

Review

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

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Abstract

In this part of the review authors discuss methods used for modification of metal oxide surfaces. On the basis of literature data it is shown, that silanization of the surfaces do not form stable supports for chromatography. On the other hand, the success of polymer modified surfaces such as polybutadiene (PBD) and polystyrene (PS) is emphasized. Permanent modification of metal oxide surfaces with Lewis bases is also widely discussed. Chromatographic properties of polymer modified surfaces of zirconia are discussed in details. The perspectives of carbon-coated metal oxide surfaces in HPLC and high temperature separations are described.

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1. Modified surfaces of metal oxides

Since unmodified metal oxides (zirconia, titania, etc.) have more complex surface chemistry than silica there have

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been many attempts to make a modified, reversed-phase surface. As pointed out in our previous review [1] there are three classes of surface modifications:

- dynamic, chemical modification—carried out by putting a strongly interacting compound in the mobile phase,
- “permanent” covalent chemical modification—e.g. silylation of the surface, or some form of direct bonding,
- physical screening—e.g. deposition of a polymer on an oxide or cladding the oxide with a carbon layer.

1.1. Dynamic modification

Dynamic modification of the surface can be used for two purposes:

- suppression of the undesired sites on the surface,
- generation of a “temporarily bonded phase on the support surface”.

Dynamic, chemical modification had been used for years for ameliorating the undesired silanophilic interactions between silica-based packings and organic bases. This was usually achieved by addition of an amine or quaternary ammonium salt to the mobile phase [2]. Since some metal oxides (titania and zirconia) can be used at much higher pH than silica there is no need to add such amine blocking agents to mobile phase. However, a form of dynamic modification of the surface is used for blocking Lewis acid sites on metal oxides. This is done by adding a hard Lewis base, e.g. inorganic phosphates, fluoride, or strong complexing agents such as EDTA, to the eluent. The dynamic modification by Lewis bases addition was examined extensively on zirconia (or modified zirconia) packings using fluoride [3,4], phosphate [5] and carboxylates [1,6–8].

The concept of the formation of a “temporary bonded phase” was invented by Hansen et al. [9] for modifying silica by using a quaternary ammonium salt in the mobile phase. Rigney attempted to do so by adding alkylphosphonates to the eluent; however, they are readily displaced by addition of other salts to the eluent [10].

1.2. Permanent covalent modification

1.2.1. Alkyl-bonded phases

There has been continued interest in forming a reversed phase on metal oxides. If a truly permanent reversed phase could be prepared on alumina, titania or zirconia that would totally sequester the Lewis sites it is likely that they would displace silica-based phases from the market. Commercially available reversed-phase zirconias and aluminas are *not* based on silanization of the surfaces. There are several concepts concerning how a permanent, stable reversed phase on a metal oxide surface can be prepared.

1.2.1.1. Silanization. Despite the fact that Schindler and Schmidbaur [11] firmly established the fact that the order of

hydrolytic stability of silicon oxide bonds is: $\text{Si-O-Si-R} \gg \text{Zr-O-Si-R} > \text{Ti-O-Si-R} \gg \text{Al-O-Si-R}$ there have been many attempts to modify metal oxide surfaces by silanization. This is not surprising since silanization chemistry as applied to silica is so well known in theory and practice. According to the above stability sequence the silanized zirconia, titania and alumina should be more susceptible to hydrolysis than modified silica supports. There are some results in the literature that confirm these expectations and there are others that seem to contradict this expectation. Not only does the instability of the Si–O–Zr bond limit the practical development of silanized zirconia surface but the structure of the hydroxyls on zirconia’s surface and their basicity [1,12] disfavor the simple silanization approach to preparing a permanent modification.

It is important to understand that most of the hydroxyls on zirconia’s surface exist in a bridged form, a form which is entirely absent from silica’s surface, and bridged hydroxyls cannot undergo silanization [1]. Alumina, zirconia and titania hydroxyls are much more basic than silica silanols [1,12]. The many attempts to silanize metal oxides began in the mid-1970s:

- Knox and Pride [13] were first to describe the surface modification of alumina by a standard silanization reaction. The efficiency of the modified alumina was reported to be comparable to that of silica. The stability of the alumina packing was not tested.
- Laurent et al. [14] found that chlorosilanes were inactive towards alumina surfaces. However, hexamethyldisilazane strongly adsorbed on the surface but subsequently when water was added to the eluent the adsorbed layer was easily displaced. Thus, Laurent et al. [14,15] advocated the use of unmodified alumina in an ion-exchange mode.
- The first, attempt to bind mono- and trifunctional silanes to zirconia surface was done by Rigney [10]. Although, coverages of up to several micromoles per square meter could be obtained the phases were unstable at high pH and rapidly lost the bonded layer.
- Later Trüdinger et al. [17] reported on the successful modification of zirconia surface with *n*-octadecyltrimethoxysilane. They used amorphous zirconia with microporous/mezoporous pore structure; however, the details of the modification procedure were not given. The resulting phase was said to be stable—withstanding 500 h at pH 12; however, no systematic stability studies were presented. The surface concentration of the C₁₈ ligands was only 1.2–1.4 $\mu\text{mol}/\text{m}^2$ [17]. This group [17] has not presented any additional work on alkyl-bonded zirconia and subsequent papers on zirconia were devoted to chromatography on polymer-coated oxides [18–20].
- Yu and El Rassi [6,21] modified a non-porous zirconia surface with monochlorooctadecylsilane and trichlorooctadecylsilane to obtain monomeric and polymeric C₁₈ phases, respectively. A great deal of the initial retention of xylene on the monomeric phase was rapidly lost at high pH but

retention stabilized at about 20% of the initial value after some 4000 column volumes of mobile phase were passed through the column. The polymeric phase lost about 40% of its initial retentivity. In a second paper, the same group described the synthesis of a range of anion-exchangers on the same non-porous zirconia [22].

- According to Tani and Suzuki [24] an attempt to modify titania with chlorosilanes failed, while with octadecyltriethoxy silane a reversed phase containing 6.16% C (w/w) was obtained. No stability tests were performed.
- Pesek and co-workers [23,25–27] modified the metal oxide surfaces with triethoxysilane to deposit a reactive silane hydride. The resulting hydride was hydrosilylated with alkenes in the presence of a chloroplatinic acid catalyst. Titania [23,25,26], alumina [25,27] and zirconia [25] were modified. The resulting phases were not studied chromatographically. In particular, no stability tests were carried out. The chromatographic results presented in the papers did not allow estimation of the performance or the stability of the packings.
- Another approach to the reversed zirconia phase was presented in a paper by Wirth et al. [28]. They modified zirconia with octadecyldimethylchlorosilane using imidazole as a catalyst. According to these authors excellent stability of the packing was observed particularly under high pH conditions. The support could withstand purging with at least 1000 column volumes of mobile phase (with no organic modifier) at pH 13 without affecting chromatographic resolution.
- Silane chemistry was also used by the Hearn and co-worker [29] to modify zirconia with 3-glycidoxypropylsilane group and then attach an imidodiacetic acid group. In addition, they bonded an isothiocyanatopropylsilane group and then reacted it with the protein concanavalin-A. The stability of the phases were not tested.
- Also according to Arenas and Foley [30] a chromatographically useful silanized alumina is not feasible due to the low stability of Al–O–Si bonds under acid conditions.

In summary, the existence of a stable silanized zirconia or metal oxide is very controversial.

1.2.1.2. Other methods.

- Bien-Vogelsang et al. [31] physically coated aluminas with polystyrene–divinylbenzene (PS–DVB), polybutadiene (PBD) and polyoctadecylsilane. The materials had excellent pH stability but rather poor chromatographic efficiency.
- Haky et al. [32–34] took advantage of the strong interactions between alumina and organic phosphonic acids. The method led to =Al–O–P–R linkages at the surface. The octadecyl phase was obtained at $3.4 \mu\text{mol}/\text{m}^2$ and used in basic solvents for a separation of basic solutes. In a subsequent paper, the C₁₈ alumina phase was used for separation of proteins and peptides [32]. Also Xiang and Blackwell used similar approach to obtain zirconia

reversed phase [35]. The same idea was used to prepare perfluoroalkyl-bonded alumina phases [36]. The disadvantage of this synthesis route is that one must avoid using phosphate or any strong hard Lewis base in the eluent. The stability of the phase has not been examined systematically. Rigney reported that alkylphosphonated phases prepared by adsorption were not stable [10].

- According to Gao et al. [16] dense, highly ordered monolayers can be prepared by the adsorption of octadecylphosphonic acid onto non-porous zirconia, titania and zirconized silica powders. The degree of order of the monolayers is comparable to self-assembled monolayers on planar surfaces. The materials made in this work were not intended for use in chromatography so their stability was not studied.
- Pesek and Lin [37] tried other modification reactions involving Grignard reagents as well as organolithium and organoaluminium compounds. Only the organolithium route could effectively bind some carbon to the surface. No significant chromatographic testing was reported.
- Another approach to obtain a reversed phase on alumina is the polymerization of 2-octadecyl-1,3-butadiene. Alumina modified in this way is commercially available from Biotech under the trade name of Unisphere Al-C₁₈ [38,39].
- Mao and Fung [40,41] synthesized an anchored polymer coating on alumina by a reaction of maleic acid and then further modifying the surface by copolymerization with octadecene. Subsequently a cross-linking agent, such as divinylbenzene was added during the polymerization process to increase the stability of the polymer. The resulting reversed phase was stable at both high (0.1 N NaOH) and low pH (0.1 N HCl). However, stability was examined only under static conditions which can be grossly deceptive. The chromatographic performance of the packing was poor but only a low quality alumina was used for coating [42].
- Hu et al. [43] used stearic acid to modify ceria-zirconia composite particles. The resulting reversed phase was not stable at extreme pHs.
- Ceria-zirconia spherules modified with alkylphosphonic acid appeared to very stable at both pH 2 and 10 [44].
- Alkylphosphonic acid modified ceria-zirconia and magnesia-zirconia were stable at pH 10 [45].

To summarize: there has been a great deal of interest in the literature to make a hydrophobically modified metal oxide surfaces. The following conclusions can be drawn:

- Most attempts with monofunctional silanizing reagents were unsuccessful. That confirms the earlier work of Schindler and Schmidbaur [11] as well as that of Arenas and Foley [30], and Laurent et al. [14].
- There are some results claiming that relatively stable phases were obtained based on trifunctional silanes. Some of these [25,27] were not confirmed chromatographically. Successful modification of metal oxide surface with trifunctional silanes does not necessarily contradict the

statements of Schindler and Schmidbaur [11] or Laurent et al. [14]. It has been well known that the silanes may form so-called horizontal polymers on the surfaces. It is then possible that even unintended surface polymerization could lead to at least some patchy covering of the surfaces. There is only one attempt reported in literature to produce self-assembled monolayers C₃₀ silanes on titania and zirconia surfaces [46]. Water coordinatively bonded to the metal oxide surface was said to promote the horizontal polymerization process [47–51]. The monolayers on titania and zirconia surfaces were characterized by higher chain order than found on amorphous substrates. This results in higher molecular shape recognition. There is, however, no report on the stability of self-assembled monolayer zirconia and titania based phases.

- An additional factor has to be taken into account; when trifunctional silanes are used for metal oxide modification as the formation of Me–O–Si–OH is possible. Such hydroxyl groups will be strongly acidic when Me is Al, Zr or Ti [52,53]. This will generate additional surface heterogeneity.
- Polybutadiene-coated metal oxides are quite well-known. Unisphere C₁₈ alumina case is an example of how a stable C₁₈ reversed phase can be made on the oxide surface. The phase is made by a polymerization of 2-octadecyl-1,3-butadiene [38]. Unisphere Al-C₁₈ has been shown to be very stable under acidic and basic conditions [39]. Also they were shown to have very uniform in particle size. The backpressure required to obtain the same flow was much lower for 8 μm Unisphere particles than for 10 μm spherical silica [39].
- A similar approach was taken by Jia et al. [54]; they synthesized a C₁₈ zirconia by copolymerization of PBD and 1-octadecene on the surface of porous microspheres.
- Another option that provides a chemically stable (pH 1–14) reversed phase is the commercial DiamonBond C₁₈ phase. This is a chemically modified (bonded) carbon-coated zirconia-based phase (see Section 1.4.).

The purpose of modifying metal oxide surface is to obtain a support having a hydrophobicity and chemical selectivity similar to that of conventional reversed-phase silicas. If we consider only non-electrolyte analytes that goal has been achieved by, e.g. coating PBD on the various oxides (alumina, zirconia [30,38,55–57], see also Fig. 3). These phases interact with *non-ionizable* solutes exclusively by reversed-phase mechanism. However, for ionizable solutes whatever surface modification is used “mixed-mode” retention mechanisms be the Lewis acid-base (ligand exchange) or Coulombic (ion-exchange) processes in addition to the reversed-phase process must be anticipated. Once the chromatographer understands the mixed-mode interaction process it can be fruitfully exploited for otherwise difficult separations because metal oxides offer much better chemical and thermal stability than silica-based supports. A number of examples of such separations are presented in this review.

1.2.2. Other permanent coatings

The strong interactions of hard Lewis acid sites on metal oxides’ surfaces with Lewis bases can be used to generate some unique and useful stationary phases. These phases can be very stable under many eluent conditions but in strongly alkaline solutions the adsorptively coated Lewis base will be displaced by the strongest Lewis base on zirconia namely hydroxide. Phosphate and fluoride were the first hard Lewis bases used to make novel stationary phases on bare zirconia. These bases have been used to block the accessible Lewis acid sites on polymer-coated zirconia phases [58]. The success of phosphate and fluoride phases led to the examination of other Lewis bases. One very useful phase uses a phosphonate analog of EDTA (see below), which, while still an anion-exchange phase, had rather different selectivity than other conventional ion-exchange phases [59] most likely due to some ligand exchange contribution to retention. Fundamentally, the same idea was used by Mao and Fung [40–42] for the synthesis of a bonded phase prepared by a reaction of maleic acid with the surface of alumina.

1.2.2.1. Phosphates. The strong interaction of phosphates with zirconia surface can be used to improve chromatographic performance. The phosphate can be used in two ways: as an eluent component and as a surface modifier, i.e. zirconia’s surface is aggressively treated with phosphoric acid and a deposit of zirconium phosphate is generated [60]. Depending on the vigorousness of the treatment (pH, concentration, temperature and time), the extent of the modification can be controlled. A nearly permanent surface modification involves refluxing the particles in dilute phosphoric acid. This results in the conversion of several layers of zirconia to zirconium phosphate. Overly aggressive treatment completely converts the oxide to the phosphate which has a layered structure whose solid is mechanically weak and useless for HPLC. At neutral pH the column can be exposed to continuous flushing with thousands of column volumes of the phosphate free mobile phase [61]. This material is a cation-exchanger, which has been used for the separation of cationic proteins and of immunoglobulins from fermentation broths [61]. The phosphated zirconia was also used for separation of aminoacids [62].

When the phosphate treatment is sufficiently extensive, it completely counteracts the effect of the hard Lewis acid sites, allowing for good chromatographic separations of hard Lewis bases. This phase is also quite stable. No change in retention or selectivity is observed up to pH 10. However, above this pH and in the absence of phosphate in the eluent, the surface phosphate groups begin to desorb, changing the properties of the material. This can be partly reversed by flushing the column with a phosphate solution at elevated temperatures. Phosphate coated zirconia which was loaded with copper ions was used to separate aminoacids and proteins by ligand exchange chromatography [63].

Polybutadiene-coated zirconia can also be modified by phosphate for the separation of peptides [58] and proteins

[64]. However, while some separations are possible this material does not function at all well or nearly as well as silica-based phases for the reversed-phase separation of peptides and proteins. The combination of the reversed phase, cation-exchange and residual ligand exchange is chromatographically too complex for large, charged bioamines. A polyphosphate-modified zirconia was found to be useful as affinity packing for immobilization and purification of biomacromolecules [65].

1.2.2.2. Fluoride. At first glance the zirconia-fluoride (F-ZrO₂) phase appears to be similar to the zirconia-phosphate phase, but F-ZrO₂ behaves very differently. First, in order to prepare F-ZrO₂, one need only flush a solution of fluoride ion through the column [3]. This phase is excellent for the separation of proteins; it possesses unique selectivity compared to other zirconia-based ion-exchange phases. Apparently, this material acts as a ligand exchange (or metal affinity) material analogous to calcium hydroxyapatite [4]. Fluoride modified zirconia was shown to have an exceptionally high capacity for proteins [4] thus it was examined for preparative protein purification [66,67]. Washing the column with fluoride solution easily regenerates it. If retention decreases, flushing the column with sodium hydroxide solution to remove any strongly adsorbed materials (e.g. proteins) and then re-equilibrating the column with a fluoride buffer fully regenerates the phase. This phase cannot be used at low pH due to the formation of hydrofluoric acid, which is detrimental to the liquid chromatograph and the detector's windows.

1.2.2.3. Phosphonate EDTA analog. The utility of the phosphate and fluoride phases led to the search for other hard Lewis bases that might provide different selectivities. The treatment of zirconia with a phosphonate analog of EDTA, ethylenediamine-*N,N'*-tetramethylphosphonic acid (EDTPA) resulted in a very useful stationary phase [59,68]. Refluxing zirconia particles in a dilute solution of EDTPA gives a cation-exchange material that has unique selectivity. It is capable of separating highly basic proteins. Proteins with *pI*s less than 6.8 are unretained on the support. Biocompatibility of the phase results in high mass recoveries for proteins. The support is commercially available (ZirChrom-PEZ). The strong Lewis acid sites on the zirconia surface are effectively blocked, producing a biocompatible stationary phase. Due to the presence of the aliphatic segment in EDTPA, EDTPA-ZrO₂ is able to separate proteins and other solutes that phosphate-zirconia cannot. This phase has been successfully used to achieve highly purified monoclonal antibodies from a cell culture supernatant with excellent recovery of biological activity [59]. In the following paper from the same group modified spray-dried zirconia microspheres with EDTPA were used for semipreparative separations of monoclonal antibodies from cell culture protein contaminants [69]. Also PBD-zirconia has been modified with EDTPA and is com-

mercially available as ZirChrom-EZ. ZirChrom-EZ presents an alternative to conventional zirconia-based reversed-phase supports for applications requiring volatile mobile phase additives. The deactivation of Lewis acid sites on the zirconia surface allows the chromatography of Lewis base analytes such as carboxylates, sulfates, and phosphates using volatile mobile phase additives such as acetate or formate which are compatible with MS detection throughout the pH range of 1–10 [214].

1.3. Introduction to polymer coating

The lack of chemical and pH stability of silica and silica-based bonded phases [70,71] has been the driving force behind the search for alternative supports [1,72]. Polymer-coated stationary phases that combine the mechanical properties of porous metal oxides, such as silica, alumina, zirconia and titania, with the versatility of organic polymers has been the major focus of this search [73–76]. Such composite materials show great potential, as ideal chromatographic supports that are mechanically and chemically stable, possess minimal non-specific adsorptivity and allow the flexible tailoring of chromatographic selectivity. The first polymer-coated material for LC was reported by Horvath et al. [77] in the 1960s. They coated a non-porous glass with polystyrene cross-linked with divinylbenzene. For chemically unstable silica, polymer coatings were deposited to shield the silica from interactions with aggressive eluents and to prevent undesirable silanophilic interactions. For the chemically more stable metal oxides, such as alumina, zirconia and titania, polymer coatings are used to mimic the different bonded-phase silane coatings used to make silica-based phases; additionally the polymer coating acts to lessen access to Lewis acid sites on the metal oxides. For example, polybutadiene resembles octyl or octadecyl groups while polystyrene possesses similar functionality to phenyl bonded silicas. Therefore, these two polymers are the most commonly used as deposits inside metal oxides [20,31,78–85]. In addition to polymers with hydrocarbon backbones (such as polybutadiene, polyacrylamides, etc.), polyethers, polysaccharides, polyamines, polynucleotides, polyamides, polypeptides and proteins, and polysiloxanes have also been immobilized on metal oxides. A survey by Petro and Berek [76] indicates that about one hundred polymers have been used for the modification of silica for liquid chromatography including all chromatographic modes (reversed phase, ion-exchange, chiral, normal phase, size exclusion, affinity, and hydrophobic interaction chromatography).

Depending on the availability of anchoring groups on the metal oxide surface these materials can be prepared either with or without covalent bonding of the polymer to the surface. Pre-synthesized polymers can be deposited directly or polymerization of monomers can be initiated and propagated on the surface [76]. Further cross-linking the chemically reactive groups in the polymer is highly desirable so as to

decrease the polymers solubility in the mobile phase and thereby enhance stability. Based on the chemical nature of metal oxide and the functionality of the polymer used, the synthetic approach is usually designed to achieve the following idealized properties:

- (1) A thin film that *does not block the pores* or restrict pore throats so as to maintain the overall pore structure and connectivity and thus give good mass transfer in the pores and in the polymer film.
- (2) A uniform coating to completely cover undesired non-specific interaction sites (such as silanol groups on silica, or Lewis acid sites on metal oxide surface).

However, polymer deposition/adsorption is a complex process governed by a very subtle balance between energetic and entropic factors [86]; in our opinion *the resulting coating is invariably patchy and uneven* to a greater or lesser extent. To adsorb the polymer on a metal oxide, we must first introduce the polymer, dissolved in a good solvent, into the pores. Polymers in good solvents configure themselves into a “Gaussian coil”—the segments expand and the polymer is swollen by the solvent. Fleer et al. [202] have shown that even after a polymer adsorbs onto a surface, the resulting coating remains significantly swollen by the solvent. The polymer segments are expanded roughly as far away from the surface as might be expected based on the polymers radius of gyration.

Even this simplified idea from polymer physics has far-reaching implications for chromatographic materials. First, the swollen polymer may not be able to enter pores whose throats are too small. Second, adsorption onto the surface may be much more hindered than one might first imagine—as long as good solvent is present, the segments still have a strong entropic driving force to extend into the solvent, and the still-swollen state of the adsorbed polymer layer can greatly limit the gain in enthalpy given by adsorption on the surface. While it is true that the good

solvent is usually removed (e.g., replaced by air or vacuum when drying), the amount of polymer that can remain at the surface may be limited by the amount that could be established while the good solvent was present. Fourth, as the good solvent is removed (e.g., by drying) the polymer can flow with the solvent and can diffuse while still wet—one cannot simply assume that the polymer will stay put in a perfectly uniform layer.

Some groups have shown that loading with increasing amounts with different amounts of polymer [75,87] increases retentivity. However, higher polymer loads lead to thicker films which ultimately block pores and lead to substantial decreases in column efficiency. Neimark et al. [88] have shown by fractal analysis that PBD does not cover the surface of silica particles with a uniform film but rather it occupies the pore space. An increase in polymer load leads to a proportional decrease of the total pore volume and specific surface area. According to these authors [88] the polymer forms unconnected (non-continuous) inclusions (or “ganglia”) which only partially fill some pores but blocks access to others. These polymer inclusions are randomly distributed throughout the pore network. An increase in polymer content leads to a decrease in the permeability of the pore network because the inclusions disrupt the pore connectivity.

It has been shown that increasing the amount of polymer (PBD/silica [89], poly(methyloctylsiloxane) (PMOS) on silica [90] as well as PBD on zirconia [91,92]) decreases the surface area and pore volume. This is shown in Table 1.

Collins et al. [90] have shown that the deposition of a liquid polymer on silica’s surface proceeds in agreement with a linear distribution model as suggested by Hanson et al. [89] for PBD coating of a wide pore silica.

According to Collins et al. this does not mean that the distribution of the polymer on the surface is uniform. Due to the small pores in HPLC packings liquid polymers exist in pores as “plugs” or “drops” [90].

Table 1
Influence of polymer load on some physical characteristic of modified supports

Reference	Data	Load, pore volume or surface area					
[89]	g PBD/g SiO ₂	0	0.047	0.098	0.156	0.187	0.302
	Pore volume (cm ³ /g SiO ₂)	0.996	0.912	0.877	0.750	0.624	0.478
	Surface area (m ² /g SiO ₂)	111	103	97	87	75	60
[88]	g PBD/g SiO ₂	0	0.045	0.089	0.135	0.158	0.232
	Pore volume (cm ³ /g SiO ₂)	1.00	0.87	0.80	0.65	0.53	0.37
	Surface area (m ² /g SiO ₂)	111	99	88	75	63	46
[90]	g PMOS/g SiO ₂	0	0.0095	0.0301	0.117	0.254	0.440
	Surface area (m ² /g SiO ₂)	389	372	347	273	197	71.6
[91]	PBD/ZrO ₂ (%C)	0	2.2	4.1	6.1		
	Pore volume (cm ³ /g ZrO ₂)	0.174	0.153	0.126	0.099		
	Surface area (m ² /g ZrO ₂)	34.1	25.9	20.5	15.0		
[92]	PBD/ZrO ₂ (%C)	0	1.53	2.68	3.91	5.59	
	Pore volume (cm ³ /g ZrO ₂)	0.17	0.15	0.13	0.13	0.094	
	Surface area (m ² /g ZrO ₂)	34.3	28.1	23.1	20.6	14.7	

Reeder et al. [93] have considered three possible models for the deposition of polymer (PBD) on zirconia's surface:

- The uniform, smooth coating model—where the polymer uniformly covers the whole surface. The thickness of the polymer layer is assumed to be uniform over the entire surface regardless of the size of the pore presenting that surface. This model assumes that the polymer has the opportunity to adsorb on any surface, and that characteristics of the adsorption isotherm (e.g., osmotic pressure gradients arising from concentration of segments at the surface) cause the adsorbed thickness to self-regulate to be fairly uniform.
- Volume proportional loading, smooth coating—where the thickness of the polymer layer is assumed to be a fixed fraction of the diameter of the pore presenting that surface since a larger pore can house a larger volume of polymer solution. This model assumes again that the polymer has the opportunity to adsorb on any surface, but this model now assumes that adsorption is very favorable and so the thickness is limited only by the local mass of polymer available to adsorb. A wider pore will contain more polymer solution, perhaps enabling a thicker coating to develop. thickness of the polymer is not uniform. The volume fraction in the pore is uniform.
- Polymer does not form a uniform layer on the pore surface, rather it forms a plug. This model assumes that adsorption is unfavorable, but as the meniscus of the solvent retreat from large pores, most of the polymer leaves the large pores. The solvent departs last from the smallest pores, and so it is assumed that the bulk of the polymer finally comes out of solution (either adsorbed or simply precipitated) in the smallest pores. decreasing the pore volume without changing the diameter.

According to these results: at low loadings PBD deposits in thin layers on the surface with no preference for filling either large or small pores. At higher loadings PBD deposits preferentially in small pores. All available porosimetric data [20,56,93,94] confirms this mechanism. The data available for PBD-coated silica [88] and zirconia [92] show that there are some differences between the two. Fig. 1 presents the influence of the relative volume of PBD (to pore volume of the support) on the blockage of pores. The slope for zirconia is roughly equal to 1 while that for silica is 2.6. That means that PBD on zirconia only removes that volume which it occupies itself and it does not block access to unoccupied pore space. In stark contrast, the same quantity of PBD on silica blocks 2.6 times more pore volume than that needed by the mass of the polymer and the polymers bulk density.

Since the amount of pore volume on silica that is blocked by a given amount of PBD is much greater than on zirconia, we infer that PBD breaks down the pore connectivity to a much greater extent on silica than on zirconia. The dependence of pore diameter and surface area on blocked pore volume shown in Fig. 2 confirms the above statement.

For silica a slow and gradual decrease of both pore diameter and surface area is observed with the increase of pore blockage. Zirconia data are different: deposition of PBD results in an increase in pore diameter. This means that PBD mainly occupies the smaller pores. Specific surface area of PBD-coated zirconia versus amount of PBD decreases faster than that on PBD-silica. On PBD-zirconia the polymer occupies the smallest pores that become inaccessible. Thus, the average diameter of pores apparently increases (as small pores are not counted in the average). On PBD-silica the polymer blocks also some unfilled pores (with perhaps higher diameters) by disrupting the pore connectivity. Thus, a slow decrease in mean pore diameter is observed upon

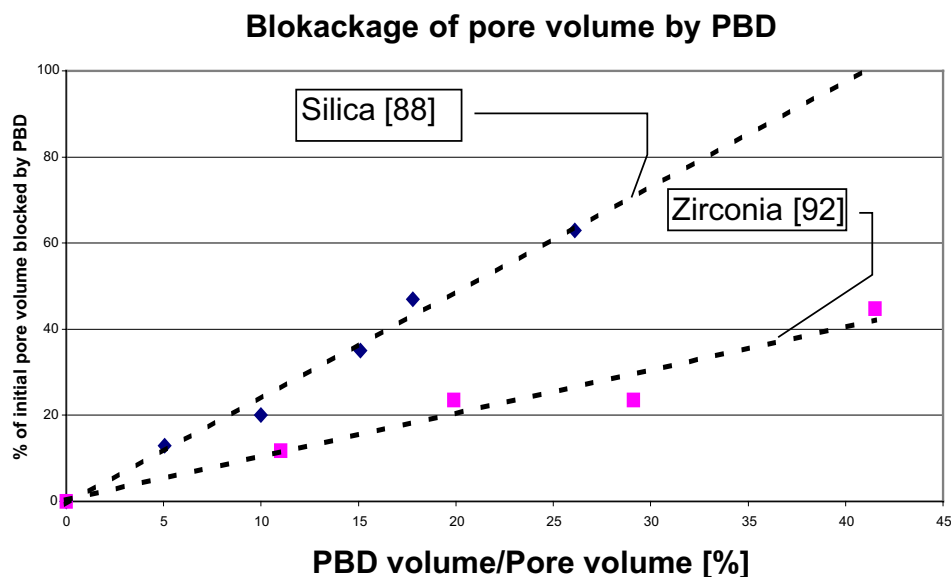


Fig. 1. Blockage of the silica and zirconia pores of by PBD (PBD volume is normalized by the initial pore volume).

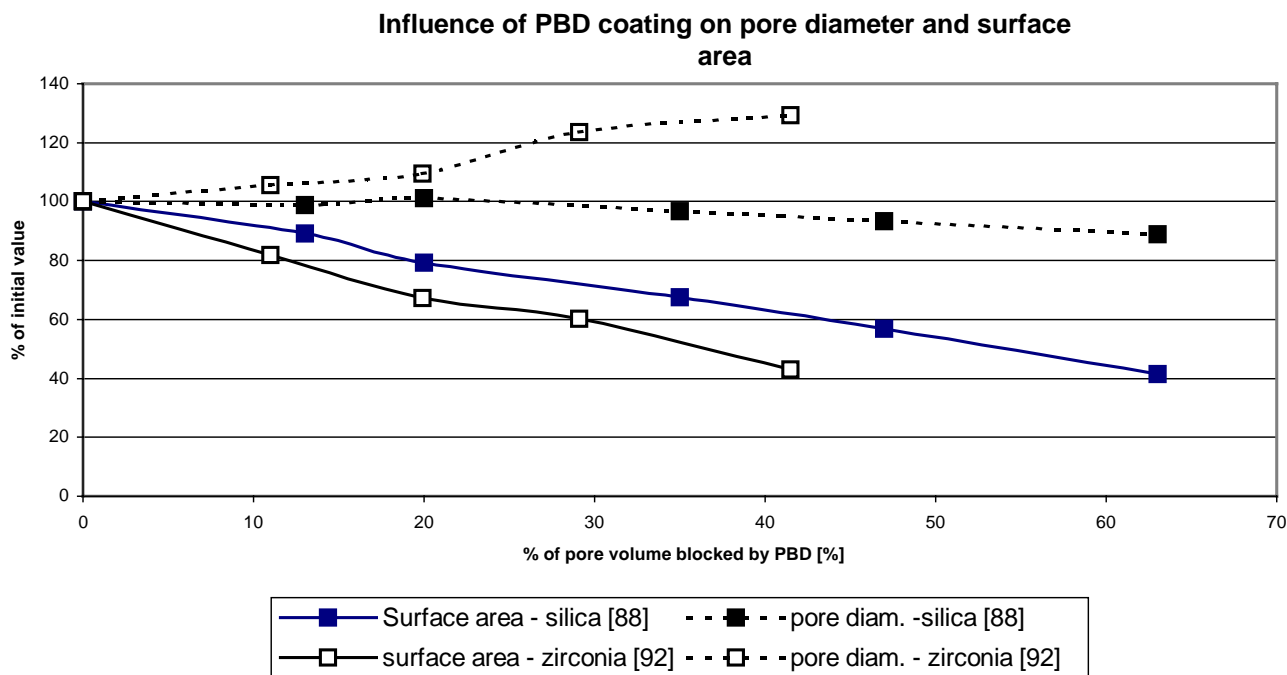


Fig. 2. Influence of PBD coating on pore diameter and surface area for silica and zirconia.

increasing the amount of PBD. Many studies have demonstrated that polymer-coated metal oxides exhibit excellent column performance with respect to column efficiency (plate count), pH stability/column life, peak symmetry for troublesome analytes and chromatographic selectivity. In stark contrast to totally polymeric phases most polymer-coated metal oxides show column efficiency comparable to silica bonded phases [74,80,87,92,95,96]. Polymer-coated alumina [20,42,74] and zirconia [20,28,80,81] have the high chemical stability of the base material while polymer-coated silica phases [87] show better pH stability than do the conventional silica bonded phases. Polymer-coated zirconia is very attractive for the separation of basic compounds owing to its excellent stability at extremely high pH and the absence of silanophilic interactions. Good shielding of silanol groups by polymers also makes polymer-coated silicas more suitable for the separation of basic solutes, peptides and proteins [20,75,87,97].

Despite the progress made in this area and the advantages pointed out above, the adoption of polymer-coated

metal oxides by practicing chromatographers has been slow. More research is needed to optimize the polymer coating processes and to fully understand the complex retention characteristics on polymer-coated metal oxides. However, commercial polymer-coated zirconias and aluminas are available. ZirChrom-PBD is a zirconia-based support while Aluspher RP-select B (Merck), Millipore PBD, and Unisphere Al-PBD (Biotage) are aluminas coated with polybutadiene; ZirChrom-PS is a polystyrene-like-coated zirconia. The properties of polybutadiene packings are described in the next section. Commercially available polymer-coated aluminas and zirconia are compared in Table 2.

1.3.1. Polybutadiene-coated metal oxides

Coating silica gels with polybutadiene has been used as a method for improving silica's stability at higher pH or for deactivation of silanophilic interactions [99]. There have been numerous studies on the use of PBD-coated oxides as reversed-phase materials [20,30,38,39,55,56,58,64,74–76,78,80,85,88,89,91–93,95,100–110]. In addition,

Table 2
Commercially available polymer modified aluminas and zirconia [38,55,78,98]

Alumina	Manufacturer	Particle diameter (μm)	(%C)	Pore size (nm)	Surface area (m^2/g)
Aluspher RP-select B	Merck	5		10	170
Unisphere PBD	Biotage	10	5.1	22.1	37
Milipore PBD	Milipore	5	7.2	9.2	110
GammaBond TM Alumina RP-1 (PBD)	ES Industries	5		8	
GammaBond TM Alumina RP-8 ^a	ES Industries	5		8	
ZirChrom-PBD	ZirChrom	3 or 5		30	30
ZirChrom-PS	ZirChrom	3 or 5		30	30

^a Alumina coated with polysiloxane polymer containing *n*-octyl groups.

polyethylene (PE)-modified titania has been described by Zaharescu et al. [111]. The properties of the PBD-silica and its use in chromatographic separations were reviewed [74–76,85]. Until recently, the focus of these studies was on the silica particles, however, many of the conclusions including the physical state (loading) of the polymer within the particle pores and their properties as a RPLC stationary phases should also apply to zirconia-based PBD phases. The preparation of PBD-alumina and zirconia are very similar to that of silica; by and large the procedure of Schomburg et al. [95] has been followed. Considerable work has been done on PBD-aluminas. Schomburg proposed the use PBD for the modification of alumina and found that the modified phases could be used with aggressive mobile phases even at pH 13. Excellent separation efficiency of PBD-alumina was found [74]. In a series of papers Arenas and Foley [30,38,55] described the reversed-phase properties of PBD modified aluminas using commercially available packings.

Simple non-electrolytes are retained on PBD-ZrO₂ exclusively by a reversed-phase mechanism. The extent of Lewis acid-base interactions on PBD zirconia for simple non-electrolytes was estimated for 26 different solutes by examining retention for mobile phases with and without fluoride. Of the 26 solutes only *p*-chlorophenol was shown to interact with the Lewis acid sites [106]. Fig. 3 illustrates similar example for 22 compounds, normalized selectivity is compared for siliceous bonded phase (Luna), polymeric packing (PLRP) and ZirChrom-PBD.

PBD modified oxides offer high pH and thermal stability: Alumina covered with hydrophobic polymer shows stability in $3 < \text{pH} < 12$ [112,113], while PBD-zirconia is stable in $1 < \text{pH} < 14$ [58,64]. PBD modified zirconia shows exceptionally high stability in alkaline conditions; there was no evidence for degradation of the support even after exposure to 1 M NaOH or at 100 °C [81,107] and then at 200 °C

[103] even during prolonged use. According to Wu et al. [114,115] PBD zirconia is stable up to 320 °C under solvating gas chromatography conditions with carbon dioxide as the mobile phase. Thus, the high thermal stability of PBD zirconia paves the road for using the packing at much higher temperatures than usually applied in HPLC. Higher temperature improves the column efficiency mainly by increasing the diffusional rate in the stationary phase. Since the increased temperature decreases the “C-term” in the Knox or van Deemter plots it enables the use of higher mobile phase flow rate. This, in turn, speeds up analysis time [107] (see also a Section 2). It has also been noted that the selectivity of PBD/zirconia changes upon increasing the column temperature [88,107,108,116].

1.3.1.1. RPLC properties of PBD-coated zirconia. The reversed-phase properties of PBD-coated zirconias have been extensively and systematically evaluated [20,56,91,92,106] and compared to conventional bonded RPLC phases such as C₁₈- and C₈-silica phases. It is generally concluded that the PBD phases have essentially the same properties as conventional phases. When the probe solutes do not interact appreciably with the surface of zirconia or silica, the retention and selectivity characteristics of PBD phases are expected to be very similar to that of the conventional bonded phases and this is indeed the case for simple non-electrolyte solutes. However, it is generally found that charged or ionizable solutes behave quite differently on PBD-coated zirconia and conventional bonded phase silicas (see, e.g. Fig. 14 in Part I). A brief discussion on the RPLC properties of the PBD phases follows.

1.3.1.2. $\ln k'$ versus n_{CH_2} . It has been repeatedly demonstrated that the $\ln k'$ in RPLC is linearly related to the number of methylene groups of a homologous series of solutes,

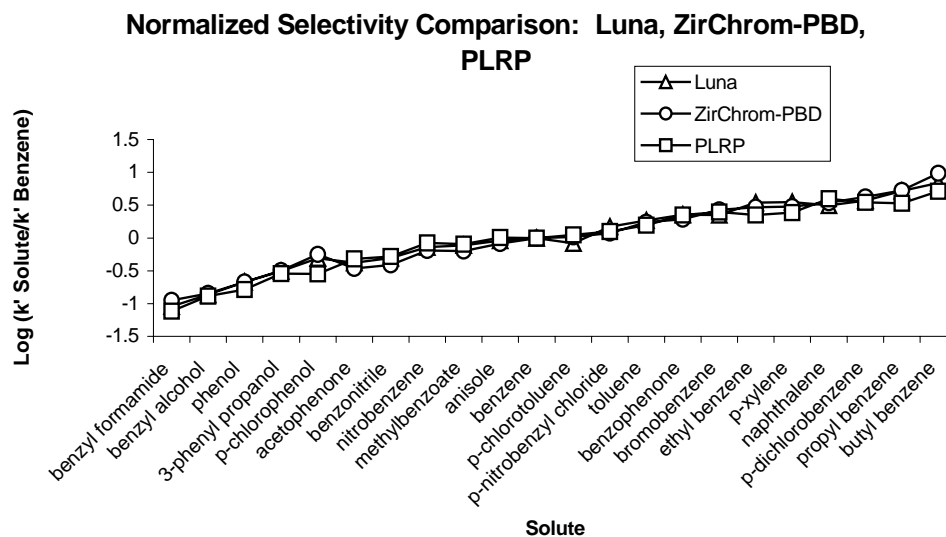


Fig. 3. Normalized selectivity comparison: Luna, ZirChrom-PBD, PLRP. LC conditions: mobile phase, 40/60 ACN–water; flow rate, 1.0 ml/min; temperature, 30 °C; injection volume, 5 μ l; detection at 254 nm.

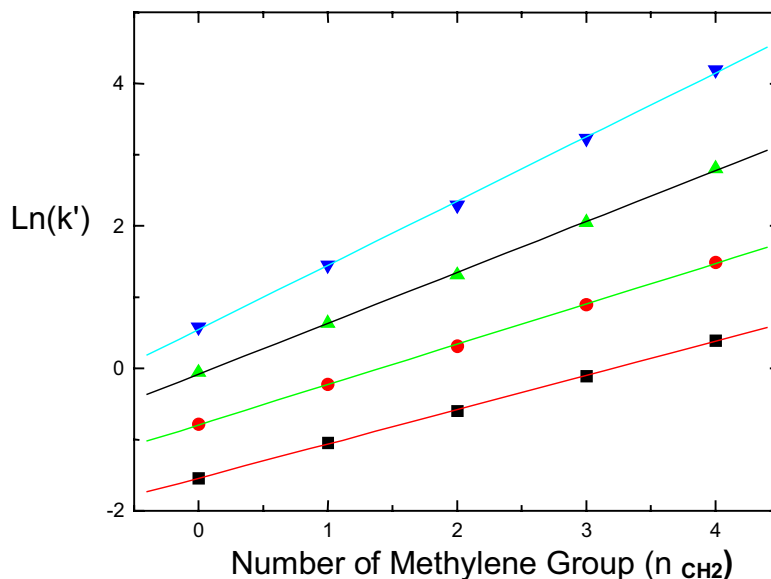


Fig. 4. Dependence of $\ln k'$ on the number of methylene groups for a homologous series of alkylbenzenes at different ACN–water mobile phase compositions. The carbon load is 1.53%. The mobile phase concentration from top to bottom: 20–50% ACN. The solutes are benzene, toluene, ethylbenzene, propylbenzene, and butylbenzene (from left to right).

and obviously retention should increase as the number of methylene groups increases [57,117]. In order to examine the reversed-phase nature of PBD phases, retention data for a homologous series of alkylbenzenes have been obtained under different carbon loads and mobile phase compositions with both ACN and MeOH modifiers [56]. Fig. 4 shows an example of the data obtained by plotting $\ln k'$ against the number of methylene groups (n_{CH_2}). This rather typical data set shows that there is a very good linear relationship between $\ln k'$ and n_{CH_2} at each mobile phase composition. This conclusion has been obtained for different carbon loads and different types of modifiers [56]. Furthermore, the slopes of the plots in Fig. 4 are not dependent on the car-

bon load (see below). Accordingly, the retention behavior observed strongly suggests that the PBD-coated zirconia is truly a reversed-phase-like material [57]. Fig. 5 shows the methylene selectivity data for zirconia, alumina, and silica. Although the packings varied rather substantially in their carbon content the methylene selectivity observed for alumina and zirconia are similar while that for C_{18} -silica is only slightly higher. We note in passing the similarity in slopes supports the concept of a partition like model of bonded phase chromatography, that is, the view that the solute molecules in the stationary phase penetrate or “dissolve” into the bonded phase and do not just contact the interface between the bonded phase and the mobile phase fluid

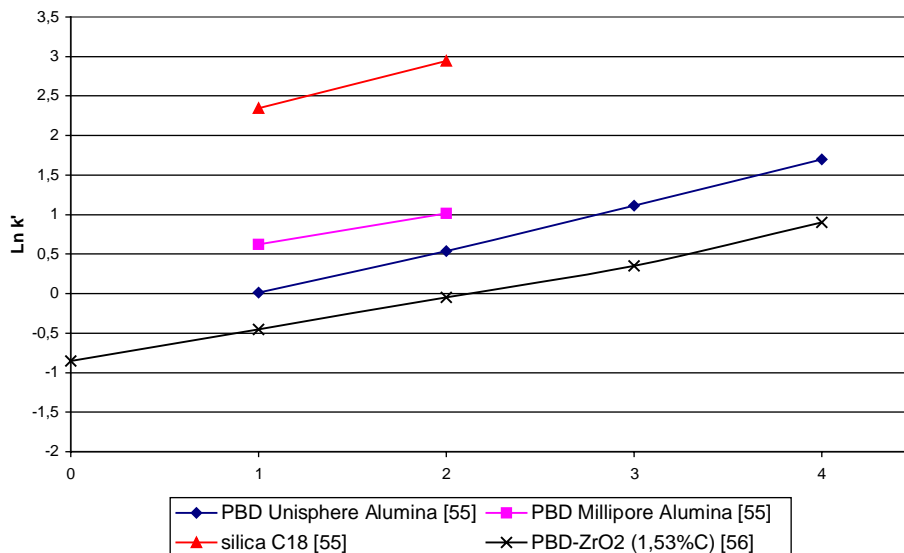


Fig. 5. Dependence of $\ln k'$ on the number of methylene groups for a homologous series of alkylbenzenes at 40% ACN–water mobile phase.

[118–121]. This argument is based on the concept that PBD coatings are very disordered and liquid-like in density. The similarity of the slopes of conventional bonded phases, PBD coatings and of distribution from aqueous–organic mixtures into bulk *n*-hexadecane suggests a very similar retention mechanism.

1.3.1.3. $\ln k'$ versus % ACN and MeOH. Another important aspect of PBD-coated zirconia phases as related to their use in RPLC is the effect of mobile phase composition on retention. This is particularly important in HPLC method development because varying the eluent composition is the principal means of optimizing the separation [56]. It is generally observed for conventional bonded phases that the retention of non-ionic species decrease monotonically as the amount of organic modifier in the mobile phase is increased. The decrease in $\ln k'$ is quasi-linear with the volume fraction of organic modifier at least over a narrow range in solvent composition [122–126].

Fig. 6 shows the dependence of $\ln k'$ of benzene and toluene for the PBD phase on mobile phase composition. First, a very good linear relationship between $\ln k'$ and the volume fraction of the organic modifiers exists from 100 to about 35% methanol. As the fraction of methanol decreases below 35%, the relationship is less linear and a change in the amount of methanol has a smaller effect on solute retention. Second, the relationship between $\ln k'$ and the percent organic for acetonitrile (ACN)–water mobile phase is approximately linear from 20 to 50% and 50 to 90% (v/v), but overall the relationship is not precisely linear. Figs. 7 and 8 present related data for PBD-alumina and PBD-zirconia.

The dependence of $\ln k'$ on PBD-alumina is parallel to that of PBD-zirconia. It is interesting to note that $\ln k'$ values for PBD-zirconia are higher than those of PBD-alumina despite the fact that the carbon loading for Millipore alumina is considerably higher. Millipore PBD-alumina has a much higher specific surface area than zirconia. Although the $\ln k'$ versus ACN or MeOH plots for PBD-alumina are available only over a much shorter range of concentrations the plots seem to have similar non-linearity as for zirconia.

To examine the mobile phase effect in more detail, the relative mobile phase sensitivity of the PBD phase to that of the ODS phase for a methylene group is also compared [56]. Fig. 9 shows the dependence of the selectivity $\ln \alpha_{\text{CH}_2}$ of a methylene unit on the volume fraction of organic modifier.

The relationship between $\ln \alpha_{\text{CH}_2}$ and percent organic is approximately linear over the whole concentration range of methanol for methanol–water mobile phase, whereas this is true only from 0 to 40% (v/v) of acetonitrile. These results are very consistent with those obtained for ODS phases by Karger et al. [127], and the mobile phase sensitivity of the PBD phase is essentially the same as the conventional ODS phase. This conclusion is also supported by the results obtained for many ODS columns and solutes [56]. This is also shown in Fig. 10.

It is evident from the above that for non-electrolytes (but not for carboxylic acids or cationic solutes) PBD-zirconia is a true reversed-phase material and behaves very similarly to conventional C_{18} -silica phases [205–208].

1.3.1.4. Effect of PBD loading on k' . Absolute retention as measured by k' should increase as more PBD is loaded on the support. Fig. 11 shows the absolute retention of benzene

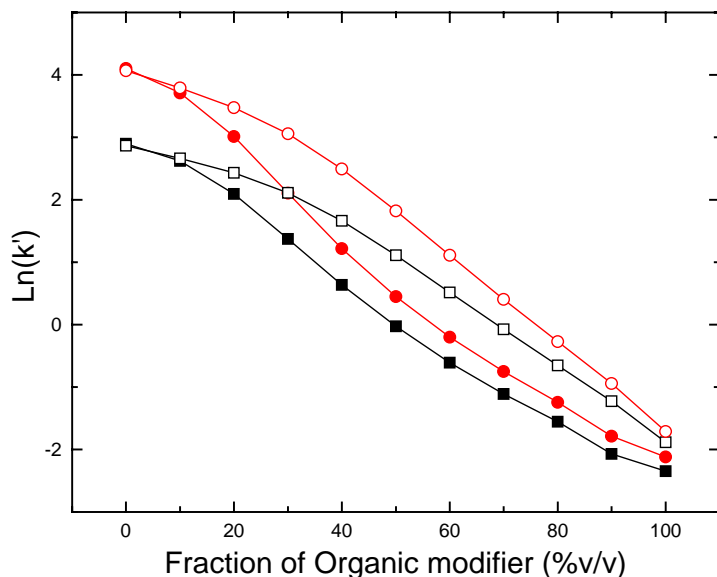


Fig. 6. Plot of logarithm of capacity factor vs. the volume fraction of organic modifiers (methanol and acetonitrile). The polymer load of the PBD phase was 5.6% carbon. The solutes are benzene and toluene. The capacity factors of both solutes at 100 and 90% organic modifiers were extrapolated by the linear relationship between $\ln k'$ and the number of methylene groups. Square symbols denote benzene, circle symbols represent toluene. Open symbols denote methanol–water mobile phase; while filled symbols denote acetonitrile–water mobile phase. Reprinted from [56].

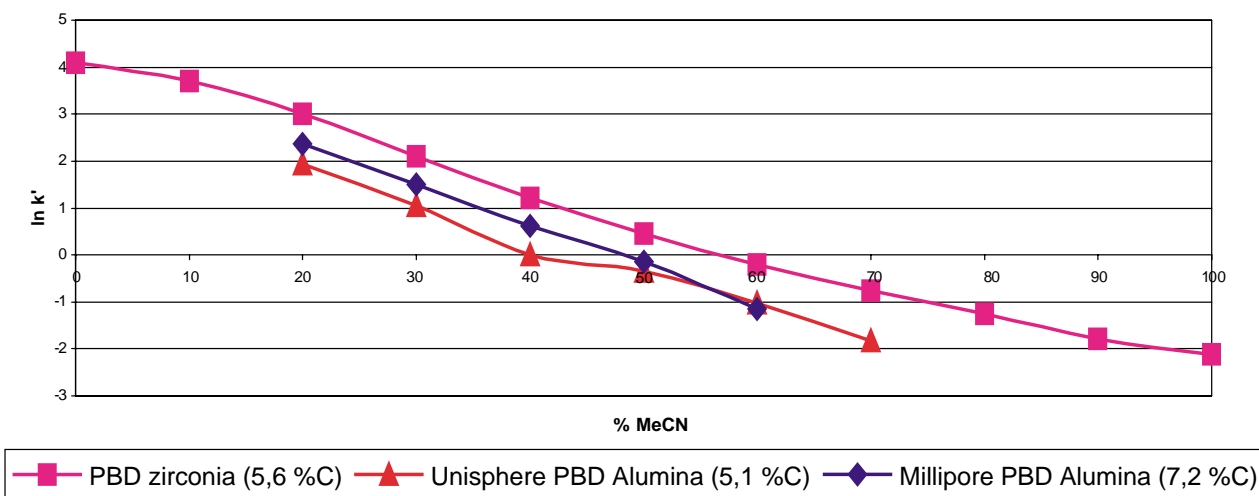
Dependence of $\ln k'$ for toluene on PBD alumina (Unisphere and Millipore) [55] and zirconia [92]

Fig. 7. Dependence of $\ln k'$ for toluene on PBD-alumina (Unisphere and Millipore) [55] and zirconia [92] for methanol–water mobile phase.

as a function of the amount of carbon on the PBD phase in two binary mobile phases [56]. It was further demonstrated [56] that the increase in the absolute retention with the carbon load can be attributed almost completely to an increase in the phase ratio and not to any change in the energetics of retention. It can be clearly seen in Fig. 11 that the absolute retention essentially increases linearly with the amount of PBD on zirconia. Berendsen and de Galan [128] have observed similar trends for the conventional RP bonded phases.

Similar properties of PBD on silica were also observed [75,85]. The retention of alkylbenzenes increased linearly with the PBD loading up to 4% (w/w), and then showed an upward swing to higher retention at much higher loads (8%). The dramatic increase in retention was explained by

the formation of bulky polymer clusters. It is noted that there is a slight upward trend in Fig. 11 at about 3% carbon load.

1.3.1.5. Effect of amount of PBD on HETP. The kinetic properties of PBD-coated zirconia have been thoroughly studied [92]. It was concluded that the PBD loading significantly affects the plate count. As predicted by the kinetic theory of chromatography and exhibited by conventional bonded ODS phases, the column efficiency is dependent on the absolute retention (k'). However, the most significant difference of the PBD phase from the conventional bonded phases is the dependence of C coefficients of the Knox equation on the PBD loading [92]. Fig. 12 shows the dependence of reduced plate height on the reduced velocity [56].

$\ln k'$ for toluene on PBD alumina (Unisphere and Millipore) [55] and zirconia [92]

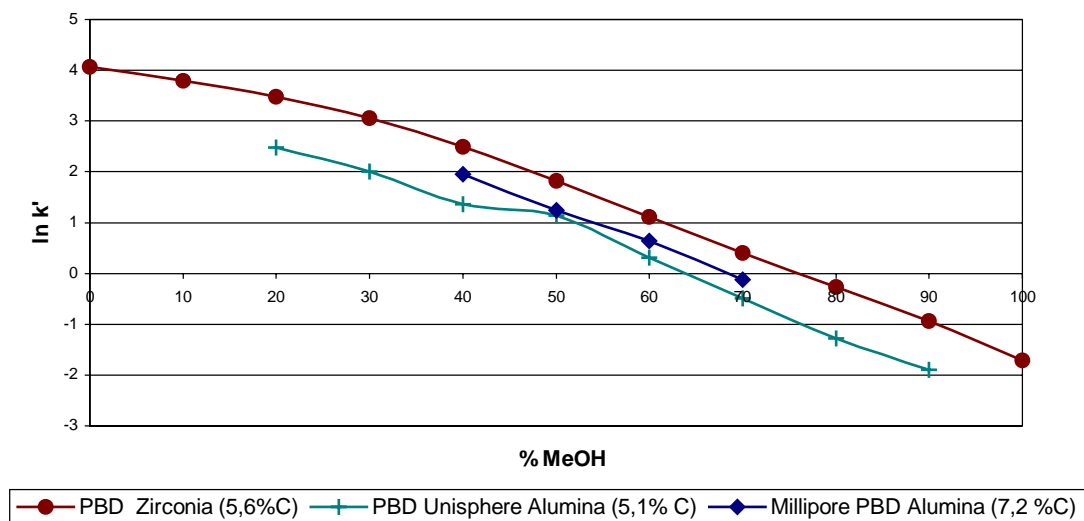


Fig. 8. Dependence of $\ln k'$ for toluene on PBD-alumina (Unisphere and Millipore) [55] and zirconia [92] for acetonitrile–water mobile phase.

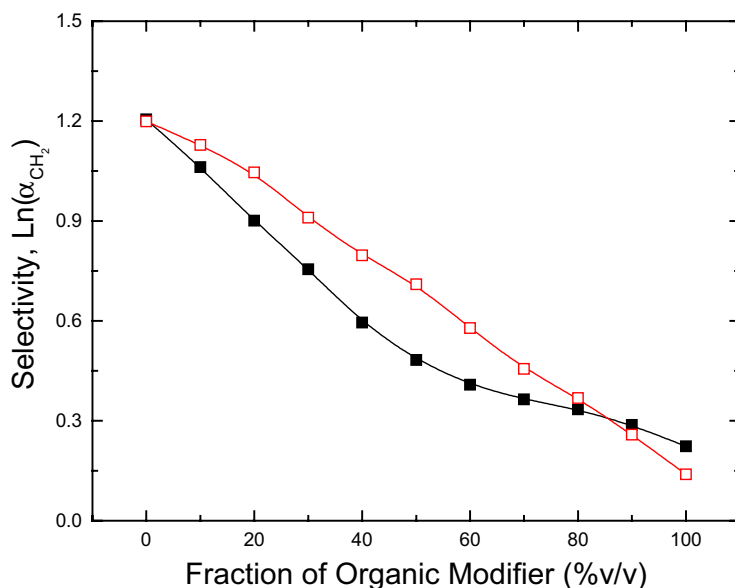


Fig. 9. Plot of the methylene group selectivity vs. the volume fraction of organic modifiers on PBD zirconia. All conditions are the same as in Fig. 6. The methylene group selectivity was computed by the slope of $\ln k'$ vs. n_{CH_2} plot. Open square and filled square denote methanol–water and acetonitrile–water mobile phases, respectively. Reprinted from [56].

The slopes of the curves at higher velocity are essentially the C coefficients. It is quite evident in Fig. 12 that the slope for the polymer load at 5.6% (weight of carbon/weight of zirconia) is significantly larger than the slopes observed at lower polymer loads. Furthermore, for a 5.6% polymer load,

the C coefficient on average is about 0.2 for solutes with k' ranging from 2 to 8. Typical C coefficients for excellent bonded phase columns are 0.03–0.05. The large C coefficient obtained with heavily loaded PBD-ZrO₂ reflects the relatively large mass transfer resistances. It was suggested

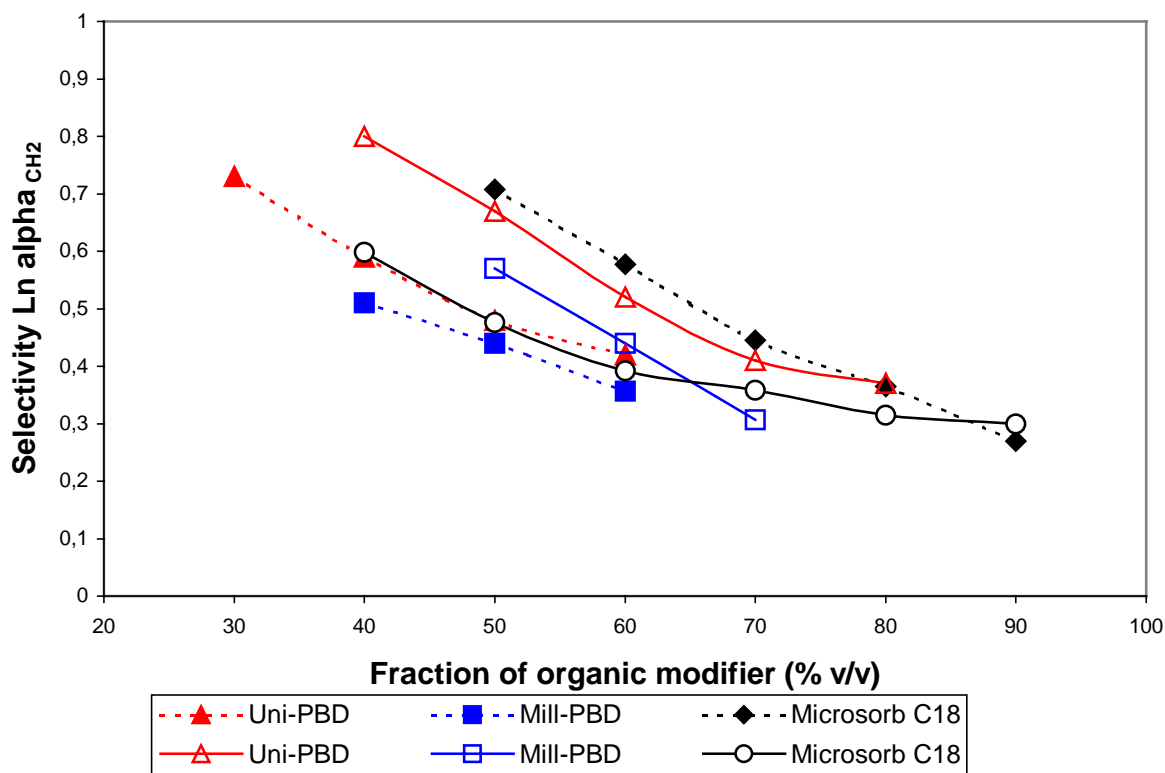


Fig. 10. Plots of the methylene group selectivity vs. the volume fraction of organic modifiers on PBD-alumina and C₁₈-silica. Open symbols for ACN–water; filled symbols for MeOH–water (based on data from [30,38,55]).

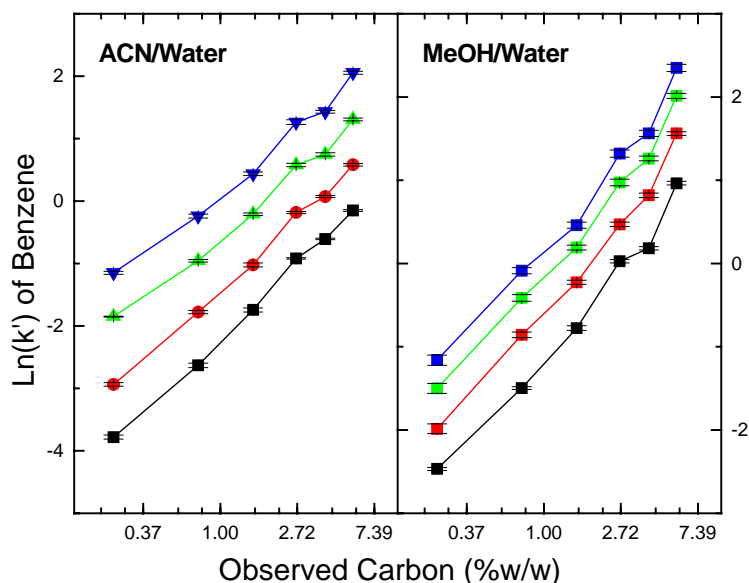


Fig. 11. Plot of the absolute retention of benzene vs. the amount of carbon on zirconia. The retention of benzene was obtained as the intercept of $\ln k'$ vs. n_{CH_2} plot. Organic fraction (% (v/v)): 20, 30, 40, and 50 from top to bottom. Left plot: acetonitrile–water mobile phase, and right plot: methanol–water mobile phase. The abscissa is in logarithm scale. The absolute retention is obtained from the intercept of $\ln k'$ vs. n_{CH_2} .

that this is due to slow diffusion inside the particles due to the polymer restriction of the pores or to the high density of the polymer (see below). The decrease in separation efficiency at very high carbon load was also observed for silica-based PBD phases [85]. It is also evident in Fig. 12 that the minimum reduced plate height is about 4, which is somewhat higher than the typical value of 2–3 obtained for the conventional bonded phases [56]. This maybe related to the particle density of zirconia particles that causes difficulty in column packing. However, it should be noted that these results were obtained with early non-commercial columns.

Current commercial zirconia-based PBD columns packed with 5 μm particles have plate counts in excess of 100,000 plates per meter and are as efficient as the best silica-based columns packed with the same size particles [92]. Fig. 13 shows a comparison of averaged efficiency for six solutes for several zirconia, alumina, silica and polymeric columns.

1.3.1.6. Effect of PBD load on LSER Coefficients. The similarity of retention of non-ionizable compounds on PBD-ZrO₂, ODS (Luna) and a polymeric type reversed-phase support (PLRP) is shown in Fig. 3. The figure shows

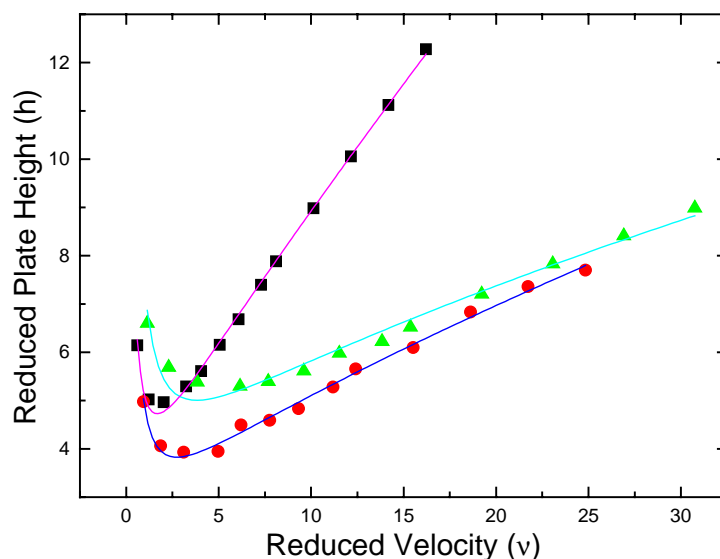


Fig. 12. Plots of the reduced plate height against the reduced velocity for column on three different carbon loads. The retention of the solutes is similar. See Table 2 of [92] for conditions. Symbols: filled square, 5.6% carbon ($k' = 2.1$ for phenyloctane); filled circle 2.68% carbon ($k' = 2.3$ for 1-phenylundecane); filled upper triangle, 1.53% carbon ($k' = 1.9$ for 1-phenylundecane). Replotted from the data [92].

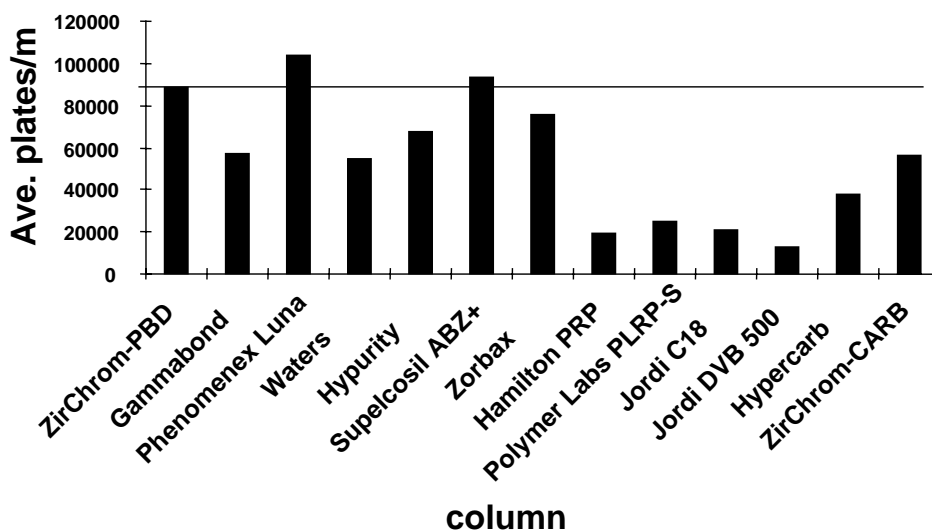


Fig. 13. Efficiency comparison using the reversed-phase test mix. Mobile phase, 65/35 acetonitrile–buffer (50 mM phosphate at pH 3.2); flow rate, 1.0 ml/min; temperature, 21 °C; detection at 254 nm; solute concentrations: uracil = 0.04 mg/ml; phenol = 0.4 mg/ml; pyridine = 0.15 mg/ml; 4-butylbenzoic acid = 0.30 mg/ml; *N,N*-dimethylaniline = 0.15 mg/ml; toluene = 4 mg/ml.

that retention of non-electrolytes on PBD-coated zirconia is purely reversed phase.

The characterization of PBD-coated zirconia phases in terms of LSERs has been thoroughly performed [106]. It was concluded that, as is the case with conventional bonded phases, the solute's size and hydrogen-bond (HB) acceptor basicity are the predominant retention determining factors, and on the whole PBD-zirconia phases closely resemble conventional chemically bonded reversed-phase materials. Interestingly, an increase in the solute's basicity decreases retention more than on conventional bonded phases, so relative to their behavior on conventional phases, strong hydrogen bases and highly dipolar analytes, when compared to non-polar solutes, are slightly less strongly retained on PBD-zirconia than on conventional phases. PBD-zirconia and conventional phases are so similar that there should be little difficulty in transferring separation methods between phases [106]. The relative retention data for 27 solutes on PBD-ZrO₂ and ODS phase also confirm the similarity of these phases [101].

The LSER fitting coefficients other than $\ln k'_0$ (the intercept of the LSER fit which depends on phase ratio) are found to be independent of the polymer load [106]. LSER fits were also carried out for polyethylene-coated alumina, zirconia and silica [129].

1.3.1.7. Effect of PBD deposition methods on properties.

The amount of PBD loaded on zirconia affects their chromatographic properties (both kinetic and thermodynamic). The PBD-coated zirconia particles described above were prepared following a procedure published by Schomburg et al. [95]. That is, they were prepared by evaporatively depositing and cross-linking PBD on microparticulate porous zirconia [20,56,90,92,106]. It has been shown that the

amount of polymer deposited is nearly linearly proportional to the amount of PBD offered to zirconia over the range 0–8% (w/w) (Fig. 14) [56].

The slope of this plot is 0.7, indicating that about 70% of the polymer offered to the zirconia is permanently incorporated. The linear relationship in Fig. 14 also suggests that the pore space is not completely filled at even the highest amount of polymer studied. This conclusion is also supported by the calculated fraction of the interior volume occupied by the polymer, by elemental analysis, BET, and phosphate adsorption data [56]. Similar results were also obtained for silica-based PBD phase [85], and about 80% of the PBD offered was permanently deposited on the silica.

The physical state of PBD within particle pores (both silica and zirconia) was the subject of many intensive studies [20,56,75,85,88,89,91]. It was concluded that PBD coating of porous particles does not result in a uniform film of polymer but rather in an inhomogeneous loading wherein the bulk polymer is not so much on the surface but rather patches or clumps of PBD occupying the pore volume [75,85,88,89]. Upon increasing the PBD loading the pores become increasingly filled but the film does not become thicker. Furthermore, Kurganov et al. [20] concluded that the PBD layer within the porous particles should have a gel-like structure that contains a lot of small pores. Although our understanding of the physical state of the PBD phase within the pores is far from complete, it is clear that the polymer layers are quite thin at low loads although small pores and small pore necks become increasingly restricted at high loads. The large mass transfer resistance, that is the high C-term observed at high polymer loads is probably related to these pore neck restrictions. This is shown in Fig. 15.

The effect of the conditions for the deposition of polymers in the preparation of polybutadiene-coated porous

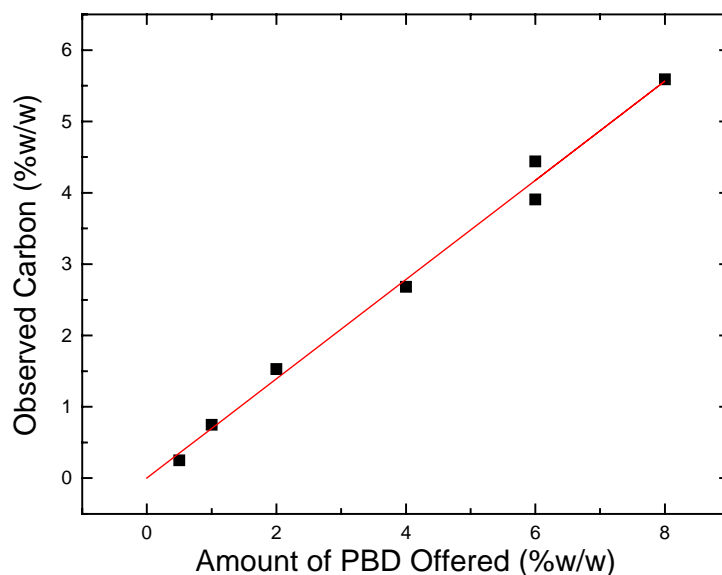


Fig. 14. Plot of the carbon load vs. the amount of PBD offered to zirconia. Both axes are denoted by weight percent relative to zirconia. The solid line is the linear regression fit with a slope of 0.7. Reprinted from [56].

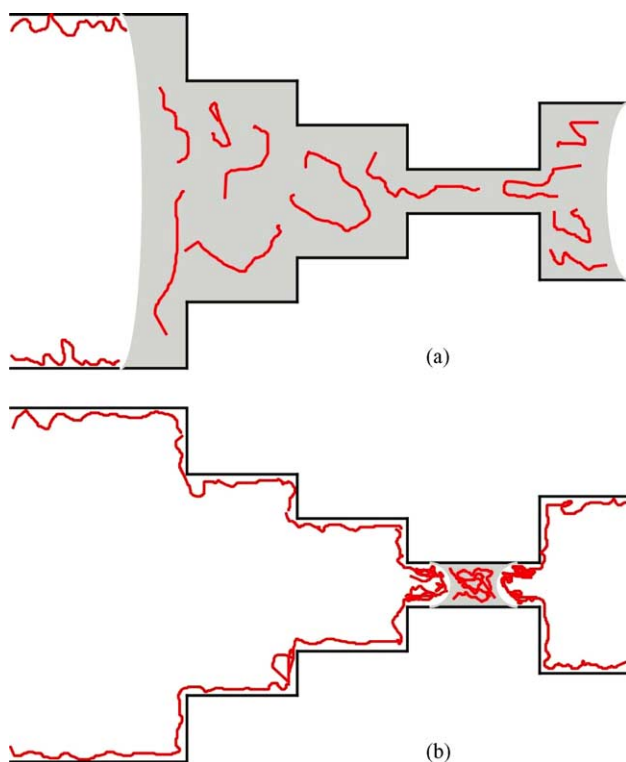


Fig. 15. Depiction of proposed mechanism for PBD deposition in porous zirconia. As the solvent is evaporated, leaving first from larger pores which are connected to the outer surface of the particle, the meniscus withdraws into smaller pores. As the meniscus withdraws polymer deposits on the surface up to some critical amount (a). The excess polymer, which is not deposited, is with the receding meniscus into smaller pores. As solvent evaporates from the smallest pores in a cluster, the polymer has nowhere to go and is deposited there (b).

zirconia particles for reversed-phase chromatography has also been evaluated [91]. Chromatographic performance is significantly degraded when the solvent is removed very slowly during the deposition process. No improvement results when the particles were loaded by using a series of small sequential loads of polymer. However, chromatographic performance improved when the particle surface is pre-coated with elemental carbon by a chemical vapor deposition (CVD) process. It is hypothesized that the polymer deposition process is controlled by the rate at which the solvent meniscus recedes during solvent evaporation, by the affinity of the polymer for the zirconia surface, and by polymer-solvent and polymer-polymer interactions.

1.3.1.8. Comparison of mixed-mode retention mechanism of organic bases on PBD-ZrO₂ and alkyl-bonded silica phases. As indicated above strong, hard Lewis bases adsorb tenaciously to the surface of zirconia. A significant fraction of the surface of PBD-coated zirconia remains quite accessible for interaction with Lewis base analytes and Lewis base constituents of buffers. The adsorption isotherm of inorganic phosphate, a very common buffer species in reversed-phase HPLC, becomes almost saturated at a surface coverage of 1.5–1.7 $\mu\text{mol}/\text{m}^2$ and independent of the adsorbate concentration of only 5 mM (ammonium phosphate) in 50/50 (v/v) acetonitrile–water on a typical commercial PBD-ZrO₂ phase [203]. It follows that the surface of this phase will have a very high negative charge over a wide pH range. In fact, such surfaces are negatively charged even at pH 2 and act as mixed-mode cation-exchange-reversed-phase media. Thus, polymer-coated zirconia phases in Lewis base buffers, at least superficially mimic, the mixed-mode retention of alkyl-bonded silicas wherein ionization of surface silanol groups provides the negative charge sites.

However, there are vast quantitative and qualitative differences in the retention processes as exhibited in the following cases:

- The fact that organic bases frequently have much larger k' values on polymer-coated zirconia [204] than on alkyl-bonded silica despite the much lower retention of purely hydrophobic species on polymer-coated zirconia due to the lower surface areas of zirconia-based phases compared to silica-based phases (see Tables 1 and 2).
- Very significant differences in band spacing and even elution sequence of organic bases on zirconia and silica-based phases [204,205,207] are commonly observed.
- The very strong effect of buffer type [205] on retention sequence of organic bases on PBD-ZrO₂ is commonly seen.
- The effect of buffer concentration on retention of organic bases on zirconia versus silica-based reversed phases [204,205] is much stronger. The slope of plots of $\ln k'$ versus $\log[\text{NH}_4^+]$, where ammonium is the displacing cation are much larger on PBD-ZrO₂ versus ODS-SiO₂.
- The exquisite sensitivity of retention of organic bases to the degree of substitution of (1°, 2°, 3° versus 4°) of the charged nitrogen site on PBD-ZrO₂ compared to alkyl-bonded silicas.
- The very strong dependence of the retention and selectivity of organic bases on polymer-coated zirconia on the degree of substitution and hydrophobicity of the cationic displacing agents [208].

Qualitatively all of the above argue strongly for a more substantial role of cation exchange versus reversed-phase processes for retention of organic bases on zirconia-based reversed phases in comparison to alkyl-bonded silica type phases. This hypothesis was validated by Yang et al. [206] in a quantitative study of the retention of a homologue series of *p*-alkyl benzyl amines as a function of the concentration of ammonium ion in the eluent. They postulated the existence of independent purely reversed phase and a “hydrophobically assisted” ion-exchange sites which lead to the following equation for the dependence of retention factor on displacer concentration for a univalent analyte:

$$k' = \frac{k'_{\text{rp}} + K_{\text{iex}}}{[\text{NH}_4^+]} \quad (1)$$

Based on Eq. (1), which fits all compounds studied from a concentration of ammonium ion of 5 mM and upwards it, was shown that frequently less than 20% of the retention of organic bases is due to the pure reversed-phase site on the PBD-ZrO₂ whereas more than 90% of the retention was due to pure reversed-phase interactions on the most silanophilic of the type B silica phases tested. This is illustrated in Fig. 16.

To summarize:

- PBD coating on zirconia is highly reproducible.
- PBD-coated oxides are chemically and thermally stable.

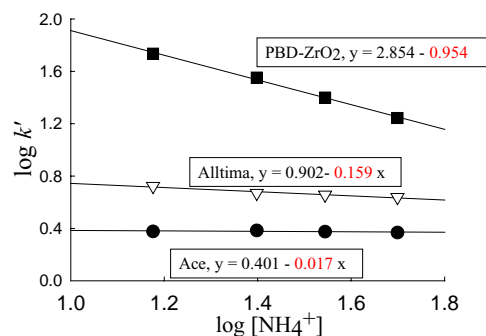


Fig. 16. Effect of counterion concentration on retention of charged species on silica-based packings and on PBD-zirconia. Conditions: 55/45 MeOH–ammonium phosphate buffer; pH = 6.0; $T = 35^\circ\text{C}$; solute: *p*-butylbenzylamine.

- PBD-coated zirconia and alumina show reversed-phase like selectivity similar to ODS-silica for non-ionizable solutes.
- Ligand exchange interactions cause low efficiency and poor peak shape for acid analytes unless an additive (a hard Lewis base) is present in the eluent.
- Reversed phase/cation-exchange mixed-mode retention is observed for basic solutes and it has a great impact on the chromatographic retention and selectivity relative to silica-based reversed phases.

1.3.2. Polystyrene coated metal oxides

While Carr and co-workers have most intensively studied PBD type zirconia-based phases they have also developed a novel family of polystyrene-coated zirconia (PS-ZrO₂) [80,130,131]. Sharygin et al. first reported PS-ZrO₂ in 1991 [132]. PS-ZrO₂ will serve as a useful alternative to PBD-ZrO₂ for analytes that cannot be resolved on PBD-ZrO₂ because the aromatic functionality of PS-ZrO₂ leads to different selectivity toward aromatic compounds. In addition, PS-coated oxide is readily modified with amine or sulfonate groups to generate ion-exchangers [130,133]. This section will briefly review the different approaches to the synthesis of PS-coated metal oxides and compare the chromatographic properties of PS-ZrO₂, PBD-ZrO₂, and other commercial RPLC supports.

1.3.2.1. Synthesis and characterization of PS-ZrO₂. As summarized in Table 3, there are basically four synthetic approaches to the preparation of PS-coated metal oxides. In Methods A and C, the copolymerization of two monomers takes place on the surface of metal oxides. Itabashi and co-workers have reported a number of polymer-coated silica sorbents by Method C, where divinylbenzene and chloromethylstyrene [134,135] or 4-vinylpyridine [136] formed a cross-linked network covering the surface of silica. Such polystyrene-coated silica gel can be further functionalized with triethylenetetramine [134] or diethyl iminodiacetate [135]. As opposed to Method C where there is no chemical bonding between the monomers and

Table 3
Comparison of synthetic approaches for preparing PS-coated metal oxides

Type	Starting material	Deposition mechanism	Synthesis scheme	Reference
A	Monomer (styrene and vinylsilane)	Chemical bonding (Si–O–M)		[79,137]
B	Pre-synthesized copolymer of styrene and vinylsilane	Chemical bonding (Si–O–M)		[20,80,82]
C	Monomer (styrene–divinylbenzene)	Physical adsorption		[134–136]
D	Polystyrene or analogs	Physical adsorption		[20]

the metal oxide surface, Method A applies styrene or its analog and another monomer, which can chemically react with the metal oxide; usually, a vinyl-substituted silane is used [79,137–139]. The chemical bonding to the surface enhances the stability and uniformity of the coated polymer on the oxide surface. The copolymerization can be achieved by either chemical initiation or γ -ray irradiation [137]. The common feature of Methods B and D is the use of a prepolymer. Kurganov et al. [20] studied both covalently bonded and adsorbed polymer coatings using polychloromethylstyrene–vinylsilane and polychloromethylstyrene, respectively.

Chromatographic separations of amine compounds with minimal tailing were achieved with both types of PS-coated supports. Lecourtier et al. [82] prepared PS-coated silica by the reaction of chlorinated silica with a living anionic polystyrene approach. However, this method results in polystyrene chains that are mainly outside the silica pores. Ghaemi and Wall [140] modified Kurganov's procedure for the synthesis of PS-ZrO₂. They hoped to prepare a uniform, thin layer of polymer on the metal oxide surface to achieve good column efficiency and complete hindrance of unde-

sired secondary interactions between the analytes and the metal oxide. However, there is no such ideal support. Some blockage of pores by the polymer is unavoidable due to the repulsive forces present when non-polar polymers are adsorbed or grafted onto highly polar metal oxide surfaces. Unfortunately, there is not enough data in the literature to allow a critical comparison of the pore structure and chromatographic performance of PS-coated oxides as prepared by the various synthetic methods.

1.3.2.2. Overview of chromatographic properties of PS-ZrO₂. Zhao and Carr [80] extensively compared the chromatographic properties of PS-ZrO₂ to those of PBD-ZrO₂. Because PBD-ZrO₂ [81] was prepared by Method D and PS-ZrO₂ by Method C, significant differences in the pore structure on the same base zirconia particles were observed between these two stationary phases. As shown in Fig. 17, the major difference between the two phases is the change in surface area in the small pore diameter region (<100 Å). Some micropores (<20 Å) seem to form on PS-ZrO₂. They may result from a presumably higher degree of cross-linking of the copolymer.

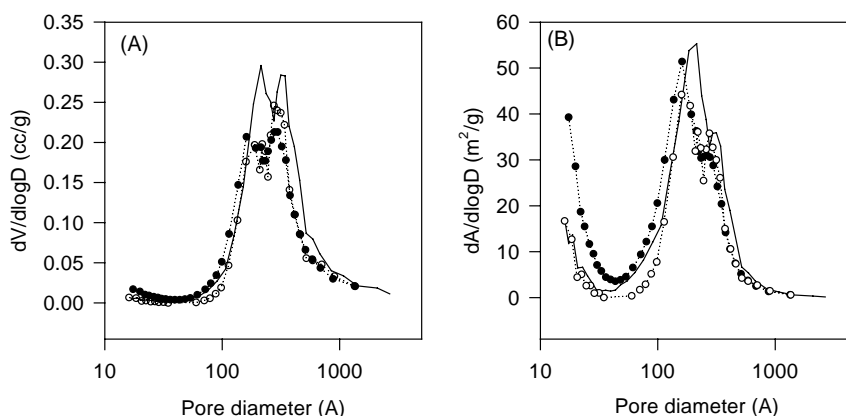


Fig. 17. Comparison of pore size distributions of PS-ZrO₂ and PBD-ZrO₂. (A) Pore volume distribution; (B) specific surface area distribution. The solid line is for bare zirconia while filled circle is for PS-ZrO₂ and open circle for PBD-ZrO₂. The carbon loadings for PS-ZrO₂ and PBD-ZrO₂ are 3.2 and 2.7%, respectively.

Also Kurganov et al. [20], who coated alumina supports with PS (Aluspher 100, Alusorb N200, and Alox T), found a significant increase in surface area. This too is probably related to the formation of micropores. The discrepancy between porosities as measured by N₂ sorbtometry (i.e. BET studies) and by inverse SEC studies indicates the possibility of swelling of the deposited polymer layer. Conversely, on PBD-ZrO₂, polymer seems to fill small pores, causing a substantial decrease in surface area. Micropores are usually undesirable because molecular diffusion and mass transfer within small pores is slow. However, flow studies (HETP versus velocity) on PS-ZrO₂ indicate minimal resistance to interphase mass transfer in the polymer coating [80]. The *C* coefficient in the Knox equation for a PS-ZrO₂ column was reported to be only 0.003. Moreover, band broadening due to slow mass transfer between mobile and stationary phases is more significant on PBD-ZrO₂ than on PS-ZrO₂ (The *C* coefficient for PBD-ZrO₂ was 0.07.) This suggests that a thinner more uniform film of PS is formed as compared to PBD or that fewer pore necks are plugged in PS-ZrO₂ than PBD-ZrO₂.

Chromatographic stability is a very important issue when a new polymer-coated metal oxide stationary phase is evaluated. In the work of Kurganov et al. [20], PS-ZrO₂ showed excellent separations and peak symmetry at both extremely acidic (0.1 M HNO₃) and basic (0.13 M NaOH) conditions. Zhao and Carr thoroughly investigated the stability of PS-ZrO₂ at pH 1 and 13 and at high temperatures (80, 120 and 160 °C) and observed no degradation of the polymer coating after flushing with thousands of column volumes of harsh mobile phases [80]. Kurganov et al. also emphasized the excellent stability of the coated oxides against the aggressive media [20]; however, the stability of their columns were not examined systematically.

1.3.2.3. Retention characteristics and chromatographic selectivity of PS-ZrO₂. Zhao and Carr have shown that PS-ZrO₂ is substantially different from PBD-ZrO₂ and

other RPLC phases even phenyl silica phases based on both the linear solvation energy relationship (LSER) approach [131] and a great number of practical separations of a variety of environmental and pharmaceutical analytes [130]. A comparison of the ratios of LSER coefficients for PS-ZrO₂ and PBD-ZrO₂ is shown in Fig. 18.

These two stationary phases are quite similar in all LSER coefficient ratios except the “*r/v*” ratio which is considerably larger for the PS phase compared to the PBD phase. The “*r/v*” ratio quantity is a measure of molecular polarizability contributions to retention. PS-ZrO₂ possesses a much greater *r/v* ratio than does PBD-ZrO₂, indicating that the π electrons of the support are involved in the retention process. This is further supported by comparing the free energy of retention per π bond (ΔG_{π}°) with the free energy of retention per methylene group ($\Delta G_{\text{CH}_2}^{\circ}$). As shown in Table 4, the ratio of $\Delta G_{\pi}^{\circ} / \Delta G_{\text{CH}_2}^{\circ}$ for PS-ZrO₂ is almost twice as large as that for PBD-ZrO₂. As a result, dramatic changes in the retention order of alkylbenzenes and polyaromatic hydrocarbons were observed on PS-ZrO₂ and PBD-ZrO₂ (see Fig. 19).

Table 4
Comparison of free energy of retention per π bond (ΔG_{π}°) vs. per methylene group ($\Delta G_{\text{CH}_2}^{\circ}$)^a

Stationary phase	(ΔG_{π}°) ^b	($\Delta G_{\text{CH}_2}^{\circ}$) ^c	$\Delta G_{\pi}^{\circ} / (\Delta G_{\text{CH}_2}^{\circ})$
PS-ZrO ₂	-299 ± 8	-140 ± 2	2.1
PBD-ZrO ₂	-248 ± 10	-232.6 ± 0.4	1.1
C ₁₈ -SiO ₂	-209 ± 8	-242 ± 2	0.9
Phenyl-SiO ₂	-133 ± 4	-128 ± 1	1.0

^a The free energy of retention of a π bond is denoted as (ΔG_{π}°) and is calculated from $-RT \ln(k'_{n+1}/k'_n)$ where *R* is the gas constant, *T* is the temperature and *n* is the number of π bond in a PAH molecule. On the other hand, the free energy of retention of a methylene group is denoted as $\Delta G_{\text{CH}_2}^{\circ}$ and is calculated the above equation where *n* is now defined as the number of methylene group.

^b Based on the data of naphthalene, anthracene, naphthacene and dibenz[*a,h*]anthracene in 50/50 acetonitrile–water at 30 °C [130].

^c Based on the data of ethylbenzene, butylbenzene, hexylbenzene and octylbenzene in 50/50 acetonitrile–water at 30 °C [130].

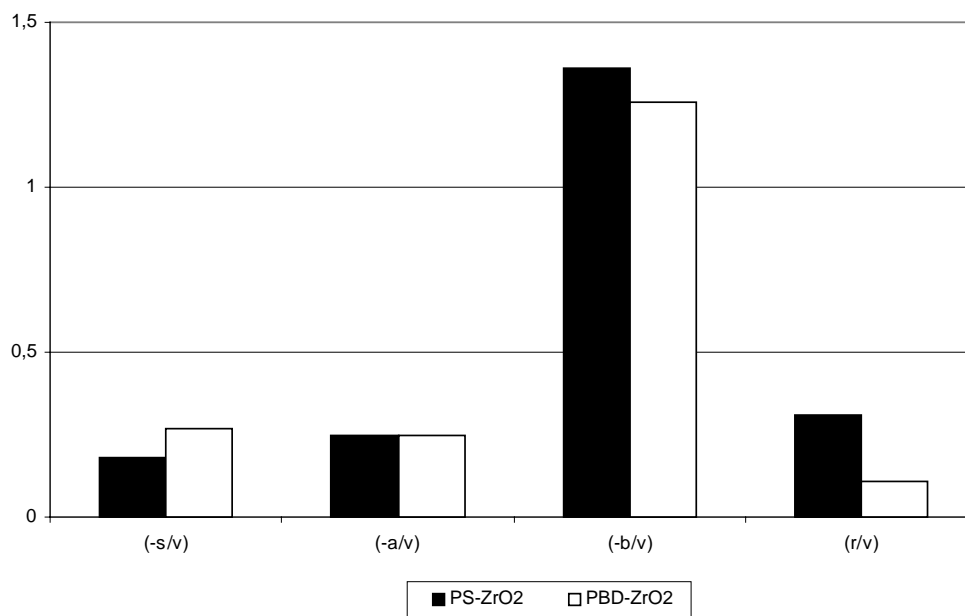


Fig. 18. Comparison of LSER coefficient ratios, s/v , a/v , b/v and r/v for PS-ZrO₂ and PBD-ZrO₂ in 50/50 acetonitrile–water. The v , a , b , s and r are the fitting coefficients of LSER equation: $\log k' = \log k'_0 + vV_2 + s\pi_2^* + a \sum \beta_2^H + rR_2$, where V_2 , π_2^* , $a \sum \beta_2^H$, $\sum \beta_2^H$ and R_2 are solute's molecular volume, dipolarity/polarizability, overall hydrogen-bond acidity, overall hydrogen-bond basicity, and excess molar refraction, respectively.

Zhao and Carr [130] show many examples of separations which indicate that the greater polarizability interactions on PS-ZrO₂ as compared PBD-ZrO₂ result in substantial differences in chromatographic selectivity for non-polar solutes. For example, PS-ZrO₂ shows much greater selectivity

than does PBD-ZrO₂ for the separation of structural isomers (phenyl toluenes, terphenyls and stilbenes) that differ in the position of phenyl groups [130]. PS-ZrO₂ was also compared to other aromatic stationary phases, such as phenyl bonded silica (phenyl-SiO₂) and polystyrene–divinylbenzene resin

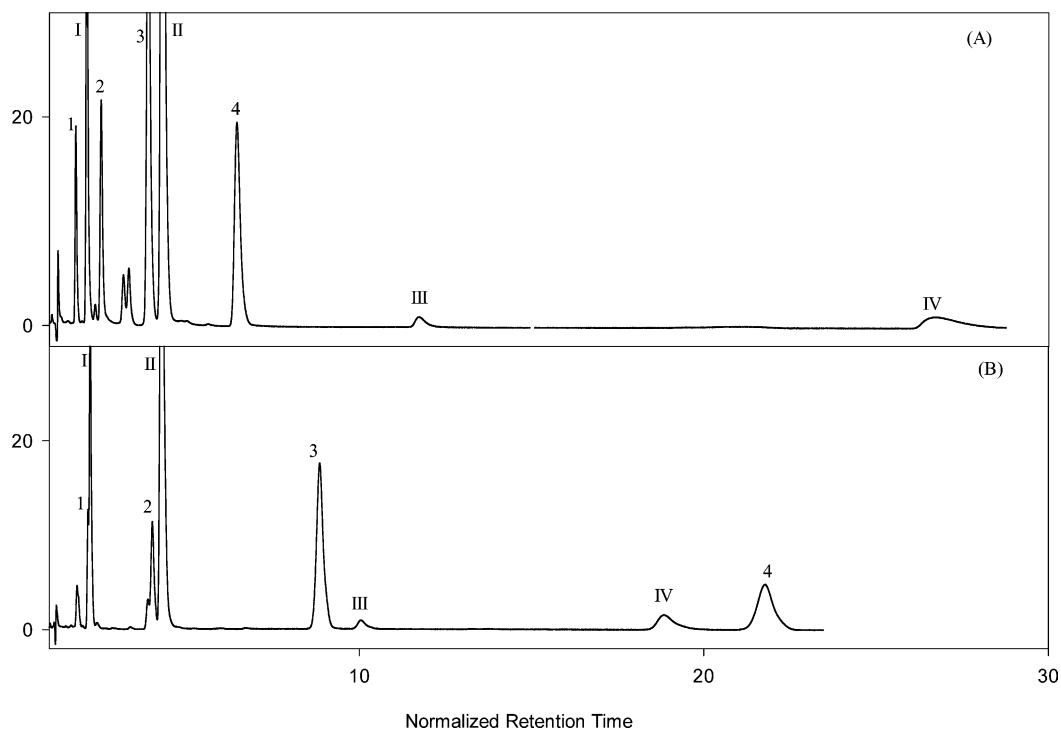


Fig. 19. Selectivity of alkylbenzenes and polyaromatic hydrocarbons on (A) PS-ZrO₂ and (B) PBD-ZrO₂. Conditions: 50/50 acetonitrile–water; $T = 30^\circ\text{C}$. Normalized retention time is calculated by the ratio of retention time to the dead time. Analytes: 1, ethylbenzene; 2, butylbenzene; 3, hexylbenzene; 4, octylbenzene; I, naphthalene; II, anthracene; III, naphthacene; IV, dibenz[*a,h*]anthracene.

(PRP-1) [130,131]. It was found that PS-ZrO₂ is similar to PRP-1 in terms of the enhanced molecular polarizability as reflected in the LSER “*r*” coefficient. (The *r/v* ratios for PS-ZrO₂ and PRP-1 in 50/50 acetonitrile–water are 0.31 ± 0.12 and 0.36 ± 0.06 , respectively.) The advantage of using PS-ZrO₂ is reflected in its effectiveness with regard to the different chromatographic selectivity and greatly reduced analysis time. In conclusion, PS-ZrO₂ will serve as an excellent candidate for difficult separation when PBD-ZrO₂ or conventional silica-bonded phases fail. Kurganov et al. [19] have also demonstrated the stability of PS-titania; however, no systematic studies on the packing stability were carried out.

1.3.3. Polyethyleneimine-coated zirconia anion-exchangers

Ion-exchange liquid chromatography is a powerful technique for the separation of both small inorganic and organic charged molecules [141–144], as well as larger bio-molecules such as: peptides, proteins and nucleic acids. Polyethyleneimine has been widely used [145–149] as a polymer coating for a variety of substrates [150–153] including silica, titania, alumina, zirconia clad-silica and porous polystyrene–divinylbenzene beads to produce ion-exchangers. However, each of these substrate materials suffers from serious operational deficiencies. For instance, polyethylene-imine (PEI) modified silica [145,149,150] suffers from poor chemical stability due to the accessibility of the underlying silica support to attack in base. Recent advances in silane chemistry have led to the development of more stable silica-based phases; however, these advances have been applied primarily to the production of reversed-phase materials [154,155] and not silica-based anion-exchangers. Polymeric ion-exchangers such as PEI coupled to polystyrene–divinylbenzene are much more chemically stable and have been reported to withstand pHs in the range of 4–12 [156]. Chemical stability is extremely important in the purification of bio-pharmaceuticals, as strongly alkali conditions are necessary for the cleaning and sterilization of columns. Polymer supports are chemically stable, but are not fully mechanically stable due to swelling or shrinking in organic solvents, at pH extremes or upon changes in mobile phase ionic strength [157]. The overriding goal of investigating the coating polyethyleneimine onto porous zirconia was to produce a more stable, high efficiency anion-exchanger.

1.3.3.1. Weak anion-exchanger (WAX). Initially two methods for coating polyethyleneimine onto porous zirconia were investigated: evaporative and adsorptive methods [158]. It was found that the type of cross-linker used to fix the polyethyleneimine to zirconia’s surface had a large effect on the hydrophobicity of the stationary phase and on its chemical and thermal stability. The use of 1,4-butanedioldiglycidylether (BUDGE) as a cross-linker [158] produced a weak anion-exchanger that was only stable from pH 3 to 9 and useful for the separation of bio-molecules

such as proteins and nucleotides; however, this stationary phase was not stable at higher pHs [159].

The elution of some inorganic anions on BUDGE cross-linked polyethylene-coated zirconia was similar (bromate < nitrite < nitrate < iodide) to that previously reported for a typical silica-based anion-exchanger [158]. Both types of coating methods produce efficient anion-exchangers, with the adsorptive coating method having somewhat better column efficiency than the evaporative method. The evaporative coating method has the advantage that it is easy to control the amount of stationary phase deposited on zirconia, and in general higher stationary phase loadings are achieved in comparison to the adsorptive method. A separation of a mixture of organic acids and inorganic anions is shown in Fig. 20 using commercially adsorptively coated zirconia.

The weak anion-exchange zirconia-based material can also be used to separate sugars using similar chromatographic conditions to those typically used on silica-based aminopropyl columns. However, the silica-based columns are known to be unstable [212,213], whereas the PEI-coated zirconia should be much more stable (Fig. 21).

1.3.3.2. Strong anion-exchangers (SAX). A chemically and thermally stable strong anion-exchanger based on zirconia has been produced using two types of cross-linkers that differ in hydrophobicity, namely: 1,10-diiododecane and 1,2-bis-(2-iodoethoxy) ethane; the second is much less hydrophobic than the first. The ionization state of these two stationary phases does not vary with pH as both are extensively quaternized. The more hydrophobic cross-linker, 1,10-diiododecane has been used for the separation of oligonucleotides and oligodeoxynucleotides [159] that differ in length by only a single nucleotide; it also is able to separate RNAs of the same length but differ by only one deoxynucleotide. In addition to being very selective, this phase was also found to be thermally and chemically stable up to column temperatures of 100 °C, and over the pH range of 1–13 [160]. The separation of the oligonucleotides resulting from the hydrolysis of Poly (G) at 100 °C is shown in Fig. 22.

The hydrophobicity of 1-10-diiododecane cross-linked PEI zirconia was found to be similar to that of a typical ODS phase [160]. Its retention was dominated by three main mechanisms, namely electrostatic, hydrophobic and Lewis acid-base interactions. The ability to run at elevated temperatures had a beneficial effect on the efficiency of ion-exchange separations and in general decreased the analysis time, primarily due to a decrease in the hydrophobic contribution to overall retention. As shown in Fig. 23, the highly hydrophobic nature of the 1,10-diiododecane cross-linked PEI-coated zirconia has been used to separate highly polarizable anions such as iodide from nitrate in the trace analysis of iodide in highly nitrate contaminated (2M) water even at a level of 1 ppm using UV detection.

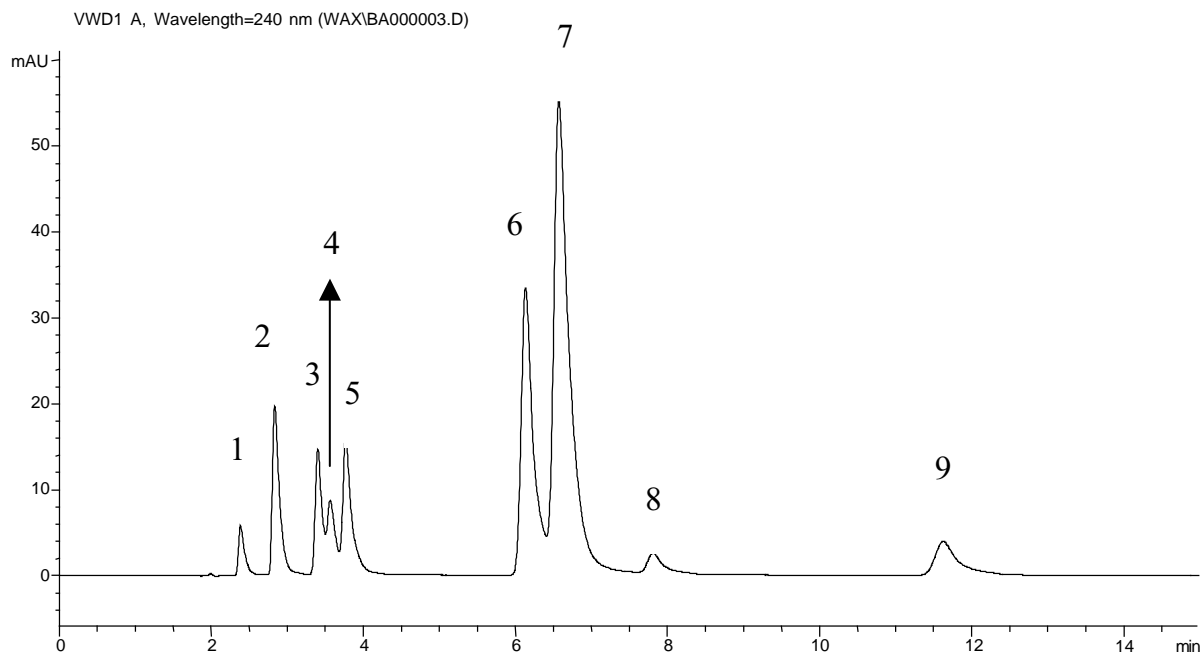


Fig. 20. Separation of a mixture of organic and inorganic anions on the adsorptively coated zirconia. Column, ZirChrom™-WAX, 150 mm × 4.6 mm i.d.; mobile phase, 45 mM ammonium phosphate dibasic at pH 8.2; flow rate, 1.0 ml/min; 240 nm detection, column temperature = 40 °C; solutes: 1, bromate; 2, nitrite; 3, benzoic acid; 4, nitrate; 5, *p*-chlorobenzoic acid; 6, *p*-bromobenzoic acid; 7, iodide; 8, *p*-fluorobenzoic acid; 9, *p*-iodobenzoic acid.

The 1,2-bis-(2-iodoethoxy) ethane cross-linker was found to be much less hydrophobic than the 1,10-diiododecane; this is beneficial in the separation of large bio-molecules such as proteins. The other primary virtue of this material is its chemical stability under alkali conditions (up to pH 13). An example of how this material could be used, which

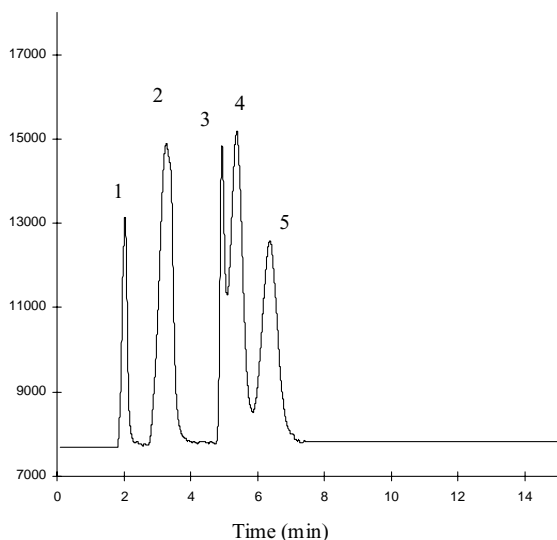


Fig. 21. Separation of sugars on weak anion-exchange column. Column, ZirChrom®-WAX, 150 mm × 4.6 mm i.d.; mobile phase, 85/15 acetonitrile–water; flow rate, 2.0 ml/min; detector, ELSD; column temperature = ambient; solutes: 1, xylose; 2, glucose; 3, sucrose; 4, lactose; 5, maltose.

capitalizes on the combination of both the cross-linked PEI stationary phase and the underlying zirconia substrate was shown in the a purification protocol developed for the efficient removal of *E. coli* 0111:B4 lipopolysaccharides (LPS) from bovine insulin samples [161]. An endotoxin clearance rate of up to 1.3×10^8 was attained, and endotoxin levels were reduced to less than 5 endotoxin units/ml even at initial contamination levels as high as 5.0×10^6 endotoxin units/ml. Furthermore, endotoxin adsorbed to the porous zirconia column was easily removed (that is, the column could be de-pyrogenated) using alkali for repeated purification cycles. This separation of LPS from insulin is the highest clearance rate ever published despite the extremely high initial level of LPS contamination. Both the 1,10-diiododecane and the 1,2-bis-(2-iodoethoxy) ethane cross-linked materials are commercially available.

1.4. Carbon-coated metal oxides

Carbon adsorbents for chromatography have been very widely studied. Several reviews describe the application of carbon in liquid and gas chromatography [162–164]. Le-boda et al. [165,166] reviewed carbon–mineral adsorbents in a two-part paper which covers the preparation of composite adsorbents as well as the surface properties and modification methods. The most common method for deposition of carbon on a surface is via the pyrolysis of a carbonaceous precursor at 700–1100 K. During the process carbon deposits (also known as coke) become localized on various parts of the adsorbent's surface; it can be homogeneously

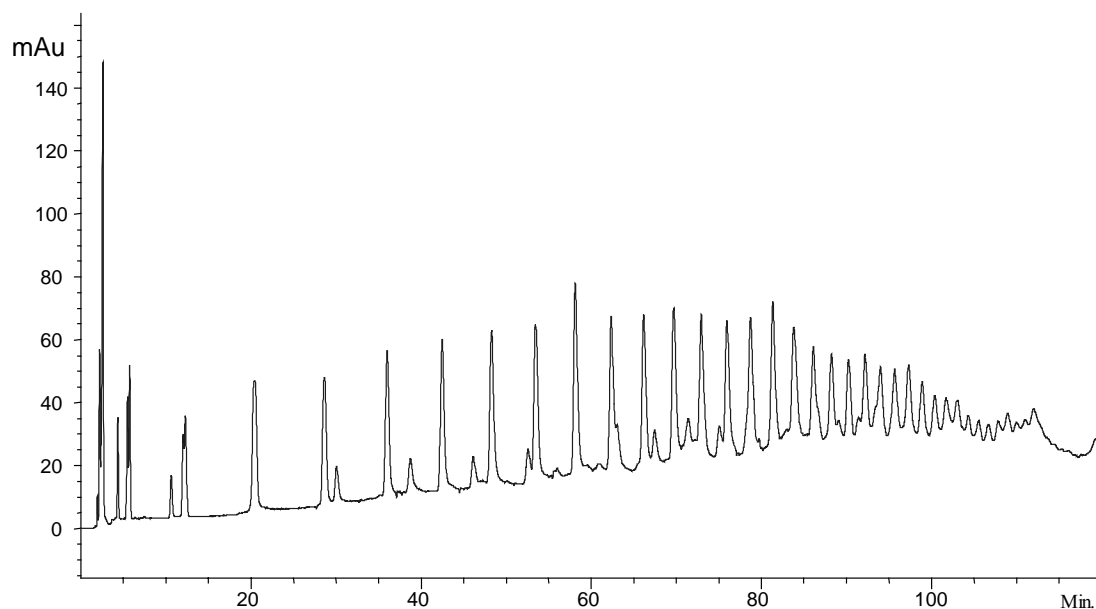


Fig. 22. The separation of the oligonucleotides resulting from the hydrolysis of Poly (G) at 100 °C. Column, ZirChrom[®]-SAX, 50 mm × 4.6 mm i.d.; mobile phase, A = 0.02 M potassium phosphate dibasic and 0.04 M NaCl at pH 8.5, B = 0.20 M potassium phosphate dibasic and 1.0 M NaCl at pH 8.5; gradient, 5–95% B over 90 min; flow rate, 1.0 ml/min; 254 nm detection, column temperature = 100 °C; solute: Poly (G) hydrolysate; injection size, 25 μ l.

dispersed or deposited in micropores and it can block mesopores [167]. Thus, there are many studies of the carbon deposition mechanism(s). The interested reader is advised to see [168] and the references therein. Despite the wealth of the research on carbon modified mineral surfaces most of the published papers are devoted to gas–solid interfaces while this review is aimed at liquid–solid interfaces and their applications in HPLC. Carbon surfaces useful for HPLC may be obtained in several ways:

- polymer infiltration of porous silica particles (acting as a template), followed by firing at approximately 1000 °C, followed by an alkaline treatment to remove the silica (leaving porous amorphous carbon) and finally high temperature firing at 2500–3000 °C to fully graphitize the remaining porous particle [169],
- deposition of a pyrocarbon layer on porous silica by passing organic vapors over particles heated to 400–700 °C [170],

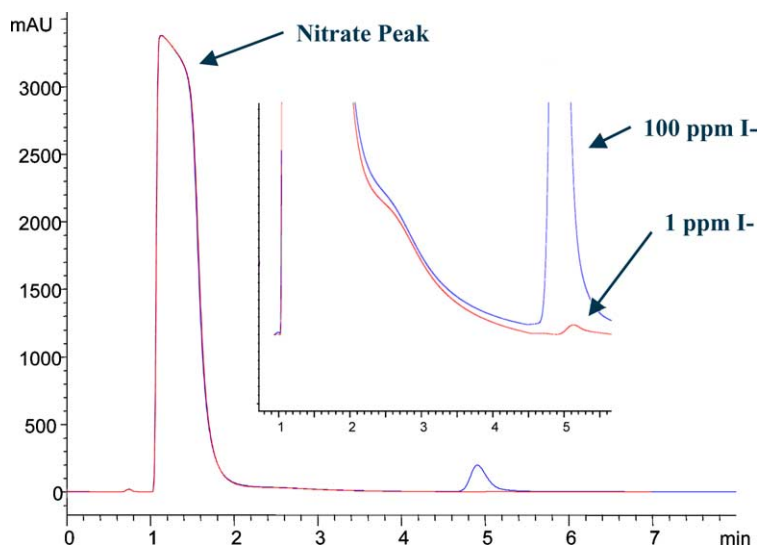


Fig. 23. Separation of highly polarizable anions: iodide from nitrate in the trace analysis of iodide in highly nitrate contaminated (2 M) water. Column, ZirChrom[®]-SAX, 50 mm × 4.6 mm i.d.; flow rate, 1.0 ml/min; mobile phase, 25 mM ammonium phosphate, 275 mM NaCl at pH 8.0; detector, 226 nm; column temperature = 30 °C; solutes: 2 M nitrate sample matrix, iodide; injection volume, 5 μ l.

- deposition of pyrocarbon layer on carbon black particles [171],
- electrochemical reduction of polytetrafluoroethylene in a lithium amalgam and thermal graphitization of the carbon particles [172],
- deposition of pyrocarbon catalyzed by nickel and other metal salts deposited on the surface [173].

The concept of coupling of metal oxides and carbon evolves from a number of useful properties shared by the materials [174]:

- both can resist the chemical attack of bases, acids and organic solvents,
- they are mechanically stable,
- they possess different and useful chromatographic selectivity.

Of the metal oxides only zirconia has been modified by carbon deposition for HPLC purposes. Thus, this part of the review will deal exclusively with zirconia-based materials. Zirconia is an ideal material to be covered with pyrogenic carbon. Pre-sintered zirconia can be heated to 700 °C without any change in pore structure [102]. Moreover zirconium(IV) sites catalyze the cracking of organic substances to carbon [174,175]. The process of chemical vapor deposition of carbon on zirconia is simple and highly reproducible; it consists of passing an organic vapor over porous zirconia particles at temperatures of ~700 °C typically at reduced pressure (~5–10 Torr) [176]. It is possible to cover up to about 97% of zirconia's surface with carbon [176]. Low-temperature glassy carbon was coated on non-chromatographic zirconia by Rittenhouse and Olesik [209]. Various organic compounds can be used as the source of carbon; the best results, in terms of chromatographic peak shape and efficiency were noted when alkanes were used for the CVD. The poorest results were observed when unsaturated hydrocarbons were used [102,176]. Another advantage of the CVD carbon-clad zirconia column is its mechanical stability of the packing compared to other types of carbon supports.

The retention mechanism is thought to be exclusively by adsorption on the rigid carbon surface. Such an adsorption process is very sensitive to the solute's shape and thus carbon surfaces are much more selective for the separation of geometrical isomers than are typical bonded phases with alkyl chains or polymer-coated metal oxides [176]. Weber and Carr [177] have shown numerous separations of geometrical isomers on C/ZrO₂ column evidencing the different selectivity of the column compared to conventional C₁₈ phases. The ability of C/ZrO₂ to separate isomers was further examined by applying them to the analysis of diastereoisomers; excellent resolving power for a wide range of analytes was found [178]. Shalliker and co-workers have shown an application of commercially available carbon-clad zirconia packings for separations of diastereoisomers of polystyrene oligomers with higher degree of resolution than reported previously [179]. The work was continued and in the next papers [180,181] the exceptional resolving power of carbon-clad zirconia for diastereoisomers was confirmed. However, the authors noted some variability in reproducibility of commercially available packing. In additional work a two-dimensional C₁₈/C-ZrO₂ system was used for the separation of closely related isomers [182].

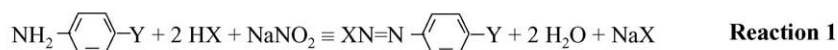
Carbon-clad zirconia differs from alkyl-bonded phases in three ways [183]:

- it is much more selective for the separation of both polar and non-polar geometrical isomers,
- it is more hydrophobic,
- in contrast to alkyl-bonded phases polar solutes are also retained through electronic (π - π) interactions.

Recently, carbon-clad zirconia was chemically modified via diazonium salt chemistry to give extraordinarily stable reversed-phase packing. The reactions are shown in Fig. 24.

The structure of the resulting phase is shown in Fig. 25.

The phase is stable from pH 0.3 to 14 at ~40 °C. The packing is also thermally stable to at least 200 °C at neutral pH (see Fig. 26). This new phase is commercially available.



Where Y = C₁₈H₃₇

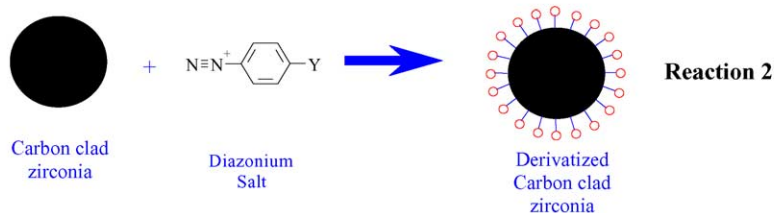


Fig. 24. Modification of carbon-coated zirconia.

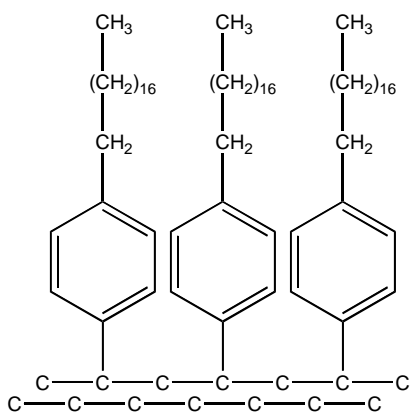


Fig. 25. Chemistry of DiamondBond C₁₈ surface.

2. High temperature separations

The high thermal and chemical stabilities of zirconia-based supports for HPLC have enabled the development of ultra-fast liquid chromatography which is based on the use of temperatures typically much higher than can be tolerated by conventional silica-based phases. The advantages of high temperature to speed-up HPLC have been theoretically demonstrated by Anita and Horvath [184]. They clearly showed that the use of elevated temperatures ought to greatly improve analysis speed by decreasing the viscosity and increasing the rate of equilibration between phases. Further, they showed that the resulting lower residence time of the analyte in the hot column could, in many cases, compensate for the increase in the rate of potential on-column analyte degradation by hydrolysis, oxidation or isomerisation reactions. However, this approach to speeding up HPLC is severely limited by the all too short useful lifetimes of bonded silica-based phases even in neutral pHs (see Fig. 6 in Part I) at temperatures

only 30–40 °C above room temperature (see Table 2 in Part I).

Temperature substantially influences the retention, efficiency and selectivity of the separation of large molecules and to lesser extent small molecules [184–186]; however, there are not many examples of the application of high temperature HPLC. Hancock et al. [210,211] studied the selectivity of peptides and proteins on “sterically protected” C₈ and C₁₈ phases. They found that using temperature along with the best gradient can greatly improve band spacing. Horvath and co-workers [184,187,188] used pellicular stationary phases to separate proteins at 120 °C. Smith and Burges [189,190] used pure water as the eluent at 200 °C for the separation of phenols and barbiturates on PS–DVB phase. Ingelse et al. [191] used water as the eluent for the separation of alcohols on an ODS column. According to Yang et al. [192] the polarity of subcritical water (200–250 °C) is similar to that of pure methanol. Therefore, pure, subcritical water can be used as the eluent for RP separations.

High temperature ultra-fast liquid chromatography requires use of an HPLC system with special attention paid to heat transfer issues as well as to extra-column effects [193–196]. An increase in column temperature from 25 to 150 °C allows considerable increases in linear velocity and as a result some separations can be done nearly 50 times faster than at ambient temperatures. The advantages of high temperature separations are illustrated in Fig. 27.

Use of high temperature greatly reduces the consumption of organic solvents; some reversed-phase separations can even be done with pure water as the mobile phase. Fields et al. [195] studied the retention of testosterone on PBD–ZrO₂ using only superheated water as the eluent. The column provided performance similar to conventional HPLC. PBD–zirconia columns were stable even when subjected to 200 atm and 150 °C for 200 h. The retention factor

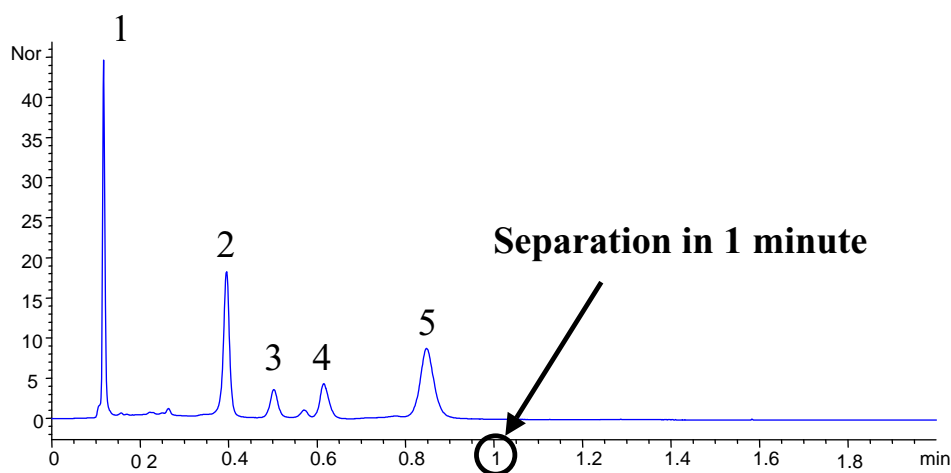


Fig. 26. High temperature separation of non-steroidal anti-inflammatories. LC conditions: column, 50 mm × 4.6 mm DiamondBond™-C₁₈; mobile phase, 25/75 ACN/40 mM phosphoric acid; pH at 2.3; flow rate, 5.5 ml/min; *T* = 150 °C; injection volume, 1 μl; detection at 254 nm; solute concentration, 0.15 mg/ml; solutes: 1, acetaminophen; 2, ketoprofen; 3, naproxen; 4, ibuprofen; 5, oxaprofen.

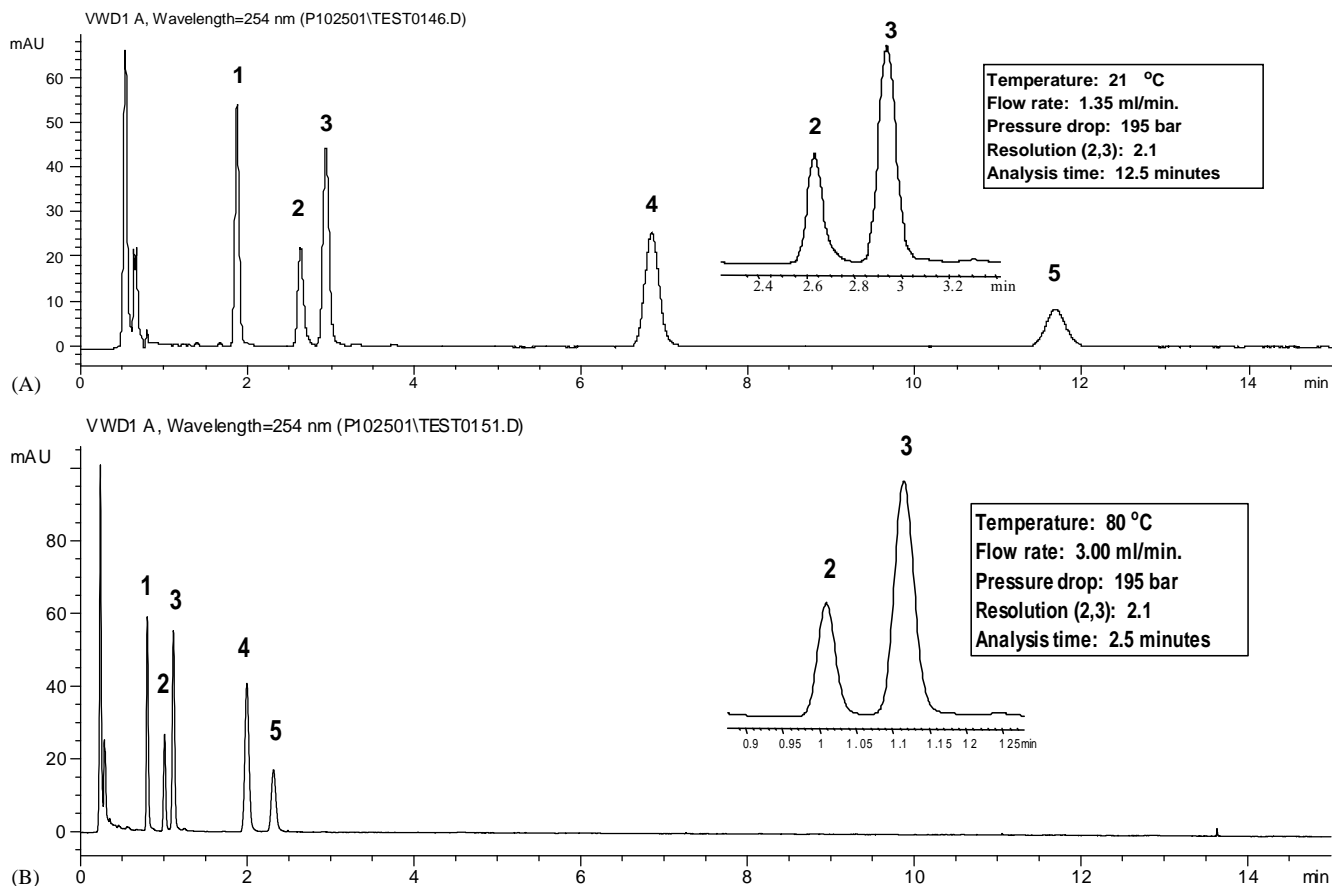


Fig. 27. Fast separation of antihistamines. LC conditions: column, 100 mm \times 4.6, ZirChrom-PBD; solutes: 1, doxylamine; 2, methapyrilene; 3, chlorpheniramine; 4, triprolidine; 5, meclizine. (A) Mobile phase; 29/71 ACN/50 mM tetramethylammonium hydroxide; pH at 12.2; flow rate, 1.35 ml/min; injection volume, 0.5 μ l; 254 nm detection; column temperature = 21 $^{\circ}$ C; pressure drop = 195 bar. (B) Same as (A), except mobile phase, 26.5/73.5 ACN/50 mM tetramethylammonium hydroxide; pH at 12.2; flow rate, 3.00 ml/min; column temperature = 80 $^{\circ}$ C; pressure drop = 195 bar.

of the test solute remains almost constant in chromatograms obtained before and after this treatment [115]. The column was also stable at a column inlet pressure of 200 atm and 260 $^{\circ}$ C. In the following paper [114] from the same group PBD-ZrO₂ was found to have substantially higher efficiency than conventional C₁₈ packed columns in solvating gas chromatography conditions with carbon dioxide as the mobile phase. The PBD modified zirconia column was used up to 320 $^{\circ}$ C without any diminution in efficiency. Wilson [197] found PBD-zirconia to be useful for the separation of pharmaceuticals using water as the eluent. Carbon-clad zirconia columns were much more retentive than PBD-ZrO₂. Wilson also noted the differences in the selectivity of the two columns. Kephart and Dasgupta used PBD and carbon-clad zirconia columns in a capillary scale reverse phase liquid chromatography system with superheated water as a mobile phase at temperatures as high as 370 $^{\circ}$ C and pressures exceeding 10,000 psi [198].

Because PBD-zirconia phases have been repeatedly shown to be stable during long-term use at both 100 $^{\circ}$ C [107] and then 200 $^{\circ}$ C [103], they were used to study the effect of temperature on selectivity. The temperature effect on selectivity depends very much on the type of solute. For

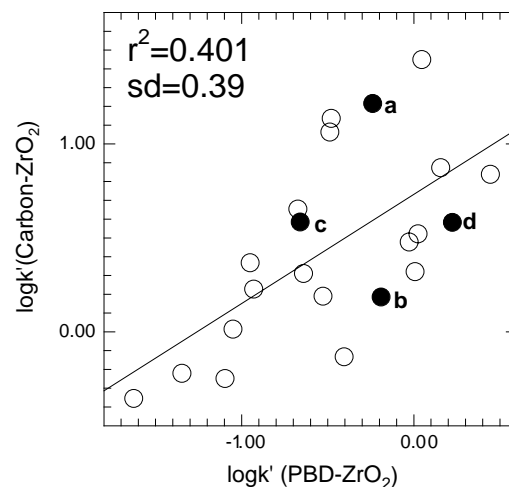


Fig. 28. Plot of $\ln k'$ on C-ZrO₂ vs. $\ln k'$ on PBD-ZrO₂ for 22 selected solutes in 50/50 acetonitrile–water mixture at 30 $^{\circ}$ C. Solid line denotes the least square line and a, b, c, d denote four different solutes (●) (from [116]).

solutes with similar structure or functional groups temperature has almost no effect. However, for solutes with very different functional groups chromatographic selectivity can vary substantially with temperature [108]. It has also been shown that high temperature can improve the column efficiency by $\sim 30\%$ mainly by increasing the diffusional rate in the stationary phase [107]. The use of increased flow rate considerably decreases the analysis time. There are many examples of substantially shorter analysis at higher temperatures with the same or better resolution [103,107]. Also aromatic phases coated on zirconia are stable at high temperatures [94].

As a variety of zirconia-based phases has been obtained, selectivity among them differs considerably. For example, although PBD-ZrO₂ and carbon-clad zirconia are both reversed-phase supports they have substantially different selectivities. This is shown in Fig. 28 [116].

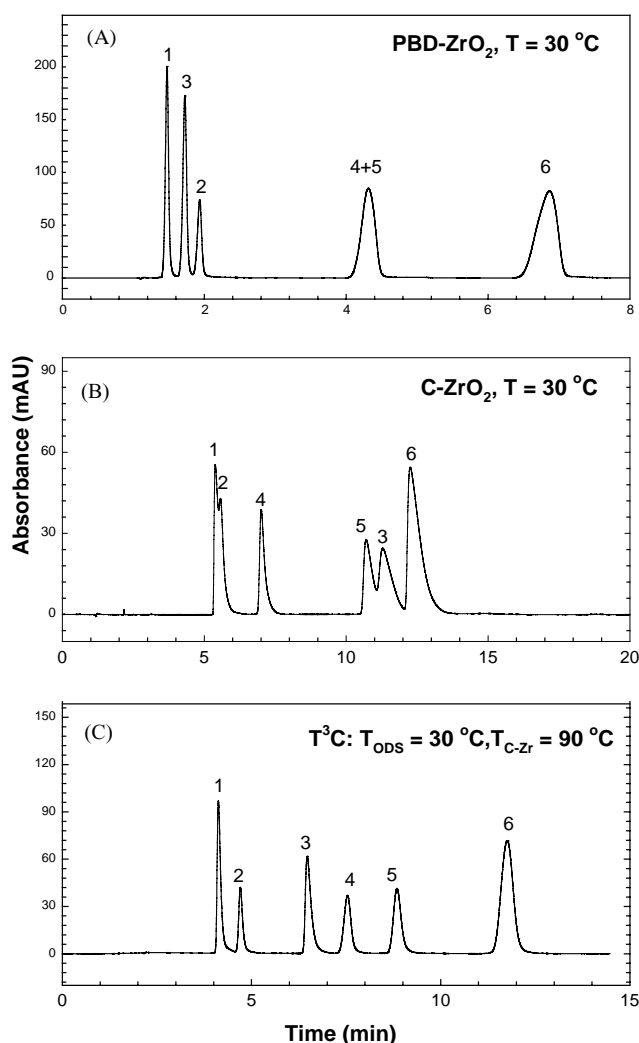


Fig. 29. Chromatograms showing the separation of aromatic compounds on (A) PBD-ZrO₂ at 30 °C; (B) C-ZrO₂ at 30 °C and (C) T³C with both columns at 30 °C. Experimental conditions: mobile phase, 40/60 acetonitrile–water; flow rate, 1 ml/min; solutes: 1, benzonitrile; 2, anisole; 3, methylbenzoate; 4, ethylbenzene; 5, *p*-xylene; 6, *n*-propylbenzene.

A minimal correlation between retention on C-ZrO₂ and PBD-ZrO₂ was found. This means that these two stationary phases possess very different selectivities. Solute a and b are on nearly a vertical line—i.e. their separation on PBD-zirconia is impossible. However, they are easily separated on carbon-coated zirconia. And conversely solutes c and d are easily separated on PBD-ZrO₂ but they coelute on C-ZrO₂. Thus, a combination of these two reversed-phase columns can have a profound effect on the separation ability of the chromatographic system.

These facts allowed the development of another application of zirconia-based phases to the new “thermally tuned tandem” column concept (T³C concept). The concept assumes the use of two tandem columns each in an independently controllable temperature zone. These columns must differ in selectivity. Selectivity of the system is “tuned” by adjusting the individual column temperatures [116]. Fig. 29 shows the effect of combining a C-ZrO₂ and PBD-ZrO₂ column on the separation of a mixture of aromatics. PBD-ZrO₂ does not separate ethylbenzene from *p*-xylene while benzonitrile and anisole coelute on the C-ZrO₂ column. The combination of two columns (i.e. two selectivities) allows the separation of both critical pairs.

The T³C concept was further developed and used for the separation of barbiturates, PTH amino acids on ODS and C-ZrO₂ columns [199], triazines and carbamate mixtures [200] and the resolution of some basic pharmaceuticals on ODS and PBD-ZrO₂ [201].

3. Conclusions

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

A number of important advantages accrue from improving the stability of the stationary phase. Perhaps the most important of these is a simplification in method development in that one stationary phase can be used over the entire pH range. This is especially important in the field of reversed-phase liquid chromatography wherein a number (3–4) different silica-based phases are offered by a single manufacturer to cover the narrow pH range from 2 to 12 and most phases cannot be used much above ambient temperature. The extremely wide pH range accessible to metal oxide supports facilitates the development of new separation methods for complex mixtures by allowing the selectivity of the ionizable compounds to be adjusted at will by the chromatographer to achieve the desired degree of resolution. Another major advantage of high stability–durability in the context of chromatographic method development is that the column can be used at higher temperatures. The use of high temperature decreases the eluent's viscosity and increases the analyte's diffusion coefficient to such an extent that it is frequent possible to decrease analysis time by a factor of 2–10 by operating the column at a higher linear velocity. In addition, high temperatures enable the new technology of stationary phase selectivity adjustment by the thermally tuned tandem column concept. Finally, the use of high temperature can sometimes completely circumvent the need for organic solvent in reversed-phase chromatography. This in turn allows the use of far UV spectroscopy (<210 nm) for detection of compounds, which do not have good chromophores at long wavelengths; and it has allowed the development of the classical gas chromatographic flame ionization detector (FID) as a detector in HPLC with pure aqueous eluents.

In this review, we described various synthetic procedures for forming metal oxides into particles useful for chromatographic separations. This included a discussion of the so-called PICA process, as well as the oil-emulsion and spray drying processes. It should be clear that under appropriate circumstances all of these processes can be used to manufacture particles with quite acceptable chromatographic properties. That is, particles of the correct overall diameter as well as the proper internal pore space and geometry to allow rapid mass transfer even when particles, which are impregnated with cross-linked polymers, are used as the stationary phase.

One of the major limitations of metal oxide particles is the fact that they cannot be chemically modified using classical silane chemistry or by adsorption of carboxylates or phosphonates to form coatings, which are adequately stable, so as to extend the pH range and temperature range accessible on silica-based phases. The recent disclosure of methods for forming carbon-carbon bonds to carbon-clad zirconia is a most promising development.

The significant advantages of metal oxide supports in comparison to silica-based supports are not purchased at no cost to do the end-user. It is a fact that the Lewis acid chemistry of metal oxides must be taken into consideration when developing new separations on such supports. Although even

the highest purity silica does contain some metal and there is no question that metals in the silica are accessible to certain analytes. Furthermore the metal components of the HPLC column especially the column, fittings and frits can cause problems the total amount of accessible metal is much lower than in any metal oxide-based stationary phase. In the case of zirconium dioxide and aluminum oxide stationary phases, one must recognize that there is a very high population (more than 3–4 $\mu\text{mol}/\text{m}^2$) of hard Lewis acid sites accessible to small analytes. When the analyte is itself a hard Lewis base (for example, the anion of a carboxylic or phosphonic acid but not amines) one will observe badly tailed peaks. This tailing is due to the sluggishness of dissociation of the coordinate covalent bond formed between the analyte and the metal center. In contrast to the presence of the small amounts of metals in silica, which are very highly variable and hard to control from batch-to-batch, the high concentration of metal sites inherent in metal oxides is quite reproducible. This situation is akin to the presence of the traces of n and p doping agents in silicon based semiconductors. Small changes for dopant have huge relative effects on the behavior of the nearly perfectly pure semiconductor. Similarly on silica-based phases extremely small amounts of metals have a great effect on the chromatography of all those analytes, which are sensitive to, metals. In particular, it well known that the presence of a metal in silica has a huge impact on silica's Bronsted acidity. In contrast, the presence of minor amounts of foreign metals in a metal oxide support has almost no effect compared to the constituent metal oxide per se.

The Lewis acid chemistry of metal oxide surfaces creates a very significant opportunity for the chromatographer for adjusting the selectivity of the separation of cationic species (for example, amines which are positively charged at pHs below their pK_a values). By addition of an appropriate buffer comprised of an anionic Lewis base (for example, one containing any of a number of carboxylic acids, phosphate or organic phosphonates) the amount of negative charge on the surface of the metal oxide can be manipulated according to the desires of the chromatographer. The negative charge in turn has a large impact on the retention of positively charged species. It is thus possible and in fact common to be able to adjust the band spacing of neutral and positively charge species whose retentions vary quite differently in response to changes in ionic strength.

3.1. Future

Given that the use of silica in chromatography has had a very long history, it should be clear that the future of metal oxides as stationary phases should be equally rich. There are many interesting avenues to explore. Among the various paths which need to be followed in the near future we envision the development of metal oxide-based monolithic stationary phases, metal oxide particles which have controlled pore sizes (there is a particular need for metal oxides with

smaller pores and higher surface areas to be used in preparative scale separations), and the exploration of metal oxide stationary phases in LC–MS. A major avenue of exploration is the development of coatings for metal oxide surfaces to fully sequester the Lewis chemistry to make the metal oxide more closely resemble silica without compromising the stability of the metal oxide. The development of chemical derivatization methods for carbon-coated on zirconia surfaces is a major step in this direction. Additional interesting avenues to explore included development of amino phases, cyano phases and diol phases to emulate the related materials on silica-based bonded phases.

At this date, a metal oxide phase that is useful for reversed-phase separations of proteins and large peptides is not available. Given the importance of bio-separations this is a major opportunity.

Finally, the stability of metal oxide phases is so great that the stationary phase is no longer the Achilles heel of the chromatographic system. In fact, the weakest link is now the column hardware. PEEK tubing and fittings cannot tolerate the high temperatures which metal oxide-based stationary phases can endure; and the metal components of the HPLC system cannot tolerate the extremely corrosive environments (hydrochloric acid, sodium hydroxide) which are readily tolerated by zirconium dioxide particles. New column hardware is needed to exploit all of the advantages inherent in metal oxide phases.

Less obvious but still very important advantages result from the ability to run separations at high temperatures. Until

recently, few chromatographers conscientiously optimized temperature in HPLC. Anita and Horvath closely examined the effect of temperature on separation speed [184]. They found that increasing the temperature affects two major parameters that influence column dynamics:

- mobile phase viscosity, and
- analyte diffusivity.

The decrease in eluent viscosity with increasing temperature (estimated at a 5–10-fold decrease for methanol on going from 20 to 200 °C) results in a tremendous reduction in column backpressure. This allows separations to be done at higher flow rates, which in turn decreases the analysis time. For example, when the same analytes are run at 30 and 100 °C, the separation at 30 °C can be run at a reasonable pressure at a flow rate of 1.0 ml/min and takes 11 min. When the same separation is run at 100 °C, the separation can be completed in less than 1 min using a flow rate of 5.0 ml/min with almost the same backpressure.

Another advantage results from the increase in analyte diffusivity with temperature. At normal operating temperatures, increasing the flow rate decreases efficiency due to slow inter-phase mass transfer. However, when the temperature is increased diffusion coefficients also increase and as a result, column efficiency remains reasonably high even at higher flow rates. It has been shown by Yen et al. [194] that on polystyrene coated zirconia column C-term for acetophenone decreases from 1.2×10^{-4} to 0.27×10^{-4} cm²/s with temperature increase from 25 to 150 °C. Therefore, at ele-

Table 5
Advantages and disadvantages of silica and metal oxide-based packings for HPLC

	Advantages	Disadvantages
Silica	Available in the widest selection of particle size, pore diameter and surface area Wide selection of types of reversed phases (C ₁₈ , C ₈ , phenyl, CN, polar embedded, fluoro) available Relatively familiar surface and modifier chemistry Many suppliers	Poor stability at high and low pHs Poor thermal stability Sensitivity to inorganic buffers even at neutral pH Sensitivity to water-rich mobile phases Highly variable population of silanol groups Many amines show tailed peaks on many silica phases Does not allow for a development of high temperature, organics mobile phase, fast HPLC
Metal oxides	Extremely high thermal stability High stability at all pHs Reversed phases commercially available (with different to siliceous supports chemistry) Well understood chemistry of zirconia's surface (surface chemistry of alumina and titania seems to be similar) Similar (PBD-modified oxides vs. ODS silica) selectivity for non-electrolytes Allow for a development of high temperature, organics mobile phase, fast HPLC Selectivity tunable by choice of buffer type Very different selectivity compared to silica for basic and acidic compounds	Available in limited selection of surface areas and pore sizes Very different surface chemistry than that of silica especially Lewis acid interactions Alumina and titania—relatively unexplored for HPLC Lack of commercially available reversed phase for titania Must have hard Lewis in eluent to separate carboxylic acids or else get no elution or extreme tailing Few manufacturers Effective use requires “unlearning” much knowledge based on silica chemistry

vated temperature a separation can be run at high velocity without serious loss in efficiency or at the cost of prohibitive backpressures [194]. Of course, even when the column can withstand elevated temperature and extremes in pH, analyte instability can be a limiting consideration [184].

The advantages and disadvantages of siliceous and metal oxide packings are summarized in Table 5.

References

- [1] J. Nawrocki, M.P. Rigney, A. McCormick, P.W. Carr, *J. Chromatogr. A* 657 (1993) 229.
- [2] J. Nawrocki, *J. Chromatogr. A* 779 (1997) 29.
- [3] J. Blackwell, P.W. Carr, *J. Chromatogr.* 549 (1991) 43.
- [4] J. Blackwell, P.W. Carr, *J. Chromatogr.* 549 (1991) 59.
- [5] J.A. Blackwell, P.W. Carr, *J. Chromatogr.* 596 (1992) 27.
- [6] J. Yu, Z. El Rassi, *J. Chromatogr.* 631 (1993) 91.
- [7] J. Blackwell, P.W. Carr, *Anal. Chem.* 64 (1992) 863.
- [8] J. Blackwell, P.W. Carr, *Anal. Chem.* 64 (1992) 853.
- [9] S.H. Hansen, P. Helbow, M. Thomsen, *J. Chromatogr.* 544 (1981) 53.
- [10] M.P. Rigney, Ph.D. Thesis, University of Minneapolis, 1988.
- [11] F. Schindler, H. Schmidbaur, *Angew. Chem.* 79 (1967) 697; F. Schindler, H. Schmidbaur, *Angew. Chem. Int. Ed. Engl.* 6 (1967) 683.
- [12] N.E. Tret'yakov, V.N. Filimonov, *Kin. Katal.* 13 (1972) 815.
- [13] J.H. Knox, A. Pryde, *J. Chromatogr.* 112 (1975) 171.
- [14] C. Laurent, H.A.H. Billiet, L. de Galan, *Chromatographia* 17 (5) (1983) 253.
- [15] C. Laurent, H.A.H. Billiet, L. de Galan, *Chromatographia* 17 (7) (1983) 394.
- [16] W. Gao, L. Dickinson, C. Grozinger, F.G. Morin, L. Reven, *Langmuir* 12 (26) (1996) 6429.
- [17] U. Trüdinger, G. Müller, K.K. Unger, *J. Chromatogr.* 535 (1990) 111.
- [18] M. Grün, A.A. Kurganov, S. Schacht, F. Schüth, K.K. Unger, *J. Chromatogr. A* 740 (1996) 1.
- [19] A. Kurganov, U. Trüdinger, T. Isaeva, K.K. Unger, *Chromatographia* 42 (3–4) (1996) 217.
- [20] A. Kurganov, V. Davankov, T. Isajeva, K. Unger, F. Eisenbeiss, *J. Chromatogr. A* 660 (1994) 97.
- [21] J. Yu, Z. El Rassi, *J. Liq. Chromatogr.* 16 (14) (1993) 2931.
- [22] J. Yu, Z. El Rassi, *J. High Resolut. Chromatogr.* 17 (1994) 705.
- [23] J.J. Pesek, M.T. Matyska, J. Ramakrishnan, *Chromatographia* 44 (9–10) (1997) 538.
- [24] K. Tani, Y. Suzuki, *J. Liq. Chromatogr. Rel. Technol.* 19 (17–18) (1996) 3037.
- [25] J.J. Pesek, V.H. Tang, *Chromatographia* 39 (11–12) (1994) 649.
- [26] A. Ellwanger, M.T. Matyska, K. Albert, J.J. Pesek, *Chromatographia* 49 (7–8) (1999) 424.
- [27] J.J. Pesek, J.E. Sandoval, M. Su, *J. Chromatogr.* 630 (1993) 95.
- [28] H.-J. Wirth, K.-O. Eriksson, P. Holt, M. Aguilar, M.T.W. Hearn, *J. Chromatogr.* 646 (1993) 129.
- [29] H.-J. Wirth, M.T.W. Hearn, *J. Chromatogr.* 646 (1993) 143.
- [30] R.V. Arenas, J.P. Foley, *Analyst* 119 (1994) 1303.
- [31] U. Bien-Vogelsang, A. Deege, H. Figge, J. Koehler, G. Schomburg, *Chromatographia* 19 (1984) 170.
- [32] J.E. Haky, A.R. Raghani, B.M. Dunn, L.F. Wieserman, *Chromatographia* 32 (1–2) (1991) 49.
- [33] J.E. Haky, S. Vemulapalli, L.F. Wieserman, *J. Chromatogr.* 505 (1990) 307.
- [34] J.E. Haky, S. Vemulapalli, *J. Liq. Chromatogr.* 13 (15) (1990) 3111.
- [35] D. Xiang, L.Y. Tang, J.A. Blackwell, *J. Chromatogr. A* 953 (2002) 67.
- [36] J.E. Haky, T.M. Blauvelt, L.F. Wieserman, *J. Liq. Chromatogr. Rel. Technol.* 19 (2) (1996) 307.
- [37] J.J. Pesek, H.D. Lin, *Chromatographia* 28 (1989) 565.
- [38] R.V. Arenas, J.P. Foley, *Am. Lab.* 26 (1994) 32.
- [39] K.B. Holland, J.M. Washington, D.C. Moe, C.M. Conroy, *Am. Lab.* 24 (1992) 51.
- [40] Y. Mao, B.M. Fung, *J. Colloid Interf. Sci.* 191 (1997) 216.
- [41] Y. Mao, B.M. Fung, *Chem. Mater.* 10 (1998) 509.
- [42] Y. Mao, B.M. Fung, *J. Chromatogr. A* 790 (1997) 9.
- [43] Y. Hu, Y. Feng, J. Wan, S. Da, L. Hu, *Talanta* 54 (2001) 79.
- [44] Y.-L. Hu, Y.-Q. Feng, S.-L. Da, *J. Liq. Chromatogr. Rel. Technol.* 24 (7) (2001) 957.
- [45] Y.-L. Hu, Y.-Q. Feng, J.-D. Wan, S.-L. Da, *J. Liq. Chromatogr. Rel. Technol.* 25 (1) (2002) 83.
- [46] M. Pursch, D.L. Vanderhart, L.C. Sander, X. Gu, T. Nguyen, S.A. Wise, D.A. Gajewski, *J. Am. Chem. Soc.* 122 (2000) 6997.
- [47] M.J. Wirth, H.O. Fatunmbi, *Anal. Chem.* 64 (1992) 2783.
- [48] M.J. Wirth, H.O. Fatunmbi, *Anal. Chem.* 65 (1993) 822.
- [49] R.W.P. Fairbank, Y. Xiang, M.J. Wirth, *Anal. Chem.* 67 (1995) 3879.
- [50] H.O. Fatunmbi, M.D. Bruch, M.J. Wirth, *Anal. Chem.* 65 (1993) 2048.
- [51] M.J. Wirth, R.W.P. Fairbank, *J. Liq. Chromatogr. Rel. Technol.* 19 (17–18) (1996) 2799.
- [52] J. Nawrocki, *Chromatographia* 31 (1991) 177.
- [53] J. Nawrocki, *Chromatographia* 31 (1991) 193.
- [54] X.N. Jia, J.J. Yang, Y.M. Zuo, *Chin. Chem. Lett.* 12 (5) (2001) 439.
- [55] R.V. Arenas, J.P. Foley, *Anal. Chim. Acta* 246 (1991) 113.
- [56] J. Li, P.W. Carr, *Anal. Chem.* 68 (1996) 2857.
- [57] L. Sun, Ph.D. Thesis, University of Minnesota, Minneapolis, MN, 1994.
- [58] L. Sun, P.W. Carr, *Anal. Chem.* 67 (1995) 2517.
- [59] A.M. Clausen, A. Subramanian, P.W. Carr, *J. Chromatogr. A* 831 (1999) 63.
- [60] W.A. Schafer, P.W. Carr, E.F. Funkenbush, K.A. Parson, *J. Chromatogr.* 587 (1991) 137.
- [61] W.A. Schafer, P.W. Carr, *J. Chromatogr.* 587 (1991) 149.
- [62] J.A. Blackwell, P.W. Carr, *J. Liq. Chromatogr.* 15 (5) (1992) 727.
- [63] J. Blackwell, P.W. Carr, *J. Liq. Chromatogr.* 15 (9) (1992) 1487.
- [64] L. Sun, P.W. Carr, *Anal. Chem.* 67 (1995) 3717.
- [65] B. Lorenz, S. Marmé, W.E.G. Müller, K. Unger, H.C. Schröder, *Anal. Biochem.* 216 (1994) 118.
- [66] A. Mullick, C.M. Grffith, M.C. Flickinger, *Biotechnol. Bioeng.* 60 (3) (1998) 333.
- [67] A. Mullick, M.C. Flickinger, *Preparative Biochem. Biotechnol.* 28 (1) (1998) 1.
- [68] A.M. Clausen, P.W. Carr, *Anal. Chem.* 70 (1998) 378.
- [69] A. Subramanian, P.W. Carr, C.V. McNeff, *J. Chromatogr. A* 890 (2000) 15.
- [70] K.K. Unger, *Porous Silica*, Elsevier, 1979.
- [71] R.K. Iler, *The Chemistry of Silica*, Wiley, New York, 1979.
- [72] K.K. Unger, in: P.R. Brown, R.A. Hartwick (Eds.), *High Performance Liquid Chromatography*, Wiley/Interscience, New York, 1989, p. 145.
- [73] A.E. Ivanov, V.V. Saburov, V.P. Zubov, *Advances in Polymer Science*, vol. 104, Springer-Verlag, Berlin, 1992, p. 135.
- [74] G. Schomburg, *LC–GC* 6 (1988) 36.
- [75] M. Hanson, A. Kurganov, K.K. Unger, V.A. Davankov, *J. Chromatogr. A* 656 (1993) 369.
- [76] M. Petro, D. Berek, *Chromatographia* 37 (1993) 549.
- [77] C.G. Horvath, B.A. Preiss, S.R. Lipsky, *Anal. Chem.* 39 (1967) 1422.
- [78] J.E. Haky, A. Raghani, B.M. Dunn, *J. Chromatogr.* 541 (1991) 303.
- [79] Y.M. Zuo, B.R. Zhu, Y. Liao, M.D. Gui, Z.L. Pang, J.X. Qi, *Chromatographia* 38 (1994) 756.
- [80] J. Zhao, P.W. Carr, *Anal. Chem.* 71 (1999) 5217.
- [81] M.P. Rigney, T.P. Weber, P.W. Carr, *J. Chromatogr.* 484 (1989) 273.

- [82] J. Lecourtier, R. Audebert, C. Quivoron, J. Liq. Chromatogr. 1 (1978) 479.
- [83] R. Abuelfafiya, J. Pesek, J. Liq. Chromatogr. 12 (1989) 1571.
- [84] J.R. Garbow, J. Asrar, C.J. Hardiman, Chem. Mater. 5 (1993) 869.
- [85] M. Hanson, K.K. Unger, G. Schomburg, J. Chromatogr. 517 (1990) 269.
- [86] G.J. Fleer, J. Lyklema, Biol. Chem. 368 (1987) 741.
- [87] H. Engelhardt, H. Löw, W. Eberhardt, M. Mauß, Chromatographia 27 (1989) 535.
- [88] A.V. Neimark, M. Hanson, K.K. Unger, J. Phys. Chem. 97 (1993) 6011.
- [89] M. Hanson, B. Eray, K. Unger, A.V. Neimark, J. Schmid, K. Albert, E. Bayer, Chromatographia 35 (7–8) (1993) 403.
- [90] K.E. Collins, M.L.M.M. Granja, R.G. Ferreira Filho, T.A. Anazawa, I.C.S.F. Jardim, Chromatographia 45 (1997) 99.
- [91] J. Li, D.H. Reeder, A.V. McCormick, P.W. Carr, J. Chromatogr. A 791 (1997) 45.
- [92] J.W. Li, P.W. Carr, Anal. Chem. 69 (1997) 2193.
- [93] D.H. Reeder, J. Li, P.W. Carr, M.C. Flickinger, A.V. McCormick, J. Chromatogr. A 760 (1997) 71.
- [94] J. Zhao, P.W. Carr, Anal. Chem. 71 (1999) 5217.
- [95] G. Schomburg, J. Köhler, H. Figge, A. Deege, U. Bien-Vogelsang, Chromatographia 18 (1984) 265.
- [96] H. Engelhardt, M.A. Cunat-Walter, Chromatographia 40 (11–12) (1995) 657.
- [97] A.D. Kataev, V.V. Saburov, O.A. Reznikova, D.V. Kapustin, V.P. Zubov, J. Chromatogr. A 660 (1994) 131.
- [98] http://www.merck.co.th/eng/product/overview/hplc_content_62.html.
- [99] G. Huhn, H. Müller, J. Chromatogr. 640 (1993) 57.
- [100] G. Schomburg, Trends Anal. Chem. 10 (5) (1991) 163.
- [101] C. McNeff, L. Zigan, K. Johnson, P.W. Carr, A.M. Weber-Main, LC–GC 18 (5) (2000) 515.
- [102] T.P. Weber, P.W. Carr, E.F. Funkenbush, J. Chromatogr. 519 (1990) 31.
- [103] J. Li, Y. Hu, P.W. Carr, Anal. Chem. 69 (1997) 3884.
- [104] Q. Tang, Y. Shen, N. Wu, M.L. Lee, J. Microcolumn Sep. 11 (6) (1999) 415.
- [105] L. Sun, A.V. McCormick, P.W. Carr, J. Chromatogr. A 658 (1994) 465.
- [106] J. Li, P.W. Carr, Anal. Chim. Acta 334 (1996) 239.
- [107] J. Li, P.W. Carr, Anal. Chem. 69 (1997) 837.
- [108] J. Li, P.W. Carr, Anal. Chem. 69 (1997) 2202.
- [109] M. Hanson, K.K. Unger, Trends Anal. Chem. 11 (1992) 368.
- [110] D.A. Hanggi, N.R. Marks, LC–GC 11 (1993) 128.
- [111] M. Zaharescu, T. Cserhádi, E. Forgács, J. Liq. Chromatogr. Rel. Technol. 20 (18) (1997) 2997.
- [112] A. Kurganov, O. Kuzmenko, V.A. Davankov, B. Eray, K.K. Unger, U. Trüding, J. Chromatogr. 506 (1990) 391.
- [113] J.R. Garbow, J. Asrar, C.J. Hardiman, Chem. Mater. 5 (1993) 869.
- [114] N. Wu, Q. Tang, Y. Shen, M.L. Lee, Chromatographia 49 (7–8) (1999) 431.
- [115] N. Wu, Q. Tang, J.A. Lippert, M.L. Lee, J. Microcolumn Sep. 13 (2) (2001) 41.
- [116] Y. Mao, P.W. Carr, Anal. Chem. 72 (2000) 110.
- [117] A.J. Bondi, J. Phys. Chem. 68 (1964) 441.
- [118] P.W. Carr, J.J. Li, A.J. Dallas, D.I. Eikens, L.C. Tan, J. Chromatogr. A 656 (1993) 113.
- [119] L.C. Tan, P.W. Carr, J. Chromatogr. A 775 (1997) 1.
- [120] P.W. Carr, L.C. Tan, J.H. Park, J. Chromatogr. A 724 (1996) 1.
- [121] J.H. Park, Y.K. Lee, Y.C. Weon, L.C. Tan, J.W. Li, L. Li, J.F. Evans, P.W. Carr, J. Chromatogr. A 656 (1993) 113.
- [122] W.R. Melander, Cs. Horvath, Chromatographia 15 (1982) 86.
- [123] P. Jandera, J. Churáček, J. Chromatogr. 91 (1974) 207.
- [124] R. Tijssen, H.A.H. Billeiet, P.J. Schoenmakers, J. Chromatogr. 128 (1976) 65.
- [125] P.J. Schoenmakers, H.A.H. Billeiet, R. Tijssen, L. de Galan, J. Chromatogr. 149 (1978) 519.
- [126] P. Jandera, J. Churáček, in: J.C. Giddings (Ed.), Advances in Chromatography, vol. 19, Marcel Dekker, New York, 1981, pp. 125–260.
- [127] B. Karger, J.R. Gant, A. Hartkopf, P.H. Weiner, J. Chromatogr. 128 (1976) 65.
- [128] G.E. Berendsen, L. de Galan, J. Chromatogr. 196 (1980) 21.
- [129] E. Forgács, T. Cserhádi, Adv. Chromatogr. 40 (2000) 359.
- [130] J. Zhao, P.W. Carr, Anal. Chem. 72 (2000) 302.
- [131] J. Zhao, P.W. Carr, Anal. Chem. 70 (1998) 3619.
- [132] L.M. Sharygin, V.M. Galkin, V.E. Moiseev, V.G. Ponomarev, V.A. Davankov, A.A.K.a.T.M. Ponomarieva, Zhur. Fiz. Khim. 66 (1991) 2677.
- [133] A.A. Kurganov, V.A. Davankov, K.K. Unger, J. Chromatogr. 548 (1991) 207.
- [134] O. Itabashi, T. Goto, Chem. Ind. (London) (1986) 287.
- [135] T.M. Suzuki, O. Itabashi, T. Goto, T. Yokoyama, T. Kimuna, Bull. Chem. Soc. Jpn. 60 (1987) 2839.
- [136] O. Itabashi, T. Goto, Chem. Ind. (London) (1988) 162.
- [137] K. Hayakawa, K. Kawase, H. Yamakita, J. Appl. Polym. Sci. 21 (1977) 2921.
- [138] A. Kurganov, A. Tevlin, V. Davankov, J. Chromatogr. 261 (1983) 469.
- [139] A. Kurganov, O. Kuzmenko, V. Davankov, B. Erany, K.K. Unger, U. Trüding, J. Chromatogr. 506 (1990) 391.
- [140] Y. Ghaemi, R.E. Wall, J. Chromatogr. 174 (1979) 51.
- [141] F.E. Regnier, Methods Enzymol. 104 (1984) 170.
- [142] H. Small, J. Chromatogr. 546 (1991) 3.
- [143] H.F. Walton, Anal. Chem. 52 (1980) 15R.
- [144] R. Wood, L. Cummings, T. Jupille, J. Chromatogr. Sci. 18 (1980) 551.
- [145] J.D. Pearson, F.E. Regnier, J. Chromatogr. 255 (1983) 137.
- [146] H. Takayanagi, T. Ito, Y. Kubo, H. Kusano, Chromatographia 25 (7) (1988) 647.
- [147] T.G. Lawson, F.E. Regnier, H.L. Wenth, Anal. Biochem. 133 (1983) 85.
- [148] J. K. Towns, F.E. Regnier, J. Chromatogr. 516 (1) (1990) 69.
- [149] N. Kitagawa, LC–GC 6 (3) (1988) 260.
- [150] R.M. Chicic, Z. Shi, F.E. Regnier, J. Chromatogr. 339 (1986) 121.
- [151] L.A. Kennedy, W. Kopaciewicz, F.E. Regnier, J. Chromatogr. 359 (1986) 73.
- [152] M.A. Strege, A.L. Lagu, J. Chromatogr. 555 (1991) 109.
- [153] R.R. Drager, F.E. Regnier, Anal. Biochem. 145 (1985) 47.
- [154] J.J. Kirkland, J.W. Henderson, J.J. DeStefano, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 762 (1997) 97.
- [155] J.J. Kirkland, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 797 (1998) 111.
- [156] M.A. Rounds, W.D. Rounds, F.E. Regnier, J. Chromatogr. 397 (1987) 25.
- [157] R. Arshady, J. Chromatogr. 586 (1991) 199.
- [158] C. McNeff, Q.H. Zhao, P.W. Carr, J. Chromatogr. A 684 (1994) 201.
- [159] C. McNeff, P.W. Carr, Anal. Chem. 67 (1995) 2350.
- [160] C. McNeff, P.W. Carr, Anal. Chem. 67 (1995) 3886.
- [161] C. McNeff, Q. Zhao, E. Almlöf, M. Flickinger, P.W. Carr, Anal. Biochem. 274 (1999) 181.
- [162] J.H. Knox, P. Ross, Adv. Chromatogr. 37 (1997) 73.
- [163] K.K. Unger, Anal. Chem. 59 (1983) 361A.
- [164] J.H. Knox, B. Kaur, in: P.R. Brown, R.A. Hartwick (Eds.), High Performance Liquid Chromatography, Wiley, New York, 1989 (Chapter 4).
- [165] R. Leboda, Mater. Chem. Phys. 31 (3) (1992) 243.
- [166] R. Leboda, Mater. Chem. Phys. 34 (2) (1993) 123.
- [167] M.S. Mel'gunov, V.B. Fenelonov, T.A. Gorodetskaya, R. Leboda, B. Charmas, J. Colloid Interf. Sci. 229 (2000) 431.
- [168] V.M. Gun'ko, R. Leboda, M. Marciniak, W. Grzegorzczak, J. Skubiszewska-Zięba, A.A. Malygin, A.A. Malkov, Langmuir 16 (2000) 3227.
- [169] J.H. Knox, B. Kaur, G.R. Millward, J. Chromatogr. 352 (1986) 3.

- [170] H. Colin, G. Guiochon, *Carbon* 16 (1978) 145.
- [171] R. Lebeda, *Chromatographia* 13 (1980) 703.
- [172] H. Colin, C. Eon, G. Guiochon, *J. Chromatogr.* 119 (1976) 41.
- [173] E. Smolková, J. Zima, F.P. Dousek, J. Jansta, Z. Plzak, *J. Chromatogr.* 191 (1980) 61.
- [174] P.T. Jackson, P.W. Carr, *Chemtech* 28 (10) (1998) 29.
- [175] R. Lebeda, A. Gierak, Z. Hubicki, A. Lodyga, *Mater. Chem. Phys.* 30 (2) (1991) 83.
- [176] T.P. Weber, P.T. Jackson, P.W. Carr, *Anal. Chem.* 67 (17) (1995) 3042.
- [177] T.P. Weber, P.W. Carr, *Anal. Chem.* 62 (1990) 2820.
- [178] P.T. Jackson, T.-Y. Kim, P.W. Carr, *Anal. Chem.* 69 (24) (1997) 5011.
- [179] A.P. Sweeney, V. Wong, R.A. Shalliker, *Chromatographia* 54 (1–2) (2001) 24.
- [180] V. Wong, A.P. Sweeney, M. Khurram, R.A. Shalliker, *J. Liq. Chrom. Rel. Technol.* 25 (3) (2002) 363.
- [181] A.P. Sweeney, P. Wormell, R.A. Shalliker, *Macromol. Chem. Phys.* 203 (2) (2002) 275.
- [182] M. Gray, G.R. Dennis, P. Wormell, R.A. Shalliker, P. Slonecker, *J. Chromatogr. A* 975 (2002) 285.
- [183] P.T. Jackson, M.R. Schure, T.P. Weber, P.W. Carr, *Anal. Chem.* 69 (3) (1997) 416.
- [184] F.D. Anita, C.J. Horvath, *J. Chromatogr.* 435 (1988) 1.
- [185] H. Chen, Cs. Horváth, *J. Chromatogr. A* 705 (1995) 3.
- [186] B. Ooms, *LC–GC Int.* 9 (1996) 574.
- [187] M.H. Chen, Cs. Horváth, *J. Chromatogr. A* 788 (1997) 51.
- [188] M.H. Chen, Cs. Horváth, *Anal. Methods Instrum.* 1 (1993) 213.
- [189] R.M. Smith, R.J. Burges, *J. Chromatogr. A* 785 (1997) 49.
- [190] R.M. Smith, R.J. Burges, *Anal. Commun.* 33 (1996) 327.
- [191] B.A. Ingelse, H.G. Jansen, C.A. Cramers, *J. High Resol. Chromatogr.* 21 (1998) 613.
- [192] Y. Yang, A.D. Jones, C.D. Eaton, *Anal. Chem.* 71 (1999) 3808.
- [193] T.S. Kephart, P.K. Dasgupta, *Anal. Chim. Acta* 414 (2000) 71.
- [194] B. Yen, J. Zhao, J.S. Brown, P.W. Carr, *Anal. Chem.* 72 (2000) 1253.
- [195] S.M. Fields, Ch.Q. Fe, D.D. Zhang, B.R. Branch, X. Jason Zhang, N. Okafo, *J. Chromatogr. A* 913 (2001) 197.
- [196] J.D. Thompson, J.S. Brown, P.W. Carr, *Anal. Chem.* 73 (2001) 3340.
- [197] I.D. Wilson, *Chromatographia* 52 (Suppl.) (2000) S28.
- [198] T.S. Kephart, P.K. Dasgupta, *Talanta* 56 (6) (2002) 977.
- [199] Y. Mao, P.W. Carr, *Anal. Chem.* 73 (2001) 1821.
- [200] Y. Mao, P.W. Carr, *Anal. Chem.* 72 (2000) 2788.
- [201] Y. Mao, P.W. Carr, *Anal. Chem.* 72 (2000) 110.
- [202] G.J. Fleer, M.A. Cohen Stuart, J.M.H.M. Scheutjens, *Polymers at Interfaces*, Chapman & Hill, London, 1993.
- [203] I. Tsukerman, M.S. Thesis, Department of Chemistry, University of Minnesota, 2003.
- [204] Y. Mao, P.W. Carr, *Anal. Chem.* 73 (2001) 4478.
- [205] Y. Hu, X. Yang, P.W. Carr, *J. Chromatogr. A* 968 (2002) 17.
- [206] X. Yang, J. Dai, P.W. Carr, *Anal. Chem.* 75 (13) (2003) 3153.
- [207] J. Dai, X. Yang, P.W. Carr, *J. Chromatogr. A* 1005 (2003) 63.
- [208] X. Yang, J. Dai, P.W. Carr, *J. Chromatogr. A* 996 (2003) 13.
- [209] C.T. Rittenhouse, S.V. Olesik, *J. Liq. Chromatogr. Rel. Technol.* 19 (17–18) (1996) 2997.
- [210] W.S. Hancock, R.C. Chloupek, J.J. Kirkland, L.R. Snyder, *J. Chromatogr. A* 686 (1994) 31.
- [211] R.C. Chloupek, W.S. Hancock, B.A. Marchylo, J.J. Kirkland, B.E. Boyes, L.R. Snyder, *J. Chromatogr. A* 686 (1994) 45.
- [212] R.J. Markovich, X.X. Qiu, D.E. Nichols, C. Pidgeon, B. Invergo, F.M. Alvarez, *Anal. Chem.* 63 (17) (1991) 1851.
- [213] I.V. Fadeeva, S.M. Staroverov, G.V. Lisichkin, *Vestnik Moskovskogo Universiteta, Seriya 2: Khimiya* 28 (3) (1987) 285.
- [214] <http://www.zirchrom.com/default.asp>.