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High-performance anion exchange of small anions with polyethyleneimine-coated porous zirconia

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Abstract

The preparation and chromatographic characterization of an ion-exchange high-performance liquid chromatographic support by deposition and crosslinking of polyethyleneimine (PEI) on the surface of porous zirconia is described. Adsorption and evaporation methods were used for coating PEI onto the zirconia particles. These two stationary phases were compared by elemental analysis, ion-exchange capacity and by chromatography. High efficiency and good selectivity were observed for inorganic and organic anions. The addition of a strong, hard Lewis base to the mobile phase dramatically improved the peak shape and efficiency of *para* benzoic acid derivatives. PEI-coated zirconia showed a distinct elution sequence for organic anions when compared to *bare* zirconia or silica-based phases. The polyamine coated zirconia was stable over a pH range from 2.75 to 9. Flow studies, using nitrite as a probe solute, showed that both coating procedures produced packed columns with good mass-transfer properties.

1. Introduction

Ion-exchange chromatography is a powerful technique for the separation of both inorganic and organic charged molecules [1–4]. Zirconium oxide (ZrO_2 , zirconia) has been shown to be a useful alternative to silica as a stationary phase in reversed-phase high-performance liquid chromatography [5–7], as an adsorbent [8] and for ligand-exchange chromatography of carboxylates [9,10]. The shortcomings of silica and derivatized bonded phases in aqueous mobile phases have been well documented [11–14]. Most significantly, silica is soluble in aqueous alkali and siloxane bonds are unstable in acidic media. In general, it lacks tolerance to harsh chemical or thermal

treatment. Above pH 8 the Si–O–Si bonds in silica hydrolyze resulting in dissolution of the phase. Zirconia exhibits good mechanical and thermal stability, and is chemically stable over the entire pH range [5,15]. Zirconia can be coated with various polymers to modify its surface interactions with solutes. Zirconia coated with polybutadiene (PBD 7) produces a stationary phase that is useful in reversed-phase separations. Polyethyleneimine (PEI) modified silica is widely used as anion exchanger [16–18]. Polymer-based stationary phases are generally stable to a wide range of mobile phase pHs. PEI-coated polystyrene–divinylbenzene (PS–DVB) supports are reported to withstand pHs in the range of 4–12 [19]. However, polymer supports are not fully mechanically stable due to swelling or shrinking in organic solvents and at extreme pHs

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and ionic strength [20]. We report here the synthesis of PEI-coated zirconia supports that are useful as stationary phases in high-performance anion-exchange chromatography (HPAEC). Two coating methods, namely adsorption and evaporation, were used to deposit PEI onto zirconia particles.

2. Experimental

All chemicals were reagent grade or better. Polyethyleneimine (PEI), average molecular mass 1800, was obtained from Polysciences (Warrington, PA, USA). 50% sodium hydroxide solution, 3,5 dinitrobenzoic acid, sodium acetate, and HPLC-grade isopropanol were obtained from Fisher Scientific (Fairlawn, NJ, USA). Aniline, *p*-nitrotoluene, *p*-cyanobenzoic acid, *p*-iodobenzoic acid, *p*-hydroxybenzoic acid, and *p*-ethylbenzoic acid, were obtained from Aldrich (Milwaukee, WI, USA). *p*-Toluenesulfonic acid, *p*-aminobenzoic acid, and *p*-nitrobenzoic acid were obtained from Eastman (Rochester, NY, USA). Sodium bromate and concentrated phosphoric acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). Sodium nitrite, benzoic acid, sodium nitrate, potassium iodide, potassium phosphate dibasic, and sodium fluoride were from Mallinckrodt (St. Louis, MO, USA). Piperazine-N, N'-bis-[2-ethanesulfonic acid] (Pipes) and tris(hydroxymethyl)aminomethane (Tris) buffers were obtained from Sigma (St. Louis, MO, USA).

All chromatograms were collected on a Hewlett-Packard 1090 (Palo Alto, CA, USA) chromatograph with a photodiode-array detector (PDA), and a Hewlett-Packard ChemStation for data collection. PEI-coated particles were packed into 5 cm × 4.6 mm I.D. stainless steel columns by the stirred upward slurry method at 5000 p.s.i. (1 p.s.i. = 6894.76 Pa) in HPLC-grade isopropanol. Water was obtained from a Barnstead Nano-Pure system, with an Organic-Free final cartridge, and boiled to remove dissolved carbon dioxide. All buffer solutions were filtered using Millipore (type HA) 0.45- μ m membrane filters prior to use.

The dead volume marker used for all experiments was the negative peak obtained upon injection of pure water. The dead volume was checked for each new mobile phase used and was found to deviate less than 3% over the course of the work.

2.1. Zirconia substrate particles

Porous zirconia particles (batch Coac-15) produced by the polymerization induced colloid aggregation [21,22] method were used as the substrate material for this work. The particles have an average diameter of 6 μ m, a surface area of 29 m²/g and an average pore size of 220 Å, as analyzed by BET nitrogen adsorption. An energetically more homogeneous surface was obtained by washing in acid and base: 85 grams of zirconia were suspended in 300 ml of 0.5 M HCl and sonicated under vacuum for 5 min. The slurry was further sonicated for 45 min and then allowed to stand for four h with occasional mixing. The particles were allowed to settle, and the liquid was decanted. The particles were collected on a sintered glass funnel and rinsed with 500 ml of water until neutral. This procedure was repeated using 0.5 M NaOH and then the particles were dried at 150°C overnight.

2.2. The coating of zirconia with PEI by adsorption

Adsorptive coating was carried out by a modification of the procedure developed by Kennedy et al. [23]. A 6-g amount of dry zirconia was placed in 30 ml of 2% (w/v) polyethyleneimine (PEI) in methanol. The mixture was then sonicated under vacuum for 1 h and swirled many times. It was then allowed to sit at room temperature for 3 h. The product was isolated on a sintered glass funnel and dried under vacuum for 30 min. The PEI was then crosslinked with 30 ml of 5% (v/v) 1,4 butanedioldiglycidylether (BUDGE) in methanol and allowed to react overnight. At the end of this period, 2 ml of triethylamine was added, and the mixture was refluxed at 70°C for 30 min. The coated and crosslinked particles were collected, washed with

100 ml of triethylamine and 300 ml of methanol, and stored in a vacuum desiccator.

2.3. The coating of zirconia with PEI by evaporation

The evaporation coating method differed from the adsorption method in that after the particles were coated with PEI, the methanol was slowly removed (over 2 h) with a rotary evaporator, using a baffled flask, at 60°C while periodically pulling a vacuum. The particles were recovered and washed as above.

2.4. Physical characterization procedures

The two PEI-coated zirconia phases were characterized by elemental analysis (C, H and N), and ion-exchange capacity. The elemental composition of the coatings was determined by M–H–W laboratories (Phoenix, AZ, USA). The anion-exchange capacity was determined by a small molecule binding assay [16]. 0.1 grams of PEI-coated zirconia were sonicated in 4 ml of methylene chloride and then centrifuged. The supernatant was decanted and replaced by 4 ml of 200 mM picric acid. After mixing, centrifugation, and decanting, unbound picric acid was washed out with four 4-ml portions of methylene chloride. Bound picric acid was released using a 5% (v/v) solution of triethylamine in methylene chloride with repeated washings and centrifugation until the supernatant was colorless. Each

washing with triethylamine was collected in a single volumetric flask. These combined washings were brought to volume with methylene chloride and assayed spectrophotometrically; the extinction coefficient of triethylamine picrate is 14 500 at 358 nm. Each sample was analyzed in duplicate and the average taken. Duplicate analyses differed by less than 5%.

3. Results and discussion

3.1. Stationary phase characterization

The two stationary phases were characterized by elemental analysis and by picric acid assay. The picric acid assay is sensitive to non-ionized primary, secondary and tertiary amines. The results of the chemical characterization are given in Table 1. As expected, the loading of PEI on the zirconia surface was higher when deposited by evaporation. The elemental analysis showed consistently higher results than the picric acid assay. The picric acid assay results are within the range of Regnier et al.'s work with polyethyleneimine (average molecular mass of 600) on silica [23]. They reported from 2.1–26 $\mu\text{mol}/\text{m}^2$ of nitrogen for eight different silicas, three aluminas, and one titania substrates. PEI-coated zirconia showed 7.2 and 10.8 $\mu\text{mol}/\text{m}^2$ of nitrogen for the adsorptive and evaporative coatings respectively. Since the theoretical molar ratio of carbon to nitrogen (C/N) for PEI is 2.0, values

Table 1
Composition of stationary phases

Phase	Elemental analysis			Picric acid assay, nitrogen ^a	PEI ^d	C/N ratio ^e
	Carbon ^a	Hydrogen ^a	Nitrogen ^a			
Adsorption ^b	50.3	113	11.0	7.2	0.23	4.52
Evaporation ^c	67.0	427	18.3	10.8	0.44	4.48

^a All results are in $\mu\text{moles}/\text{m}^2$ of zirconia surface.

^b Prepared by the adsorption method as described in text.

^c Prepared by the evaporation method as described in text.

^d Micromoles of PEI per square meter on the zirconia surface by elemental analysis, assuming 42 nitrogen per PEI molecule.

^e Carbon to nitrogen mole ratio by elemental analysis.

greater than 2.0 provide information about the amount of crosslinking. The results of the carbon to nitrogen ratio indicated that both methods produced phases with similar extents of crosslinking. BUDGE has an elemental formula of $C_{10}H_{18}O_4$. If we assume that PEI has a formula $(C_2H_5N)_n$ and both ends of BUDGE are linked with PEI, then ca. 50% of the amine groups on the surface of both phases are crosslinked.

3.2. Chromatographic characterization

The two stationary phases showed high efficiency for the chromatography of small anions. An example separation of nitrite and nitrate (reduced plate height of 2.8 for each solute) on the adsorptively coated column is shown in Fig. 1. The separation of a mixture of organic acids on the same column is shown in Fig. 2. Results for the chromatography of a number of small anions on both columns and a measure of the reduced plate height, are given in Table 2. The evaporatively coated phase showed more retention towards anions than the adsorptively coated phase, but generally had lower efficiency. The height-over-area method was used to calculate the number of theoretical plates and reduced plate height:

$$N = 2\pi (H t_R / A)^2 \quad (1)$$

$$h = L / (N d_p) \quad (2)$$

where N is the number of theoretical plates, H is the height of the peak, t_R is the retention time, A is the area of the peak, h is the reduced plate height, and d_p is the average diameter of the particles. The height-over-area method typically gives more conservative estimates than the inflection-point or half width methods, especially for skewed peaks [24].

The elution order of solutes contained in Table 2 was as expected. Aniline, which is cationic at pH 7.0, eluted before any of the other solutes. 4-Nitrotoluene, which has no net charge, eluted slightly after aniline but before any of the anionic solutes. The fact that both aniline and

4-nitrotoluene were retained at all indicates there must be some hydrophobic interaction of the solutes with the stationary phase. The inorganic anions listed in Table 2 eluted in the same order (bromate < nitrite < nitrate < iodide) as on a typical silica-based anion exchanger [25]. However, nitrite, nitrate and iodide exhibited a distinctly different elution order on bare zirconia [9]. This suggests that the PEI coating at least partially blocks interactions with the bare zirconia substrate. Both benzoic acid and 3,5-dinitrobenzoic acid showed poor efficiency compared to the inorganic anions.

The capacity factors of anions on both the adsorptively and evaporatively coated columns followed the loading of stationary phase on the substrate zirconia. The increase in capacity factor was more accurately predicted by the elemental analysis results than the anion-exchange capacity. The picric acid assay showed that the evaporatively coated particles had 49% more PEI, while elemental analysis indicated a 66% higher loading. The average increase in capacity factor for the anionic solutes listed in Table 2 was 82% with a standard deviation of 5%. If we assume that both columns are identical except for the phase ratio then the capacity factor on the evaporatively coated column (k'_e) and the adsorptively coated column (k'_a) should be related as:

$$k'_e = (\phi_e / \phi_a) k'_a \quad (3)$$

where ϕ is the phase ratio. As shown in Fig. 3, Eq. 3 is followed very nicely for the anionic solutes. Least-squares analysis of the anionic solutes gives $n = 6$, $(\phi_e / \phi_a) = 1.72$, S.D. = 0.17, and intercept = 0.16 ± 0.25 . We note that the intercept is statistically zero as predicted by Eq. 3, and the slope is in fairly good agreement with elemental analysis (72% versus 66%, respectively). We also note that the cationic solute, aniline, is less retained on the more heavily loaded evaporatively coated column. Donan exclusion from the more positively charged surface is stronger on the more heavily coated particles resulting in a lower capacity factor for the cationic solute.

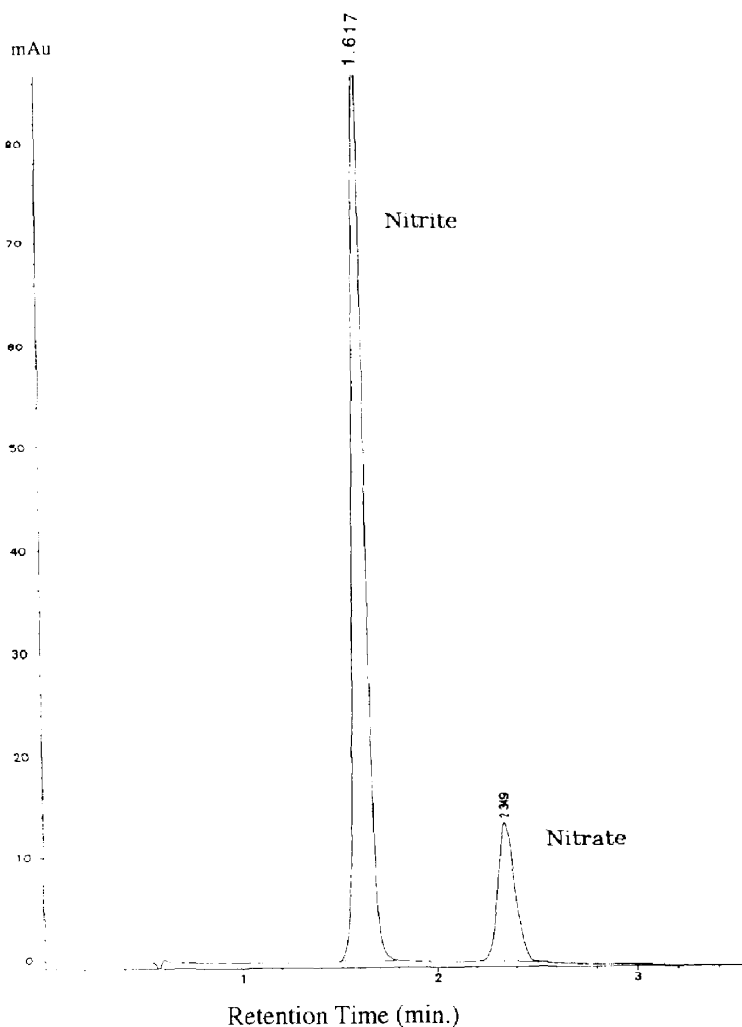


Fig. 1. The separation of nitrite and nitrate on adsorptively coated PEI-zirconia. Column, 5 cm \times 0.46 cm I.D.; mobile phase, 500 mM sodium acetate at pH 7.0; flow rate, 1.0 ml/min; column temperature, 35°C; injection volume, 5 μ l; solute concentration, 10 mM; detection at 240 nm.

3.3. The effect of adding fluoride to the mobile phase

The surface chemistry of zirconia is very complex [22]. It has been demonstrated that zirconia interacts strongly with hard Lewis bases [25,26]. Without additives in the mobile phase, strong hard Lewis bases, like benzoates, elute with poor peak shapes. This is primarily due to

interactions between the carboxylate group (Lewis base) and bare zirconia (Lewis acid), leading to a mixed mode retention [27]. The addition of fluoride, a very strong, hard Lewis Base, to the mobile phase helps control Lewis acid–base interactions of solutes and peak shape often improves dramatically (see Table 3). To investigate Lewis acid–base interactions with the bare zirconia surface in the PEI-coated zirconia,

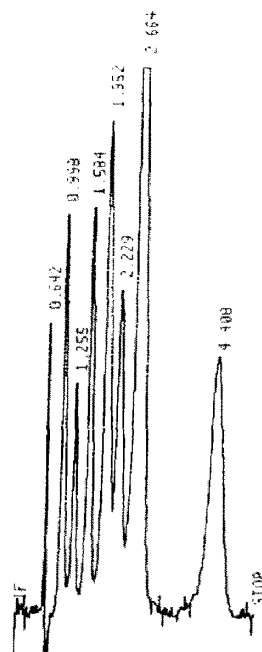


Fig. 2. The separation of organic acids on evaporatively coated PEI-zirconia. Column, 5 cm \times 0.46 cm I.D.; mobile phase, 100 mM sodium acetate at pH 7.0; flow rate, 1.0 ml/min; column temperature, 35°C; injection volume, 5 μ l; solute concentration, 10 mM; detection at 240 nm. Solutes and their retention times in minutes: Phenylphosphonic acid (0.998), benzoic acid (1.255), *p*-nitrobenzoic acid (1.584), diphenylphosphate (1.952), *p*-chlorobenzoic acid (2.229), *p*-hydroxybenzoic acid (2.664), *p*-iodobenzoic acid (4.408), dead-volume peak (0.642).

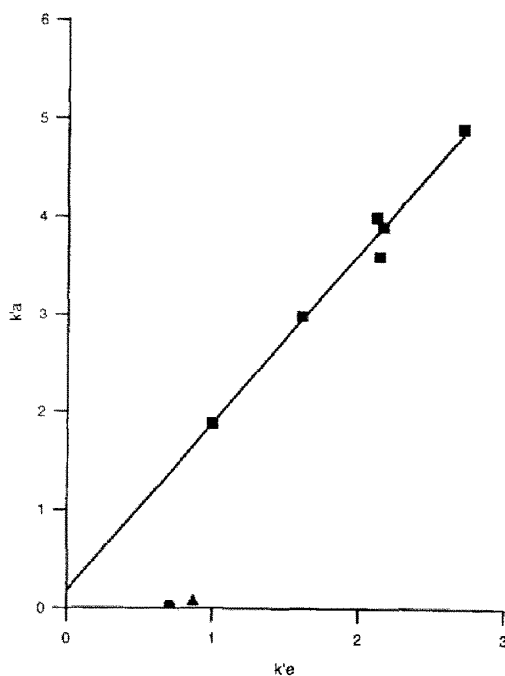


Fig. 3. Plot of capacity factors for the evaporatively and adsorptively coated columns for solutes contained in Table 2. ■ = Anions, ● = cation, ▲ = neutral.

six different *p*-benzoic acid derivatives were examined with and without fluoride in the mobile phase. The addition of fluoride to the mobile phase produced a dramatic improvement in the

Table 2
Comparison of adsorptively and evaporatively coated columns

Solute	$k'_{\text{adsorption}}$	$k'_{\text{evaporation}}$	Reduced plate height ($h_{\text{ads.}}$)	Ratio $h_{\text{ads.}}/h_{\text{evap.}}$
Aniline	0.70	0.03	3.9	0.6
4-Nitrotoluene	0.87	0.98	4.3	0.5
Potassium bromate	1.00	1.89	3.8	0.8
Sodium nitrite	1.61	2.97	2.8	0.8
3,5-Dinitrobenzoic acid	2.12	3.99	7.9	0.8
Benzoic acid	2.15	3.59	10.4	1.1
<i>p</i> -Toluenesulfonic acid	2.17	3.90	4.4	0.6
Sodium nitrate	2.72	4.90	2.8	0.9
Potassium iodide	5.26	—	3.8	—

Mobile phase for both columns, 0.5 M sodium acetate at pH 7.0; column temperature, 35°C; flow rate, 1.0 ml/min; solutes concentration 10 mM; injections, 5 ml; detection, 240 nm.

Table 3
Effect of fluoride in the mobile phase

Solute	k'		Reduced plate height ^b	Ratio h^b/h^a
	Fluoride ^a	No fluoride ^b		
<i>p</i> -Cyanobenzoic acid	2.12	3.52	27.7	4.1
<i>p</i> -Aminobenzoic acid	2.75	13.6	83.3	11.6
<i>p</i> -Nitrobenzoic acid	2.87	5.74	23.8	4.0
<i>p</i> -Ethylbenzoic acid	3.39	6.59	55.6	6.7
<i>p</i> -Hydroxybenzoic acid	6.52	15.2	83.3	11.6
<i>p</i> -Iodobenzoic acid	10.8	19.2	36.2	4.4

^a Mobile phase, 0.5 M sodium acetate, 100 mM sodium fluoride at pH 7.0; column temperature, 35°C; flow rate, 1.0 ml/min; solute concentration, 10 mM; injections, 5 μ l; detection, 240 nm.

^b Same as footnote a, but no fluoride in the mobile phase.

peak shape and efficiency for all solutes. Fluoride addition also had an effect on the selectivity towards *p*-aminobenzoic acid. The capacity factor changed from 13.6 with no fluoride in the mobile phase to 2.75 with fluoride. A chromatogram of *p*-hydroxybenzoic acid with and without fluoride in the mobile phase is shown in Figs. 4 and 5. A comparison of the two chromatograms shows an 11-fold improvement in efficiency and no peak tailing when fluoride was present in the mobile phase.

3.4. A comparison of a strong silica-based, bare zirconia, and PEI-coated zirconia ion exchangers

The differences in retention under similar mobile phase conditions between PEI-zirconia, silica-based and bare zirconia ion exchangers is shown in Table 4. There was very little retention and no discernible dependence of retention on pK_a for the silica-based system. The para benzoic acids were strongly retained on bare zirconia

Table 4
Comparison of silica-based, bare zirconia and PEI-coated zirconia anion exchangers

<i>Para</i> benzoic acid	pK_a	Capacity factor (k')		
		Spherisorb 5 SAX ^a	Bare zirconia ^b	PEI-coated zirconia ^c
Nitro	3.44	0.2	4.6	5.7
Cyano	3.55	0.2	4.3	3.5
Iodo	4.00	0.3	13.7	19.2
Ethyl	4.35	0.4	18.1	6.6
Hydroxy	4.58	0.2	24.7	15.2
Amino	4.85	0.3	50.5	13.6

Para benzoic acid derivatives on silica-based, bare zirconia, and PEI-coated zirconia columns. column temperature, 35°C; flow rate, 1.0 ml/min; solute concentration, 10 mM; injections, 5 μ l; detection, 254 nm.

^a Mobile phase, 100 mM Pipes at pH 6.8 [9].

^b Mobile phase, same as footnote a [9].

^c Mobile phase, 100 mM Pipes at pH 7.0;

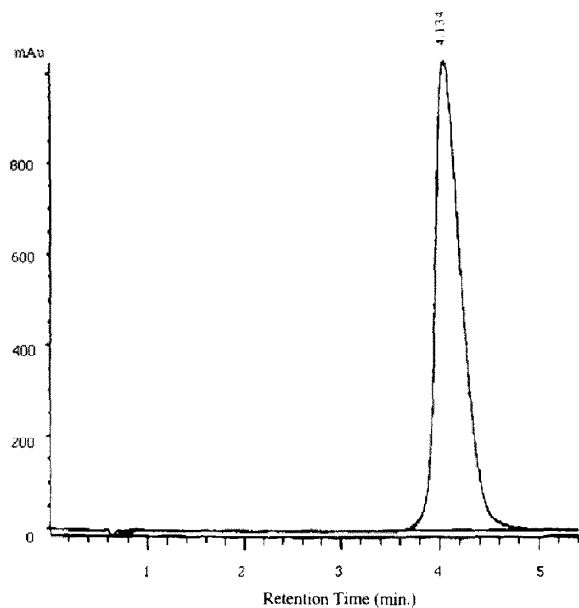


Fig. 4. The elution of *p*-hydroxybenzoic acids on adsorptively coated PEI-zirconia. Column, 5 cm × 0.46 cm I.D.; mobile phases, 100 mM Pipes buffer at pH 7.0 containing 100 mM sodium fluoride; flow rate, 1.0 ml/min; column temperature, 35°C; injection volume, 5 μl; solute concentration, 10 mM; detection at 254 nm.

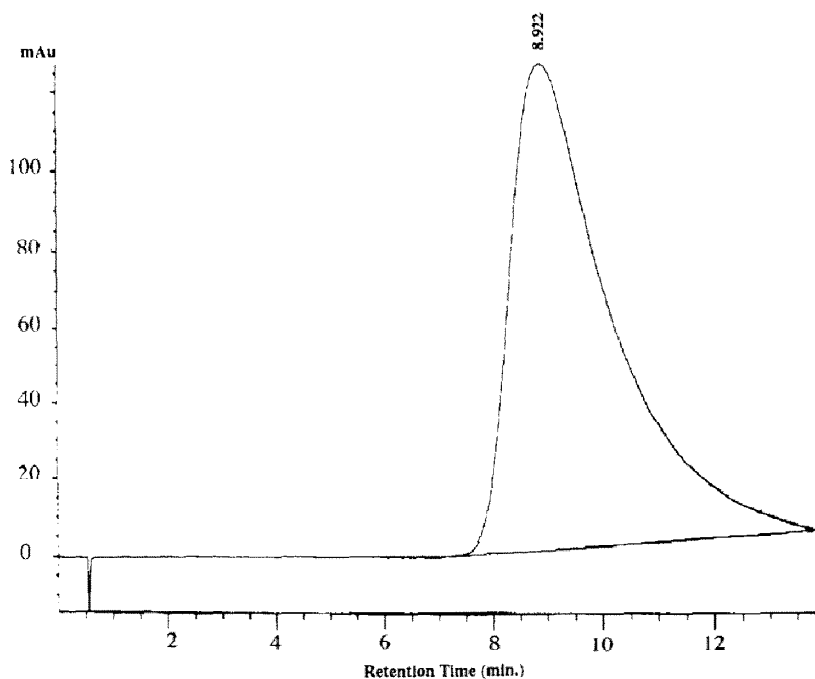


Fig. 5. The elution of *p*-hydroxybenzoic acids on adsorptively coated PEI-zirconia. All conditions as in Fig. 4, except for absence of 100 mM fluoride.

with capacity factors ranging from 4 to 50. The elution order for the *bare* zirconia column showed a strong correlation between pK_a and capacity factor. This is primarily due to the strength of the Lewis acid–base interactions between the carboxylate groups and the zirconia surface. In general, the higher the pK_a value the stronger the Lewis acid–base interaction and the greater the capacity factor. The PEI-coated zirconia also showed a wide range in capacity factors for the *para* benzoic acids, but there was no apparent correlation with pK_a . The PEI-coated zirconia exhibited an elution order for *para* benzoic acids different from either the silica-based or *bare* zirconia columns.

3.5. Flow study of PEI-zirconia columns

A flow study was done to evaluate the coefficients of the Knox–Bristow [28] equation:

$$h = Av^{0.33} + B/v + Cv \quad (4)$$

where h is the reduced plate height, v is the reduced velocity and A , B , and C are the fitting

coefficients. Typical coefficient values for a good column are $A < 1$, $B \approx 2$ and $C < 0.2$. A diffusion coefficient (D_m) of 2×10^{-5} cm²/s [29] was used for the calculation of reduced velocity. This coefficient was estimated by analogy to the observed diffusion coefficient of potassium nitrate at room temperature. This value gave a reasonable estimate of the B coefficient, which is related to longitudinal diffusion within the column, and should have a value of about 2 [30]. Nitrite was used as the probe solute for the study and the results, along with the fit line, are shown in Figs. 6 and 7. The coefficients were evaluated using multi-variate regression and are given in Table 5. Coefficient A is related to the column packing, and C is related to the mass transfer within the column [24]. The results indicate that both columns exhibit good mass-transfer characteristics.

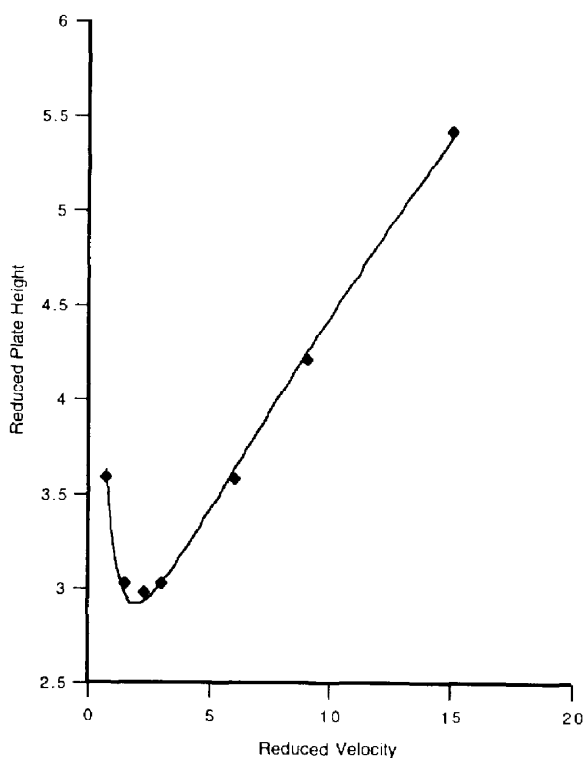


Fig. 6. Flow study on adsorptively coated PEI-zirconia. Column, 5 cm \times 0.46 cm I.D.; mobile phase, 100 mM sodium acetate at pH 7.0; flow rate, 1.0 ml/min; column temperature, 35°C; injection volume, 5 μ l; solute concentration, 10 mM; detection at 240 nm. \blacklozenge = Nitrite probe solute.

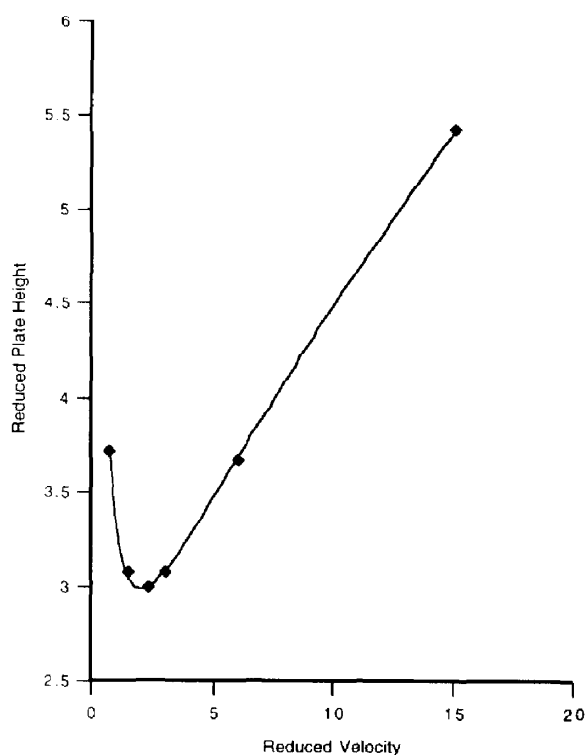


Fig. 7. Flow study on evaporatively coated PEI-zirconia. All conditions as in Fig. 6.

3.6. Acid and base stability

The acid and base stability of the PEI-coated zirconia was tested for each column. Stability studies did not use pHs above 9 as PEI becomes deprotonated, resulting in a loss of retention of anions. Future work using quaternized PEI coating will investigate the stability over a wider range of pHs. The evaporatively coated column was tested at pH 2.75 and pH 9.00 using potassium iodide as the probe solute with a phosphate buffer mobile phase. At each pH the ionic strength was kept constant at 0.20. The evaporatively coated phase proved to be stable under both acidic and basic conditions. The results are shown in Fig. 8. The iodide anion is very polarizable and is well retained at pH 2.75 with a k' around 7, while at pH 9.00 the k' dropped to slightly above 2. This large decrease in retention at pH 9.00 suggests that the PEI is becoming deprotonated. A chromatogram of iodide at pH

Table 5
Knox–Bristow coefficients

Column	A	B	C
Adsorptive	1.5 ± 0.05	1.6 ± 0.06	0.10 ± 0.01
Evaporative	1.5 ± 0.02	1.7 ± 0.02	0.10 ± 0.004

Mobile phase, 100 mM Pipes buffer, 100 mM NaF at pH 7.0; column temperature, 35°C; flow rate, 1.0 ml/min; solute concentration, 10 mM; injections, 5 µl; detection, 254 nm.

9.00 using the evaporatively coated column is shown in Fig. 9. The adsorptively coated column was initially tested at pH 5 using nitrite as a probe and then exposed to phosphate buffer mobile phases in the pH range of 3 to 9 in 1 pH increments. 500 ml of each pH was run through

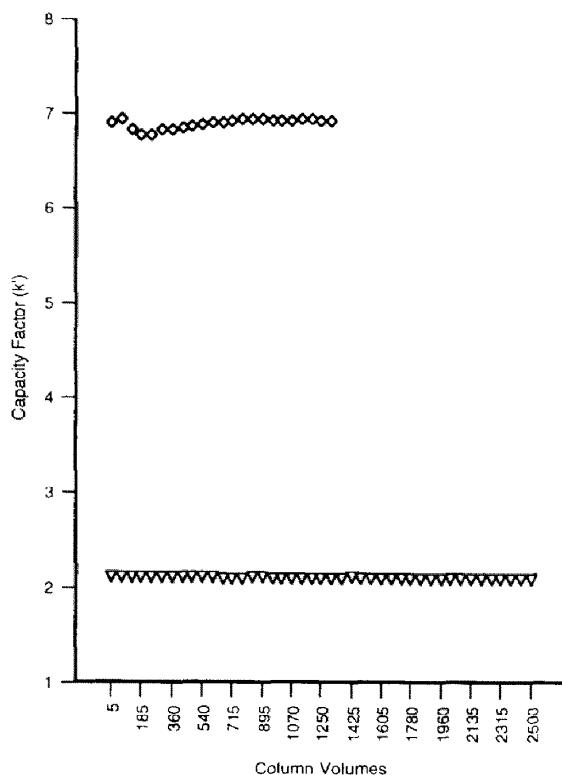


Fig. 8. Acid and base stability study on evaporatively coated PEI-zirconia. Column, 5 cm × 0.46 cm I.D.; mobile phases, 200 mM potassium phosphate dibasic at pH 2.75 (▽), 67 mM potassium phosphate dibasic at pH 9.0 (◇); flow rate, 1.0 ml/min; column temperature, 35°C; injection volume, 5 µl; solute concentration, 10 mM; detection at 240 nm.

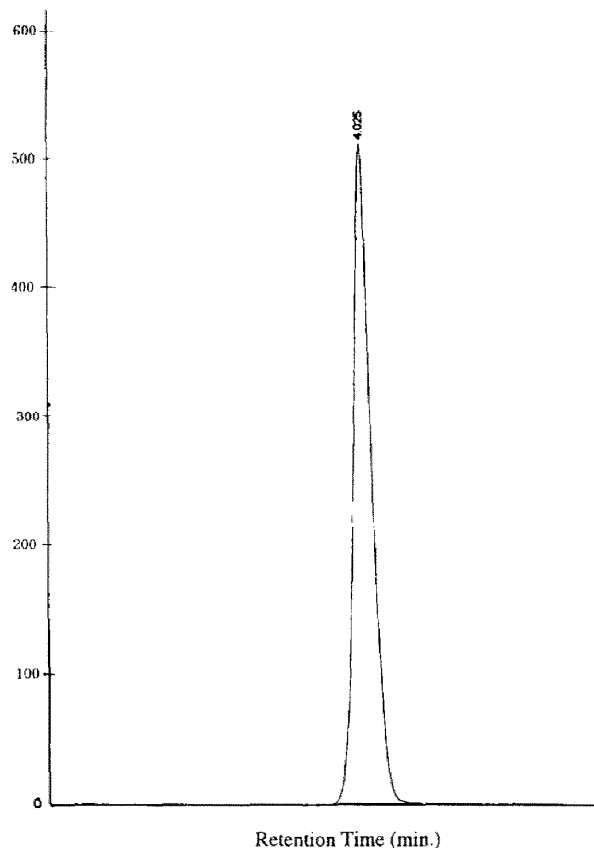


Fig. 9. The elution of potassium iodide on the evaporatively coated PEI-zirconia. Column, 5 cm × 0.46 cm I.D.; mobile phases, 10 mM Tris buffer, 500 mM sodium acetate at pH 9.0; flow rate, 1.0 ml/min; column temperature, 35°C; injection volume, 5 µl; solute concentration, 10 mM; detection at 240 nm.

the column for a total of 3.5 l. Nitrite was then tested again at pH 5 with a less than 1% change in the capacity factor.

4. Conclusions

Zirconia may be coated with polyethyleneimine by both adsorptive or evaporative methods to produce efficient anion-exchange stationary phases for use in high-performance liquid chromatography. The evaporative coating method allows for higher loading of PEI onto the zirconia surface. Both columns were efficient in

the separation of small inorganic and organic anions. Due to the Lewis acid–base chemistry of zirconia, the addition of fluoride to the mobile phase produced as much as an 11-fold increase in chromatographic efficiency for benzoic acid derivatives. PEI-coated zirconia showed a distinct elution order of *para* benzoic acids from *bare* zirconia or silica-based columns. Inorganic anions such as nitrite, nitrate and iodide had the same elution order as on a silica-based anion exchanger, but was opposite to that of *bare* zirconia. A flow study to evaluate the coefficients of the Knox–Bristow equation showed that both PEI-zirconia columns had good mass-transfer characteristics. Both columns were chemically stable from pH 2.75 to 9.

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