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PREPARATION AND EVALUATION OF INORGANIC ANION-EXCHANGE SORBENTS NOT BASED ON SILICA[*]

R. M. CHICZ, Z. SHI and F. E. REGNIER*

Department of Biochemistry, Purdue University, West Lafayette, IN 47907 (U.S.A.)

SUMMARY

The use of alternate macroporous (greater than 200 Å) inorganic support materials in the preparation of pellicular anion-exchange packings was explored. Alumina, magnesia, titania, and a zirconia-coated silica were chosen for comparison with silica. The stationary phase attached to the support surfaces was an adsorbed polyethyleneimine, crosslinked with 1,4-butanediol diglycidyl ether. Packing materials were characterized by static elemental analyses, chromatographic retention, static loading capacity and pH stability. Titania, alumina, and zirconyl-clad silica packings were found to be substantially more stable under alkaline conditions than silica-based materials. The data show that the stationary phase was successfully bonded in all cases and functioned in anion-exchange chromatography. When the surface area and pore diameter of these alternate materials is equivalent to silica, there is little difference in chromatogrpahic properties.

INTRODUCTION

Porous silica is currently the most widely used support for the preparation of high-performance liquid chromatographic (HPLC) packings, because it is available in a wide variety of particle sizes, pore diameters, pore volumes, and surface areas. In addition, silica surfaces are easily derivatized and are mechanically stable to several hundred atmospheres of pressure. The single greatest limitation of silica matrices is poor alkaline stability.

The oxides of aluminium, titanium, and zirconium have also been shown to produce chromatographic matrices capable of adsorption[1–4]. The possibility that these materials might have greater alkaline stability has stimulated interest in their use as chromatographic supports. Unfortunately, little work has been done on the coupling of organic stationary phases to these alternate inorganic materials.

A further limitation on experimentation with materials not based on silica has been the difficulty in obtaining commercially produced wide-pore media in the particle size range 5–20 μm. Advances in the synthesis of inorganic materials with uniform structures[5–7] would suggest that the limited commercial availability of HPLC-

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grade inorganic supports other than silica is not based on technical problems in their synthesis.

Pivotal in the use of alternate inorganic support materials as chromatographic media is the ability to couple organic stationary phases to their surfaces. In this study, adsorption was examined as a means of applying anion-exchanging stationary phases to titania, magnesia, alumina, and zirconia-treated silica. These support materials were coated by the adsorbed polyethyleneimine (PEI) coating method developed by Alpert and Regnier[8] for silica-based supports. Theoretically, this adsorption technique should be equally applicable to supports other than silica if they have a negatively charged surface. Stationary-phase ligand density, loading capacity, column stability, and separation characteristics of materials not based on silica were compared with those of a standard silica support.

MATERIALS AND METHODS

Support materials

Vydac 101TPB (17-μm spherical, 300 Å) silica was used as the standard against which the other inorganic supports were compared. Vydac was a gift from The Separations Group (Hesperia, CA, U.S.A.). The 398–840-μm (controlled-pore, 400 Å) alumina and titania were gifts of Corning Biological Products (Medfield, MA, U.S.A.) as was the 37–70-μm particle diameter (220 Å pore diameter) controlled-pore glass (CPG). The 10-μm (300 Å) magnesia was a gift of Pennsylvania Glass & Sand (Berkeley Springs, WV, U.S.A.), while the 5-μm alumina (spherical, 150 Å) was purchased from EM Labs. (Elmsford, NY, U.S.A.). Zirconyl-treated silica was prepared using the 17-μm Vydac silica.

Reagents

The crosslinker, 1,4-butanediol diglycidyl ether (BUDGE), was purchased from Aldrich (Milwaukee, WI, U.S.A.). Polyethyleneimine-6 (PEI-6) (MW *ca.* 600) was obtained from Polysciences (Warrington, PA, U.S.A.). Inorganic reagents (for eluents and assays) and solvents (methanol, acetone, triethylamine, etc.) were of AR grade or comparable quality.

Proteins

Proteins used as chromatographic test materials were egg white ovalbumin (OVA) (Grade V) and bovine serum albumin (BSA) (Fraction V) and proteins used for binding assays were BSA (Fraction V) and hemoglobin (Hb) (crude powder, Type II). All proteins were purchased from Sigma (St. Louis, MO, U.S.A.).

Instrumentation

HPLC separations were performed on a Varian 5500 pumping system (Varian Instrument Group, Walnut Creek, CA, U.S.A.) equipped with a Micromeritics 786 variable-wavelength UV detector (Micromeritics, Norcross, GA, U.S.A.). Proteins were monitored at 254 nm. Data were collected on a Linear Model 1200 recorder (Linear Instruments, Reno, NV, U.S.A.). The system was fitted with a Valco Model C6U injector with a 100-μl injection loop (Valco Instruments, Houston, TX, U.S.A.). A Varian Series 634 UV–VIS spectrophotometer was used to measure absorbancies in the picric acid and protein-binding assays.
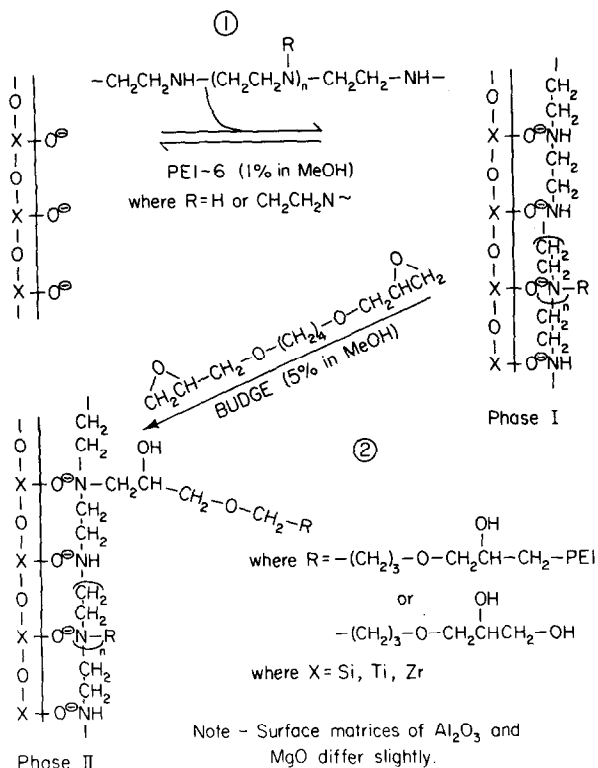
①

R
|
$-CH_2CH_2NH-(CH_2CH_2N)_n-CH_2CH_2-NH-$

PEI-6 (1% in MeOH)

where R = H or $CH_2CH_2N\sim$

Phase I

BUDGE (5% in MeOH)

②

where R = $-(CH_2)_3-O-CH_2\overset{\text{OH}}{\underset{|}{CH}}-CH_2-PEI$

or

$-(CH_2)_3-O-CH_2\overset{\text{OH}}{\underset{|}{CH}}CH_2-OH$

where X = Si, Ti, Zr

Note – Surface matrices of Al_2O_3 and MgO differ slightly.

Phase II

Fig. 1. Synthetic route to an adsorbed PEI stationary phase. The process takes place in two steps: (1) adsorption of PEI-6 onto the support material; and (2) crosslinking of the PEI-6 polymer in order to stabilize the stationary phase further.

Synthesis of packing materials

Application of stationary phases to inorganic materials was achieved by a modification of the procedure used by Alpert and Regnier[8] (Fig. 1). The procedure outlined below applies to the synthesis of gram amounts of packing material. Support material was suspended and agitated in 10 ml of 1% (w/v) PEI-6–methanol solution. After this mixture was allowed to stand at room temperature for 30 min, the polyamine-coated support material was collected in a sintered-glass funnel and dried under vacuum for 30 min. Crosslinking was achieved by suspending coated support materials in 10 ml of a 5% (v/v) solution of BUDGE in methanol. After 12 h at room temperature (without agitation), the mixture was heated on a steam bath for 30 min. The coated (crosslinked) support material was then filtered on a sintered-glass funnel, washed alternately with methanol and triethylamine, dried under vacuum and stored in a dessicator.

Zirconia coating

A modification of the method developed by Marsh and Tsao[9] was used to apply a surface coating of zirconia to silica. Silica was suspended in 10 ml of a zirconate–chelating agent solution. The zirconate–chelating agent solution was pre-

pared by combining 2,4-pentanedione, zirconium isopropoxide, and toluene in a 1:3:3 (v/w/v) mixture. The solution was degassed and allowed to react for 1 h. The zirconyl-clad silica was next vacuum-dried overnight and then placed in a muffle furnace at 500°C for 8 h. The zirconyl-clad silica was repeatedly washed with 2-propanol then vacuum dried and stored in a dessicator.

Chemical analysis of packings

Stationary-phase ligand density, *i.e.* the total ion-pairable amine, was estimated by the picric acid assay of Alpert and Regnier[8]. Both BSA- and Hb-binding capacity assays were performed as described by Kopaciewicz *et al.*[10].

Column packing procedure

Column packing materials were slurried in 2-propanol (2%, w/v) and packed at 5000 p.s.i. with a pneumatic pump (Haskel, Burbank, CA, U.S.A.), as described by Pearson and Regnier[11]. Approximately 90–110 ml of solvent was pumped through the columns with the packing pump. The large particle diameter CPG, alumina, and titania were dry-packed.

Chromatography

A 100-μg mixture of OVA and BSA was eluted from the anion-exchange test columns at a flow-rate of 1 ml/min with a 20-min linear gradient ranging from a 0.01 *M* Tris buffer (pH 7.5) to a 0.01 *M* Tris buffer (pH 7.5) containing 0.5 *M* sodium chloride.

pH Stability of packings

Aliquots of supports (100 mg) with blue dye-labelled stationary phase[12] in 1 *M* potassium hydroxide were heated to 100°C for 1 h and then kept in the 1 *M* potassium hydroxide for 24 h at room temperature. Stability of the packing was assessed by two criteria: (1) the leaching of the blue amine layer into solution; and (2) the dissolution of base material. Total dissolution of the base silica produced a flocculent blue precipitate of the organic coating with no mechanical strength.

RESULTS AND DISCUSSION

A schematic representation of the two-step process by which PEI-6 is adsorbed on an inorganic surface and cross-linked to form a continuous film with BUDGE is given in Fig. 1. Since concentrated solutions of PEI tend to clog the pores of a support[13], a 1% solution of PEI in methanol was used to minimize the possibility of forming a thick coating. BUDGE was used as the crosslinking agent because the molecule is sufficiently long to form a bridge between adjacent adsorbed amine molecules[14] and, when used in low concentration, it does not add significantly to the hydrophobicity of the stationary phase. BUDGE can react with adsorbed polyamine species in two ways: (1) both oxiranes in the crosslinker can react with amines in a crosslinking reaction; or (2) only one oxirane reacts with an amine and the other oxirane is ultimately hydrolyzed to a diol (see Phase II in Fig. 1). The illustration of a surface shown in Fig. 1 applies equally to silica, titania, and zirconia. Although the surface of alumina and magnesia is different from that of silica (Fig. 2), a similar
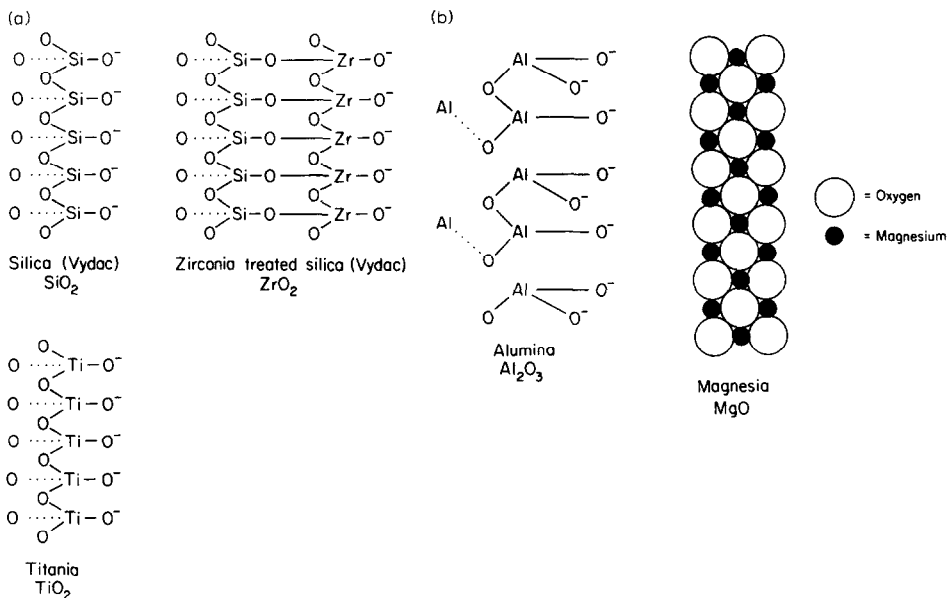
Fig. 2. Schematic representation of the surfaces of inorganic matrices. (a) The oxides of silicon, titanium, and zirconium represent similar oxygen ligand densities due to the crystal lattice structures formed by the common coordination number. (b) Differences in the charge density at the surfaces of magnesia and alumina are due to coordination number.

reaction scheme can be conceptualized for polyamine adsorption on alumina and magnesia.

Physical properties of the various supports are listed in Table I. Owing to the limited commercial availability of microparticulate inorganic support materials other than silica, we studied a wide range of particle sizes. Data for support surface area were provided by manufacturers in all cases except for the titania and zirconyl-clad Vydac, which were obtained by the BET method[15]. Static binding assays, elemental

TABLE I

PHYSICAL PROPERTIES OF SUPPORT MATERIALS

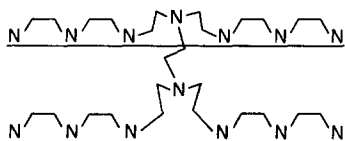| Support material	Particle size (μm)	Pore diameter (Å) given by manufacturer	Surface area (m²/g) given by manufacturer	Particle shape
Vydac	15–20	300	157	S*
CPG	37–74	222	130	I**
Magnesia	10	300	185	I
Alumina	5	150	70	S
Alumina	398–840	400	–	S
Titania	398–840	400	15	S
Zirconia-coated Vydac	15–20	226	141	S

* Spherical particle.
** Irregular particle.

Fig. 3. Stationary phase–surface interactions. PEI-6 has fourteen nitrogens per molecule. For simplicity, PEI is represented schematically without ethylene groups or protons and with a primary:secondary:tertiary amino group ratio of approximately 1:2:1.

ports with the standard Vydac 101TPB.

A scheme for the interaction of the polyamine stationary phase with inorganic support surfaces is proposed in Fig. 3. It is likely that only the primary and secondary amino groups of PEI-6 interact electrostatically with the inorganic support, since tertiary amines are sterically hindered from making direct contact with surfaces. Ion-pairing capacities (IPC) and intrinsic loading capacities (mg protein/g packing) are presented in Table II. The IPC data show that magnesia adsorbed approximately twice as much PEI as the Vydac silica. The remaining supports, with the exception of titania, adsorbed amounts of PEI equivalent to silica. Previous studies have demonstrated that the BSA-binding capacity of a column material is a function of three variables: (1) stationary-phase density (μmoles N/m^2); (2) surface area of the material; and (3) pore diameter of the material[16]. This suggests that the low loading capacity of the titania-based column material is due to its limited surface area, as indicated by the small ion-pairing capacity of the material. In contrast, the ratio of BSA-loading capacity to IPC for the 150-Å alumina is smaller than that of the titania-based material. This is because the smaller pores in the alumina support restrict access of BSA to some of the internal surfaces of the support[16]. The ratios of protein bound to materials other than silica to the binding capacity of the standard Vydac silica material was the same within experimental limits for hemoglobin (Hb) (Table II) and BSA.

The carbon and nitrogen content of the polyamine-coated column materials was determined by elemental analysis (Table III). Estimation of the number of PEI

TABLE II

IPC AND INTRINSIC LOADING CAPACITY OF COATED SUPPORT MATERIALS

Support	IPC $\left(\dfrac{\mu mol\ N}{g\ support}\right)$	BSA_{iec} $\left(\dfrac{mg\ BSA}{g\ support}\right)$	Hb $\left(\dfrac{mg\ BSA}{g\ support}\right)$
Vydac	436	110	45
CPG	267	84	46
Magnesia	580	98	43
Alumina (5 μm)	297	50	18
Alumina	393	6	17
Titania	42	26	9
Zirconia	342	80	33

TABLE III

ELEMENTAL ANALYSIS

Support material	Total carbon (%)	Total nitrogen (%)	Carbon from PEI-6 (%)	Carbon from BUDGE (%)	Average No. of nitrogen atoms derivatized
Vydac	4.62	1.53	2.6	2.0	4.3
CPG	3.14	0.71	1.2	1.9	8.8
Magnesia	4.61	1.41	2.4	2.2	5.1
Alumina (5 μm)	1.41	0.38	0.6	0.8	6.6
Alumina	3.47	0.72	1.2	2.3	10.5
Titania	3.33	1.01	1.7	1.6	5.2

TABLE IV

COMPARISON OF ION PAIRING CAPACITY PER UNIT OF SURFACE AREA

Support material	$\dfrac{\mu Moles\ N}{m^2}$	Comparison with silica (%)
Vydac silica	2.78	100
Magnesia	3.14	113
Titania	2.80	101
Zirconia	2.43	87
Alumina	4.24	153

TABLE V

RETENTION TIMES OF OVALBUMIN AND BOVINE SERUM ALBUMIN ON PEI-COATED INORGANIC SUPPORTS

Support	Retention time (min)	
	OVA	BSA
Alumina (5 μm)	7.8	16.2
CPG	5.4	7.5
Magnesia	7.9	9.0
Zirconylclad silica	8.9	19.5
Titania	9.5	12.1
Silica	9.5	12.9

nitrogen atoms derivatized by oxiranes was based on these elemental analyses. Only primary and secondary amino groups that are not ion-paired with surface functional groups may participate in the reaction with oxiranes. The extent of reaction between PEI and oxiranes varies according to the support, indicating that there are differences in the extent of polyamine ion-pairing in the coating process.

Differences in the coordination chemistry of surface atoms influenced both the amount of polyamine adsorbed/unit surface area and the extent of crosslinking. For example, silica, titania, and zirconia all have coordination numbers of 4 and their oxides are believed to have similar adsorption characteristics. According to Wells[17], the crystal lattice structure of magnesia would lead it to behave in a similar fashion, even though magnesium has a coordination number of 2. In contrast, alumina, with a coordination number of 3, has 37% more surface oxygen than the other materials[18] and would be expected to provide for higher polyamine loading. General substantiation of these hypotheses is seen in Table IV. The supports with similar oxygen ligand densities (silica, titania, zirconia, and magnesia) adsorbed equivalent amounts of PEI per square metre of surface, whereas alumina adsorbed 53% more PEI per square metre than these materials.

Large differences in the particle diameter of these supports made comparison of their chromatographic performance difficult. However, retention times were compared and found to be similar for the different supports (Table V). Differences found for the zirconyl-clad silica and 5-μm alumina were probably the result of perturba-
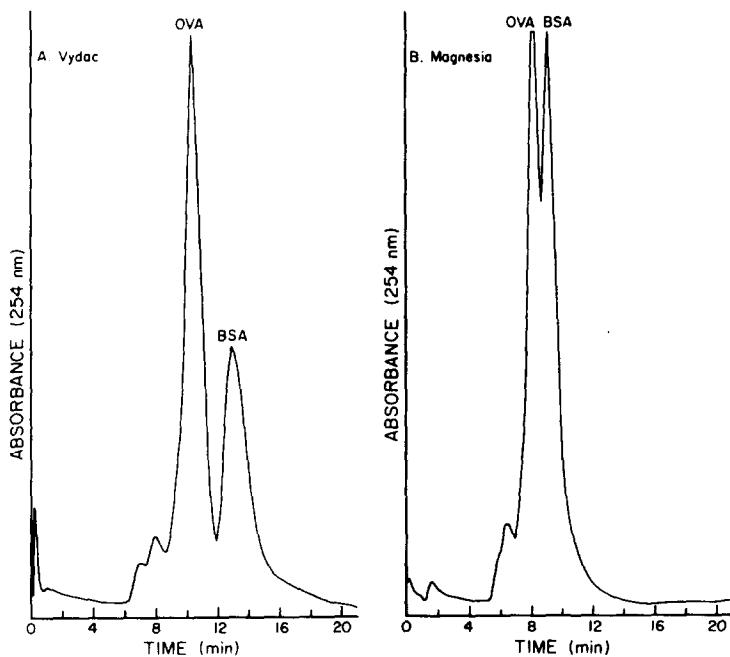

Fig. 4. Chromatographic resolution of a mixture of OVA and BSA on (A) silica- and (B) magnesia-based anion-exchange columns.

tions in crosslinking the stationary phase. The support surface can alter the cross-linking reaction by influencing the ratio of primary to secondary amino groups sequestered at the surface. A comparison of Vydac silica and magnesia showed these two materials to be similar in terms of chromatographic performance (Fig. 4).

Stability at basic pH is reported to be one of the advantages of these alternate support materials[3,10,19]. In an effort to confirm these reports, qualitative stability studies at alkaline pH were carried out on polyamine-coated support materials, derivatized with a blue dye. Leaching of the blue polyamine coating was used as a measure of coating stability. Titania, alumina, and zirconia-coated silica showed little loss of polyamine stationary phase as judged visually in a 24-h test at pH 14. Even boiling the supports in 1 M potassium hydroxide produced no visual change. CPG, Vydac silica, and magnesia dissolved within 30 min in boiling 1 M potassium hydroxide, leaving a flocculant blue mass of PEI coating.

CONCLUSIONS

Our studies demonstrate that (1) adsorbed polyamine coatings provide a route to the preparation of stable anion-exchange stationary phases on a variety of inorganic support materials, (2) bonding anion-exchange coatings to inorganic materials is relatively simple, and (3) the actual chemical composition of the support has little influence on the performance of the coating. Furthermore, there is a correlation between the specific adsorption of polyamine and the density of adsorbing groups on the surface of the support. Therefore, supports of similar surface charge density, surface area, and pore diameter should produce equivalent separation media irrespective of the support matrix.

Our pH stability studies confirmed that (1) titania, alumina, and zirconyl-clad silica are all more alkali stable than silica and (2) alkaline conditions will not erode adsorbed polyamine coatings. Thus, it may be concluded that alumina, titania, and zirconyl-clad silica supports with adsorbed stationary phases are all potentially useful materials for operation under alkaline conditions.

ACKNOWLEDGEMENTS

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