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Physical and chemical characterization of a porous phosphate-modified zirconia substrate

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ABSTRACT

A phosphate modification of previously described porous zirconium oxide high-performance liquid chromatographic support particles has been developed. Modification of the surface with inorganic phosphate alleviates the irreversible adsorption of proteins on the native oxide surface and makes the surface more biocompatible. X-ray photoelectron spectroscopic, solid-state ^{31}P NMR, elemental analysis, pH stability and ^{32}P phosphate release studies that have been used to characterize physically the surface of these modified particles are reported.

INTRODUCTION

We have recently reported on the development of acid- and base-stable porous zirconium oxide spherules as support particles for high-performance liquid chromatography (HPLC) [1,2] and previous work on the chromatographic utility of zirconium oxide, silica and glasses which contained an overlayer of zirconia was reviewed. The support particles consist of monoclinic zirconia crystals and can be produced with diameters ranging from 5 to 50 μm and pore diameters from 60 to 415 Å. The mechanical, chemical and thermal stability of monoclinic zirconia is the basis for our interest in developing and investigating porous zirconia spherules as HPLC supports. Zirconia particles with large pore diameters (>300 Å) are now available, making the investigation of protein separations on zirconia possible.

Zirconia particles have the extremely high pH stability typical of organic polymeric supports but maintain the high mechanical stability of silica and alimunia supports. Static pH stability studies [2] showed no evidence of dissolution of the zirconia particles at any pH (1–14), whereas more than 1% (w/w) of a 100-mg sample of alumina dissolved in 900-ml aliquots of aqeuous solutions at any pH < 3 or > 12. The use of silica-based supports is limited

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to the Ph range 2–8 owing to their high solubility outside this range. Attempts to inhibit the dissolution of silica by the use of saturation columns [3], zirconia cladding [4] or acid–base treatment [5] have been only partly successful. Clearly, zirconia has distinct advantages over alumina and silica for both high- and low-pH applications.

Polymer-based supports have been developed which are stable over a much broader pH range than are silica and alumina. Such supports are, however, subject to shrinking and swelling, which may restrict diffusion [6] and cause higher column backpressures. The use of organic modifiers is also necessary to wet the polymer effectively [7]. These effects have adverse consequences on column efficiency.

Another disadvantage of silica-based supports is the strong interaction of amines with the residual silanol groups on the silica surface [8], leading to peak tailing, poor efficiency, low recoveries and hysteresis effects. Zirconia has no strong interactions with amines and their efficient separation is possible without the use of mobile phase additives [2].

Although zirconia has no strong interactions with amines, it does have a high affinity for many anions, especially phosphate [9,10]. Indeed, even a brief exposure of zirconium oxide to a dilute phosphate solution converts the support from an anion to a cation exchanger at pH 8. This can be reversed by rinsing the support with strongly alkaline solutions. Exposure of zirconia to samples containing certain anions such as phosphate will thus alter its chromatographic properties. This characteristic requires that such anions be removed from the sample matrix in a "clean-up" step or that their adsorption be blocked so as to maintain reproducible retention behavior of the zirconia. The presence of strong adsorption sites on the heterogeneous surface of the zirconia is also evident by the strong adsorption of certain anionic solutes in all modes of chromatography on zirconia. Elution of carboxylic and phosphonic acids requires the presence of an oxyanion as an additive in the mobile phase to avoid unacceptable peak broadening and low recoveries [1].

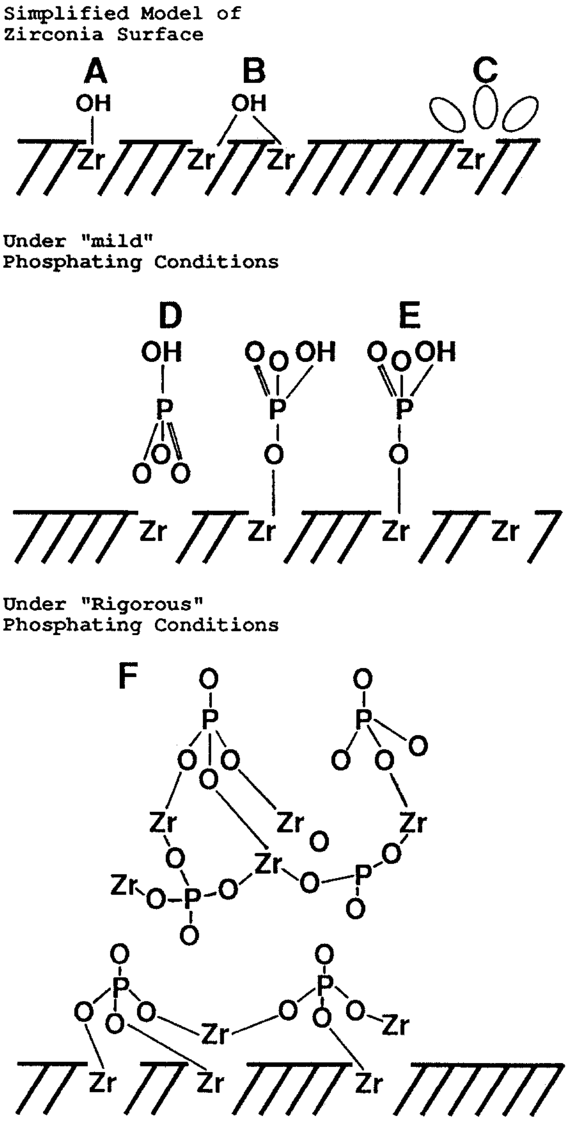

Fig. 1. Proposed models for the surface species of various phosphate-treated zirconias. (A) terminal hydroxide group; (B) bridging hydroxide group; (C) uncoordinated Lewis acid site; (D) physisorbed phosphate group; (E) esterified phosphate group covalently bound to the surface; (F) multi-layer zirconium phosphate area resulting from partial dissolution of the zirconia matrix.

The surface of the zirconia particles is complex and several surface species have been identified, as depicted in Fig. 1. Infrared studies of zirconia have confirmed the presence of at least two types of surface hydroxyl species [11–13]: a terminal hydroxyl (A), similar to the free silanol of silica, and a bridging hydroxyl (B), in which a single oxygen is shared by two zirconium atoms. As with other metal oxides, these surface hydroxyl groups are partly responsible for the amphoteric nature of the surface. They may be protonated or deprotonated depending on the solution pH, giving the zirconia anion- or cation-exchange properties, respectively:

$$\text{Zr–OH}_2{}^+ \rightleftharpoons \text{Zr–OH} + \text{H}^+ \rightleftharpoons \text{Zr–O}^- + 2\text{H}^+$$

Coordinatively bound water molecules may also contribute to the ion-exchange properties of the zirconia. The point of zero charge, *i.e.*, the pH at which zirconia has no net charge, is between 8 and 9.2 for the material used in these studies [1].

In addition to surface hydroxyls, the presence of coordinatively unsaturated zirconium(IV) ion sites (C in Fig. 1) on zirconia was confirmed by the use of a nitroxyl ion paramagnetic probe and ESR [14]. These very strong Lewis acid sites are similar to those found on alumina and are probably responsible for the strong interactions of certain anions with the surface of the zirconia.

These interactions persist even when bare zirconia particles are coated with a polymeric phase such as polybutadiene [1]. Whereas non-polar molecules such as benzene behave as expected, oxyanions such as benzoic acid did not elute from polybutadiene-coated zirconia. Ordinarily such polar molecules are less retained than non-polar solutes on a non-polar surface such as polybutadiene. These observations show the strength and persistence of surface interactions with the surface on zirconia despite the presence of a polymer coating. Indeed, it is unlikely that any polymer coating procedure will successfully block all of the adsorption sites. Rigney [1] found continued phosphate adsorption despite thick polybutadiene coatings of up to 1.7 nm. Similarly residual silanol groups continue to play a role in chromatographic separations on silanized supports despite years of work to alleviate these interactions [8].

The separation of proteins on zirconia has become feasible with the development of particles whose pores are large enough to avoid steric exclusion of these large molecules. Obviously, if the separation of small solutes containing a single oxyanion functionality requires the presence of inorganic phosphate in the mobile phase to block the strong interactions with the surface of zirconia, the separation of large biopolymers containing many such groups will be extremely problematic. Indeed, elution of myoglobin and bovine serum albumin from zirconia required a pH 10 buffer and then only partial separation was achieved. Very poor efficiency and peak shapes were observed despite the presence of phosphate in the mobile phase [1].

The aim of this work was to modify the surface of the zirconium oxide spherules reported previously [1,2] in order to remove the undesirable oxyanion interactions without compromising the mechanical and chemical stability of the particles. The strong affinity of zirconia for phosphate and the low solubility of zirconium phosphate [15] in water made a phosphate modification of the surface a reasonable approach. A phosphate modification of zirconia that effectively blocks the strong sites responsible for the oxyanion interactions would provide a more "bio-compatible" stationary phase, suitable for the separation of proteins and other biologically important molecules. The widespread use of the calcium phosphate hydroxyapatite in protein chromatography and zirconium phosphate for transition metal ion spearations also suggested that a phosphate-modified zirconia would be of interest as a chromatographic support.

This paper described the study of the preparation and physico-chemical characterization of phosphate-modified zirconia particles. Their chromatographic properties and utility in the separation of proteins are described in the following paper [29].

EXPERIMENTAL

Water was purified using a Barnstead Nanopure deionizing system with an organic free cartridge and a 0.2-μm final filter. All water was boiled and cooled prior to use in order to remove dissolved carbon dioxide. All chemicals were of analytical-reagent grade or better. Hydrochloric acid [7647-01-0] was obtained from EM Science (Gibbstown, NJ, USA), 50% sodium hydroxide solution [1310-73-2] from Curtin Matheson Scientific (Houston, TX, USA), tris(hydroxymethyl)aminomethane (Tris) [77-86-1] from Sigma (St. Louis, MO, USA), potassium phosphate dibasic [7758-11-4] and potassium chloride [7447-40-7] from Mallinkrodt (Paris, KY, USA), sodium acetate [127-09-3] and sodium borate [1330-43-4] from Fisher Scientific (Fairlawn, NK, USA), phenylphosphonic acid [1571-33-1] and zirconium phosphate from Pfaltz & Bauer (Waterbury, CT, USA) and phosphoric acid [7664-38-2] from J.T. Baker (Phillipsburg, NJ, USA). Carrier-free [^{32}P]-orthophosphate was purchased from Amersham (Arlington Heights, IL, USA).

Zirconia particles were prepared by a proprietary process at the Ceramic Technology Center of 3M. Several batches of the zirconia particles with various physical properties were used throughout this work. The typical particle had a pore diameter of about 300 Å as determined by mercury porosimetry and a BET surface area of about 120 m^2/g. The particles are well defined spherules and the chief difference in the particles used here is the average particle size. For this work, larger particles (>20 μm) were used, but small (5-μm) well sized particles can be obtained for chromatographic purposes by particle size classification methods.

All particles were chemically pretreated before further modification in order to minimize any differences in the surface chemistry of the different batches of zirconia particles. Pretreatment with acid or base is known to affect the retention characteristics of inorganic supports, especially alumina [16]. Galkin *et al.* [17] noted differences in the reactivity of various zirconia surfaces toward phosphoric acid based on the number of condensed and free hydroxyls. In this study, all particles were immersed for 1 h in 0.1 *M* hydrochloric acid followed by 1 h in 0.1 *M* sodium hydroxide solution at room temperature and were swirled periodically. At the beginning of both the acid and base treatments, the particles were thoroughly degassed by sonication and application of a vacuum. The treatment was carried out in closed high-density polyethylene bottles rather than glass flasks to prevent precipitation or adsorption of dissolved silicates on the particles. In order to avoid the adsorption of carbonates, freshly boiled and cooled deionized water was used and the lower carbonate-containing 50% saturated sodium hydroxide solutions rather than sodium hydroxide pellets were used to prepare all alkaline solutions. The particles were thoroughly rinsed two or three times with 500-ml portions of deionized water after both the acid and base treatments.

During both the acid and base treatments, most of the particles settled to the bottom of the flask within about 10–15 min. However, up to about one third of the particles remained suspended for periods longer than 2 h in the water rinses, especially after the base treatment, probably owing to charging of the particles. The high ionic strength of the acidic and basic solutions supresses the repulsion between the particles. For expediency, larger particles (>20 μm) were rinsed in a sintered-glass funnel. A portion of the smaller particles was sacrificed with each water rinse rather than waiting for all of the particles to settle. The particles were either immediately subjected to the phosphate modification described below or they were dried in a vacuum oven at 60°C and stored in capped bottles.

The phosphate modification was carried out as follows: 15–25 g of pretreated zirconia particles were placed in a 500-ml round-bottomed flask. About 250 ml of 0.1 *M* phosphoric acid containing 1.0 *M* potassium chloride were added and the resultant slurry was thoroughly degassed by sonication and application of a vacuum. The potassium chloride was added to minimize any charging effects but other particles have been prepared without the addition of the salt and similar phosphorus to zirconium ratios were obtained. The potassium ion may also have a significant effect on the interlayer distance of any localized crystalline zirconium phosphate that might form. The slurry was warmed using a heating mantle such that the phosphoric acid solution refluxed for 4 h. Mixing was induced by "bumping" of the particles during reflux and by occasional swirling. Particles prepared in this manner are referred to as ZrP(0.1) and were used in all chromatographic studies unless indicated otherwise; the (0.1) denotes that the particles were treated with 0.1 *M* phosphoric acid and 1.0 *M* potassium chloride for 4 h.

A similar phase was also prepared in which 1.0 *M* phosphoric acid solution was substituted for the 0.1 *M* phosphoric acid–1.0 *M* potassium chloride solution. These particles were refluxed for only 1 h and are referred to as ZrP(1.0). Both the ZrP(0.1) and ZrP(1.0) particles had a "glue-like" appearance after phosphoric acid treatment. They became free-flowing particles after rinsing with HPLC-grade water and drying in vacuum.

Elemental analysis was done by placing 5-g samples of 100–400-μm zirconia particles in a filter flask containing 200 g of the appropriate phosphoric acid solution. The slurry was then subjected to vacuum in order to remove air from the pores and wet the surface with the acid solution. After degassing, the flask was maintained at the desired temperature for the time period indicated. When the desired reaction time had elapsed, the particles were thoroughly rinsed with distilled water and dried for 24 h at 80°C. The surface area of each sample was determined by the BET nitrogen adsorption method. A portion of each sample was dissolved in hydrofluoric acid and analyzed by inductively coupled plasma (ICP) atomic emission spectroscopy. It should be noted that rinsing with water or neutral salts such as potassium chloride does not remove adsorbed phosphate from the surface of zirconia. Hence the elemental analysis studies (see below) probably represent the sum of both the adsorbed phosphate and covalently bound phosphate.

The pH stability of the phosphate modified zirconia particles in alkaline and acidic aqueous media was assessed in a static solubility study. About 1.5 g of the phosphate-modified zirconia [ZrP(0.1)] were weighed and placed in 500 ml of an aqueous solution at a known pH. Aqeous solutions of pH 1 and 3 were prepared by addition of hydrochloric acid to freshly boiled HPLC-grade water until the desired pH was obtained. Aqueous solutions of pH 11, 12 and 13 were likewise prepared by addition of carbonate-free sodium hydroxide to HPLC-grade water. Aqueous solution of pH 5, 8 and 10 were buffered with 10 m*M* sodium acetate, Tris and sodium borate buffers, respectively. Aliquots of 10 ml of the supernatant were collected from each of the samples decribed above after 1, 2, 4, 8 and 16 days. The pH of the solutions was redetermined after the last sample collection. The concentrations of phosphorus and zirconium in each of the supernatant samples were determined by ICP analysis. Samples were filtered to remove particulates using a Millipore Type HA 0.2-μm filter prior to analysis.

Phosphate-release and -exchange studies were performed using [^{32}P]phosphate-treated particles. About 7 g of zirconia (415 Å, 10–20-μm particles) were combined with 5 ml of HPLC-grade water and the resultant slurry was thoroughly degassed using house vacuum and sonication for about 5 min. The slurry was the added to a premixed solution of 1.0 ml of 1.0 mCi [^{32}P]phosphate in 100 ml of 0.10 *M* phosphoric acid–1.0 *M* potassium chloride and refluxed as before. After cooling, the mixture was filtered through Whatman No. 1 filter-paper on a Buchner funnel and washed with 2 l of 5 m*M* Tris (pH 8). The radioactivity remaining in aliquots of successive rinses was measured to determine when the inerstitial [^{32}P]phosphate had been removed. The radioactivity in the third rinse was less than 0.1% of that in the first, at which point rinsing was terminated. However, it is very likely that a significant amount of physisorbed phosphate remains after rinsing. The beads were dried overnight in a vacuum oven. A 200-mg amount of the dried particles was distributed into each of 24 tared 1.5-ml microcentrifuge tubes.

To each triplicate set of tubes was added 1.0 ml of 0.10 *M* phosphate buffer at one of eight different pHs from 1 to 14. The tubes were thoroughly vortexed and mixed continuously on a rocker. At various times after the addition of buffer, the tubes were removed from the rocker, centrifuged for 3 min at 6000 rpm and 50-μl aliquots of the supernatant were removed and added to 10.0 ml of scintillation fluor (Aquasol) for counting. Radioactivity was determined with a Packard Tricarb scintillation counter. Buffer was replaced to maintain a constant volume in the sample tubes. After the aliquots had been taken and the buffer replaced, the tubes were vortex mixed to resuspend the beads and returned to the rocker. Following removal of the 13-h aliquot, the tubes were centrifuged and, the buffer was decanted, discarded and replaced with fresh buffer. Subsequent samples of this supernatant buffer were removed and counted as described above. As ^{32}P has a relatively short half-life, all supernatant samples were counted consecutively on the same day at the end of the experiment to simplify the calculations.

X-ray photoelectron spectroscopy (XPS) was peformed on a Physical Electronics Industries (Eden, Prairie, MN, USA) Mode 555 ESCA/Auger system. Spectra were obtained with an Mg Kα source (15 kV, 2 mA) and a cylindrical mirror analyzer using a 100-eV pass energy for survey scans (1000–0 eV binding energy) and a 25-eV pass energy otherwise. Particles were held in place using aluminum tape.

Solid-state ^{31}P magic angle spinning (MAS) NMR spectra were obtained on an IBM Instruments NR/100 Fourier transform NMR spectrometer with the IBM Instruments NMR solids accessory. The instrument was calibrated with methyltriphenylphosphonium bromide at 20.43 ppm (*vs.* phosphoric acid at 0.00 ppm).

RESULTS AND DISCUSSION

Preparation and surface coverage

The extent of phosphate modification as a function of phosphoric acid concentation, reaction temperature and reaction time is shown in Table I. Not surprisingly, the phosphorus to zirconium P/Zr ratio increases with increasing phosphoric acid concentration and with increasing temperature. However, an analogous increase in the P/Zr ratio with time is only noted at the higher temperature of 100°C. This could be the result of kinetic limitations in the esterification of the phosphate with the surface hydroxyls. The fact that the amount of phosphate incorporated into the zirconia is independent of the reaction time at the lower temperature suggests that under these conditions phosphate is merely adsorbed on the zirconia surface and does not react to form covalent zirconium–phosphate bonds. The lack of any covalently bound phosphate in the materials produced at low temperature and at reaction times of the order of hours was confirmed by solid-state ^{31}P NMR studies (see below).

A theoretical maximum monolayer surface coverage of 4.9 m^2/g of phosphate on zirconia was calculated by assuming a face-centered cubic packing arrangement with a packing efficiency of 74% and a phosphate ion surface contact area of 25 Å^2. This assumes the most efficient packing geometry possible and hence probably overestimates the actual surace coverage. The surface contact area of the phosphate was calculated from the Van der Waals radius of oxygen and the average angle between the oxygen and phosphorus in phosphate. This value is considerably less than the 6.98 and 5.82 $\mu mol/m^2$ measured for the 1-h, 100°C, 1 molal phosphoric acid-treated and the 4-h, 100°C, 0.1 molal phosphoric acid-treated zirconia, respectively. This clearly suggests that at least some of the phosphate is incorporated into some form of zirconium phosphate and is neither adsorbed on the surface nor covalently bound surface sites.

It should be noted that elemental analysis does not distinguish between covalently bound phosphate and phosphate which is merely adsorbed on the surface. The high affinity of zirconium phosphate and phosphate ion has been observed in this laboratory and elsewhere [18].

BET surface area measurements were done to determine the effect of the modification on the surface

TABLE I

ELEMENTAL ANALYSIS OF VARIOUS PHOSPHATE-MODIFIED ZIRCONIAS

H_3PO_4 concentration (mol/kg)	Temperature (°C)	Time (h)	Specific surface area[a] (m^2/g)	P/Zr molar ratio[b]	Phosphate Surface coverage ($\mu mol/m^2$)
0.00	25	1	116	0.004	0.00
0.01	25	1	115	0.032	1.98
0.10	25	1	104	0.057	4.08
1.00	25	1	110	0.063	4.32
0.00	25	4	113	0.004	0.00
0.01	25	4	116	0.034	2.14
0.10	25	4	121	0.061	3.80
1.00	25	4	117	0.065	4.04
0.00	100	1	124	0.004	0.00
0.01	100	1	124	0.032	1.88
0.10	100	1	114	0.070	4.57
1.00	100	1	109	0.001	6.98
0.00	100	4	105	0.004	0.00
0.01	100	4	110	0.045	3.00
0.10	100	4	117	0.089	5.82
1.00	100	4	111	0.149	9.41

[a] As determined by BET nitrogen adsorption measurements.

[b] As determined by ICP spectroscopy.

area of the particles. As shown in Table I, there are only insignificant random changes in the surface area of the particles on treatment with phosphoric acid. This indicates that the pore structure of the particles is unaffected by the modification, *i.e.*, no significant decrease in pore volume has taken place.

Stability testing

In order to obtained a preliminary idea of the stability of the phosphate-modified zirconia, ZrP (0.1) was exposed to several solutions of various pH and the amounts of zirconium and phosphorus released were determined by ICP analysis of the supernatant and is shown in Fig. 2. An obvious relationship between the amount of released phosphate and the alkalinity of the solution is noted. Very little phosphate is desorbed in acidic solution whereas more than half of the total phosphate is desorbed at pH 13. The instability of zirconium phosphate at high pH has been noted previously [19].

The curves in Fig. 2 suggest a fast desorption process that is essentially complete by the second day, followed by a much slower release that continues throughout the duration of the study. This is especially evident in the neutral and alkaline pH solutions. After 16 days at pH 12.7, only *ca.* 3.1 μmol/m^2 of phosphate had desorbed from the surface. Even at high pH, the affinity of zirconia for phosphate is significant and indeed only half of the amount of phosphate incorporated into the phosphate-modified zirconia matrix had desorbed (see Table I).

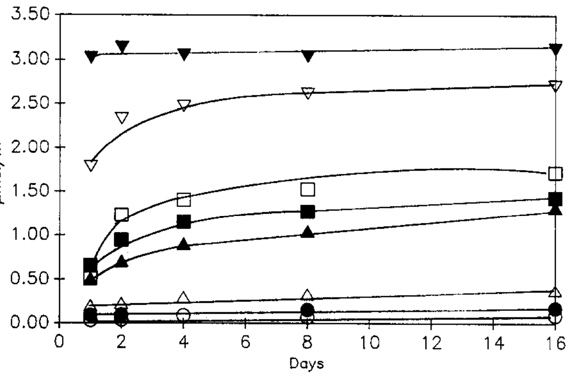

Fig. 2. Static pH stability of phosphate-modified zirconia , ZrP (0.1). Amount (μmol) of phosphate released into solution per squate meter of zirconia as a function of time and pH. ○ = pH 1.39 hydrochloric acid; ● = pH 3.18 hydrochloric acid; △ = pH 5.01, 10 m*M* sodium acetate buffer; ▲ = pH 7.95, 10 m*M* Tris buffer; □ = pH 9.83, 10 m*M* sodium borate buffer; ■ = pH 8.56 sodium hydroxide solution, originally pH 10.78; ▽ = PH 11.68 sodium hydroxide solution; ▼ = pH 12.68 sodium hydroxide solution.

Zirconium levels in all of the supernatants from the study above, except for the Tris-buffered (pH 8.00) samples shown in Fig. 3, were below the detection limit of 0.025 μg/ml of zirconium for ICP analysis. A similar study was undertaken on unmodified zirconia particles and no detectable zirconium was found. We assume that the Tris buffer is responsible for the presence of detectable amounts of zirconium in the supernatant. The three hydroxyl groups of the Tris may have enough chelating ability to solubilize the otherwise insoluble zirconium phosphate species in alkaline solutions. The stability of the unmodified zirconia particles in 10 m*M* Tris medium (pH 8.00) was then assesed by the method described above. No zirconium was found in the supernatant within the detection limits of the technique. This strongly suggests that the phosphate modification involves the breaking of zirconium–oxygen bonds in the zirconia matrix, thereby making the surface zirconia atoms susceptible to complexation by Tris. This is supported by the high surface coverages of phosphates determined earlier.

The effect of phosphate on the solubility of the support was investigated by adding 10 m*M* potassium phosphate to the Tris-buffered solution described earlier. As shown in Fig. 3, the added phosphate did indeed suppress the solubility of the zirconium phosphate but the level of zirconium solubility is still unacceptable. Based on this information,

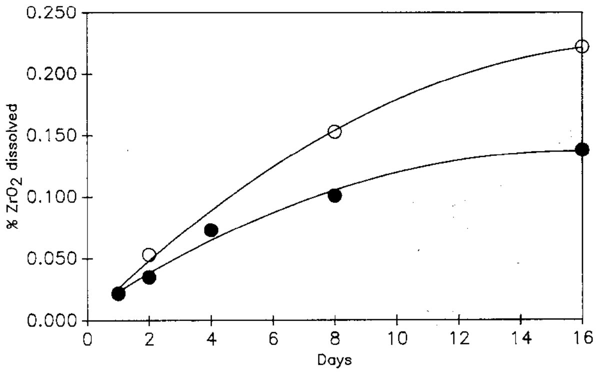

Fig. 3. Static pH stability study of phosphate-modified zirconia, ZrP(0.1). Amount (wt.%) of zirconia dissolved. ○ = pH 7.85, 10 m*M* Tris buffer; ● pH 7.98, 0 m*M* Tris, 10 m*M* potassium phosphate solution.

it is not advisable to use Tris or similar chelating buffers in the mobile phase with this support.

In order to assess the lability of the surface, the exchange rate of phosphate from radiolabeled ZrP (0.1) in buffered 0.2 *M* phosphate solutions was studied as a function of pH (see Experimental). As shown in Fig. 4, a considerable amount of phosphate (*ca.* 1.3 μmol/m^2) is exchanged for unlabeled solution-phase phosphate from pH 1 to 10. This is despite the fact that very little phosphate ($<$0.25 μmol/m^2) was found in the acidic solutions by ICP in the pH stability study described earlier. The data from this stability study are replotted *vs.* pH in Fig. 5 to be more analogous to the exchange experiment. It seems that at pH 10 and above, the competition between the solution-phase phosphate and hydroxide ion begins to favor the hydroxide ion. This results in the upward curvature of radioactive phosphate lost from the surface in Fig. 4 and the amount of phosphate which becomes soluble at pH 10 and above as seen in Fig. 5. The enhanced exchange rate of radiolabeled phosphate in acid suggests that a portion of the phosphate on the surface is labile and subject to exchange with phosphate in solution at lower pH. Note that no enhanced solubility of phosphate at lower pH was found in the stability study (Fig. 5).

Spectroscopic studies

Electron spectroscopy is a powerful surface analysis technique for the identification of elements and their chemical state [20]. Previous studies [21–23] have shown that the relative binding energy shifts of the oxygen and zirconium bands are dependent on the crystalline nature of the zirconium phosphate. Satellite structures and "shake-up" phenomena of the P 2*s* and Zr 3*d* bands are also influenced by the nature of the zirconium phosphate.

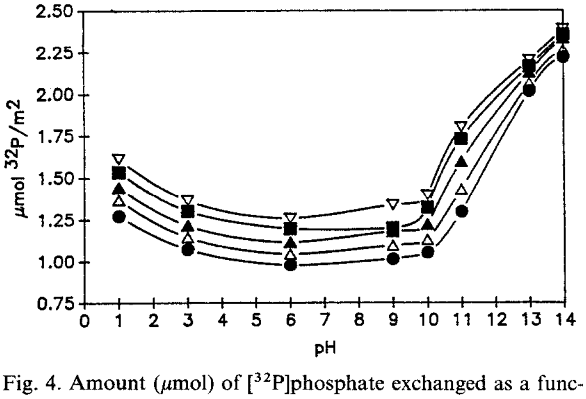

Fig. 4. Amount (μmol) of [^{32}P]phosphate exchanged as a function of pH. ● = 2 days; △ = 4 days; ▲ = 8 days; ■ = 18 days; ▽ = 29 days. Data for 7 g of radiolabeled ZrP(0.1) in 0.2 *M* potassium phosphate solutions buffered to the indicated pH.

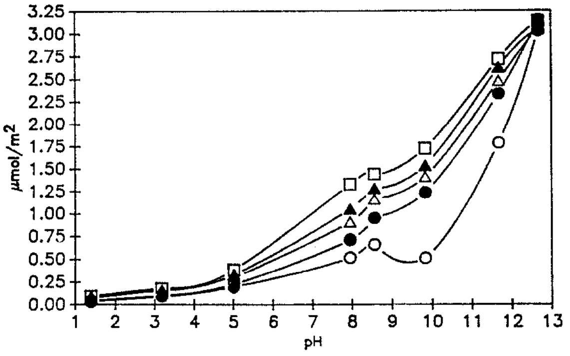

Fig. 5. Amount (μmol) of phosphate released as a function of pH. ○ = 1 day; ● = 2 days; △ = 4 days; ▲ = 8 days; □ = 16 days. Conditions as in Fig. 2.

Neither the oxygen nor the zirconium bands in the spectra showed any asymmetry which might indicate the presence of more than one oxygen or zirconium state. It is likely that the contribution of the zirconia substrate in the phosphate-modified phases to the oxygen and zirconium bands masked any contribution from the phosphate surface species. However, close examination of the expanded spectra of the P 2*p* bands did show two distinct phosphorus chemical states in the phosphate-modified zirconia phases. The two phosphorus states are consistent with the presence of a covalently bound zirconium phosphate species on the surface and an adsorbed phosphate species.

MAS-NMR has proved to be a powerful technique for the characterization of solid [24–26]. The ^{31}P nucleus is NMR active (S = 1/2) with 100% abundance and thus is easily studied by NMR. Several solid-state ^{31}P NMR spectroscopic studies of α-Zr(HPO$_4$)$_2$ · H$_2$O [27] and γ-Zr(HPO$_4$)$_2$ · 2H$_2$O [28] and also amorphous zirconium phosphate have been reported. Solid-state ^{31}P NMR spectra of the phosphate-modified zirconia were obtained in order to characterize better the surface species present (see Fig. 6).

Amorphous zirconium phosphate gels exhibit three resonances at -11.8, -19.3 (major) and

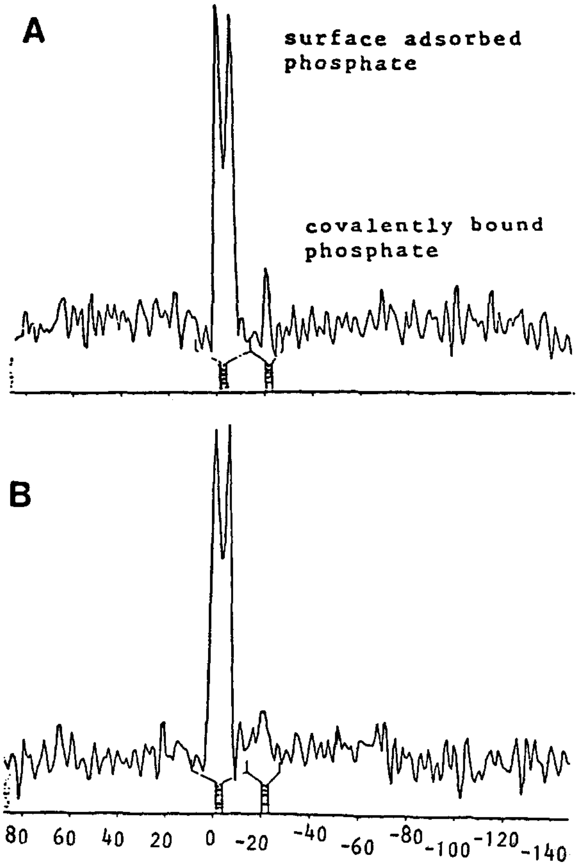

Fig. 6. Solid-state ^{31}P MAS-NMR spectra of phosphate-modified zirconia. (A) ZrP(1.0); (B) ZrP(0.1).

−25.5 ppm [27]. Despite its two crystallographically distinguishable phosphate groups, crystalline α-zirconium phosphate has a single resonance at −16.6 ppm [27] (−18.7 ppm [28]). The ^{31}P NMR spectrum of γ-zirconium phosphate does, however, contain two distinct resonances at −9.4 and −27.4 ppm [28] of equal intensity, suggesting that there is a significant chemical difference between the two phosphate groups in γ-zirconium phosphate and not simply a crystallographic difference.

No quantitative comparisons can be made between the two phosphate-modified zirconia phases ZrP(0.1) and ZrP(1.0) as different numbers of scans were used to obtain the two spectra. Quantitative comparisons between resonances in the same spectrum are also not possible as the relaxation times of the different phosphate species were not determined.

Based on these NMR spectra, it is not possible to distinguish between phosphate esterified onto surface hydroxyl groups and phosphate bound into a multi-layer zirconium phosphate matrix. NMR does, however, clearly show the presence of covalently bonded phosphate and phosphate adsorbed on the surface of the particles. Assignment of the covalently bound phosphate was made by comparison with crystalline zirconium phosphate. This information, combined with the kinetic effects noted in Table I, shows that phosphate adsorbs rapidly to the surface (under 3 h) at room temperature but the formation of covalent phosphate bonds requires the more rigorous conditions of lower pH, higher temperature (100°C) and longer exposure times.

^{31}P MAS-NMR spectroscopy was also used to investigate the alkaline instability of the phosphate-modified zirconia. As solid-state ^{31}P NMR spectroscopy is capable of distinguishing between adsorbed and covalently bound phosphate, it can be used to determine whether covalently bound phosphate is hydrolyzed or if the physically adsorbed phosphate is stripped from the surface by exposure to base.

The insure a good signal-to-noise ratio, particles with a larger surface area (50 m^2/g, 45-μm particles, 100 Å mean pore diameter) were used in this study. The pulse delay time of the instrument was also optimized to maximize the signal corresponding to the covalently bound phosphate. The particles were pretreated and modified with 1.0 *M* phosphoric acid as described earlier. These particles were statically exposed to 0.1 *M* sodium hydroxide solution for 3 days. Spectra of the two samples were obtained on the same day using the same instrument parameters. In this way the integrated peak areas of the two resonances corresponding to the surface-adsorbed phosphate and the covalently bound phosphate can be directly compared between the phosphate-modified zirconia and the base-treated phosphate-modified zirconia.

Examination of the spectra shown in Fig. 7 clearly shows that the covalent phosphate bonds were hydrolyzed by the alkaline conditions and that a large amount of the adsorbed phosphate desorbed.

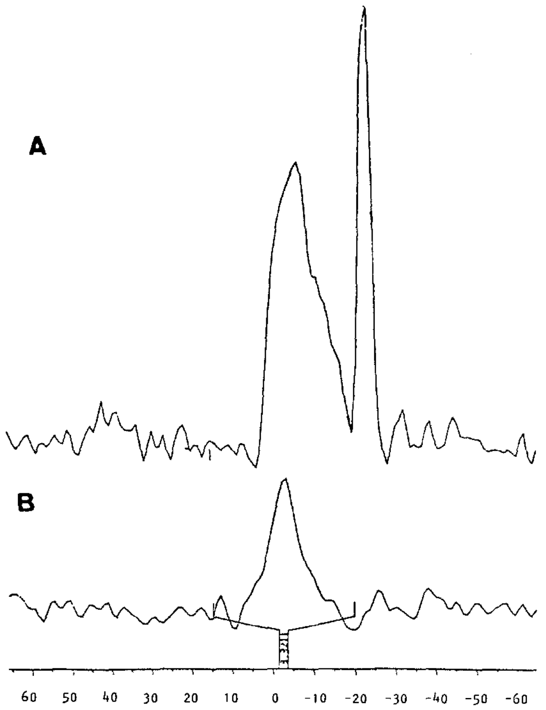

Fig. 7. Solid-state ^{31}P MAS-NMR spectra of ZrP(1.0). (A) Untreated; (B) after exposure for 3 days to 0.1 *M* sodium hydroxide solution.

CONCLUSIONS

Several spectroscopic techniques were used in the course of this work to characterize the surface species on phosphate-modified zirconia particles. These surface species are responsible for the chromatographic properties of the particles.

A simplified scheme of what we believe are the likely surface species on the unmodified zirconia particles is given at the top of Fig. 1. The terminal (A) and bridging hydroxyl (B) groups and the coordinatively unbound zirconium Lewis acid sites (C) are shown. Under the "mildest" phosphating conditions, *i.e.*, neutral pH, room temperature and short contact times, phosphate adsorbs on the surface of the particles (D), especially on the Lewis acid sites. At acidic pH and higher temperatures, esterification of the phosphate with surface hydroxyls (E) takes place as the kinetic bariers are overcome. Under the aggressive conditions of low pH, high phosphate concentration, high temperature and long reaction times, dissolution of the bulk zirconia matrix results with the "reprecipitation" of zirconium phosphates on the surface. Phosphate ions sorb on any exposed zirconium atoms in the precipitated zirconium phosphate.

Unfortunately, it is not feasible to remove the adsorbed phosphate from the surface of the particles to determine the amount of adsorbed phosphate, as the conditions necessary for the complete removal of the adsorbed phosphate also hydrolyze the covalently bonded phosphate. Thus elemental analysis cannot provide information on the presence or relative amounts of covalently bound phosphate. The solid-state ^{31}P NMR studies clearly show the presence of covalently bound phosphate and thus the surface is not merely covered with adsorbed phosphate. The elemental studies also show that the extent of the modification exceeds a monolayer. This suggests that the model depicted in Fig. 1F is reasonable. The release of zirconium ions into solution in the pH stability study of phosphate-modified zirconia in Tris buffer (Fig. 3) also suggests that the bulk zirconia matrix is attacked by phosphate.

It the support is to be regeneratable, the bulk zirconia support must not be involved, *ie.*, the modification must be a true surface modification which does not involve the breaking of zirconium oxygen bonds in the bulk zirconia matrix. ICP elemental analysis studies have shown that both the 0.1 and 1.0 *M* phosphoric acid treatments involve breaking zirconium–oxygen bonds in the bulk zirconia matrix and hence loss of the bulk support will occur on treatment with sodium hydroxide. Further study is needed to determine if it is possible to limit the modification to a monolayer and still block the sites responsible for the specific anion interactions.

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REFERENCES

1 M. P. Rigney, *Ph.D. Dissertation*, University of Minnesota, Minneapolis, MN, 1988.
2 M. P. Rigney, E. F. Funkenbusch, T. P. Weber and P. W. Carr, *J. Chromatogr.*, 499 (1990) 291–304.
3 J. G. Atwood, G. J. Schmidt and W. Slavin, *J. Chromatogr.*, 171 (1979) 109–115.
4 R. W. Stout, S. I. Sivakoff, R. D. Ricker, H. C. Palmer, M. A. Jackson and T. J. Odiorne, *J. Chromatogr.*, 352 (1986) 381–397.
5 J. Köhler and J. J. Kirkland, *J. Chromatogr.*, 385 (1987) 125–150.
6 F. Nevejans and M. Verzele, *Chromatographia*, 20 (1985) 173–178.
7 L. D. Bowers and S. Pedigo, *J. Chromatogr.*, 371 (1986) 243–251.
8 R. E. Majors, in Cs. Horváth (Editor), *High Performance Liquid Chromatography: Advances and Perspectives*, Vol. 1, Academic Press, New York, 1980.
9 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*, United Nations, New York, 1958, paper 1832, pp. 3–16.
10 C. B. Amphlett, *Inorganic Ion Exchangers*, Elsevier, Amsterdam, 1964.
11 P. A. Agron, E. L. Fuller, Jr., and H. F. Holmes, *J. Colloid Interface Sci.*, 52 (1975) 553–561.
12 T. Yamaguchi, Y. Nakano and K. Tanabe, *Bull. Chem. Soc. Jpn.*, 51 (1978) 2482–2487.
13 N. E. Tret'yakov, D. V. Pozdnyakov, I. M. Oranskaya and V. N. Filimonov, *Zh. Fiz. Khim. (Engl. Transl.)*, 44 (1970) 596–600.
14 E. V. Lunina, A. K. Selivanovskii, V. B. Golubev, T. Y. Samgina and G. I. Markaryan, *Zh. Fiz. Chim. (Engl. Transl.)*, 56 (1982) 415–418.
15 G. Hevesy and K. Kimura, *J. Am. Chem. Soc.*, 47 (1925) 2540–2544.
16 H. Engelhardt and H. Elgass, in Cs. Horváth (Editor), *High Performance Liquid Chromatography: Advances and Perspectives*, Vol. 2, Academic Press, New York, 1980, pp. 57–111.
17 V. M. Galkin, L. M. Sharygin, V. E. Moiseev and V. P. Pyshkin, *Izv. Akad. Nauk SSSR, Neorg. Mater. (Engl. Transl.)*, 19 (1983) 1199–1202.
18 C. B. Amphlett, *Inorganic Ion Exchangers*, Elsevier, Amsterdam, 1964.
19 A. Clearfield, in A. Clearfield (Editor), *Inorganic Ion Exchange Materials*, CRC Press, Boca Raton, FL, 1982, pp. 1–74.
20 L. C. Feldman and J. W. Mayer, *Fundamentals of Surface and Thin Film Analysis*, Elsevier, New York, 1986.
21 G. Alberti, U. Costantino, G. Marletta, O. Puglisi and S. Pignataro, *J. Inorg. Nucl. Chem.*, 43 (1981) 3329–3334.
22 S. Pignataro, G. Marletta, O. Puglisi, U. Costantino and G. Alberti, *J. Electron Spectrosc. Related Phenom.*, 25 (1982) 49–57.
23 G. Marletta, O. Puglisi, S. Pignatori, G. Alberti and U. Costantino, *Polyhedron*, 2 (1983) 157–162.
24 D. J. O'Donnell, V. J. Bartuska, A. R. Palmer and D. W. Sindorf, *Am. Lab.*, 18 (1986) 96–111.
25 B. C. Gerstein, *Anal. Chem.*, 55 (1983) 781A.
26 B. C. Gerstein, *Anal. Chem.*, 55 (1983) 899A.
27 K. Segawa, H. Nakajima, S. Nakata, S. Asaoka and H. Takahashi, *J. Catal.*, 101 (1986) 81–89.
28 N. J. Clayden, *J. Chem. Soc., Dalton Trans.*, (1987) 1877–1881.
29 W. A. Schafer and P. W. Carr, *J. Chromatogr.*, 587 (1991) 149.