made	134	0.3	8.7		[114]
Fisher Sci.	<10				[115]
YMC	48		10	7	[116]
Aldrich (anatase)	10		0.2		[22]
Titania 100A	52	0.20	12.5	5	[21]
Titania YMC-Ti-100	51	0.16	12.3	5	[76,82,114]
Titania YMC-Ti-300	16	0.12	30	5	[82]
Titania YMC-Ti-1000	<5	0.12	100	10	[82]
Titania	177	0.48	10.5	3.8 (2.8–10.4)	[117,83]
Titania (dried at 200 °C)	126	0.3	11.1	4.0	[118,119]
Titania (dried at 200 °C)	111	0.3	8.7	3.7	[120]
Sachtopore	67	0.22	11.2	5.9	[23]
Titania (PICA)	36.7	0.30	32.2	3.5 ± 0.5	[77]

Table 7
Sachtopore® titania

Pore diameter (Å)	Specific surface area (m²/g)	Specific pore volume (ml/g)	Particle diameters (μm)
60	100	0.2	3, 5, 10, 20, 40, 80
100	55	0.21	3, 5, 10, 20, 40, 80
300	15	0.12	3, 5, 10, 20, 40, 80
2000	<5	n.d.	3, 5, 10, 20, 40, 80

Strongly dehydroxylated titania has only 0.5 –OH/nm² (0.83 μmol/m²). Such highly dehydroxylated surfaces dissociatively adsorb water [102], as does zirconia [1].

The heterogeneity of the surface also arises from the presence of Ti^{4+} sites; according to Hadjiivanov and Klissurski [102] titania surface contains three kinds of Ti^{4+} sites (Lewis acid sites) and two kinds of hydroxyls. Winkler and Marmé [23] state that there are no reports on the presence or absence of such Lewis acid sites on HPLC-grade titania particles.

The maximum number of titratable protons on titania is equal to 2.79 μmol/m² (1.79 OH/nm²) [110,111]. The fluoride adsorption capacity of titania was found to be 2.82 μmol/m² [110] (which is much lower than that observed for zirconia: ∼8 μmol/m² [112,113]) while acetate adsorption capacity was determined at 3.32 μmol/m² [111] and the amount of chemisorbed water was found to be 3,81 μmol/m².

Much of the work cited here was done on non commercial research grade titania or non HPLC-grade titania. Table 6 shows the variety of materials used.

Titania is commercially available in a wide range of pore and particle diameters as Sachtopore's HPLC-grade adsorbent for normal phase chromatography. Sachtopore titanias for HPLC are listed in Table 7.

4. Methods of preparation of metal oxide particles for liquid chromatography

4.1. Overview, and limits to scope

Two recent reviews [121,122] have described the characteristics essential for the practical use of ceramic packing materials in liquid chromatography. First, the particles should be on the order of 5 μm in diameter, as monodisperse as possible in diameter, and either spherical or of compact enough shape so as to be easily packed in a column. For the most part, to ensure column efficiency, modern synthesis strategies for LC materials have focused on trying to produce spherical particles. It should be noted, though, the first LC materials and many older materials were produced as irregular particles and then post-synthetically processed and classified to select packable fractions. Scott [17] notes that carefully milled and classified particles are often acceptable, even if they are not spherical as first prepared. Nevertheless, we will see that synthesis strategies are now available to produce packable particles without the need for milling, thereby substantially increasing the yield and ease of synthesis, as well as the reproducibility, of the particles.

The second general requirement for LC applications is that the internal mass transfer resistance be minimized to diminish peak broadening. There are a great many materials discussed in the literature and on the market that boast of very high surface areas, but the LC practitioner must be aware that the total surface area is not the only feature of interest. To establish acceptably fast intraparticle diffusion, the pores within the particles should be in the "mesopore" range (tens to hundreds of Angstroms), and large molecule chromatography (such as proteins or polymers) require that the most constricted regions (i.e. pore necks) should be no less than about 100 Å. Such a porous material, if assembled from primary particles on the order of hundreds of Angstroms, will typically have a surface area on the order of tens of square meters per gram. Higher specific surface area materials usually present micropores (several to tens of Angstroms). For reasonably large solutes, micropores might not even contribute significantly to the column capacity—the solute will not access the surface area in the smaller pores. Even if the solute could access that surface, though, there is a significant danger that at least some of the micropores will cause serious intraparticle diffusion resistance, which will broaden peaks and reduce column efficiency. It might be possible, of course, to produce very high specific surface area materials

with only large pores (e.g. a very open, light scaffold structure), but it is not yet clear whether such materials can be made in a packable form or whether, if they could be made, the packing would be stable under ordinary LC conditions. However, we will, consider two such prospective materials below.

The two above general requirements for LC applications of particles rule out many current methods of preparation of porous ceramics, since most recent work has focused on producing microporous materials, not necessarily spherical or monodisperse in particles, for gas separations and gas phase catalysis. In recent years, there have been thousands of papers and patents on the production and use of ceramic colloids, but here we will select only the main lines of work relevant to liquid chromatography. Moreover, there is a very large body of work on methods of surface modification of such particles, but this section will focus on the synthesis of the "base" particle.

4.2. Background—historical approaches to the production of porous ceramic particles

It is useful, to survey briefly the broad background upon which this limited field is based [123]. The production of ceramic colloids has a large and venerable history. Silica is, of course, well known for the variety of microstructures that can be made, and silica colloids are among the oldest "advanced materials". Silica colloids are typically made in large scale, often continuous, processes involving the controlled precipitation through pH modulation of sodium silicate solutions. Several key companies (DuPont, EkaNobel, Nalco, PQ (Nyacol), Bayer, Monsanto, and Nissan.) produce on the order of 100,000 t per year of silica colloid for applications in gas separations, catalyst support, polymer filling and strengthening, rheological control, raw material for other, more "advanced" materials, and as described below for liquid chromatography [124].

Alumina [125,126], titania [127] and zirconia [128] colloids are also produced on large scale (though not as large as silica) for use as catalysts, pigments, and for structural ceramics. The production method is generally hydrothermal precipitation, sometimes in continuous processes with recycling. The production of "nanostructured", frequently porous, ceramic materials for separations and catalysis has been rejuvenated in the last two decades by the application of "sol–gel" methods—which generally indicates the production of solid materials from solutions or suspensions, but has come more and more in the last two decades to indicate the production of ceramics from metal-organic precursors in solution (e.g. metal alkoxides) [129]. Among the many reviews of this field, that of Lin and Deng [130] on porous materials is particularly relevant to the metal oxide materials of interest in this review. The bulk of the "sol–gel" work, though, has focused on the synthesis of membranes and particles for gas separations and catalysis, but specific applications to the production of LC materials will be discussed below.

4.2.1. Background: methods of preparation of silica LC particles

Silica is the ceramic whose synthesis is best understood and provides the greatest number of structures suitable for LC [17,26]. The development of methods for LC particle production with silica establish a useful framework against which we can understand the production of LC particles of other metal oxides. Indeed, we will see below that LC materials of zirconia, titania, and alumina follow patterns well established with silica, so we will briefly review what is known about these processes with silica.

A schematic illustration of colloid aggregation methods is shown in Fig. 8.

Silica for LC can be made in one of four ways: (1) controlled pore glass, (2) gelation of silicate salt solutions, (3) controlled aggregation of a stable silica colloidal sols, either through reactive aggregation, spray drying, or oil emulsion drying strategies (vide infra), and (4) hydrolysis of organosilanes (particularly, alkoxysilanes). The first method, relying on the phase separation in the melt state of multicomponent silicate glasses, can not be viewed as generally available for other metal oxides (which are not nearly as good glass formers as silica), so we will not focus on controlled pore glass method here (though an example of recent work along these lines is available [131]). The other three methods, though, are entirely general and can be used with other metal oxides.

Silica "gel" LC particles are made on a very large scale by the acidification with sulfuric acid of sodium silicate solutions. This method is based on both the solubility of silica (as a function of pH) and on the colloidal stability of the dispersed solid phases that appear. The starting solution of sodium silicate is stable at high pH, where dissolved silica is present in the form of complex polyanions and (depending on pH and concentration) on highly negatively charged, colloidally stable silica colloids. Upon reducing the pH, the polyanions undergo condensation polymerization, producing more colloidal particles. Moreover, the colloids become destabilized as the surface charge decreases (as the ionic strength increases with the addition of acid). The choice of ions and the composition, the introduction of additives (e.g. adsorbing or complexing molecules, cosolvents to modulate the dielectric constant) and even the order and method of mixing can dramatically affect the size distribution and pore structure of the solid phases that are made. In general, the solid phase so made is only metastable and is subject to various aging processes. For instance, it is common to allow the gel to strengthen and its pore structure to be refined (generally, decreasing the surface area somewhat) by allowing "syneresis" with aging. During the syneresis process, the macromolecular and colloidal structure of the gel adjusts in a process similar to Ostwald ripening. The principle is that portions of the structure that present a higher chemical potential (higher curvature, less stable bonding configuration) dissolve, while more stable structures (lower curvature, more stable bonding) are formed. This process may be accelerated and guided by hydrothermal treatment with additives

Aqueous suspension of ceramic colloid

Spray drying

Oil emulsion

Polymerization

Droplets sprayed in air

Droplets emulsified

Water-soluble monomers added

Dried

Water extracted into oil, dried

Polymer precipitated (collecting colloid)

Sintered

Washed, sintered

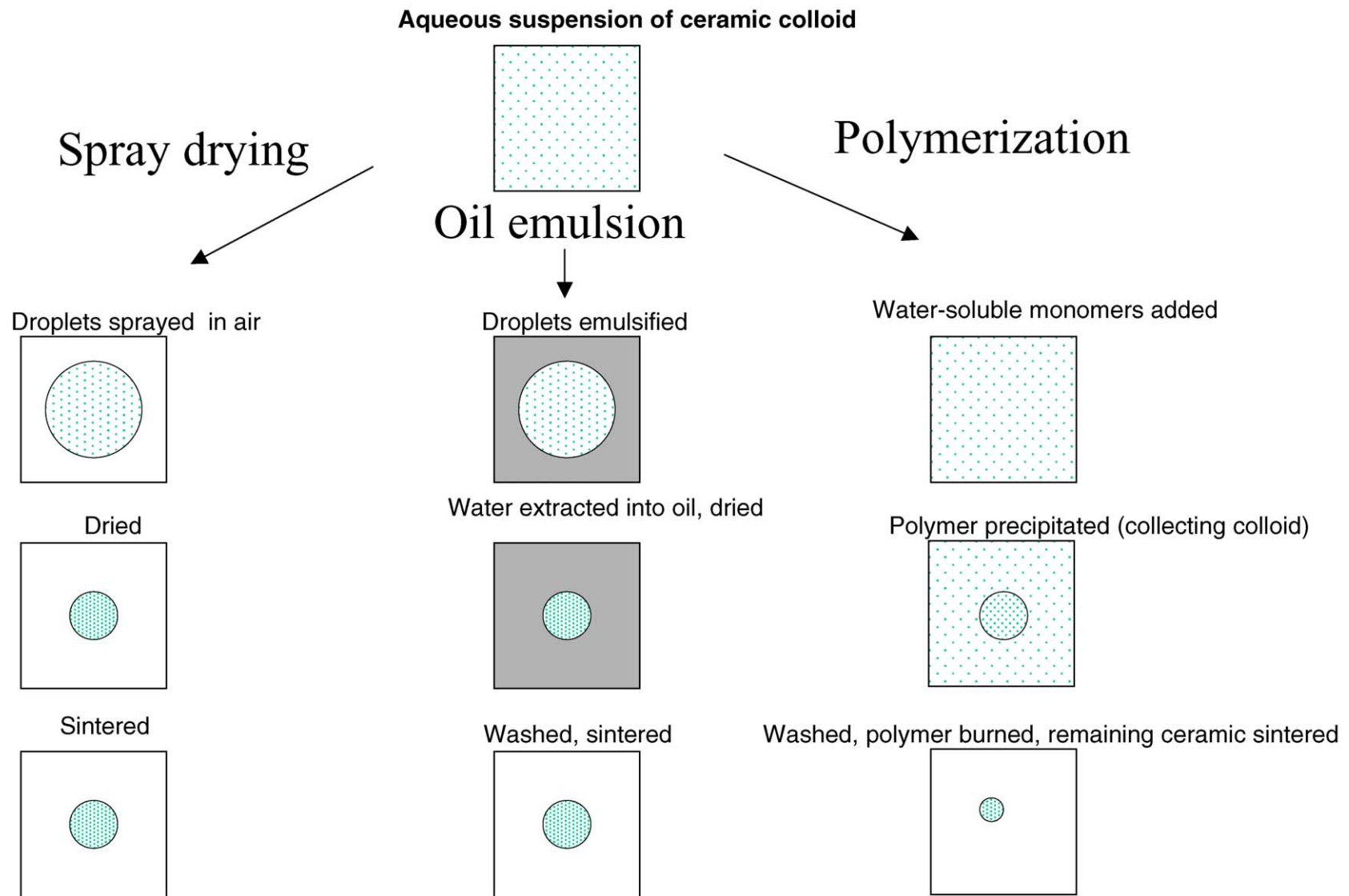Washed, polymer burned, remaining ceramic sintered

Fig. 8. Schematic illustration of colloid aggregation methods.

that serve to tailor the pore structure. The silica gels shrink during syneresis and expel water.

The procedure used to dry the gel also strongly affects the pore structure, since the hydrated solid is still reactive enough and weak enough to undergo considerable shrinkage and pore reshaping under the influence of capillary forces and decreasing water potential. It is also common to subject the dried material to further heating (sintering) to allow further pore reconstruction. It is important to note that while sintering and shrinkage usually go hand-in-hand, they are not necessarily the same thing. The goal of many sintering treatments is *not* primarily to shrink the particles, but rather to strengthen the particles and to allow small micropores to disappear while forming more pore space in the mesopore and macropore range. We will see an example below where sintering produces virtually no shrinkage, but rather only pore reconstruction, of zirconia.

In the past, micron-sized irregularly shaped silica gel particles were made by milling and particle size classification to make smooth packable particles (though rarely actually spheres). There are few, if any, HPLC-grade silicas made by this approach any longer.

The third approach to making silica LC particles is controlled aggregation. Two approaches have been taken: reactive aggregation and aggregation in dispersed droplets. The first was pioneered by Iler and McQueston [132] in a process they termed "coacervation". A stable aqueous sol of colloidal silica particles of several hundred Angstroms diameter is mixed with monomers that are initially miscible but which, upon polymerization, phase separate and thereby gather the colloidal particles into spherical aggregates that can be surprisingly monodisperse in size. For instance, urea and formaldehyde can be added to an acidic sol. The acid catalyzes the formation of a urea-formaldehyde resin, which separates from the aqueous media. When the hardened particles of polymer which contain the colloid are burned to remove the polymer, what is left is porous silica made of the original colloids packed in a spherical microparticle. This process offers a high degree of monodispersity and near perfect sphericity, and it also offers particles of pore size and strength controlled largely by the size of the original colloid. Aggregation in dispersed droplets includes spray drying and oil emulsion. In both techniques, a stable aqueous colloidal sol is dispersed into droplets either in the gas phase (spray drying) or in another liquid phase (oil emulsion). After this dispersion, removal of water from the sol causes the colloidal particles to connect together into a gel. These techniques will be discussed further below.

The fourth approach to making silica HPLC particles provides the precursor in the form of an alkoxide rather than as polyanions of silicic acid. Generally, a silicon alkoxide (e.g. tetra-*n*-butoxy silane) is dissolved in an alcohol, and water is added along with an acid or base catalyst to begin hydrolysis and condensation reactions. It is important to understand that all alkoxysilanes are oligomeric unless distilled immediately before use and that their properties de-

pend greatly on their trace water content. The alcohol is ordinarily present in sufficient quantity to ensure miscibility of the (initially) hydrophobic alkoxide and the water. Depending on the composition, this reaction can produce a gel much like that produced by the acidification of silicate solutions, it can produce an "aerogel" of immense surface area (but microporous), or it can produce porous particles. With each of these synthesis strategies, two problems must be dealt with when producing LC particles.

First, how to make spherical particles. One way to ensure that particles are spherical is to rely on the action of liquid–gas or liquid–liquid interfaces to minimize surface energy by adopting a spherical shape. Thus, spherical porous silica particles have frequently been made by spray drying. This method involves the dispersal of the reactive solution or of a colloidal suspension through a small nozzle as a fine mist of liquid (or aerosol if a colloid is spray dried into a hot drying gas. The particles are formed by reaction and drying in the gas phase. Typically, a rather broad range in particles are produced although the particle size of the most abundant particles can be tuned by varying the concentration of the reactants or colloid used as well as the nozzle size and other parameters. The particles are spherical although a fraction of the particles in a particular batch may not be spherical depending on drying conditions. The particles made by thsi method must be classified (by air or liquid sedimentation/elutriation) to give a useably narrow range in particle sizes for HPLC.

Another common route is to mechanically disperse the aqueous gelling silicate, or silane solution or a silica sol (stable colloidal suspension) as an emulsion of micron-scale droplets within an hot, immiscible oil phase. Typically a long chain alcohol is added to aid water transport and speed up the drying process. Due to a better match between the viscosity of the gelling (usually polar) phase and the oil, the size distribution can often be more easily controlled with this method—however, the typical range of particle size is comparable to the spray drying method, and size classification is invariably needed. This strategy is of general use in the synthesis of other metal oxides as well. It is important to note, though, that the particle size distribution may be quite broad; it is important either to pay particular attention to the emulsification conditions to regulate droplet size or to ascertain whether particle classification is needed after synthesis.

Another strategy to produce spherical particles is to regulate the appearance and aggregation of the solid phase during gelation. Stober et al. [133] first showed that, under certain conditions, the hydrolysis of silicon alkoxides to produce spherical, unaggregated, porous silica particles up to 2 μm in diameter. This behavior is exhibited in conditions where either nucleation of the solid phase occurs in a brief burst, followed by slow growth from solution with little aggregation, or where the aggregation of the particles favors the formation of monodisperse aggregates. The principle advantage of this approach is the *remarkable monodispersity* of the size distribution and the ease with which it is produced.

In general, spheres of this type show pores from 10 to 50 Å in diameter [122]. Unger et al. [134] have shown that the particle size accessible can be extended up to about 10 μm in diameter by the continued addition of alkoxide in a second step, after the formation of the original particles. In the first step, the particles are formed by the base-catalyzed hydrolysis of silicon alkoxide, much as described by Stober et al. [133]. In the second step, though, the suspension is provided with extra nutrient to produce larger spheres. Sathyagal and McCormick [135] explained the physical chemical conditions required to succeed in this "continued growth", while avoiding secondary nucleation of another set of (smaller) particles. Moreover, although it is traditional to ensure a single phase at the beginning of reaction, Barder and DuBois [136] showed that when producing Stober-like spheres it can actually be advantageous to use so little alcohol that the alkoxide and water are not, at first, totally miscible. When less alcohol is used (allowing two phases at the beginning of reaction), the ultimate particles can be made larger, without decreasing the yield. Though it would be difficult to predict a priori exactly what compositions or processing conditions would produce this behavior for other metal oxides.

The second major problem when producing LC particles has been in the area of pore size refinement. In addition to the traditional methods of syneresis and manipulations of sintering conditions, several groups have shown the advantage of incorporating "pore formers" in the processing. For instance, sodium chloride can be deliberately incorporated into the drying gel, so that crystallizing salt can template the pore spaces. The salt is, of course, easily removed after drying and sintering. Unger et al. [122] also demonstrated the use of alkylamines with alcohol cosolvent to make the pores of Stober-like spheres unimodal and mesoporous. Alcaraz [137], and Alcaraz and Holmgren [138] showed that the pore size can be refined to make them unimodal in mesopore and macropore range by treating with mineralizing agents such as sodium hydroxide or carbonate, organic complexing agents at elevated temperature. We might consider this process as related to the pore refining processes of syneresis and sintering, and more fundamentally to the physical chemistry of Ostwald ripening.

There are new prospects to produce more open pores, yet high specific surface area forms of metal oxides. For instance, Unger et al. [76] and Schuth [139] showed that silica and other metal oxides of the "MCM41" structure (templated by a hexagonal liquid crystal with pores on the order of 50 Å) can be ground and classified, packed, and used for LC of small molecules. This method did not yield spheres, but the authors showed that the powder could be size classified to 5–10 μm particles that they could be packed. Other work directed toward producing optical diffraction materials (e.g. [140]) that have ca. 2000 Å pores and 75% porosity has shown that metal oxides can be formed in the interstices of packed latex particles to produce exceedingly low density, high surface area, yet large pore materials. It remains unknown at this time, though, whether such materials can

routinely be made into spheres and then packed into LC columns with high plate counts that can be operated for prolonged times at typical flow rates and back pressures.

On the other extreme of pore size control is the quest for *nonporous* materials for ultrafast chromatography. Unger et al. [134] showed that, if desired, the porous Stober-like spheres can be made nonporous in the deposition of subsequent material. It can be reasoned that controlled deposition of material might be used to accomplish subtler goals. One might deliberately block micropores while leaving meso- and macropores open, or one might seek to strength a very high specific surface area, large pore material by a measured deposition of strengthening material without blocking pores.

The distribution of pore space through the silica particle has also received considerable attention in the field of "pellicular" or "porous shell" materials [141,142] but these topics are beyond the scope of this paper except as they are relevant to the "grafting" methods described.

It is worthwhile to make several general comments as we turn our attention to the synthesis of non-silica materials. The methods for particle size and pore size control have been, in general, much more thoroughly explored for silica than for any of the metal oxides. However, virtually any method that shows promise for silica synthesis should also be considered in the synthesis of metal oxides. Indeed, in the sections that follow, we will point out clear similarities. If a method has not yet been explored, it should not be concluded that the method has no promise—it is simply that there has been less (and less focused) attention to the range of particle and pore size control for the purposes of metal oxide liquid chromatography.

Only two fundamental differences exist that will, in general, limit one's ability to try "silica" methods with other metal oxides. First, the solution chemistry of each metal oxide is, of course, different owing to the varying acid–base thermochemistry and kinetics, so the precise conditions that will give rise to a desired colloid or gel structure will differ. Silica solution chemistry is uniquely well-explored owing to a long history of interest in fields ranging from materials science to geochemistry, and the solution chemistry of other metal oxides is less well explored (but growing rapidly thanks to the surge of interest in "sol–gel" methods to make new nano- and microstructures and to make coatings and fibers of ceramics). Second, silica is almost unique in its propensity to form amorphous structures. This means that, in general, the silica will be less prone to crystallize and will be generally easier to sinter gently to refine pore structure and establish strength without the major changes in pore structure often caused by crystallization. However, there are many documented cases of success at producing useful pore structures with crystallizing metal oxides (vide infra). It is often desirable to carefully control sintering conditions to encourage the formation of very small crystals (e.g. smaller than the desired pore size of ca. 100 Å) so that the pore walls are relatively smooth. This is not essential, though. Commercially available alumina phases, in particular, show crystals

that are on the same order of the pore size. The crystallinity of the metal oxides also implies that a polycrystalline structure may need to be sintered more rigorously than for the analogous silica structure to establish particle strength and to try to remove micropores.

Another prefatory comment is in order. Since LC particles can be made in a great number of ways (vide supra) with very different resulting particle and pore structures, it is important when comparing particles of different metal oxides to avoid comparing apples and oranges. For instance, the fact that a given silica phase shows higher porosity than a given zirconia phase should not be taken to imply that all silica phases with have higher porosity than any prospective zirconia phase. The methods of synthesis have simply *not* been explored thoroughly enough to make such a deduction. When possible, comparative studies should include high resolution scanning electron micrographs of the particles to establish whether the particle and pore structure are similar enough in nature to warrant quantitative comparison. That warning stated, there are at least two very informative comparisons between stationary phases [21,82].

Finally, it is important to remember that for LC purposes, total surface area and total porosity are *not* the key measures of interest. Zeolites and microporous silica aerogels, for instance, have surface areas of hundreds of square meters per gram and the aerogels can have fractional porosities above 90%—but they are quite useless for LC if they are not stable and if the pore size is so small as to give too high an intraparticle diffusion resistance at particle sizes practical for LC.

4.3. Zirconia

Zirconia colloid is made by the dissolution of a zirconium salt (e.g. a chloride) at pH where its solubility is high and then inducing precipitation by a shift of pH. Bleier and Cannon [143] showed that the hydrolysis of the zirconyl ion, following dissolution of the chloride salt, creates a sufficient pH shift to allow precipitation of zirconia colloids even without the need for addition of other components. We can regard this approach—the preparation of a salt solution, followed by precipitation of the oxide—as related to the sodium silicate method.

One major difference, though, is that the zirconia precipitates in microcrystalline rather than amorphous form. Colloids that are fairly monodisperse in size, with averages of 100–1000 Å, can easily be made with this type of process and are commercially available.

Mesoporous, spherical zirconia LC particles have been made by causing such colloids to gel in a droplet. Trudinger et al. [13] demonstrated a procedure, used by a number of other investigators [82,144–146], to first form the colloids from an aqueous salt solution and then to mechanically disperse the aqueous sol into an emulsion in an oil phase. As water is extracted from the aqueous droplets, the colloids gel to form a mesoporous structure. This produces ca. $10 \mu m$

spheres with pores ranging from 100 to 300 Å. Carr and co-workers [147] used a similar procedure, with a commercially available colloid and modified by rheological control of the aqueous phase and by the introduction of a reactive binder to enhance the stability and monodispersity of the resulting microspheres (however, they tended to make spheres on the order of $50 \mu m$ in diameter). Shalliker et al. [148–154] used a similar procedure, enhancing the rate and perhaps stability of the gel structure with the addition of urea. Moreover, the addition of sodium chloride as a pore former and the manipulation of sintering conditions were shown to help tailor the pore structure, in analogy to the effects seen with silica, increasing the pore size up to 600 Å. This addition of porogen or pore size controlling agent should be applied to other techniques as well. (The use of other metal oxides as pore formers has also been considered, see Section 4.6.)

Carr et al. [17,135,147,155–163] took an approach related to Iler's to make monodisperse, micron-scale spherical microparticles out of commercially available colloid through the addition of urea and formaldehyde. Pore structure and particle size variations were investigated as preparation and sintering conditions were manipulated, and the most striking result was that very strong, very porous (up to 50% porous), very large pore (200 Å minimum aperture) monodisperse spherical particles could be made. Even very severe sintering caused little overall collapse of the pore volume; instead, small pores were consumed and large pores were retained. This behavior contrasted markedly with the sintering and shrinkage of zirconia colloidal aggregates produced by more conventional methods, such as the oil emulsion method.

The production of zirconia powders using the hydrolysis of alkoxides is well established for the purpose of making green bodies for structural ceramics (e.g. engine parts, sensors), particularly since the alkoxide approach affords an easy way to incorporate other components such as ceria, which enhance the mechanical toughness of the final ceramic part (e.g. [164]). There has been little work to date on the manipulation of the alkoxide method to make LC particles. It is unclear, though, whether such materials would be superior to the mesoporous materials made from colloids.

Just as Stober had shown that the alkoxide method could yield monodisperse silica microspheres, Lerot et al. [165] showed that it could yield monodisperse zirconia microspheres that could be controlled from 0.1 to $2.5 \mu m$ in diameter, with a surface area of ca. $4 m^2/g$ after sintering. Lerot found that to achieve micron-scale, distinct spheres, it was necessary to modify the alkoxide with a fatty acid to reduce the rate of hydrolysis. Carr and co-workers [166] found that the micropores of these spheres could be removed by judicious sintering, while keeping the spheres unaggregated, to make nonporous zirconia for ultrafast chromatography [167]. DiMaggio et al. [164] showed that in the absence of the fatty acid it is possible to make zirconia and zirconia–ceria gels from the alkoxides with an average pore size of 100 Å, but these are not spherical particles.

4.4. Titania

As with zirconia, Trudinger et al. [13] have demonstrated that titanyl chloride hydrolysis followed by forming emulsion in oil can be used to make mesoporous spheres of titania. Yamashita et al. [144] demonstrated a similar method, but using titanium alkoxide hydrolysis rather than the chloride method to make the titania colloid and by adding urea and amine to the synthesis (reportedly to help produce spherical particles). A patent awarded to Sachtleben in 1997 [168] claims the formation of porous titania with surface area of about $55 \, m^2/g$ (with pores of $100 \, \text{Å}$ and particle sizes from 5 to $80 \, \mu m$) and with a crystal size of $100 \, \text{Å}$ through the hydrolysis of titanyl sulfate solutions. Though this patent describes their use as polymerization catalysts, it may be a related to the production method for their commercially available titania LC particles. Takahashi and co-workers [169,170], and Tani and co-workers [103,171], in a manner similar to Stober, hydrolyzed titanium alkoxides to make spherical ca. $5 \, \mu m$ particles with pores of ca. $50 \, \text{Å}$ that offer prospects for LC use. Sato et al. [169] added stearic acid to the gelling system can help increase the pore size from ca. 50 to $200 \, \text{Å}$.

The coacervation method pioneered by Iler and developed by Carr and co-workers has been adapted to the titania system by Jiang and Zuo [77] to make spheres 2–6 μm in diameter with pores ca. $300 \, \text{Å}$.

4.5. Alumina

Though there is a great deal known about the production of microporous alumina for catalyst applications (including alkoxide routes and even the production of $500 \, \mu m$ porous γ-alumina spheres directly from the corrosion of aluminum [172]), relatively little has been published on the production of alumina LC materials. A patent awarded to Biotage in 1990 [173] demonstrates the use of cosolvents and the manipulation of solution conditions to control the size and habit and state of aggregation of aluminum hydroxide crystallizing from alkali metal aluminate solutions. One figure in this patent resembles Biotage chromatographic particles—intergrown, platelike crystals to make a particle of about 10 μm in diameter with ca. $1000 \, \text{Å}$ pores.

Separately, Palkar [172] has shown the prospects for a novel synthesis route, directly oxidizing aluminum metal to make porous (ca. $50 \, \text{Å}$) aluminum hydroxide spheres (though his spheres are a bit large, at ca. $500 \, \mu m$ diameter).

4.6. Mixed metal oxides

There are a number of solution preparations of metal oxides mixed at the molecular level with silica. Relatively few of them pay particular attention to the production of spherical particles which might be useful for LC. However, Kaneko et al. [174] and Zhang and co-workers [175,176] report the production of coprecipitated sodium silicate plus aqueous metal chlorides, followed by pH shift to make mixed oxide powders. Though Kaneko did not, Zhang et al. used an oil emulsion method, these particles might to make a stable packing (ca. $10 \, \mu m$ diameter, with pores 20–100 Å in diameter). Honda et al. [177] report an example of a silica/titania/polymer composite with claims of unique chromatographic performance.

What is more interesting along this line, though, are efforts to capitalize on the mesoporous structure presented by one metal oxide synthesis, but presenting a very different metal oxide surface. We will refer to this as "grafting". This is somewhat distinct from surface modification, as by silanization, since the goal of grafting is to provide a fully dense coating of the new metal oxide. Iengo et al. [178] report grafting zirconia and silica on alumina using metal alkoxides. Barder and DuBois [179] and Barkatt and Macedo [180] report the use of metal alkoxides, acetates, and acetylacetonates to graft metal oxides onto silica. Finally, Kirkland et al. [142] have shown that it is possible to graft porous silica layers made of silica colloid onto silica particles, and there is every reason to expect that sintering and pore restructuring methods could be used to make these grafts dense.

Earlier there were several attempts to obtain chromatographic supports with different surface chemistry: one of first was the group of Aue and co-workers [181] who used volatile chlorides of several metals ($AlCl_3$, $FeCl_3$, $SnCl_4$) and later $CrCl_3$ [182] for the reaction with silica silanols. The bonded metals were hydrolyzed and used in HPLC as "metal oxides with silica geometry". The modified silica showed different retention behavior than plain silica surfaces. However, a mixed structure of the oxides means also a different chemistry of the surface. Mixed oxides are very well known in catalysis and they can show radically different surface properties than initial components. First of all the acidity of the surface, in most cases, increases [18–20]. Kaneko et al. [174] coprecipitated mixed oxides and they found significant changes of the surface chemistry, e.g. very strong acid sites were observed for silica doped with zirconia, alumina and titania. This increased acidity of surface hydroxyls of zirconia–silica oxide was applied in ion exchange and ion-exclusion chromatography by Ohta et al. [183–186]. Alumina and zirconia modified silica ion exchangers were used for simultaneous separation of mono- and divalent cations [186]. Zirconia–silica and zirconia–magnesia supports were prepared by the oil emulsion method [185]. Although Kaneko et al. [174] reported a slight acidity of silica–magnesia supports, according to Zhang et al. [175,176,187] the zirconia–magnesia preparation does not show any acidity. As these two supports were calcined at $600 \, °C$ they are composed of tetragonal zirconia [175,176,187]. The magnesia–zirconia surface was dynamically modified with alkylphosphonate to give a surface coverage of $6.1 \, \mu mol/m^2$. The resulting surface appeared to be very stable in both acidic and alkaline conditions; over 8000 column volumes of purge did not change the retention of dimethylaniline or biphenyl.

It is worth recalling that different metal oxides have different solubilities, so it is possible to use phase separation of metal oxides to create "porogen" structures—with a more soluble oxide that can be dissolved to leave pores. Controlled pore glasses, of course, represent this sort of approach. Also notable is the use of silica to generate leachable pore space in zirconia [222].

4.7. Effect of synthesis method on the pore structure

Although there have been a number of reports comparing the pore structure and chromatographic performance of LC particles of different composition and from different preparation methods, there have been very few that compared LC particles that were "homologous" in the sense that they were similar in most respects but differed only in, for instance, the method of preparation. One series of papers [135,147,155–163] affords a comparison of the type of pore structure, sintering behavior, diffusion behavior, and chromatographic performance of mesoporous zirconia made of the same colloid through two different methods to make spherical aggregates—that using an oil emulsion (cf. [13]) versus that using polymerization method (cf. [132]). This series of papers demonstrated that the polymerization method (first described by Iler and McQueston [132] and demonstrated for chromatography by Kirkland [188] has the potential to produce much more porous particles, offering significantly less intraparticle diffusion resistance, than does the more traditional oil emulsion method. This method can easily produce spheres from 1 to 10 μm in diameter with a fractional porosity up to 50% and with a pore size on the order of the size of the colloid used (e.g. a 100 Å colloid gives 50–150 Å pores, a 1000 Å colloid gives 750–1200 Å pores). Indeed, the polymerization-produced zirconia, though significantly strengthened by sintering, shrank very little at temperatures that virtually collapsed the emulsion-produced zirconia [161]; instead, the pores were made larger, both strengthening the particle and providing less diffusional resistance for LC.

5. 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 non-electrolytes 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.

5.1. 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:

$$M–OH + H^+ \leftrightarrow M–OH_2^+ \quad K_1^{int}$$

$$M–OH + OH^- \leftrightarrow M–O^- + H_2O \quad K_2^{int}$$
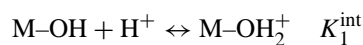
where K_1^{int} and K_2^{int} are the intristic ionization constants.

According to the model of Yates et al. [189] H^+ and OH^- are the potential determining ions. When the number of positively ($M–OH_2^+$) charged species is equal to number of negatively ($M–O^-$) charged species then the surface has a zero net charge. The pH of the point of zero charge (pH_{pzc}) depends on ionization constants:

$$pH_{pzc} = 0.5(pK_1^{int} + pK_2^{int})$$

Practically, when $pH < pK_1^{int}$ metal oxide will act as an anion exchanger while at $pH < pK_2^{int}$ 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 [94,95,190]. 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). Isoelectric points of metal oxides are listed in Table 8.

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 [61,62]) 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) [16]. The chromatographer can choose to:

Table 8
Isoelectric points of metal oxides of chromatographic interest

	Skeleton density (g/cm^3) [1,76]	Isoelectric point					
		[82]	[1]	[76]	[23]	[109]	[191]
Zirconia	5.8	10–13	6.4–7.25	8	–		
Alumina	3.5	7	8.5–9.1	7	8.1		8.7
Titania	4.0	5	5.9	5	5.6	6.5	
Silica	2.2	3	3	3	2.2		

- 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 [1,63,74,75] 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 [23]. Very similar separations can be done on alumina and zirconia [13,76,82]. Normal phase chromatography for alumina, titania and zirconia was compared by Kurganov et al. [82] and Grün et al. [76]. 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 [192,193].

Important fundamental studies on the LC separation of inorganic anions on alumina were carried out by Schmitt and Pietrzyk [194]. 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 strength, counter-ion, analyte concentration as well as mobile phase solvent composition. Strong affinity of alumina for sulfates and carboxylates was also noted [194]. Similar strong affinity of titania to carboxylates is also reported by Tani and Kubojima [117]. Detailed adsorption studies of halide ions on alumina were recently carried out by Szczepaniak and Kościelna [195]. Ion-exchange properties of alumina were examined and used for the separation of inorganic ions

[91,196–199], of heroin samples [200] and a mixture of proteins [201]. The ion-exchange properties of titania [117,202] and zirconia [75] 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 [75,194]. 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 [1,112,113,117,203,204]. Al$_2$O$_3$ is even used for removal of excessive concentrations of fluoride from drinking water [203]. Metal impurities in silica have a large effect on the acidity (pK_a) of neighboring SiOH sites. Residual metals exist in even the new type B high purity silicas. The National Institute of Standards and Technology Standard Reference Material for checking HPLC reversed-phase columns contains a metal complexant (quinizarin) as a probe solute [208].
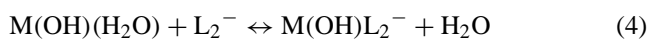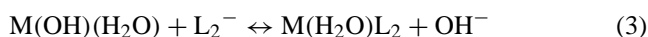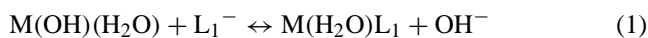
5.2. Ligand exchange

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. [21] have determined the amount of adsorbed water on metal oxides by TGA (for from ambient temperatures ambient to 350 °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 $\mu mol/m^2$ for alumina. However, according to earlier findings of Moterra and co-workers [205] coordinated water is removed from zirconia below 200 °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 $\mu mol/m^2$ [206]. Jaroniec et al. [21] found 22.1 $\mu mol/m^2$ of adsorbed water on titania surface while Ridrígez et al. [109] found only 3.82 $\mu mol/m^2$.

The following chemical equilibria have been used to describe the ligand exchange reactions on metal oxide surfaces [1,112]:
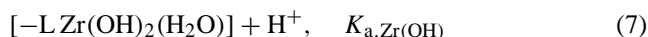
$$M(OH)(H_2O) + L_1^- \leftrightarrow M(H_2O)L_1 + OH^- \tag{1}$$

$$M(OH)(H_2O) + L_1^- \leftrightarrow M(OH)L_1^- + H_2O \tag{2}$$

$$M(OH)(H_2O) + L_2^- \leftrightarrow M(H_2O)L_2 + OH^- \tag{3}$$

$$M(OH)(H_2O) + L_2^- \leftrightarrow M(OH)L_2^- + H_2O \tag{4}$$

$$M(OH)L_1^- + L_2^- \leftrightarrow M(OH)L_2^- + L_1^- \tag{5}$$

Where M represents the metal oxide metal, and 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)–(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.
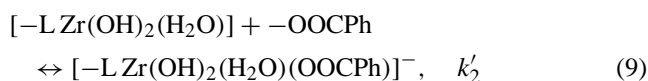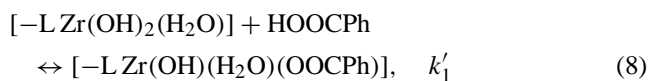
The chemistry of benzoic acids adsorption on ligand exchange media prepared by impregnating Zr(IV) ion into an iminodiacetic ion exchangers are nicely explained by Yucchi et al. [207]. According to their work an increase in pH induces benzoic acid dissociation:

$$HOOCPh \leftrightarrow {}^-OOCPh + H^+, \quad K_a \tag{6}$$
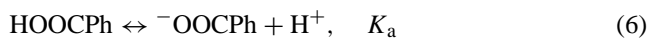
At higher pH the stationary phase also dissociates:

$$[-L\,Zr(OH)_2(H_2O)] + H^+, \quad K_{a,Zr(OH)} \tag{7}$$

Two retention paths are possible:

$$[-L\,Zr(OH)_2(H_2O)] + HOOCPh$$
$$\leftrightarrow [-L\,Zr(OH)(H_2O)(OOCPh)], \quad k_1' \tag{8}$$

$$[-L\,Zr(OH)_2(H_2O)] + {}^-OOCPh$$
$$\leftrightarrow [-L\,Zr(OH)_2(H_2O)(OOCPh)]^-, \quad k_2' \tag{9}$$

Following the generally known dependencies it is possible to write a general equation for k':

$$k' = \left(k_1' + \frac{k_2'\,K_{a,Zr(OH)}}{[H^+]} \right)$$
$$\times \left(\frac{1 - 1/[1 + 10^{(pK_{a,Zr(OH)}+pH)/2.5}]}{1 + K_a/[H^+]} \right) \tag{10}$$

The $\log k_1'$ is almost constant (it is independent of substituents (such as nitro-, chloro-, dimethylamine) on the aromatic ring for a series of benzoic acids), $\log k_1'$ corresponds to the reaction of neutral benzoic acid with Zr(IV). while $\log k_2'$ corresponded to coordination of benzoate anion to Zr(IV). According to Yucchi et al. benzoate's Lewis basicity is well correlated with the Brönsted basicity, thus the $\log k_2'$ is correlated to the pK_a of the benzoic acids. Findings of Yucchi et al. [207] fully confirm earlier results of Blackwell and Carr [209,211].

As shown above the possibility for ligand exchange requires the presence of coordinatively bonded water on the Lewis acid sites on the surface. Ligand exchange does not take place on silica *per se*, because it does not contain σ-coordinated water.

The strength of the interaction of a Lewis base with the *hard* Lewis acid sites characteristic of zirconia, titania and alumina depends on the electron density and the polarizability of the base. Lower polarizability and higher electron density lead to stronger interaction. The strength of interactions of the bases is as follows:

phosphate > fluoride > citrate > sulfate > acetate

> formate > nitrate > chloride.

An eluotropic strength scale for of Lewis bases (based on their ability to displace benzoate anions) has been developed for zirconia [1,209].

Blackwell and Carr [211] found a linear correlation between $\log k'$ and the pK_a of the benzoic acids and that was also confirmed by Yucchi et al. [207]. The slope of the correlation was found to vary with pH as predicted by Blackwell and Carr [211]. Conversely no such correlation was found for the same solutes on siliceous-based strong anion exchanger. This supports the view that the correlation of $\log k'$ and pK_a for the benzoic acids is related to the Lewis acidity of the zirconia support and thus is due to ligand exchange and not anion-exchange chromatography.

Blackwell compared the relative Lewis acidities of zirconia and alumina [1,210,211]. According to his results alumina is not as strong a Lewis acid as is zirconia. It has been speculated that titania behaves similarly to zirconia and alumina on the basis of similar interactions with benzoic acids, phosphonates, phosphates and carboxylic acids [1,111,117,212]. According to Grün et al. [76] titania exhibits a pronounced Lewis acidity while according to van Veen [213] Ti^{4+} sites do not appear to be as reactive as Al^{3+} sites. The ligand exchange behavior of titania was also

40 mM Acetate pH 4

N = 260
A_s = 3.35
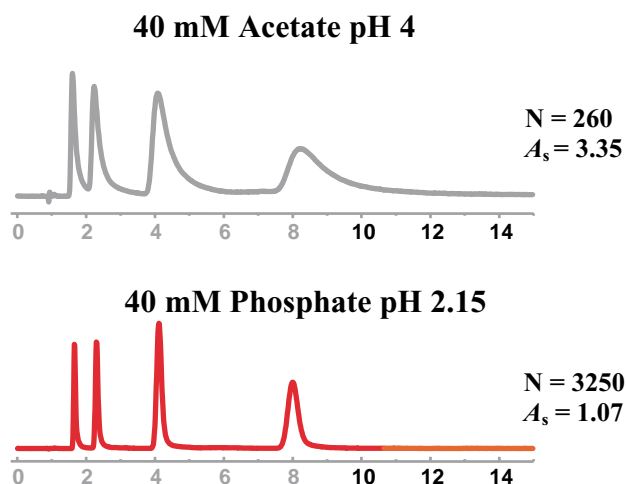
40 mM Phosphate pH 2.15

N = 3250
A_s = 1.07

Fig. 9. Separation of alkoxyacids on PBD zirconia. *HPLC conditions*: 25% ACN, 40 mM above additive, 5 mM NH₄F; 0.6 ml/min; 30 °C; 254 nm detection.

examined by Yu et al. [202]. The existence of strong interactions between titania and phosphates and phospholipids were proposed for as the mechanism of selective enrichment of phospholipids from biological samples [214].

Blackwell and Carr carefully examined the importance of ligand exchange process on chromatographic retention on zirconia [211]. The sites will strongly interact with *hard* Lewis bases such as fluoride, hydroxide or carboxylates [215]. Reader is referred to Pearson theory [223]. Also titania and alumina strongly interact with carboxylic acids [82,194,216]. The maximum amount of anion adsorption is likely to occur at pH = pK_a [1,216]. Thus, when the chromatographed analytes are hard Lewis bases we expect very poor chromatographic performance unless a stronger Lewis base is present in the eluent. For example, the presence of fluoride or phosphate in the mobile phase greatly improves the chromatography of Lewis bases [112,113]. Fig. 9 shows the chromatography of alkoxyacids. As the carboxylic acids strongly interact even with PBD-coated surface an addition of Lewis base is necessary to improve the efficiency of the separation. The figure shows that phosphates are stronger Lewis bases thus they block the available Lewis sites and the acids can be separated more efficiently.

Fluoride cannot be used at very low pH as it dissolves zirconia [112,113] and alumina [190,194].

Since Lewis acid sites are still available on PBD modified zirconia surfaces protein carboxyl groups can and do strongly interact with the sites. PBD-coated zirconia, silica and alumina [217] were compared for irreversible adsorption of proteins. No elution of proteins was observed from zirconia and silica supports with 0.1% trifluoroacetic acid containing mobile phase. This was attributed to the stronger Lewis acid sites on zirconia as compared to alumina. Addition of citric or phosphoric acid made elution of proteins from native zirconia supports possible [218]. The presence of fluoride, phosphate, polyvalent organic ligands

or organophosphonates in the mobile phase allowed separation of a wide variety of acidic, neutral and basic proteins on bare zirconia surfaces [219]. The retention is caused by a combined ion exchange and ligand exchange mechanism. Thus, elution can be controlled via the concentration of the Lewis base or by ionic strength.

An example of a mixed mode retention showing the behavior of some antihistamines on PBD–ZrO₂ is presented in Fig. 10. 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.

Phosphate modified PBD zirconia shows both reversed phase and cation-exchange properties even under acidic mobile phase conditions [220]. 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 Fig. 11. An increase in buffer concentration from 20 to 100 mM decreased the retention times by more than 50% [221].

pH is another variable that is used to control retention when ion exchange is the main retention mechanism. This is shown in Fig. 12. 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.

5.3. The effect of Lewis base buffers on the chromatography of cationic analytes

The discussion below is devoted to the chromatographic differences between reversed-phase silica-based supports and PBD modified zirconia. Chromatography on both supports differs greatly only for ionizable solutes. Based on the discussion above it should be clear that when a strong hard Lewis base buffer is present in the eluent the chromatography of cationic molecules such as the majority of pharmaceuticals will be greatly influenced by the adsorption of the Lewis base component of the eluent onto zirconia's surface. In this section we show the effect of phosphate on the chromatography of a set of nine antihistamine drugs on both a common type of ODS phase and a PBD–ZrO₂ phase. The structures of the nine antihistamines and their pK_a values are given in Fig. 13 and the chromatography of the compounds on ODS and PBD–ZrO₂ are shown in Fig. 14.

One of the initial most interesting observations is that despite the much lower phase ratio of the PBD zirconia phase compared to the ODS phase and that in the same eluent

Chromatography of Antihistaminic Drugs

Analytes:
1. Theophylline
2. Albuterol
3. Ephadrine
4. Norephedrine
5. Chlorpheniramine
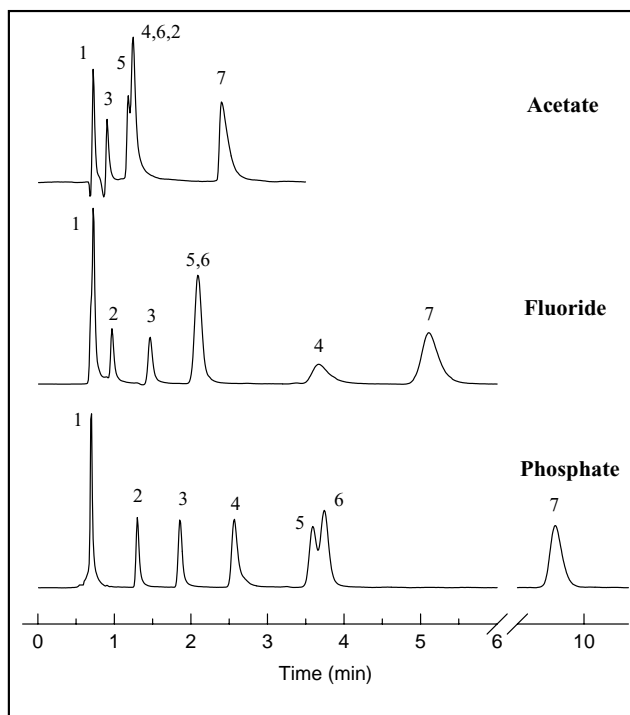6. Diphenhydramine
7. Promethazine

Fig. 10. Chromatograms of test basic solute mixture on PBD–ZrO$_2$ 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 °C. (1) Ammonia acetate; (2) ammonia fluoride; (3) ammonia phosphate dibasic.
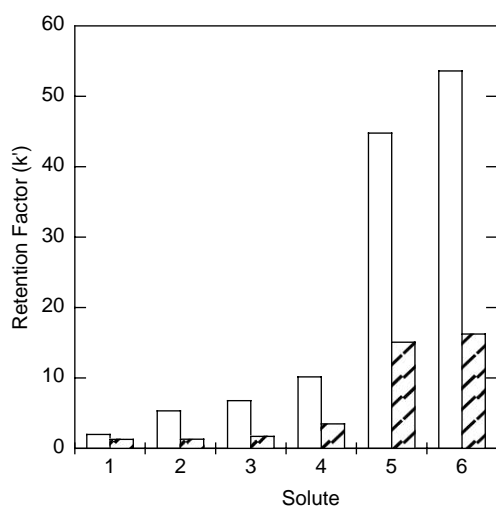

Fig. 11. Effect of ionic strength on the retention factors of test basic solutes on PBD–ZrO$_2$. Mobile phase, 30% ACN/buffer at pH 7; flow rate, 0.8 ml/min, 40 °C, 210 nm, *Open bars*: 20 mM ammonia phosphate monobasic. *Full bars*: 100 mM ammonia phosphate monobasic. *Solutes*: (1) lidocaine; (2) norpseudoephedrine; (3) tryptamine; (4) quinidine; (5) amitriptyline; (6) nortriptyline.

nonpolar compounds such as alkylbenzenes are much less retained on PBD than on ODS the retention of these basic drugs is substantially greater on the PBD zirconia phase than the ODS phase. Further the elution sequence and the band spacings are radically different on ODS and PBD. As shown in Fig. 15 there is almost no relationship between the retention on the ODS phase and the PBD zirconia phase in this eluent. Also shown in Fig. 15 is a plot of the enthalpy of retention on the PBD zirconia phase versus the enthalpy of retention on the ODS phase. These enthalpies were obtained by studying the retention factors as a function of temperature on both phases. Here again there is almost no relationship between the two thermodynamic parameters.

Basic chromatographic concepts would tell us that the mechanism for retention on the two phases is most certainly different despite the fact that ODS and PBD–ZrO$_2$ are both reversed-phase type materials.

The fundamental difference in the mechanisms of retention on these two phases is most clearly revealed by the data of Fig. 16 in which the logarithm of the retention factor is plotted versus the logarithm of the phosphate concentration in the eluent. Here we see that the slope of the relationship on the ODS phase is significantly lower than the slope of the relationship on the PBD phase. What this means is that there
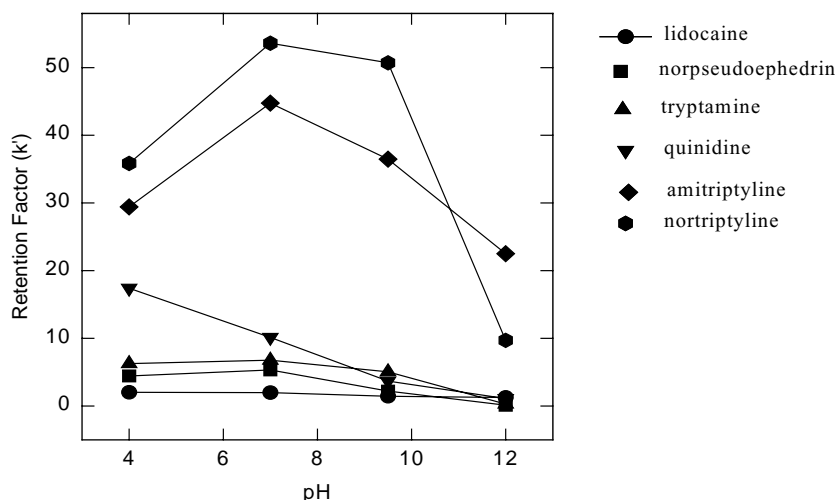
Fig. 12. Effect of mobile phase pH on the retention factors of basic solutes on PBD–ZrO$_2$. Mobile phase, 30% ACN + 20 mM ammonia phosphate buffer adjusted to different pH, except for pH 12 that was obtained with 20 mM of sodium phosphate buffer; flow rate, 0.8 ml/min, 40 °C, 210 nm.

is a much greater contribution of ion-exchange interactions to the retention of these positively charged molecules on the PBD–ZrO$_2$ phase than on the ODS phase. It is the much greater contribution above ion exchange which causes the basic molecules to be more retained on the PBD phase than on the ODS phase. It also causes the tremendous difference in elution sequence on the two types of reversed phases.

At this point, it might seem that the adsorption of phosphate on to the surface of ZrO$_2$ is a significant impediment to doing chromatography on PBD–ZrO$_2$. It might appear that one must control the concentration of phosphate much more precisely when using the PBD phase than when using the

ODS phase; however, as shown by the data in Fig. 17 this is not true. One merely needs to control the phosphate concentration by approximately a factor of two better when using the PBD phase than when using ODS phase. This will be discussed in details in Section 1.3.1.7 in Part II. Much more importantly, as revealed in the window diagram optimization shown in Fig. 18, one can obtain a much better separa-
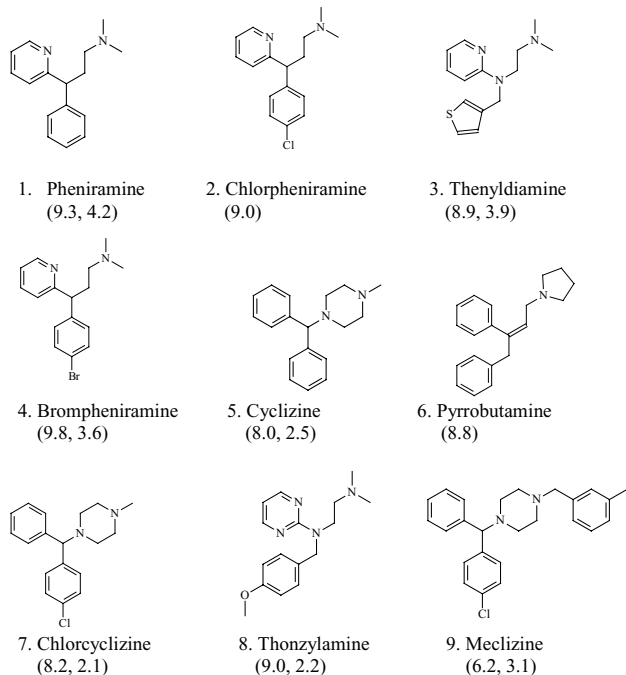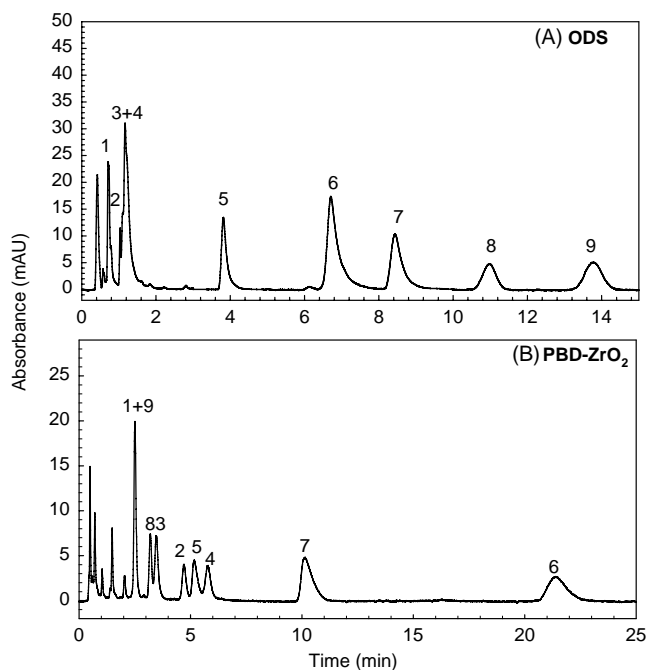

Fig. 14. Chromatograms showing the separation of antihistamines on (A) ODS column and (B) PBD–ZrO$_2$ column. *Experimental conditions*: temperature, 30 °C; mobile phase, 40/60 acetonitrile/25 mM potassium phosphate buffer at pH 7.0; flow rate, 1 ml/min; detection, 254 nm. *Solutes*: (1) pheniramine; (2) chlorpheniramine; (3) thenyldiamine; (4) brompheniramine; (5) cyclizine; (6) pyrrobutamine; (7) chlorcyclizine; (8) thonzylamine; (9) meclizine.
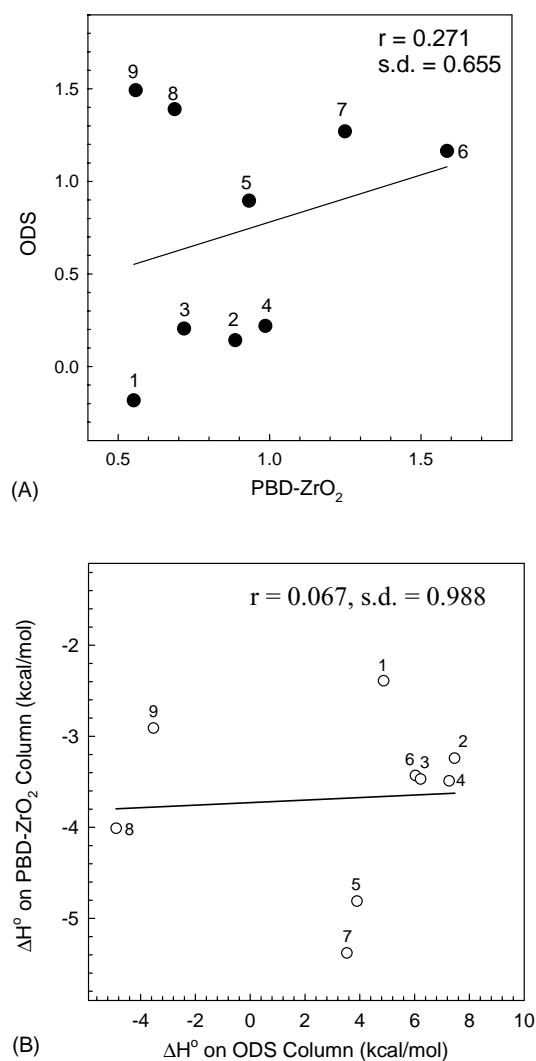

1. Pheniramine (9.3, 4.2)
2. Chlorpheniramine (9.0)
3. Thenyldiamine (8.9, 3.9)
4. Brompheniramine (9.8, 3.6)
5. Cyclizine (8.0, 2.5)
6. Pyrrobutamine (8.8)
7. Chlorcyclizine (8.2, 2.1)
8. Thonzylamine (9.0, 2.2)
9. Meclizine (6.2, 3.1)

Fig. 13. Structures and pK_a values of antihistamines.

Fig. 15. (A) Plot of $\log k'$ on PBD–ZrO$_2$ vs. $\log k'$ on ODS for the antihistamines. (B) Plot of the apparent ΔH° on PBD–ZrO$_2$ column vs. apparent ΔH° on ODS column for the antihistamines.
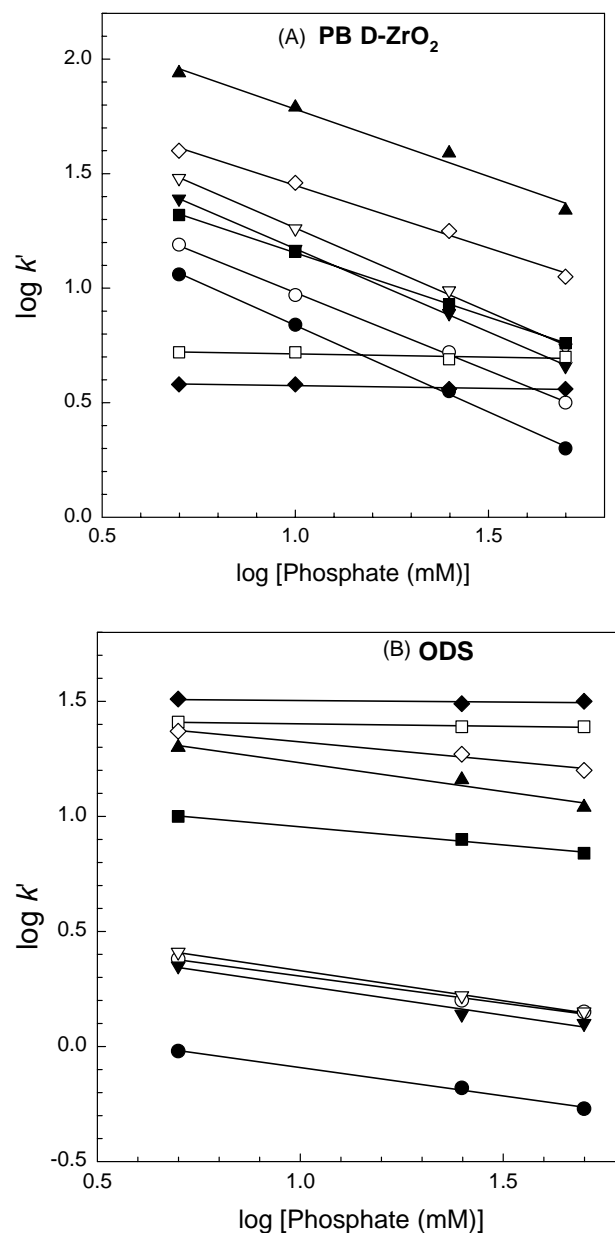

Fig. 16. Plot of $\log k'$ vs. logarithm of phosphate buffer concentration in milimole on (A) PBD–ZrO$_2$ column and (B) ODS column. Experimental conditions: mobile phase, 40/60 acetonitrile/potassium phosphate buffer at pH 7.0; temperature, 30 °C. *Solutes*: (●) pheniramine; (○) thenyldiamine; (▼) chlorpheniramine; (▽) brompheniramine; (■) cyclizine; (□) thonzylamine; (◆) meclizine; (◇) chlorcyclizine; (▲) pyrrobutamine.

tion on the PBD–ZrO$_2$ phase than on the ODS phase. There are clearly several concentrations of phosphate at which a resolution >1.0 can be obtained on the PBD–ZrO$_2$ phase whereas the maximum possible resolution obtainable on the ODS phase is slightly less than 0.5.

The point of this discussion is that the adsorption of phosphate and other hard Lewis base anions on the surface of zirconia can be used to "tune" a separation whereas the effect of such buffers on retention on ODS phases is very much less. Thus, the apparent complexity of zirconia's surface chemistry can frequently be turned to the knowledgeable chromatographers advantage.

One last point is to be made in connection with the adsorption of hard Lewis bases on the surface of zirconia in the context of the chromatography of cationic molecules and that is frequently the case that the loading capacity of the PBD–ZrO$_2$ phase is greater than the loading capacity of a conventional ODS phase despite the fact that the overall sur-

face area of the zirconia phase is considerably lower than the surface area of the conventional silanized silica-based phase (see Fig. 19). This is due to the fact that on either type of phase overload occurs when the basic molecules have consumed a significant fraction of the negatively charged sites on the surface. The amount of negative charge on zirconia's surface due to the adsorption of Lewis base buffers is much greater than the amount of negative charge due to the presence of underivatized residual dissociated silanol groups.

$\Delta[C]$ of phosphate corresponding to 1% change in κ'

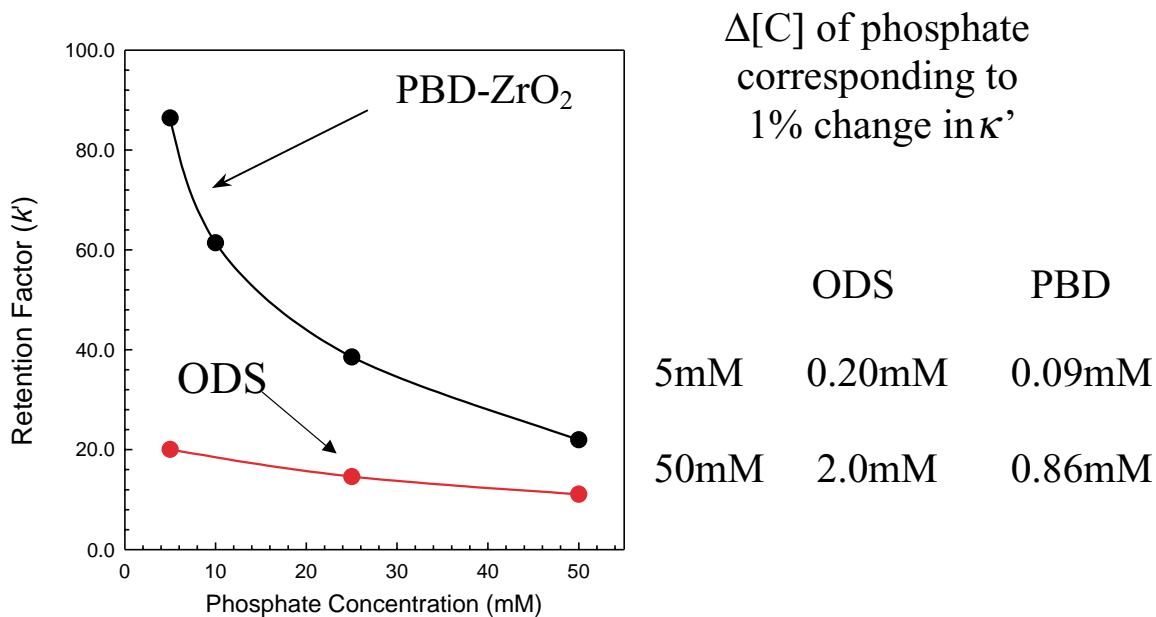|	ODS	PBD
5mM	0.20mM	0.09mM
50mM	2.0mM	0.86mM

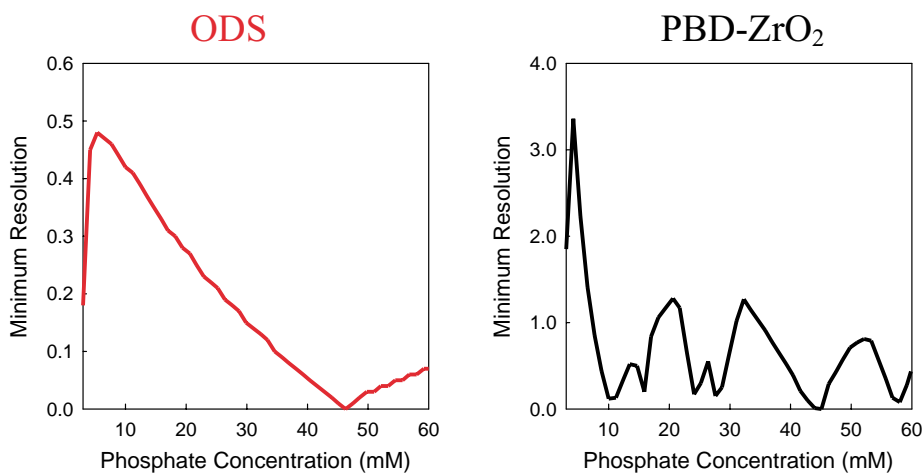Fig. 17. Effect of buffer concentration on retention of pyrrobutamine.

Fig. 18. Effect of buffer concentration on resolution (nine antihistamines)—window diagram optimization of PO_4^{3-} concentration for antihistamines.
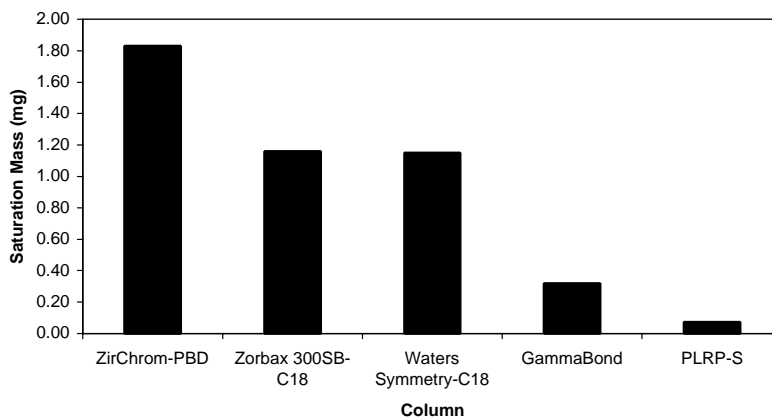

Fig. 19. Sample loadability of ZirChrom®-PBD for basic drugs compared to other "stable" reversed-phase columns. The sample: nortriptyline. The retention factor was kept constant across all columns by adjusting the strength of the mobile phase. Saturation mass: calculated by the method used by Snyder [92]. *LC conditions*: all columns (150 mm × 4.6 mm i.d.) packed with stationary phases with similar specifications as ZirChrom®-PBD (3 μm particle size, 300 Å pore size). Mobile phase, ACN/phosphate buffer, pH 7.0; temperature, 30 °C; flow rate, 1.0 ml/min; detection at 254 nm.

The best approach to method development on PBD–ZrO$_2$ depends on the class of compounds to be separated:

Acidic compounds: phosphate (>20 mM) is a necessary component of the eluent. For low pH separations phosphoric acid should be used. For separations at pH > 4 the addition of fluoride might be useful. (Ammonium salts of both Lewis bases are preferred for solubility and other reasons.) However, when phosphate or fluoride are added, they block Lewis acid sites but they generate at the same time a strong negative charge on the surface, i.e. cation-exchange interactions tremendously intensify. To avoid this acetate may be used instead of phosphate or fluoride. Adjusting the ionic strength and pH of buffer can control the extent of cation exchange.

Basic compounds: high pH may be used (up to pH 14) at pH lower than 10 addition of phosphate, fluoride or another Lewis base anion may be useful to optimize retention. The ionic strength and pH of the buffer will have a great effect on the band spacing. To maximize selectivity differences relative to ODS phases the ionic strength should be kept low (< 10 mM). Furthermore in contrast to ODS retention decreases as pH is elevated above the analyte's pK_a.

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