

CHROM. 22 061

PREPARATION AND EVALUATION OF A POLYMER-COATED ZIRCONIA REVERSED-PHASE CHROMATOGRAPHIC SUPPORT

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SUMMARY

The preparation of a reversed-phase high-performance liquid chromatographic support by deposition and cross-linking of polybutadiene on the surface of microporous zirconia is described. It is demonstrated that acidic solutes undergo very strong interactions with the zirconia surface even in the presence of a thick polymer layer. These interactions can be minimized by the use of phosphate in the mobile phase, in which case the small solute retention characteristics of the polymer coated zirconia are comparable to alkylsilane-derivatized silica-based reversed-phase supports. Most importantly, the polymer-coated zirconia was stable in alkaline solution. There was no evidence for degradation of the support even when it was exposed to a mobile phase of 1 *M* sodium hydroxide at 100°C. In contrast, polybutadiene-coated alumina was not stable under these conditions.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) is the most common mode of HPLC for many reasons, including its applicability to the separation of solutes of very different polarity, molecular weight and chemical functionality. Another important factor in the widespread use of RP-HPLC is the convenience, versatility and high efficiency of silica-based, microparticulate, bonded-phase chromatographic supports. The popularity of silica as a chromatographic support results from the cumulative impact of its excellent mechanical strength, availability in a wide range of pore sizes, nearly ideal pore structure (and concomitant mass transfer properties) and the great versatility of silanization chemistry for altering the chemical properties of its surface. However, silica gel and derivatized bonded phases also have significant limitations, most notably their solubility in aqueous alkaline media and lack of tolerance to harsh treatments both chemical and thermal. The dissolution of silica in aqueous mobile phases is widely recognized and has been extensively documented^{1–6}. The stability of silica-based bonded-phase supports is also limited in acidic mobile phases due to acid-catalyzed hydrolysis of the siloxane bonds (Si–O–Si) that anchor the bonded-phase to the silica surface^{7–9}. As an example of their lack of

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robustness, routine sanitization measures used extensively in biochemistry, such as cleaning with hot alkali, cannot be applied to silica-based columns. The inherent instability of silica-based supports is a particularly significant limitation for preparative-scale work. The need for extreme stability, chemical and otherwise, in preparative-scale columns is inherent in the high cost of the columns and the expense involved in developing large scale separations. Clearly it is very desirable in preparative-scale work to be able to rejuvenate contaminated columns. This often requires the use of very harsh reagents and conditions which can damage silica-based columns.

Although the great majority of work in RP-HPLC has been done using modified silica, a variety of other support materials have been investigated in an effort to develop alternatives to silica and silane chemistry to circumvent the above mentioned limitations. Among these are rigid and semi-rigid organic polymer supports¹⁰⁻¹², graphitized carbon¹³⁻¹⁵, and modified alumina¹⁶⁻²².

Although hydrous zirconia and zirconium phosphate have been used as ion-exchange supports, there has been only one report on the use of spherical, microparticulate zirconia as a reversed-phase chromatographic support²³. This work involved use of a low-surface-area zirconia (9 m²/g even after hydrothermal treatment) that was dynamically modified with a hydrophobic quaternary amine. No attempt was made to characterize the chromatographic properties of the zirconia nor to develop a permanent means of surface modification. A reversed-phase zirconia support has also been prepared by direct precipitation of an organophosphate or organophosphonate with zirconium(IV)²⁴⁻²⁶. This latter approach was used to prepare layered crystalline reversed-phase chromatographic supports of the formula Zr(O₃POR)₂, where R = butyl, lauryl, octylphenyl or octadecyl^{27,28}. Although these materials had reversed-phase characteristics, attempts to use particle sizes of less than 180 μm led to bed compaction and excessive back-pressures²⁵.

We have previously described the properties and alkaline stability of porous microparticulate zirconia²⁹. The goal of the present work was to produce a polymer-coated zirconia reversed-phase material with alkaline stability comparable to that of the unmodified zirconia.

EXPERIMENTAL

Chromatographic supports

A variety of samples of porous zirconia were used during the course of this work. The identity and physical properties of these materials were described previously²⁹. A 15 cm × 0.46 cm I.D. column packed with γ-RP1 polybutadiene-coated alumina was obtained from ES Industries (Marlton, NJ, U.S.A.). A sample of 5-μm Spherisorb 5AY alumina (Phase Separations, Norwalk, CT, U.S.A.) was also used.

Chemicals

All reagents used were obtained from commercial sources and were reagent grade or better, unless noted below. Polybutadiene (PBD), molecular weight 4500, 45% vinyl was obtained from Aldrich (Milwaukee, WI, U.S.A.). Polybutadiene standards of molecular weight 439, 982, 2760 and 22 000 were obtained from Polysciences (Warrington, PA, U.S.A.). Dicumyl peroxide (DCP) was obtained from Alfa (Danvers, MA, U.S.A.). Methanol, 2-propanol, acetonitrile and tetrahydrofuran (THF)

were CHROMAR grade obtained from Mallinckrodt (St. Louis, MO, U.S.A.). The water used in all experiments was from a Barnstead Nano-Pure system with a "Organic-Free" final cartridge. All chromatographic mobile phases were filtered through 0.45- or 0.22- μm filters prior to use. Water for use in the preparation of high pH mobile phases was boiled prior to use to remove carbon dioxide.

Apparatus

The chromatographic apparatus, column hardware and column packing procedures were described previously²⁹.

Modification procedure

The modification procedure used was based on that of Schomburg and co-workers^{30,31}. In all cases, the support was boiled in carbon dioxide-free water to fully hydrate the surface, dried at 125°C under vacuum for 12 h and cooled in a desiccator over P₂O₅ prior to modification. Four different modification procedures were used and are described below.

Procedure A. Zirconia with a high carbon load was prepared by adding 50 ml of pentane containing 0.55 g of PBD to 3.5 g of the support. The slurry was then placed in an ultrasonic bath and a vacuum applied for approximately 5 min. DCP (0.01 g) was added and the slurry again placed in an ultrasonic bath and a vacuum applied. The pentane was removed by vacuum (aspirator) and the material dried at 70°C *in vacuo* for 12 h. The coated support was heated in a tube furnace at 200°C for 2 h with a constant nitrogen purge. After removing the coated/cross-linked support from the tube furnace and allowing it to cool, it was washed successively with 200 ml of pentane, toluene, methylene chloride, THF, methanol and methanol-water (50:50).

Procedure B. Zirconia with a low carbon load was prepared by adding 35 ml of pentane containing 0.09 g of PBD to 3.5 g of the support. The slurry was then placed in an ultrasonic bath and a vacuum applied for approximately 5 min. A 10-ml volume of pentane containing 0.002 g of DCP was added and the slurry again placed in an ultrasonic bath and a vacuum applied. The slurry was then placed in a shaker bath at room temperature for 1 h after which the supernatant was removed by filtration. The material was heated in a tube furnace at 200°C for 4 h under nitrogen, washed as described in Procedure A and dried at 70°C for 12 h.

Procedure C. Zirconia with an intermediate carbon load was prepared by adding 50 ml of pentane containing 0.27 g of PBD to 3.0 g of the support. The slurry was then placed in an ultrasonic bath and a vacuum applied for approximately 5 min. A 10-ml volume of pentane containing 5.2 mg of DCP was added. The methodology of Procedure A was then followed.

Procedure D. A 20-ml volume of pentane containing 0.2 g of PBD was added to 3.5 g of support. The slurry was placed in an ultrasonic bath and a vacuum applied for approximately 5 min. The pentane was removed by an aspirator and the material dried at 70°C *in vacuo* for 12 h. The PBD was cross-linked by irradiation with an ESI Research accelerated-electron beam at a dosage of 5 Mrad at 175 kV by the Process Research Laboratory of the 3M Company (St. Paul, MN, U.S.A.). The cross-linked PBD-coated support was then washed as described in Procedure A.

Stability tests

The stability of the modified supports was determined by monitoring the retention of a set of non-polar test solutes with time in a mobile phase of methanol-0.1 *M* sodium hydroxide (50:50) at a temperature of 50°C. The columns used were 5 cm × 0.46 cm I.D. and were packed as described previously²⁹. A 5 cm × 0.46 cm I.D. column was also packed with PBD-coated alumina obtained by unpacking a 15 cm × 0.46 cm I.D. ES Industries γ RP-1 column.

The stability of zirconia-PBD and alumina-PBD in 1 *M* sodium hydroxide at 100°C was determined as described previously²⁹.

Chromatographic evaluation

Retention data were obtained at a flow-rate of 1 ml/min and a column temperature of 40°C unless otherwise noted. The system dead volume used in the calculation of capacity factors was determined by injection of ²H₂O, or by the method of Knox³². Volumes of 2, 5 or 10 μ l of a 1 mM solute solution were injected.

Static phosphate adsorption

A 10-ml volume of 10 mM phosphoric acid was added to 0.1 g of support and the mixture ultrasonicated under vacuum for 20 min. After 12–24 h, an aliquot of the supernatant was withdrawn, filtered through a 0.45- μ m filter and the phosphorous content determined by inductively coupled plasma emission spectrometry (ICP-ES). The amount of phosphate adsorbed was determined from the difference in this measurement and that of a phosphoric acid blank.

RESULTS AND DISCUSSION

Initial attempts to produce a zirconia-based reversed-phase were made by modifying the surface by reaction with organosilanes under a wide variety of conditions. Although reversed-phase supports of moderate coverages were obtained, silanized zirconia was not stable in aqueous mobile phases due to the susceptibility of the Zr-O-Si bond to hydrolysis. This instability is not surprising given the instability of Si-O-Si bonds and the fact that the replacement of a silicon atom in a siloxane bond by a heteroatom in general produces M-O-Si bonds which are less stable than the Si-O-Si bond³³.

Other attempts were made to produce monomeric zirconia-based reversed phases by reaction of zirconia with organozirconium compounds and by adsorption of organophosphonates, however, in all cases the resulting phases were not stable in alkaline media.

Preparation of polymer-coated zirconia

Stable zirconia-based reversed phases were prepared by depositing and cross-linking polymers on the zirconia surface. In addition to stability, another advantage of polymer-coated reversed phases is the ability to prepare materials with widely varying carbon loads, irrespective of the population of reactive sites on the surface or the stability of bonds to the surface. As shown by the data in Table I, the amount of carbon on the support surface is easily varied over an order of magnitude by adjusting the amount of polybutadiene offered to the support. It is important to take into

TABLE I
COATING OF ZIRCONIA WITH POLYBUTADIENE

%PBD ^a	Preparation method ^b	%C ^c	d_f ^d
0.88	C	1.2	0.22
2.7	C	2.1	0.39
2.9	B	0.84	0.19
4.0	B	2.9	0.60
5.0	D	2.3	0.43
5.0	C	4.6	0.85
5.0	C	4.5	0.83
5.0	C	4.3	0.79
15.7	A	7.7	1.7
15.7	A	7.5	1.7

^a %PBD = %PBD (w/w) in solution, with respect to weight of zirconia.

^b For details, see Experimental section.

^c %C = %carbon (w/w) on coated support as determined by conventional C,H,N analysis.

^d d_f = film thickness (nm), as determined from zirconia surface area and amount of immobilized PBD (assumes no change in PBD density as a result of immobilization and cross-linking).

account the surface area of the zirconia supports when considering the carbon analysis data in Table I. When normalized to the support surface area, these data cover a range which includes "typical" carbon loads of commercial silylated SiO₂ reversed-phase supports. Note that under the conditions given above the modification process is reproducible as reflected by the agreement among the carbon analysis data obtained on different supports prepared from the same lot of zirconia with the same amount of polybutadiene.

Clearly, the carbon load (or film thickness) of the coated supports increases upon increasing the amount of PBD added to zirconia, although this increase is not linear with the amount of PBD offered, as shown in Fig. 1. This is not surprising since results of static adsorption experiments indicate that approximately 30 mg PBD/g zirconia represents monolayer coverage on a zirconia support with 50 m²/g of surface

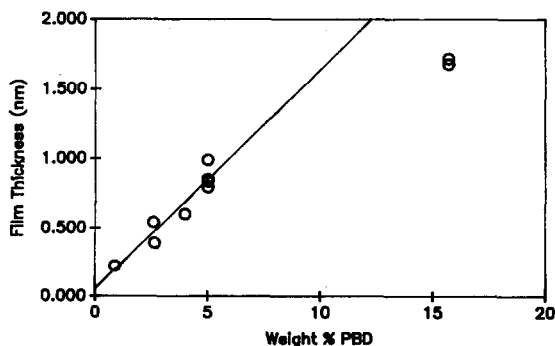


Fig. 1. Variation of PBD film thickness with %PBD. Zirconia-PBD prepared according to procedures A and C. Weight% PBD and film thickness have the same meaning as in Table I.

area. When the PBD coverage exceeds a monolayer, it is likely that washing with pentane prior to cross-linking removes a greater fraction of the PBD deposited on the zirconia surface than it does when coverages are lower. It is also quite possible that geometric considerations dictate less effective cross-linking as coverage exceeds a monolayer. Both of these factors lead to lower immobilization "efficiency" with increasing amounts of applied PBD.

It is, of course, unavoidable that as the amount of polymer on the surface is increased, there will be a concomitant decrease in the surface area of the support as the thickness of the polymer layer reduces the effective mean pore diameter of the support. This loss in surface area with increasing polymer film thickness is demonstrated by the data of Table II. This phenomenon is not limited to polymer-coated supports; it is well documented for silylated supports³⁰. When zirconia-PBD was prepared as described in Procedure A, almost all of the surface area was lost. Microscopic inspection of the support particles showed a very irregular outer surface and a high degree of particle aggregation. By contrast, at carbon loads approximating monolayer coverage the loss in surface area is comparable to that encountered in silanization of silica with octadecylsilane. There is no evidence of an irregular polymer layer or particle aggregation.

As noted in the experimental section, the PBD cross-linking reaction was initiated by either a chemically generated free radical, or via electron-beam irradiation of the coated sample in the absence of a chemical initiator. The extent of cross-linking, as well as the course of the cross-linking reaction, was followed by monitoring the C=C stretching band at approximately 1640 cm^{-1} in the infra-red spectrum. Fig. 2 shows diffuse reflectance infrared Fourier transform (DRIFT) spectra of a sample cross-linked with DCP at avarious exposure times at 200°C . A 60% decrease in absorbance at 1640 cm^{-1} occurs during the first hour of treatment at 200°C and after 21.5 h the absorbance is decreased by more than 90%. We do not believe that this extremely low apparent degree of unsaturation is due solely to a high cross-linking efficiency, rather we think that some oxidative degradation may occur in addition to the cross-linking reaction. A spectrum of the sample cross-linked by irradiation with a high energy electron beam is shown in Fig. 3. The extent of cross-linking is comparable to that achieved with DCP-initiated cross-linking.

Column efficiency

One of the potential limitations of polymer-coated chromatographic supports is

TABLE II
VARIATION IN SURFACE AREA OF POLYBUTADIENE-COATED ZIRCONIA

%C ^a	Preparation method ^b	d_f ^a	SA_b ^c	SA_a ^d
2.9	B	0.19	50.4	38.9
5.0	C	0.85	60.6	29.3
7.7	A	1.7	50.4	4.0

^a As defined in Table I.

^b For details, see Experimental section.

^c SA_b = surface area of zirconia before immobilization of PBD.

^d SA_a = surface area of PBD-coated zirconia.

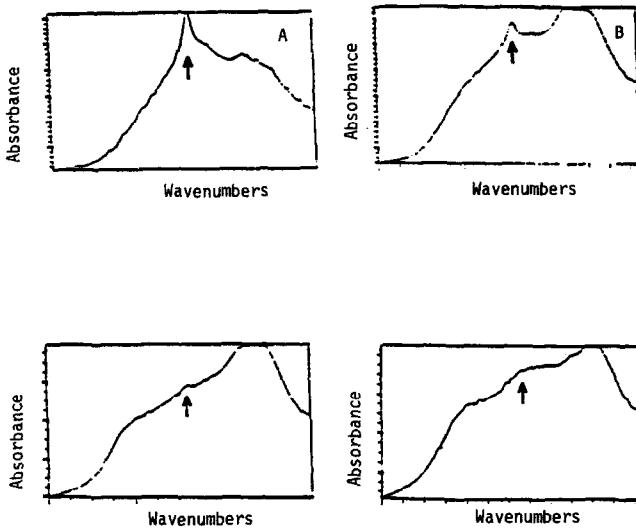


Fig. 2. DRIFT spectra of zirconia coated with 50 mg/g of PBD. A = 0 h at 200°C; B = 1 h at 200°C; C = 4 h at 200°C; D = 21.5 h at 200°C. C=C stretching bands at 1640 cm^{-1} are indicated by the arrows.

a loss in chromatographic efficiency that may result from restricted mass transfer of solutes in a polymer layer. We investigated the extent of this problem by determining the efficiency of supports coated with varying amounts of PBD. Plots of plate height, h , vs. linear velocity, u , for PBD-zirconia with two different PBD film thicknesses are shown in Fig. 4. Note that varying the film thickness by a factor of approximately three has an observable effect on the efficiency of the chromatographic support. This effect is evidenced by the fact that the support with a $0.43\text{-}\mu\text{m}$ PBD film reaches its minimum h of 2.9 at a mobile phase velocity of about 0.042 cm/s , whereas the support

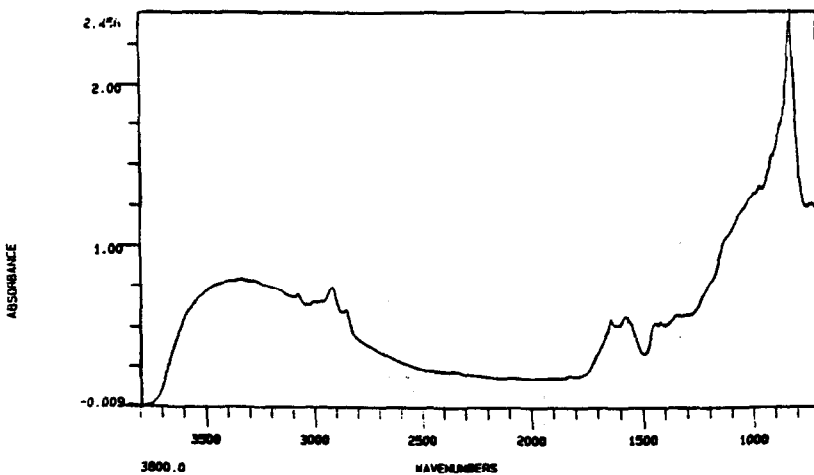


Fig. 3. DRIFT spectrum of zirconia coated with 50 mg/g PBD and crosslinked by high energy electron beam. Dosage = 5 Mrad at 175 kV.

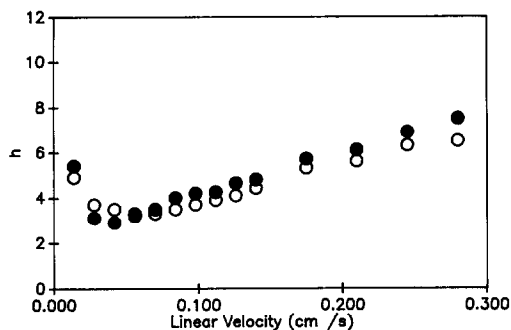


Fig. 4. Efficiency of PBD-coated zirconia. Solute octanophenone. Mobile phase methanol-water (60:40) Column temperature 25°C. PBD film thickness: ○ = 0.15 nm; ● = 0.43 nm.

with a 0.15- μm PBD film reaches its minimum h of 3.3 at a mobile phase velocity of 0.056–0.07 cm/s. Both of these effects would result if restricted mass transfer in the polymer film was a significant factor in determining the chromatographic efficiency. The smaller h_{opt} exhibited by the support with the 0.43- μm film is not consistent with this conclusion, however, this data was obtained on a lot of zirconia with a fairly broad particle size distribution and a high level of very small ($< 2 \mu\text{m}$) particles such that the column beds were rather unstable. It is likely that the poorer optimum efficiency exhibited by the support with the 0.15- μm film is a consequence of the geometry of the packing bed and not related to the film thickness. Despite evidence of the adverse effect of the polymer layer on efficiency, it should be recognized that these supports do have an h_{opt} of about 3. Columns packed from lots of zirconia with a narrower particle size distribution routinely have h_{opt} of less than 3.

Stability of modified zirconia

The results of stability testing on PBD-coated supports prepared as described in Procedures A–D, and on a PBD-coated alumina support are shown in Fig. 5. For all of the zirconia–PBD columns there was an initial 2–5% decrease in the retention of ethylbenzene after which there was no further decrease. In the case of the alumina–PBD column, the stability test was terminated after 8000 column volumes of methanol–0.1 M sodium hydroxide (50:50) were passed through the column due to the appearance of double peaks and a 20% increase in column pressure. The alumina column was disassembled and the column bed examined. A void of approximately 2–3 mm had formed at the head of the column, and there was obvious channelling along the walls of the column. When the PBD-coated alumina column was unpacked, aggregated particles of diameter greater than 44 μm were observed. None of the zirconia columns exhibited any voids, nor any other indication of instability upon exposure to 30 000 column volumes of the alkaline mobile phase.

The data in Table III show that the carbon content of the supports decreased after treatment at high pH. Note that the carbon content of the aggregated $> 44 \mu\text{m}$ alumina–PBD particles was approximately 15% higher than that of the 5- μm alumina–PBD. Coupled with the results of the chromatographic evaluation, the carbon analysis indicates a loss of uncross-linked or partially cross-linked polymer in the initial stages of the stability test despite the preliminary washing. Sample C was

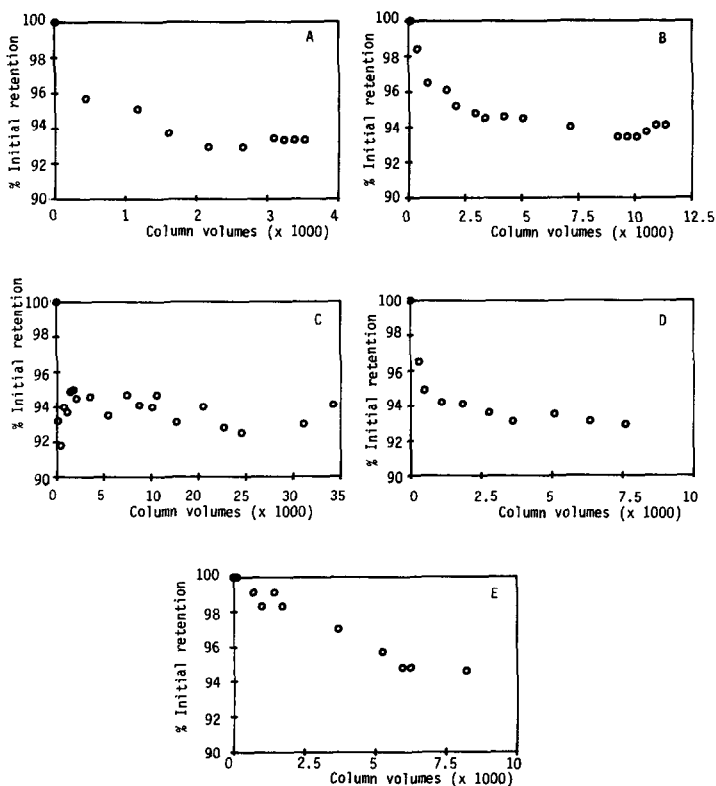


Fig. 5. Chromatographic evaluation of the alkaline stability of PBD-coated supports. A = zirconia-PBD prepared by Procedure A; B = zirconia-PBD prepared by Procedure B; C = zirconia-PBD prepared by Procedure C; D = zirconia-PBD prepared by Procedure D; E = Al₂O₃-PBD.

TABLE III

CARBON CONTENT OF POLYBUTADIENE-COATED SUPPORTS BEFORE AND AFTER TREATMENT WITH AN ALKALINE ELUENT

Sample	%C _b ^a	%C _a ^b
A	7.7	6.7
B	0.8	0.6
C	2.5	2.1
D	2.3	1.7
Al ₂ O ₃	3.8	3.0
Al ₂ O ₃ > 44 μm		3.5

^a Carbon content of support as determined by conventional C, H, N analysis.

^b Carbon content of support after exposure to methanol-0.1 M sodiumhydroxide (50:50) at a flow-rate of 1 ml/min and a column temperature of 50°C. Volume of mobile phase passed through each column is shown in Fig. 5.

Soxhlet extracted with toluene for 24 h prior to testing its stability in an attempt to remove partially cross-linked oligomers, but this treatment had no apparent effect on the initial decrease in retention, nor on the loss of carbon.

A third measure of stability was to assay the column effluent for zirconium by ICP-ES. This evaluation was carried out for samples C and D in Table III. Zirconium was absent at the level of detectability of $0.03 \mu\text{g/ml}$. Even if zirconium was present at the detection limit, this corresponds to a cumulative loss of less than 0.001% of the mass of zirconium originally present in the column. No determination of aluminum in the effluent of the PBD-coated alumina column was made, however, the peak dou-

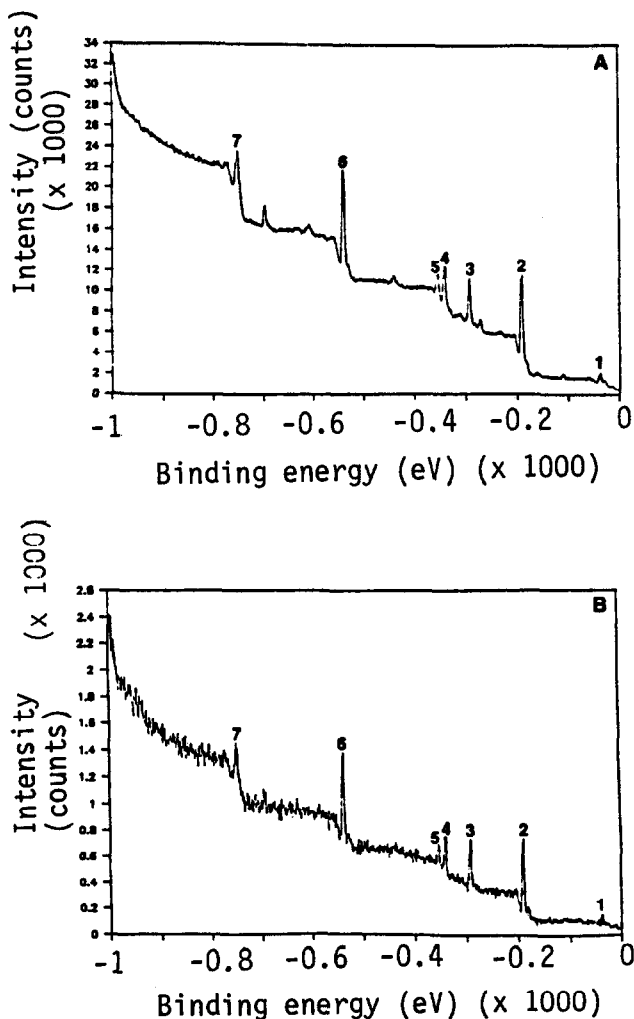


Fig. 6. ESCA spectra of zirconia-PBD. A = Spectrum of sample A before alkaline stability test; B = spectrum of sample A after 30 000 column volumes of methanol-0.1 *M* sodium hydroxide (50:50). Column temperature 50°C. Peaks: 1 = Zr 4p; 2 = Zr 3d; 3 = C 1s; 4 = Zr 3p 5/2; 5 = Zr 3p 1/2; 6 = O 1s; 7 = O Auger.

bling, increased column pressure and presence of a substantial void at the head of the column after the stability test all indicated that alumina dissolved during the course of the experiment.

Electron spectroscopy for chemical analysis (ESCA) spectra of a sample of zirconia-PBD before and after the stability test described above are shown in Fig. 6. Once again, there is no evidence for any gross changes in the zirconia-PBD after treatment at extreme pH.

In addition to the stability test described above, the stability of zirconia-PBD and alumina-PBD prepared by procedure C was evaluated by exposing columns packed with these supports to a mobile phase of 1 *M* sodium hydroxide at 100°C. The results of ICP analysis of the column effluents are given in Table IV. No zirconium was found in the column effluent. In contrast, it is obvious from the data in Table IV that alumina dissolves to a significant extent during the first hour of exposure to these conditions. In fact, after 3.25 h of exposure the amount of aluminum found in the effluent corresponded to loss of more than 10% of the alumina originally present in the column. The dissolution of alumina was confirmed by visual inspection of the column, which revealed a void of several mm as well as channeling along the column walls. Initially, it was surprising that the dissolution of such a large amount of alumina was not accompanied by a collapse of the column bed, but the alumina has a relatively low porosity (*ca.* 50%) so that a large amount of alumina would have to dissolve before the column bed would collapse.

The retention of a reversed-phase test probe, ethyl benzene, actually increased by approximately 15% on both columns after this treatment. Although this increase in retention is not understood, it does point out that, unlike a conventional monomeric bonded phase column in which dissolution of the inorganic support must be accompanied by loss of bonded phase, dissolution of the support of a polymer-coated column does not necessarily result in loss of stationary phase. Given the high degree of cross-linking of the polymer coating and its insolubility in typical reversed-phase eluents, it is likely that the polymer would remain in the column even as the inorganic column bed dissolves. This is a possible explanation for the existence of the large (aggregated > 44 μm) particles in the alumina column after exposure to alkaline conditions. Exposure to alkaline conditions causes dissolution of alumina particularly from the more accessible outer surface of a bead. The relative amount of PBD on

TABLE IV

EXPOSURE OF POLYBUTADIENE-COATED SUPPORTS TO 'STERILIZING' CONDITIONS

Column	Time (h) ^c	Al ($\mu\text{g/ml}$) ^a	Si ($\mu\text{g/ml}$) ^a	Zr ($\mu\text{g/ml}$) ^a
Al ₂ O ₃ -PBD	1	680	13.6	— ^b
	3.25	243	3.3	— ^b
Zirconia-PBD	1	— ^b	1.6	— ^b
	3.25	— ^b	2.1	— ^b
Zirconia, unmodified	1	— ^b	2.4	— ^b
Blank (1 <i>M</i> NaOH)		— ^b	1.5	— ^b

^a $\mu\text{g/ml}$ in column effluent as determined by ICP-ES.

^b Less than the detection limit by ICP-ES.

^c Time of exposure to 1 *M* NaOH at a flow-rate of 1 ml/min; *T* = 100°C.

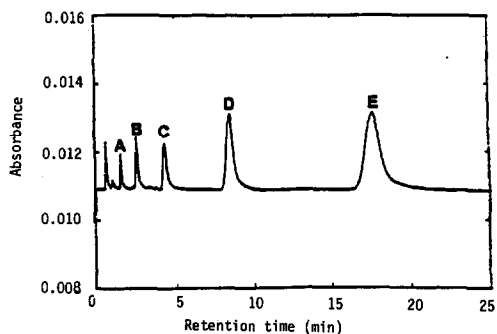


Fig. 7. Separation of alkyl aryl ketones on zirconia-PBD. Column = 5 cm \times 0.46 cm I.D. packed with 3.5- μ m zirconia with 0.39 nm PBD film. Column temperature 25°C. Mobile phase methanol-water (50:50). Flow-rate 1 ml/min. Peaks: A = propanophenone; B = butanophenone; C = pentanophenone; D = hexanophenone; E = heptanophenone.

the outer surface will thereby increase and particle aggregation occurs. This conclusion is supported by the high carbon content of these aggregates relative to the 5- μ m particles recovered from the column as well as by the fact that a significant loss in reversed-phase retention is not observed despite the dissolution of a substantial amount of alumina.

The results described above strongly suggest that a meaningful evaluation of the alkaline stability of an inorganic-based polymer-coated chromatographic support must be based not only on the retention of non-polar test solutes, but also on analysis of the effluent for dissolved support.

Retention characteristics

A typical separation of several alkyl aryl ketones on a zirconia-PBD support is shown in Fig. 7. As expected for a reversed-phase support, the solutes elute in order of increasing alkyl carbon number. The hydrophobicity of the zirconia-PBD support is clearly indicated by the linearity of the plot of log capacity factor (k') versus alkyl carbon number for these solutes, shown in Fig. 8. Similar plots for alkylbenzenes and

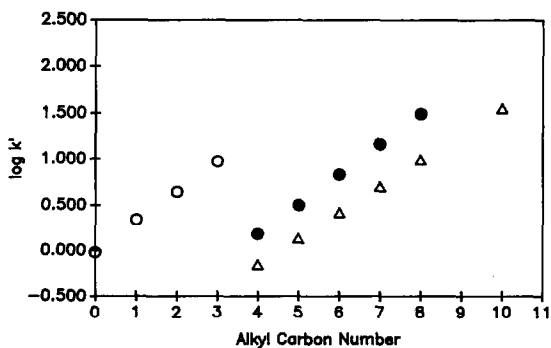


Fig. 8. Reversed-phase retention on zirconia-PBD. Mobile phases as indicated in figure; other chromatographic conditions as in Fig. 7. \circ : Alkylbenzenes, methanol-water (50:50), slope = 0.33, intercept = -0.011, $r = 0.9993$; \bullet : alkylarylketones, methanol-water (50:50), slope = 0.33, intercept = -1.14, $r = 0.9999$; Δ : alkylarylketones, methanol-water (60:40), slope = 0.28, intercept = -1.30, $r = 0.9999$.

for the alkyl aryl ketones in a stronger mobile phase are shown in Fig. 8. In view of the more polar nature of the alkyl aryl ketones, the alkylbenzenes were more retained than the alkyl aryl ketones at any alkyl carbon number as expected for reversed-phase retention. Increasing the methanol content of the mobile phase decreased the retention of the alkyl aryl ketones and also led to a lower slope in the plot of $\log k'$ vs. carbon number; this is also consistent with a reversed-phase retention mechanism.

The general retention characteristics of zirconia-PBD relative to a monomeric octyl-silane support were further evaluated in a pH 3 phosphate-containing mobile phase using the solvatochromic comparison method³⁴ with a set of 18 solutes. The overall conclusion from the solvatochromic comparison experiment is that there are no dramatic qualitative differences in the retention characteristics of the zirconia-PBD column relative to a conventional RP-HPLC column. This is, at least in part, due to the fact that the conditions chosen for this comparison minimized the effect of solute interactions with the zirconia support. Under different chromatographic conditions the effect of these interactions becomes quite evident. This is demonstrated by the data in Table V which summarizes the retention of several test solutes on two different zirconia-PBD columns. These data show only slight differences in the retention of a variety of uncharged non-polar solutes on a given column in the three

TABLE V

CHROMATOGRAPHIC EVALUATION OF PBD-COATED ZIRCONIA COLUMNS

Capacity factors for a 5 μ l injection of a 1 mM test solute solution determined at a flow-rate of 1 ml/min and a column temperature of 40°C.

Solute	Capacity factors					
	Column 1 ^a			Column 2 ^b		
	Mobile phase			Mobile phase		
	1 ^c	2 ^d	3 ^e	1 ^c	2 ^d	3 ^e
Benzene	1.03	1.02	0.93	0.16	0.16	0.05
Toluene	2.14	2.16	2.04	0.40	0.40	0.20
Ethylbenzene	4.24	4.15	4.02	0.78	0.76	0.70
Nitrobenzene	0.68	0.66	0.54	0.11	0.12	0
Fluorobenzene	1.09	1.10	1.00	0.19	0.18	0.13
Chlorobenzene	2.70	2.70	2.50	0.54	0.52	0.44
Bromobenzene	3.60	3.60	3.36	0.74	0.72	0.63
Iodobenzene	5.65	5.65	5.30	1.18	1.16	1.03
Benzoic acid	-f	-f	-0.23	-f	-f	-0.22
Cinnamic acid	-f	-f	-0.17	-f	-f	-0.21
Phthalic acid	-f	-f	-0.23	-f	-f	-0.19
Phenyl phosphonic acid	-f	-f	-0.22	-f	-f	-0.21

^a 5 cm \times 0.46 cm I.D. ZrO₂-PBD column with 0.43 nm PBD film.

^b 5 cm \times 0.46 cm I.D. ZrO₂ column with 0.15 nm PBD film.

^c Mobile phase 1: methanol-water (40:60).

^d Mobile phase 2: methanol-0.1 M NaCl (40:60).

^e Mobile phase 3: methanol-0.1 M phosphate buffer pH 7 (40:60).

^f No elution observed in 1 h at 0.0005 a.u.f.s.

different mobile phases, indicating that these solutes are retained by a reversed-phase retention mechanism.

In contrast, elution of benzoic acid, phthalic acid, cinnamic acid and phenyl phosphonate was not observed at all in methanol-water (40:60), even in the presence of 100 mM sodium chloride. This indicates that these solutes were either irreversibly adsorbed onto the zirconia support, or were so strongly retained that their retention times were too long and the peaks too broad to permit detection. When phosphate was added to the mobile phase, these solutes eluted with negative capacity factors. We previously reported that the presence of phosphate in the mobile phase converts microporous zirconia into a cation exchanger²⁹, and these data indicate that solute elution occurred prior to the dead volume as a result of electrostatic exclusion of the negatively charged solute from the pores of the negatively charged support.

Note that the data in Table V show no evidence that solute-zirconia interactions were diminished on the support with a thicker (0.43 nm) film. In fact, although the data are not given, this same effect was observed on supports with PBD films as thick as 1.5 nm. Clearly, there is a very strong interaction between these anionic solutes and the zirconia support which the PBD film does not completely inhibit, regardless of its thickness. This is consistent with the expectation that deactivation of surface sites does not necessarily accompany the deposition of a polymer film since in many cases no specific interaction or reaction with the surface sites occurs. By contrast, a substantial "deactivation" which was dependent on film thickness was reported with silica coated with PBD³¹.

The apparent accessibility of anion-exchange sites on the zirconia surface even after deposition of a thick polymer film was confirmed by determining the static phosphate adsorption capacity of several supports with different PBD film thicknesses. The results of this experiment, which are summarized in Table VI, show that some reduction in phosphate adsorption accompanies the deposition of a PBD film on the zirconia surface. Fig. 9 shows that the amount of phosphate adsorbed decreases linearly with PBD film thickness for films of less than 0.58 nm. The amount of phosphate decreased from 3.9 $\mu\text{moles}/\text{m}^2$ on bare zirconia to 2.3 $\mu\text{moles}/\text{m}^2$ on zirconia-PBD with a 0.58 nm PBD film. Note that this data encompasses a PBD molecular

TABLE VI
STATIC PHOSPHATE ADSORPTION ON ZIRCONIA-PBD

Support	PBD (mol. wt.)	d_f^a	$\mu\text{moles}/\text{m}^2^b$
Zirconia	No PBD	0	3.93
Zirconia	400	0.1	3.54
Zirconia	2700	0.43	3.42
Zirconia	900	0.24	3.22
Zirconia	4500	0.22	3.21
Zirconia	4500	0.45	2.74
Zirconia	4500	1.7	2.35
Zirconia	22 000	0.58	2.30

^a d_f = PBD film thickness in nm.

^b $\mu\text{moles}/\text{m}^2$ = μmoles of phosphate adsorbed/ m^2 zirconia.

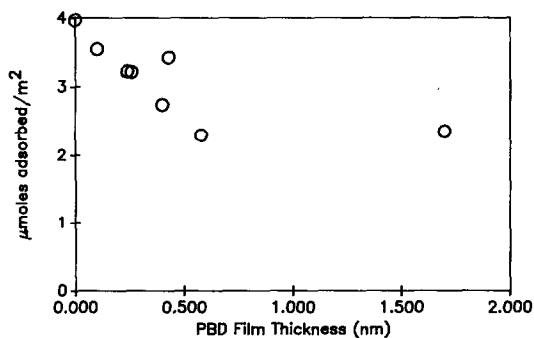


Fig. 9. Dependence of phosphate adsorption on PBD film thickness d_f and $\mu\text{moles}/\text{m}^2$ as defined in Table VI.

weight range from 400 to 22 000 daltons. The value of $2.3 \mu\text{moles}/\text{m}^2$ appears to represent the lower limit of phosphate adsorption, as increasing the PBD film thickness by a factor of three did not result in any further reduction in the amount of phosphate adsorbed on the zirconia-PBD support. The significance of this lower limit is not clear. The independence of the amount of phosphate adsorbed on the molecular weight of PBD clearly indicates that the $2.3 \mu\text{moles}/\text{m}^2$ of "unblockable" anion-exchange sites are not a consequence of a simple size exclusion effect, that is, they are not sites which are located in micropores or other regions of the zirconia particle which are accessible to inorganic phosphate but inaccessible to PBD because of its larger size. These unblocked sites are analogous to the silanol groups which remain underivatized on silica after reaction with small organosilanes (*i.e.*, endcapping reagents). The resistance of these silanol sites to derivatization has been explained in terms of geometric effects related to the highly fractal nature of the silica surface³⁵. Since the surface of zirconia is also highly fractal, it is likely that local geometric factors (as opposed to geometric factors such as exclusion from pores) are involved in the fact that a finite number of surface sites remain accessible even after deposition of an extremely thick PBD film. Another possible explanation for the adsorption of $2.3 \mu\text{moles}/\text{m}^2$ of phosphate even in the presence of a heavy PBD load is that a PBD monolayer covers a certain fraction of the zirconia surface leading to the initial decrease in phosphate adsorption and that additional PBD layers, which are not in intimate contact with the zirconia surface, do not further restrict access to the surface.

The chromatographic results and the results of the static phosphate adsorption experiment both clearly indicate that the zirconia-PBD support has substantial anion-exchange capacity. The presence of accessible anion-exchange sites leads to mixed-mode retention mechanisms which complicate the chromatography of acidic solutes such as carboxylic acids and organophosphonates. One possible strategy for inhibiting the interaction of these solutes with the zirconia surface is to use phosphate-containing mobile phases so that inorganic phosphate competes for anion-exchange sites with the solute molecules. This approach is analogous to the use of amine-containing mobile phases to improve the chromatography of amines on silica-based reversed phases.

The data in Table VII demonstrate the effect of various concentrations of inorganic phosphate on the chromatography of several test solutes. If the retention of

TABLE VII
EFFECT OF PHOSPHATE CONCENTRATION ON RETENTION ON PBD COATED ZIRCONIA^a

Solute	1 mM H ₃ PO ₄ ^b		10 mM H ₃ PO ₄ ^c		100 mM H ₃ PO ₄ ^d	
	<i>k'</i> ^e	<i>N</i> ^f	<i>k'</i>	<i>N</i>	<i>k'</i>	<i>N</i>
Benzene	0.58	242	0.60	205	0.47	278
Benzoic acid	0.64	43	0.53	82	0.24	263
Toluene	1.0	179	1.1	159	0.84	216
Toluic acid	0.90	37	0.72	79	0.40	187
Benzylamine	0.32	196	0.36	211	0.23	273
Phenyl phosphonic acid	— ^g		— ^g		0.13	43

^a Column, 5 cm × 0.46 cm I.D. packed with 10 μm–15 μm zirconia–PBD; flow-rate, 1 ml/min; column temperature, 40°C; injection volume, 5 μl; solute concentration, 1 mM.

^b Mobile phase: methanol–1 mM H₃PO₄ (50:50) adjusted to pH 3.

^c Mobile phase: methanol–10 mM H₃PO₄ (50:50) adjusted to pH 3.

^d Mobile phase: methanol–100 mM H₃PO₄ (50:50) adjusted to pH 3.

^e *k'* = capacity factor.

^f *N* = 5.54 (*t_r*/*W*)².

^g No elution observed at *k'* < 100.

these solutes were due solely to a reversed-phase process, benzoic acid and *p*-toluic acid should elute before benzene and toluene, respectively, as a result of their greater polarity. In fact, in 1 mM phosphate at pH 3, benzoic acid is slightly more retained than benzene, while toluene is more retained than toluic acid. In 10 mM and 100 mM phosphate, benzoic acid and toluic acid elute before benzene and toluene, respectively. Clearly, in the presence of even a small amount of phosphate in the mobile phase, there are few anion-exchange sites present on the zirconia–PBD support. This finding is consistent with the reported conversion of zirconia from an anion exchanger to a cation exchanger upon treatment with inorganic phosphate³⁶. In 1 mM phosphate, the benzoic acid and toluic acid peaks are tailed and broader than the benzene and toluene peaks as reflected in the number of theoretical plates. (It should be noted that the column used for this experiment was packed with a 10–15 μm zirconia–PBD support that had a non-uniform particle size distribution. As a result the column efficiency was low. The comparison of the benzoic acid and toluic acid peaks to the benzene and toluene peaks is still valid.)

The chromatography of benzoic acid and toluic acid improved upon increasing the phosphate concentration so that in the presence of 100 mM phosphate the plate counts determined from these peaks are comparable to those determined for benzene and toluene. Note that phenyl phosphonic acid is irreversibly adsorbed in both the 1 and 10 mM phosphate mobile phases, and elutes as a broad peak in 100 mM phosphate. The chromatography of benzylamine is comparable to that of benzene. It elutes sooner than benzene as is expected for a less hydrophobic solute and has a slightly wider peak, but there is no evidence that it exhibits the strong interactions with zirconia–PBD that acidic solutes do under these conditions. Other inorganic phosphate species, including pyrophosphate and tripolyphosphate, were also used as mobile phase modifiers without appreciably different results from those described above for orthophosphate. Mobile phase additives other than phosphate will most

likely be less effective in controlling the interaction of these solutes with the zirconia surface since their interaction with the surface is weaker than that of phosphate. Results obtained with acetic acid as a mobile phase modifier confirmed this expectation.

Clearly, a more desirable approach for controlling the interactions with the zirconia surface would be to deactivate the surface permanently by incorporation of a blocking agent in the modification procedure. An attempt was made to do so by adsorbing allylphosphonate onto the anion-exchange sites both before and after deposition of the PBD film and to then "anchor" the allylphosphonate by cross-linking it with the PBD. The initial attempt involved adsorbing the allylphosphonate onto unmodified zirconia from methanol and then depositing a PBD film according to Procedure C given above. The effectiveness of the allylphosphonate deactivation was determined by examining the chromatography of benzoic acid and other solutes in methanol-H₂O (50:50) mobile phase. Benzoic acid was irreversibly adsorbed on an untreated zirconia-PBD support under these conditions. The initial result on the allylphosphonate-deactivated support was encouraging in that elution of benzoic acid was observed, however, there was still strong anion-exchange retention as evidenced by a capacity factor of 6.2 for benzoic acid compared to a value of 0.3 for benzene. Additional injections of benzoic acid were made and the retention increased in a regular fashion upon each injection as shown in Fig. 10. The extent of increase in retention was dependent on the amount of injected benzoic acid. If no injections were made, there was essentially no increase in the retention of benzoic acid with time. These observations indicate that the benzoic acid displaced allylphosphonate from the zirconia surface thereby exposing more sites and causing an increase in anion-exchange retention with each subsequent injection. The same approach to deactivation was also attempted using octylphosphonic acid, but once again, deactivation was incomplete and the retention of benzoic acid increased upon repetitive injections.

Both of the supports prepared by adsorbing an organophosphonate and then depositing a PBD film had carbon loads which were much lower than expected based on the amount of PBD offered. This could be due to desorption of the adsorbed

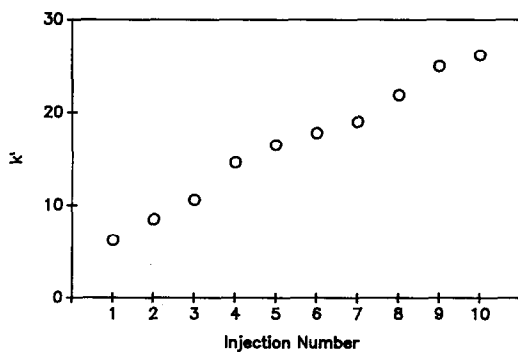


Fig. 10. Retention of benzoic acid on deactivated zirconia-PBD. Column, 5 cm \times 0.46 cm I.D. packed with 10–15 μ m zirconia coated with allylphosphonate and PBD; mobile phase, methanol–10 mM H₃PO₄ (50:50) adjusted to pH 3; flow-rate 1 ml/min; column temperature, 40°C; injection volume, 5 μ l; solute concentration, 1 mM.

phosphonate into pentane during the wash step in the PBD deposition procedure, to less adsorption of PBD onto the non-polar zirconia-organophosphonate surface from pentane, or to the masking of specific sites on the zirconia surface onto which PBD adsorbs. To avoid any or all of these possible problems, the deactivation was attempted by depositing the PBD film and then adsorbing allylphosphonate onto the surface from methanol, however, this approach also gave low carbon loads and an unstable, incompletely deactivated surface.

Although a stable, completely deactivated zirconia-PBD support was not prepared, deactivation of the zirconia surface with an unsaturated organophosphonate, such as allylphosphonate, followed by deposition and cross-linking of a polymer film should be a viable approach to the preparation of a permanently deactivated zirconia-PBD. Experimental problems which need to be addressed are the appropriate organophosphonate to use and the appropriate solvents and conditions from which to adsorb the organophosphonate and PBD in order to maximize surface coverage and deactivation.

CONCLUSIONS

The preparation of a polymer-coated zirconia-based support has been described. Polybutadiene was immobilized on porous microparticulate zirconia and cross-linked chemically or by irradiation with high-energy electrons. The coated, cross-linked support has very high alkaline stability which we believe is superior to that of any inorganic-based chromatographic support previously reported, and probably adequate for any conceivable use.

Zirconia-PBD has very similar retention characteristics to alkyl-silica bonded phases with respect to the retention of small solutes in a phosphate buffer at pH 3. Under other conditions, particularly when phosphate is absent from the mobile phase, acidic solutes are very strongly retained due to interactions with (basic) anion-exchange sites on the surface of the zirconia support. These interactions can be controlled to some extent by the addition of phosphate to the mobile phase.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contributions of E. F. Funknenbusch, D. A. Hanggi, Scott Culler and Paul Martin of the 3M Company. This work was supported in part by grants from the University of Minnesota Center for Biological Process Technology and the 3M Company.

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