

CHROM. 21 788

## PHYSICAL AND CHEMICAL CHARACTERIZATION OF MICROPOROUS ZIRCONIA

M. P. RIGNEY<sup>a,\*</sup>, E. F. FUNKENBUSCH<sup>b</sup> and P. W. CARR

*Department of Chemistry, University of Minnesota, 207 Pleasant St. S.E., Minneapolis, MN 55455 (U.S.A.)*

---

### SUMMARY

Small (< 10  $\mu\text{m}$ ) microporous zirconia has been prepared and evaluated as an alkaline stable high-performance liquid chromatographic support. Zirconia is an amphoteric metal oxide which exhibits both anion- and cation-exchange properties depending on the solution pH and the nature of the buffer. The affinity of zirconia for polyoxy anions, particularly the effect of phosphate on the properties of the zirconia, is examined. Most significantly, the exceptional stability of zirconia, even under extreme conditions, is demonstrated and compared to the stability of alumina.

---

### INTRODUCTION

At present, 3- and 5- $\mu\text{m}$  microparticulate silica gels, and related bonded phase supports are the most versatile and advanced chromatographic supports available. 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 hence mass transfer properties), the great versatility of the silanization chemistry and the availability of reagents for altering the chemical properties of its surface. However, silica gel also has a number of significant limitations, particularly its solubility in aqueous alkaline media.

The dissolution of silica in aqueous mobile phases is widely recognized and has been extensively documented<sup>1–6</sup>. The stability of silica-based bonded phase supports is also limited in acidic solution due to the acid-catalyzed hydrolysis of the siloxane bonds (Si–O–Si) anchoring the bonded phase to the surface<sup>7–9</sup>. Silica and bonded phase supports can be stabilized to alkali by acid and base pretreatment of the silica<sup>10</sup>, the use of a sacrificial presaturation column<sup>11</sup>, and by cladding the silica surface with alkaline-stable metal oxides such as zirconia and titania<sup>12–20</sup>. Although all such measures improve the stability, and in some cases the support can be used at a pH as high as 10, the stability is not as great as one could desire for a number of applications, particularly for biological separations.

---

<sup>a</sup> Present address: Ecolab, Inc., Ecolab Center, St. Paul, MN 55102, U.S.A.

<sup>b</sup> Present address: 3M Company, 3M Center, St. Paul, MN 55144, U.S.A.

A variety of substrates have been evaluated in an effort to develop chemically stable chromatographic supports. For example, rigid and semi-rigid organic polymers, particularly polystyrene-divinylbenzene (PS-DVB) supports, have been widely studied as alternatives to silica-based reversed-phase supports<sup>21-24</sup>. The two major advantages of PS-DVB supports in comparison to silica-based supports are their excellent stability over a broad pH range, and the absence of silanol groups on their surface. However, reversed-phase chromatography on these support materials is often characterized by asymmetric peaks and undesirably long retention times, particularly for aromatic solutes. Although improvements have been made with these supports, the chromatographic efficiency attainable on PS-DVB supports is generally not comparable to the efficiencies attainable on silica-based bonded phase microparticulate supports<sup>21,24</sup>.

Carbon (in the form of charcoal) was widely used as an adsorbent in the early days of chromatography, particularly in the displacement and frontal modes<sup>25-29</sup>. Since that time various carbonaceous adsorbents have continued to attract interest as reversed-phase chromatographic supports, primarily because of their excellent stability over a broad pH range. However, it was clear even in the early work of Tiselius cited above<sup>27-29</sup> that the surface of charcoal is very heterogeneous as reflected in peak asymmetry and irreversible adsorption of many solutes. The most homogeneous form of carbon is graphite, but most forms of graphite have a very low surface area or are too fragile for use as a high-performance liquid chromatographic (HPLC) support. Knox and Gilbert<sup>30</sup> developed a method of preparing porous graphitic carbon (PGC) by pyrolysis of a phenol-formaldehyde polymer coated on silica followed by dissolution of the silica "template" and thermal treatment to improve surface homogeneity. PGC has adequate mechanical stability for use as an HPLC support, but the surface remains somewhat heterogeneous and is likely to be subject to the type of long term oxidation common to all carbon surfaces.

The search for a material which possesses the desirable mechanical and physical properties of silica but with improved aqueous stability has also led to the preparation and evaluation of alumina-based stationary phases. Alumina has historically been widely used as a support for normal phase adsorption chromatography, although its use has not been as widespread as that of silica due at least in part to the greater complexity of the adsorption mechanism on alumina. Recently, high-performance ion-exchange and reversed-phase supports have been prepared by coating polymers onto the surface of alumina<sup>31,32</sup>, and these supports have shown considerable improvement relative to silica with respect to alkaline stability.

We believe there are potential advantages to the use of an inorganic support, particularly for applications at the preparative and process scale, and our objective is to develop and characterize a microparticulate, zirconia-based support with the desirable physical and mechanical properties of silica, but with chemical stability superior to any other inorganic support. We are also interested in chemically modifying this support for use in a variety of chromatographic modes, including reversed-phase (RP) HPLC<sup>33</sup>.

Zirconia and zirconium phosphate have been widely used as ion-exchange chromatographic supports, particularly in the nuclear industry for applications such as the treatment of reprocessing solutions<sup>34</sup>, high-level waste treatment, recovery of fission products and high-temperature ion-exchange separations. The attributes of

zirconia and zirconium phosphate that have made them particularly appealing in these applications are their extreme chemical and thermal stability. For example, hydrous zirconia is stable to water treatment at temperatures as high as 300°C<sup>35</sup>, and zirconium phosphate has also been used at temperatures as high as 300°C for extended periods of time<sup>36,37</sup>.

The porous zirconia spherules used in this work were comprised of monoclinic zirconia crystals. Crystalline zirconia has an extremely high melting point (2700°C), exceptional resistance to attack by both acids and alkalis, and excellent mechanical properties. The surface of zirconia, like most metal oxides, is highly hydroxylated. The existence of hydroxyl groups on the surface of zirconia has been confirmed spectroscopically<sup>38-41</sup>. Two distinct hydroxyl infrared bands have been reported<sup>38,39,41</sup> and have been assigned to surface bridging and terminal hydroxyl groups. We expect that the bridging hydroxyl, being strongly polarized by two zirconium(IV) ions will be more acidic while terminal hydroxyls more basic.

As with other metal oxides, the surface hydroxyl groups on zirconia control the surface chemistry, particularly with respect to the acid-base properties. Zirconia is an amphoteric metal oxide. The isoelectric point (IEP) and/or point-of-zero charge (PZC), range from less than 3 to higher than 10<sup>42-48</sup>. It is obvious from the wide range of these values that the source, type, chemical and thermal pretreatment and method of measurement can have a profound effect on the apparent acidity and basicity.

The presence of acidic and basic groups on the surface of zirconia is reflected in its cation- and anion-exchange properties. Zirconia has anion-exchange properties in neutral and acid solution and cation-exchange properties in alkali solutions<sup>49-51</sup>. Zirconia has a unique selectivity for polyoxy anions such as borate, carbonate, phosphate, sulfate, chromate, arsenate, arsenite, molybdate and tungstate<sup>52</sup>. A particularly interesting property, first observed by Amphlett *et al.*<sup>48</sup> is that treatment of zirconium oxide with phosphoric acid converts it to zirconium phosphate and that the treated support displays cation-exchange properties at 2 < pH < 14.

There has only been one previous report on the use of zirconia as a HPLC support<sup>53</sup>, and this work involved the use of a low-surface-area zirconia (9 m<sup>2</sup>/g even after hydrothermal treatment) that was dynamically modified with a hydrophobic quaternary ammonium salt. No attempt was made to characterize the chromatographic properties of the zirconia nor to develop a permanent means of surface modification.

On the basis of this brief review it is obvious that, despite more desirable chemical stability, the potential of zirconia as an HPLC support has not been evaluated. The objective of the present paper was to study the properties of unmodified zirconia as a prelude to other studies in which its surface is chemically modified<sup>33</sup>.

## EXPERIMENTAL

### Chemicals

All reagents were obtained from commercial sources and were reagent grade or better unless noted below. Triphenyl phosphine oxide, diphenyl phosphoric acid and diphenyl phosphinic acid were obtained from Aldrich (Milwaukee, WI, U.S.A.). Phenyl phosphonic acid was obtained from Alfa Chemicals (Danvers, MA, U.S.A.). The disodium salt of nicotinamide adenine dinucleotide, reduced form (grade III) was

obtained from Sigma (St. Louis, MO, U.S.A.). Methanol and 2-propanol 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 an "Organic-Free" final cartridge. All chromatographic mobile phases were filtered through 0.45- or 0.22- $\mu\text{m}$  filters prior to use. Water for use in the preparation of solutions for the alkaline stability tests can be boiled to remove carbon dioxide prior to use.

### Chromatographic supports

The porous zirconia spherules were provided by the Ceramic Technical Center of 3M Co. (St. Paul, MN, U.S.A.). The spherules were prepared by a proprietary process, fired at high temperature (typically 600°C), and sized by air classification. During the course of this work several different lots of zirconia with widely different physical properties were used. Some of the relevant physical properties, as determined by the BET nitrogen adsorption isotherm technique, are summarized in Table I.

The alumina used was from two sources: Woehlm acidic alumina, activity grade 1 obtained from Alupharm Chemicals (New Orleans, LA, U.S.A.), and 5- $\mu\text{m}$  Spherisorb 5AY alumina (Phase Separations, Norwalk, CT, U.S.A.). The physical properties of these supports are also given in Table I.

### Apparatus

HPLC studies were carried out on one of two chromatographic systems. System 1 consisted of a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 1090L liquid chromatograph with a DR5 binary solvent delivery system and a filter photometric detector with a 254-nm filter installed. For stability testing with alkaline mobile phases an expanded pH range kit was installed. Absorbance data were digitized, integrated and plotted using a Hewlett-Packard Model 3393A integrator interfaced to the chromatograph via a HP-IL interface loop.

System 2 consisted of an IBM Instruments (Danbury, CT, U.S.A.) Model 9533 liquid chromatograph, a Rheodyne Model 7125 injection valve and an IBM Model 9522 fixed-wavelength UV detector with a 254-nm filter installed. Absorbance data were typically digitized and stored on an IBM 9000 computer using the chromato-

TABLE I  
PROPERTIES OF POROUS ZIRCONIA SPHERULES

NA = Data not available.

Sample	Particle size ( $\mu\text{m}$ )	Surface area ( $\text{m}^2/\text{g}$ )	Pore diameter ( $\text{\AA}$ )	Porosity (%)
ZrO <sub>2</sub> 1	50	50	100	NA
2	30-40	30	106	30
3	1-40	41	150	48
4	20-40	50	24	47
5	5-10	55	146	53
6	<8	61	96	45
7A	5-10	62	95	35
7B	10-15	62	95	35
Al <sub>2</sub> O <sub>3</sub>	5	92	132	NA

graphic applications package (CAP), version 1.4 provided by IBM. Chromatograms from System 2 were printed either on the IBM 9000 printer or on a Hewlett-Packard Model 7470 plotter using a BASIC program to read the data files and drive the plotter.

System 2 was used with a Tracor Model 965 photoconductivity detector for the chromatography of inorganic anions on zirconia. In order to make a differential conductivity measurement, the photoreactor coil was disconnected and a "T" placed in the eluent stream between the pump and the injector to divert mobile phase into the reference cell of the conductivity detector.

For experiments in which chromatographic supports were exposed to hot (100°C) 1 M sodium hydroxide, an Altex (Berkeley, CA, U.S.A.) Model 110A pump and a Fisher (Pittsburgh, PA, U.S.A.) Isotemp oven were used.

The columns used in the experiments were packed in 5 cm × 0.46 cm I.D. or 5 cm × 0.21 cm I.D. column blanks of 316 stainless-steel (316 SS). Two different end fitting configurations based on ¼-in., 316 SS Parker-Hanifin end fittings were used. Type 1 used an unmodified end fitting with a ¼ in. × ⅜ in. 2-μm titanium frit. Type 2 used a 2-μm 316 SS screen in a specially modified Parker-Hanifin end fitting as described by Sadek<sup>54</sup>. Particles greater than 15 μm diameter were packed by preparing a dilute slurry of the support material in methanol or isopropyl alcohol, and then drawing this slurry through a column with a type 2 end fitting by house vacuum (or aspirator). Particles smaller than 15 μm were packed at 6000–9000 p.s.i. from a methanol or isopropyl alcohol slurry using a stirred upward slurry packing technique.

#### *Acid-base titrations*

The titrations of zirconia and silica with 0.1 M hydrochloric acid and 0.1 M sodium hydroxide were performed by preparing a 10-mg/ml slurry of the support in 20% (w/v) sodium chloride in a beaker thermostatted at 30°C and blanketed with a flow of nitrogen. The titrant was dispensed in increments of 0.025 ml with a 2.5-ml capacity Radiometer pH Stat autoburette, and after each addition the pH of the slurry was measured with an Orion Model 801 pH meter equipped with an Orion Ross combination pH electrode.

#### *Stability testing*

The stability of zirconia and alumina at pH 1, 3, 10, 12 and 14 was examined under static conditions by adding 100 mg of support to 900 ml of the appropriate solution and monitoring the appearance of zirconium or aluminum in the supernatant solution. Solutions at pH 10, 12 and 14 were 0.0001, 0.01 and 1.0 M carbon dioxide-free sodium hydroxide, respectively. Solutions at pH 1 and pH 3 were 0.1 M hydrochloric acid and 0.001 M hydrochloric acid, respectively. The solutions were stored at ambient temperature and were agitated twice per day. Testing was performed at various time intervals by withdrawing 10 ml from the supernatant solution, filtering through a 0.45-μm filter and determining the concentration of zirconium or aluminum by inductively coupled plasma emission spectrometry (ICP-ES). The amount of zirconia and alumina dissolved was determined as follows:

$$\% \text{ zirconia dissolved} = \frac{\text{zirconium}_f \cdot V_s \cdot 123.22 \cdot 100\%}{91.22 \cdot M_z}$$

where  $\text{zirconium}_f = \mu\text{g/ml}$  of zirconium found by ICP-ES;  $V_s = \text{ml}$  of solution before sample aliquot is removed;  $M_z = \text{mass}$  of zirconia in sample solution.

$$\% \text{ alumina dissolved} = \frac{\text{aluminum}_f \cdot V_s \cdot 101.96 \cdot 100\%}{26.9815 \cdot 2 \cdot M_A}$$

where  $\text{aluminum}_f = \mu\text{g/ml}$  found by ICP-ES;  $V_s = \text{ml}$  of solution before sample aliquot is removed;  $M_A = \text{mass}$  of alumina in sample solution.

The stability of zirconia under "sterilizing conditions" was determined by exposing a 5 cm  $\times$  0.46 cm I.D. zirconia column (type I end fitting) to a mobile phase of 1 *M* sodium hydroxide for 3.25 h at 100°C and determining the amount of zirconium in the column effluent by ICP-ES.

## RESULTS AND DISCUSSION

The proprietary procedure for preparing porous microparticulate zirconia produces very regular spherical particles. Initially, zirconia with a mean particle diameter as large as 50  $\mu\text{m}$  was produced, however, considerable progress has been made through the course of the work in the production of small zirconia particles. Mean particle diameters in the 5–10- $\mu\text{m}$  range can now be prepared routinely. The mechanical stability of the zirconia particles appears to be comparable to that of silica. Columns have been slurry packed at pressures as high as 9000 p.s.i. and operated under extreme conditions for long periods of time without any evidence of mechanical instability.

The surface areas given in Table I are low when compared to the surface areas of the silica supports typically used in HPLC, however, it is important to recognize when considering the surface area data that the true density of monoclinic zirconia is approximately 5.8 g/ml<sup>55</sup>, whereas the true density of a common commercial silica packing is approximately 2.3 g/ml (ref. 2). Due to its higher density, the surface area of zirconia is comparable to that of silica when considered in terms of surface area per unit volume. It has been estimated that a high-performance silica column usually has a surface area to volume ratio of 125 m<sup>2</sup>/ml<sup>56</sup>. By comparison, a typical zirconia column has a ratio of 122 m<sup>2</sup>/ml<sup>57</sup>.

The particle size distribution for a 5–10- $\mu\text{m}$  fraction of zirconia is shown in Fig. 1. A typical pore size distribution, determined by mercury intrusion porosimetry, is shown in Fig. 2. Note that the size distribution is fairly uniform although there is some skewing of the distribution towards small particle diameters. Because of the high density of zirconia the size classification of the particles has been somewhat problematic, however, considerable improvement continues to be made in the classification of zirconia particles and hence narrower size distributions can be obtained.

As noted above, the reported acid–base properties of zirconia vary over a wide range. Many previous reports<sup>58–61</sup> examined the acid–base properties of zirconia by adsorption of acidic and basic compounds from the gas phase, however, in most chromatographic applications zirconia will be used in a partially aqueous environment, therefore the determination of its acid–base properties in aqueous media seemed most relevant to its end use. Consequently the acid–base properties of zirconia were briefly studied by titration in aqueous media. Titration of zirconia with hydrochloric

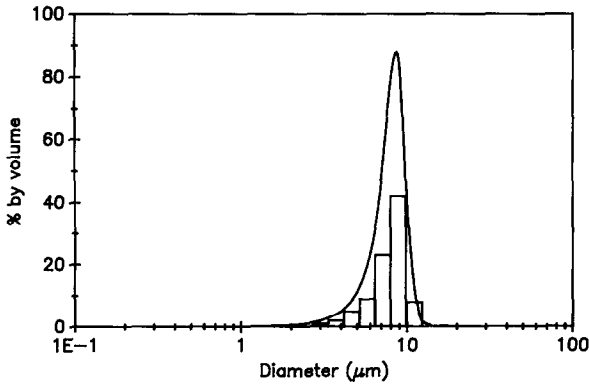


Fig. 1. Particle size distribution of zirconia sample 7A from Table I.

acid gave no indication of any strongly basic sites. As shown in Fig. 3, a titration with sodium hydroxide gave very similar results to those obtained for silica, when the data are presented per unit area. The pH of the 1% aqueous suspension of zirconia was 5.5, compared to a pH of 4.2 for the 1% suspension of silica. These titration results are consistent with other reports in the literature<sup>47</sup>.

In addition to its acid-base properties, another consideration relevant to the derivatization and chromatographic use of zirconia is the concentration of adsorptive sites on its surface. The concentration of these sites was roughly estimated from the thermogravimetric analysis curve (TGA) shown in Fig. 4. Based on the water loss determined by TGA, a value of  $9.8 \mu\text{mol}/\text{m}^2$  of active sites is obtained if we arbitrarily assume that the mass decrease from 205–455°C is due primarily to the loss of strongly hydrogen-bonded water and that the mass decrease from 455–810°C results from the condensation of neighboring zirconium hydroxides. This is not a truly quantitative

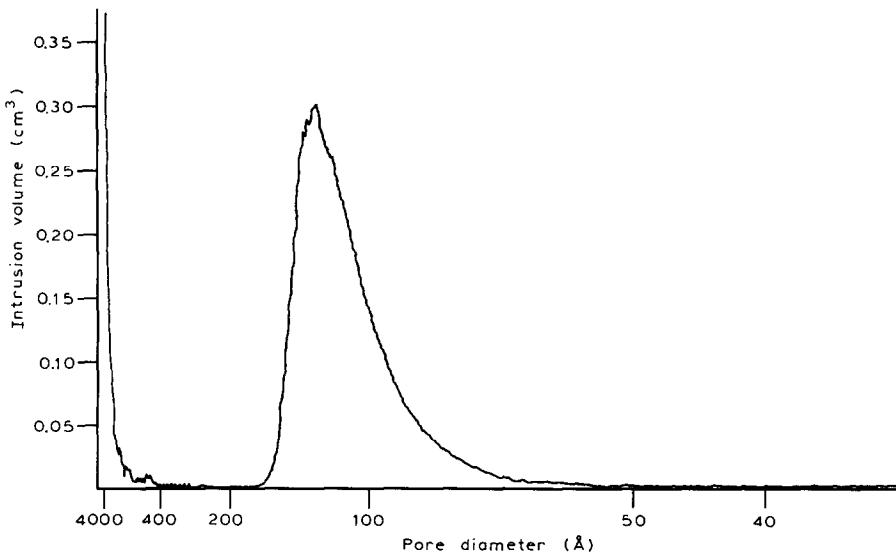


Fig. 2. Pore size distribution of zirconia sample 5 from Table I.

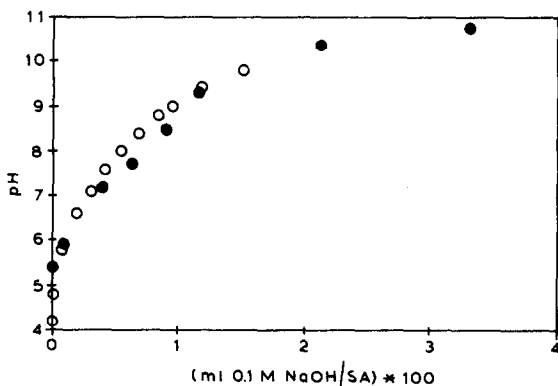


Fig. 3. Titration of zirconia ( $ZrO_2$ , ●) and silica (Nuclcosil 100-30, ○) with 0.1 M NaOH. SA = Surface area.

measure of the surface population of  $ZrOH$  groups but it does provide a rough estimate of the hydroxyl content of the zirconia surface. Estimates of the surface hydroxyl population based on adsorption of organic and inorganic phosphates are of the same order of magnitude and indicate that  $9.8 \mu\text{mol}/\text{m}^2$  is an upper limit.

As already noted, the primary motivation for evaluating zirconia as a support material is its stability at extreme pH values, particularly under alkaline conditions. Therefore, its stability from pH 1–14 was determined under static conditions and compared to that of alumina. Alumina was chosen as a “reference” because it has been studied and is being used as an alkaline-stable alternative to silica as a chromatographic support material. No comparison was made to silica since it is known to dissolve in aqueous alkaline media<sup>1–6</sup>. The results of the static stability test for zirconia, as well as for alumina are shown in Fig. 5. Note that there was no zirconium detected in the supernatant solutions at any pH (detection limit =  $0.03 \mu\text{g}/\text{ml}$ ). By contrast, detectable levels of aluminum were found at pH 1, 3, 12 and 14. The superior stability of zirconia relative to alumina at  $\text{pH} \geq 12$  and  $\text{pH} \leq 3$  is obvious. Although it is very likely that different forms and sources of alumina will have different relative

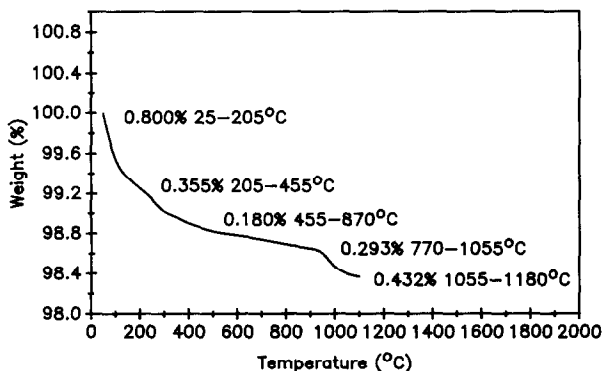


Fig. 4. Thermogravimetric analysis of zirconia.



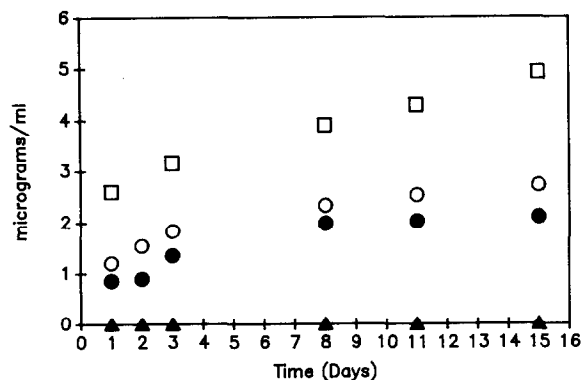


Fig. 5. Alkaline stability of zirconia and alumina. ▲ = ZrO<sub>2</sub>, pH 1-14; □ = Al<sub>2</sub>O<sub>3</sub>, pH 14; ○ = Al<sub>2</sub>O<sub>3</sub>, pH 12; ● = Al<sub>2</sub>O<sub>3</sub>, pH 3.

stabilities, it is still clear that in terms of stability in aqueous mobile phases at extreme pH (either acidic or basic), zirconia is the material of choice, not silica or alumina. It should be noted that the static conditions used in this experiment constitute a much less stringent test of stability than the dynamic conditions inherent in typical chromatographic usage.

The stability of zirconia under alkaline conditions was further tested by exposing a column of zirconia particles to a mobile phase of 1 *M* sodium hydroxide at 100°C for 3.25 h at a flow-rate of 1 ml/min. There was no zirconium detected in the column effluent after this treatment. Even if zirconium were present at just below the detection limit (0.03 μg/ml) this corresponds to the dissolution of less than 0.001% of the mass of zirconia originally present in the column under these very extreme conditions.

Given that the extreme stability of the zirconia makes it a viable alternative inorganic support, it is important to understand its surface chemistry. The ion-exchange retention of a variety of organic ionic solutes on zirconia was studied in order to develop a better understanding of its surface properties. The capacity factors ( $k'$ ) for these test solutes under a variety of conditions are given in Table II. Several qualitative conclusions can be made based on this data. Zirconia acts as an anion exchanger at pH 5 in an acetate buffer and at pH 8 in a Tris buffer. Note that as the pH is increased from 5 to 8 the retention of anionic solutes decreased as expected. However, even at pH 8 there was still no retention of cationic solutes. At pH 9.2 in a borate buffer, only cations are retained on the zirconia. Although zirconia has been reported to have a very strong interaction with borate (and other oxygen containing polyanions), the addition of borate did not cause any irreversible changes in the properties of the zirconia. This is indicated by the agreement between the capacity factors obtained in acetate buffer before and after exposure of the column to borate, as shown in Table II. The data in Table II also show that at pH 8 in Tris buffer, treatment of the column with 5 *mM* phosphate resulted in retention of only cations. This conversion of zirconia from an anion exchanger to a cation exchanger at pH 8 by treatment with phosphate was not readily reversible as evidenced by the fact that overnight flushing with a Tris buffer containing no phosphate followed by a redetermination of the retention of the test solutes in Tris buffer at pH 8 (again with no phosphate present in the mobile phase)

TABLE II  
ION-EXCHANGE PROPERTIES OF ZIRCONIA

Column: 5 cm × 0.21 cm I.D. packed with 30–40 μm zirconia. Mobile phase: 100 mM aqueous buffer as specified in table heading. Flow-rate: 1 ml/min. Column temperature: 25°C.

Solute	$pK_a$	Acetate (pH 5) <sup>a</sup>		Tris (pH 8) <sup>b</sup>			Borate (pH 9.2)
		A	B	A	B	C	
Benzylamine	9.3	0	0	0	1.9	1.9	0.3
Diphenyl cyclam	12 ( $pK_1$ )	0	0	0	6.0	6.0	1.0
<i>p</i> -Nitrobenzoic acid	3.4	2.2	2.3	0	0	0	0
Benzoic acid	4.2	5.8	6.2	1.7	0	0	0
<i>cis</i> -Cinnamic acid	3.9	7.0	6.7	2.3	0	0	0
<i>trans</i> -Cinnamic acid	4.4						
Hydroxybenzoic acid	4.5 ( $pK_1$ )	14.8	14.6	8.6	0	0	0
	9.3 ( $pK_2$ )						
Acetophenone		0	0	0	0	0	0

<sup>a</sup> A = Before exposure to borate buffer; B = after exposure to borate buffer.

<sup>b</sup> A = Before exposure to phosphate buffer; B = 50 mM phosphate in mobile phase; C = no phosphate in mobile phase after extensive flush with Tris buffer.

gave essentially the same results as those obtained in Tris buffer with 5 mM phosphate present in the mobile phase. Thus, although the acid–base titration data gave no indication of amphoteric acid–base properties, the ion-exchange results do show both anion- and cation-exchange properties, depending on the solution pH and the nature of the buffer. The transition from an anion exchanger to a cation exchanger occurs between pH 8 and pH 9.2.

An example of a separation of four weak organic acids at pH 5 in an acetate buffer is shown in Fig. 6. The efficiency of the zirconia column was determined as a function of flow-rate in the same mobile phase. The results are given in Fig. 7. Note that there is a strong dependence of efficiency on flow-rate, with the efficiency decreasing as the flow-rate increases. The efficiencies achieved are comparable to those observed for ion exchange on unmodified alumina<sup>62</sup> and silica<sup>63</sup>.

The retention of a variety of inorganic anions on zirconia in a 10 mM acetate buffer at pH 5.0 is summarized in Table III. Note that the oxyanions  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{BrO}_3^-$ ,  $\text{B}_4\text{O}_7^{2-}$  and  $\text{S}_2\text{O}_3^{2-}$  are strongly retained on zirconia, as expected given the strong interaction between zirconia and oxygen-containing anions which was noted

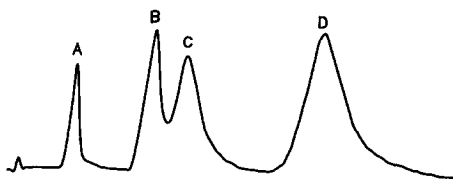


Fig. 6. Anion-exchange separation on zirconia. 5 cm × 0.46 cm I.D. column packed with 10–15 μm zirconia. Mobile phase: 10 mM acetate buffer at pH 5.0. Flow-rate: 1 ml/min. Column temperature: 40°C. A = *p*-Nitrobenzoic acid; B = benzoic acid; C = cinnamic acid; D = hydroxybenzoic acid.

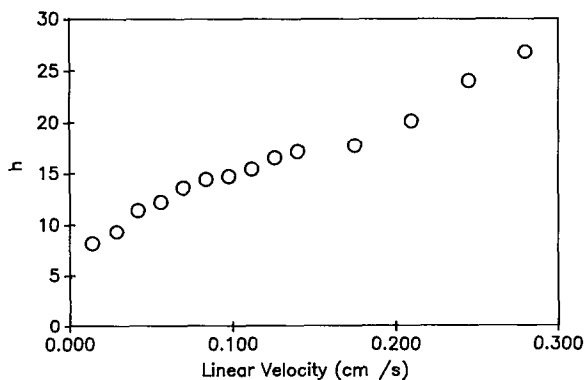


Fig. 7. Efficiency of zirconia column as determined from width at half height of hydroxybenzoic acid peak ( $k' \approx 25$ ). Chromatographic conditions are given in Fig. 5.

above. The halides elute in the order  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$  which is consistent with their elution order on polystyrene-divinylbenzene  $\text{R}_4\text{N}^+$  type ion exchangers<sup>64,65</sup>. The elution sequence for these anions on alumina is reversed<sup>66</sup>. The chromatographic efficiencies determined for these anions were comparable to those reported for the organic acids.

As already noted when describing the ion-exchange behavior of zirconia, phosphate has an essentially irreversible effect on the properties of zirconia. This strong interaction with phosphate has many implications for the use of zirconia as a chromatographic support and we have therefore examined the adsorption of several different phosphate species on zirconia. The adsorption of NADH onto the zirconia surface was studied a static adsorption experiment in the summarized in Table IV. Clearly NADH does adsorb onto zirconia.  $3.5 \mu\text{mol/g}$  adsorbed under the experimental conditions, although this is not necessarily an upper limit. Zirconia pretreated with inorganic phosphate did not adsorb NADH to a measurable extent. Washing the zirconia with  $0.1 M$  sodium hydroxide after phosphate treatment gave a product which adsorbed some NADH, although not as much as the untreated zirconia. This is not surprising since it is known that phosphate is stripped from zirconium phosphate at high pH. Washing the phosphate-treated zirconia with  $0.1 M$  nitric acid had no apparent effect, that is, it did not adsorb NADH after washing

TABLE III  
RETENTION OF INORGANIC ANIONS ON ZIRCONIA

Column:  $5 \text{ cm} \times 0.46 \text{ cm I.D.}$  packed with  $10\text{--}15 \mu\text{m}$  zirconia. Mobile phase:  $10 \text{ mM}$  acetate buffer, pH 5. Flow-rate:  $1 \text{ ml/min}$ . Injection volume:  $20 \mu\text{l}$ . Solute concentration: *ca.*  $15 \text{ mM}$ . Detector: Tracor 965 photoconductivity detector.

Solute	$k'$	Solute	$k'$
Chloride	2.2	Sulfate	6.2
Bromide	3.0	Bromate	6.5
Iodide	3.5	Tetraborate	10.6
Nitrate	4.9	Thiosulfate	15.0

TABLE IV  
NADH ADSORPTION ON ZIRCONIA

$\mu\text{mol}$  of NADH adsorbed onto 100 mg of zirconia from 5 ml of 100 mM pH 8 Tris buffer.

Sample treatment <sup>a</sup>	$\mu\text{mol}$ NADH added	$\mu\text{mol}$ NADH adsorbed	NADH Adsorbed $\mu\text{mol/g ZrO}_2$
None	0.5	0.15	3.5
100 mM phosphate, pH 8, NaOH wash <sup>b</sup>	0.5	0.28	2.8
100 mM phosphate, pH 8	0.5	0	0
100 mM phosphate, pH 8, HNO <sub>3</sub> wash <sup>c</sup>	0.5	0	0
100 mM phosphate, pH 8, NaOH wash <sup>d</sup>	0.5	0.082	0.82
100 mM borate, pH 8	0.5	0.25	2.5

<sup>a</sup> 100 mg of support were washed with  $2 \times 2$  ml of the designated buffer before addition of NADH.

<sup>b</sup> Sample of zirconia with  $3.5 \mu\text{mol/m}^2$  adsorbed NADH washed with  $1 \times 0.2$  ml of 1 M phosphate, pH 8.

<sup>c</sup> 100 mg of phosphate-treated zirconia were washed with  $2 \times 2$  ml of 0.1 M HNO<sub>3</sub> before addition of NADH.

<sup>d</sup> 100 mg of phosphate-treated zirconia were washed with  $2 \times 2$  ml of 0.1 M NaOH before addition of NADH.

with 0.1 M nitric acid. This finding is consistent with the known stability of zirconium phosphate in acidic solutions. Inorganic phosphate was able to displace some fraction of previously adsorbed NADH. The presence of borate reduced the extent of NADH adsorption, but not as dramatically as phosphate.

Additional experiments were done to more clearly define the variables effecting the adsorption of oxyphosphorus compounds onto zirconia. In these experiments, the static adsorption of phenylphosphonic acid, diphenyl phosphate, diphenyl phosphinic acid and triphenyl phosphine oxide on zirconia was studied under a variety of solvent conditions. As shown by the data in Table V, all three compounds with an hydroxyl group adsorb to nearly the same extent in methanol. In carbon dioxide-free water diphenyl phosphate and diphenyl phosphinic acid adsorb to a greater extent than in methanol, presumably due to their lower solubility in water. In the absence of a strong adsorption competitor it is not surprising that the solubility

TABLE V  
STATIC ADSORPTION OF ORGANOPHOSPHORUS COMPOUNDS ON ZIRCONIA

$\mu\text{mol}$  of compound adsorbed from 100  $\mu\text{M}$  solution onto 100 mg of zirconia. Determined by decrease in absorbance of solution at 214 nm after 1 h of exposure to zirconia.

Compound	$\mu\text{mol adsorbed per g zirconia}$		
	50 mM Tris	CO <sub>2</sub> -free water	Methanol
Phenyl phosphonic acid	3.2	3.3	2.0
Diphenyl phosphate	0	0.9	1.7
Diphenyl phosphinic acid	0	2.6	2.0
Triphenyl phosphine oxide	0	0	0

would determine the extent of adsorption. The adsorption of diphenyl phosphate was significantly reduced in water relative to methanol. In 50 mM Tris buffer only phenyl phosphonic acid adsorbed to a measurable extent. The Tris, present at a much higher concentration than the adsorbates, is preferentially adsorbed except in the case of the phenyl phosphonic acid which has two hydroxyl groups. We have interpreted this as evidence for the formation of a "bidentate" surface complex. In fact, the formation of bidentate surface complexes between phosphonic acids and metal oxides has previously been demonstrated spectroscopically<sup>67,68</sup>. In general, these adsorption results seem to indicate that hydroxyl groups on the organophosphorus compounds are essential for adsorption and that adsorption of phosphonates, with two hydroxyl groups per molecule is stronger than that of other phosphate species.

## CONCLUSIONS

The evaluation of porous microparticulate zirconia spherules is reported. The physical and mechanical properties of these spherules make them appropriate for use as HPLC supports. Zirconia has both anion- and cation-exchange properties, depending on solution pH and buffer composition. Inorganic phosphate adsorbs onto zirconia and alters its anion-exchange properties, transforming it from an anion exchanger below pH 9 to a cation exchanger. Organophosphates and organophosphonates, particularly the latter, are strongly adsorbed on zirconia. Most importantly, it has been demonstrated that these porous zirconia spherules are stable at pH 1–14 under static conditions and that their alkaline stability is superior to that of chromatographic alumina.

## ACKNOWLEDGEMENTS

This work was supported in part by grants from the University of Minnesota Center for Biological Process Technology and 3M Company.

## REFERENCES

- 1 R. E. Iler, *The Chemistry of Silica*, Wiley-Interscience, New York, 1979.
- 2 K. K. Unger, *Porous Silica (Journal of Chromatography Library, Vol. 16)*, Elsevier, Amsterdam, 1979.
- 3 R. P. W. Scott, *Adv. Chromatogr.*, 20 (1982) 167.
- 4 C. T. Wehr and R. E. Majors, *LC·GC, Mag. Liq. Gas Chromatogr.*, 5 (1987) 942.
- 5 A. Wehrli, J. C. Hildenbrand, H. P. Keller, R. Stampeli and R. W. Frei, *J. Chromatogr.*, 149 (1978) 199.
- 6 K. Krummen and R. W. Frei, *J. Chromatogr.*, 132 (1977) 27.
- 7 J. J. Glajch, J. J. Kirkland and J. Köhler, *J. Chromatogr.*, 384 (1987) 81.
- 8 K. K. Unger, N. Becker and P. Roumeliotis, *J. Chromatogr.*, 125 (1976) 115.
- 9 C. Horvath, W. Melander and I. Molnar, *Anal. Chem.*, 49 (1977) 142.
- 10 J. Köhler and J. J. Kirkland, *J. Chromatogr.*, 385 (1987) 125.
- 11 J. G. Atwood, G. J. Schmidt and W. Slavin, *J. Chromatogr.*, 171 (1979) 109.
- 12 P. P. Wickramanayake, A. Chatt and W. A. Aue, *Can. J. Chem.*, 59 (1981) 1045.
- 13 R. Aigner-Held, W. A. Aue and E. E. Pickett, *J. Chromatogr.*, 189 (1980) 139.
- 14 W. H. Tomb and H. H. Weetall, *U.S. Pat.*, 3 783 101 (1974).
- 15 R. W. Stout and J. J. DeStefano, *J. Chromatogr.*, 326 (1985) 63.
- 16 R. W. Stout, *U.S. Pat.*, 4 600 646 (1986).
- 17 D. R. Marsh and G. T. Tsao, *Biotechnol. Bioeng.*, 18 (1976) 349.
- 18 H. H. Weetall and N. B. Havewala, *Biotechnol. Bioeng.*, 3 (1972) 241.
- 19 R. W. Stout, S. I. Sivakoff, R. D. Ricker, H. C. Palmer, M. A. Jackson and T. J. Odiorne, *J. Chromatogr.*, 352 (1986) 381.

- 20 A. Barkatt and P. B. Macedo, *U.S. Pat.*, 4 648 975 (1987).
- 21 J. R. Benson and D. J. Woo, *J. Chromatogr. Sci.*, 22 (1984) 386.
- 22 F. Nevejans and M. Verzele, *Chromatographia*, 20 (1985) 173.
- 23 L. D. Bowers and S. Pedigo, *J. Chromatogr.*, 371 (1986) 243.
- 24 T. Hanai, in S. Hara, S. Mori and T. Hanai (Editors), *Chromatography —The Separation System*, Maruzen, Tokyo, 1981, p. 121.
- 25 H. G. Cassidy, *J. Am. Chem. Soc.*, 62 (1940) 3073.
- 26 H. G. Cassidy and S. E. Wood, *J. Am. Chem. Soc.*, 63 (1941) 2628.
- 27 A. Tiselius and L. Hagdahl, *Acta Chem. Scand.*, 4 (1950) 394.
- 28 L. Hagdahl, R. J. P. Williams and A. Tiselius, *Ark. Kem.*, 7 (1952) 1.
- 29 A. Tiselius, *Kolloid-Z.*, 105 (1943) 101.
- 30 J. H. Knox and M. T. Gilbert, *U.K. Pat.*, 7 939 449 (1979); *U.S. Pat.*, 4 263 268 (1979); *G.R.F. Pat.*, P2 946 688-4 (1979).
- 31 U. Bien-Vogelsang, A. Deege, H. Figge, J. Köhler and G. Schomburg, *Chromatographia*, 19 (1984) 170.
- 32 R. M. Chicz, Z. Shi and F. E. Regnier, *J. Chromatogr.*, 359 (1986) 121.
- 33 M. P. Rigney, T. P. Weber and P. W. Carr, *J. Chromatogr.*, 484 (1989) 273.
- 34 E. R. Russel, A. W. Adamson, J. Schubert and G. E. Boyd, *United States Atomic Energy Commission Report CN-508* (1943).
- 35 C. B. Amphlett, L. A. McDonald and M. J. Redman, *J. Inorg. Nucl. Chem.*, 6 (1958) 236.
- 36 N. Michael, W. D. Fletcher, D. E. Croucher and M. J. Bell, *Westinghouse Report CVNA-135* (for the United States Atomic Energy Commission) (1961).
- 37 C. B. Amphlett, L. A. McDonald and M. J. Redman, *J. Inorg. Nucl. Chem.*, 6 (1958) 220.
- 38 P. A. Agron, E. L. Fuller, Jr. and H. F. Holmes, *J. Colloid Interface Sci.*, 52 (1975) 553.
- 39 T. Yamaguchi, Y. Nakano and K. Tanabe, *Bull. Chem. Soc. Jpn.*, 51 (1978) 2482.
- 40 N. E. Tretyakov, D. V. Dozdneyakov, O. M. Ornskaya and V. N. Filiminov, *Zh. Fiz. Khim.*, 44 (1970) 1077.
- 41 A. J. Tench and R. L. Nelson, *Trans. Faraday Soc.*, 63 (1967) 2254.
- 42 E. J. W. Verwey, *Rec. Trav. Chim.*, 60 (1941) 625.
- 43 S. Mattson and A. J. Pugh, *Soil Sci.*, 38 (1934) 229.
- 44 G. W. Smith and T. Salman, *Can. Met. Q.*, 5 (1966) 93.
- 45 G. A. Parks, *Adv. Chem. Ser.*, 67 (1967) 121.
- 46 G. A. Parks, *Chem. Rev.*, 65 (1964) 177.
- 47 H. Kita, N. Henmi, K. Shimazu, H. Hattori and K. Tanabe, *J. Chem. Soc., Faraday Trans. I*, 77 (1981) 2451.
- 48 C. B. Amphlett, L. A. McDonald and M. J. Redman, *J. Inorg. Nucl. Chem.*, 6 (1958) 236.
- 49 C. B. Amphlett, *Inorganic Ion Exchangers*, Elsevier, Amsterdam, New York, 1964.
- 50 K. A. Kraus and H. O. Phillips, *J. Am. Chem. Soc.*, 78 (1956) 249.
- 51 C. B. Amphlett, in *Proceedings of the Second International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958, P/271*, United Nations, Geneva, 1958, p. 17.
- 52 K. A. Kraus, H. O. Phillips, T. A. Carlson and J. S. Johnson, in *Proceedings of the Second International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958, P/1832*, United Nations, Geneva, 1958, p. 3.
- 53 Y. Ghaemi and R. A. Wall, *J. Chromatogr.*, 174 (1979) 51.
- 54 P. C. Sadek, *Ph.D. Thesis*, University of Minnesota, Minneapolis, MN, 1985.
- 55 R. Stevens, *Zirconia and Zirconia Ceramics*, Publication No. 113, Magnesium Elektron, Twickenham, 1986.
- 56 K. K. Unger and U. Trudinger, in P. R. Brown and R. A. Hartwick (Editors), *High Performance Liquid Chromatography*, Wiley, New York, 1989, Ch. 3, p. 159.
- 57 T. P. Weber, personal communication.
- 58 E. Paukstis, R. I. Soltanov and E. N. Yurchenko, *React. Kinet. Catal. Lett.*, 19 (1982) 105.
- 59 K. Esumi and K. Meguro, *Bull. Chem. Soc. Jpn.*, 55 (1982) 315.
- 60 T. Yamaguchi, Y. Nakano and K. Tanabe, *Bull. Chem. Soc. Jpn.*, 51 (1978) 2482.
- 61 N. E. Tretyakov, D. V. Dozdneyakov, O. M. Ornskaya and V. N. Filiminov, *Zh. Fiz. Khim.*, 44 (1970) 1077.
- 62 C. Laurent, H. A. H. Billiet and L. de Galan, *Chromatographia*, 17 (1983) 253.
- 63 R. L. Smith and D. J. Pietrzyk, *Anal. Chem.*, 56 (1984) 610.
- 64 J. S. Fritz, D. T. Gjerde and C. Pohlandt, *Ion Chromatography*, Hüthig, Heidelberg, 1982.
- 65 F. C. Smith, Jr. and R. C. Chang, *The Practice of Ion Chromatography*, Wiley, New York, 1983.
- 66 G. L. Schmitt and D. J. Pietrzyk, *Anal. Chem.*, 57 (1985) 2247.