Journal of Chromatography, 120 (1976) 321–333

CHROM. 8909

USE OF OXIRANES IN THE PREPARATION OF BONDED PHASE SUPPORTS*

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(First received September 12th, 1975; revised manuscript received January 12th, 1976)

SUMMARY

A variety of stationary phases were bonded to silaceous supports through an intermediate silane coupling agent, γ-glycidoxypropylsilane. Consequently, a series of ion-exchange, hydrophobic, and hydrophilic supports were prepared that are stable, withstand high mobile phase velocities, and have sufficient porosity to allow the partitioning of biopolymers. Liquid–liquid partition chromatography of proteins was achieved with phosphate buffers while the separation of a series of aromatic compounds was accomplished on the same columns with hexane.

Anion- and cation-exchange supports were found to have hemoglobin ion-exchange capacities similar to classical cross-linked dextrans while allowing separation speeds 10–20 times those of carbohydrate gel supports.

INTRODUCTION

Organic stationary phases have been coupled to inorganic supports through three different types of reactions, *viz.* (1) esterification of surface silanols to form an Si–O–C bond[1], (2) silylation of surface silanols to form an Si–O–Si–C bond[2], and (3) Grignard coupling to form an Si–C bond[3]. Since the Si–O–C bond readily hydrolyzes[4], the utility of silyl ester bonded stationary phases is seriously diminished when an aqueous mobile phase is used. The ease of preparation and hydrolytic stability of Si–O–Si–C bonded phases have resulted in expanded use of silylation for the preparation of bonded phase supports. Though the Grignard coupled bonded phases are reported to be very stable, they have not gained wide acceptance. This may be the result of difficulty in their preparation.

A number of different Si–O–Si–C bonded supports have been prepared by reacting an organosilane of the general formula Y_3–Si–P_s with an inorganic support, where P_s is the stationary phase and Y is an alkoxy, phenoxy, or halogen group[3]. The general formula of the bonded phase that results from reaction of one of these organosilanes with a silaceous material is $\equiv SiP_s$. Unfortunately, there are many

* Journal Paper No. 6022 from the Purdue University Agricultural Experiment Station.

stationary phases that would have chromatographic utility but are not commercially available as the organosilane.

This paper reports a technique for coupling a variety of stationary phases (P_s) to supports through the use of an intermediate silane coupling agent. The primary function of the coupling agent is to provide a bond between the inorganic support and the stationary phase (P_s). This is accomplished through the addition of P_s groups to γ-glycidoxypropylsilyl bonded phase supports. The resulting composite has the general chemical formula

$$\equiv SiCH_2CH_2CH_2OCH_2\underset{\displaystyle OH}{\underset{|}{C}}H\text{–}CH_2P_s.$$

EXPERIMENTAL

Equipment

Liquid chromatography was carried out with an Isco Model 314 pumping system (Instrument Specialties, Lincoln, Neb., U.S.A.), a Perkin-Elmer Model NFC 254 ultraviolet (UV) detector (Perkin-Elmer, Norwalk, Conn., U.S.A.), and a Precision Sampling Model 420 inlet (Precision Sampling, Baton Rouge, La., U.S.A.) with 1-m stainless-steel columns of 2 mm I.D.

The ion-exchange chromatography was on an Isco Model 384 pumping system with a Disc Model 706 sample injection valve (Disc Instruments, Costa Mesa, Calif., U.S.A.) and a Perkin-Elmer Model NFC 254 UV detector. Stainless-steel columns of 0.6 m $\times$ 5 mm I.D. were used.

Gas chromatography was on a Varian 1200 gas chromatograph with a flame ionization detector and an on-column injection port. Coiled stainless-steel columns 6 ft. $\times$ 1/8 in. O.D. were used.

Reagents

The bonded phase supports were prepared with controlled porosity glass (CPG) from Corning (Medfield, Mass., U.S.A.) and γ-glycidoxypropyltrimethoxysilane (Silane Z-6040) and γ-aminopropylamine (A-1100) from Dow Corning (Midland, Mich., U.S.A.). Tetraethylenepentamine, diethylamine, tetraethylene glycol, heptanol, dimethylaminoethanol, diethylaminoethanol, glycerol, boron trifluoride etherate, cyanoacetic acid, diethyl phthalate, phthalic anhydride, 1-phenylethanol, benzyl alcohol, and the *n*-alkanes were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). McReynolds' ten probes, Porasil C, and polyethylene glycols 400, 1540, 750, 4000 and 20,000 were obtained from Anspec (Ann Arbor, Mich., U.S.A.). Benzoic acid, benzaldehyde, acetophenone, and 2-phenylethanol were from Matheson, Coleman & Bell (Norwood, Ohio, U.S.A.). Benzophenone and nitrophenyl acetate were from Eastman (Rochester, N.Y., U.S.A.). Hemoglobin, albumin, chymotrypsin, pancreatin, chymotrypsinogen A, and the nucleotides were from Sigma (St. Louis, Mo., U.S.A.).

Procedures

Ion-exchange capacities. Anion-exchange capacities were determined using

1 ml of anion-exchange support that had been washed several times with 0.01 *M* sodium phosphate buffer (pH 8.0). One hundred milligrams of hemoglobin in 5 ml of the above pH 8.0 phosphate buffer were added to the ion-exchange support followed by vortex mixing. After sedimentation of the support, excess buffer containing unbound protein was aspirated from the support. Following three washes with 5 ml of the 0.01 *M* sodium phosphate buffer (pH 8.0), hemoglobin was released from the anion-exchange support with 5 ml of 0.4 *M* sodium phosphate (pH 4.0). The solution containing the released hemoglobin was decanted into a 100-ml volumetric flask and the support washed twice more with 5-ml portions of the pH 4 releasing buffer. The combined washings containing released protein were made up to 100 ml and the hemoglobin concentration determined by absorbance at 410 nm.

Cation-exchange capacity was obtained in an identical manner except that the binding of hemoglobin to the cation-exchange support was achieved in 0.01 *M* sodium acetate buffer (pH 4.0) and release was achieved with 0.4 *M* sodium phosphate buffer (pH 8.0).

Preparation of γ-glycidoxypropylsilyl supports. A 5% aqueous solution of the silylation reagent was prepared by adding γ-glycidoxypropyltrimethoxysilane dropwise to water, keeping the pH of the solution between 5.5 and 5.8 with 10^{-3} *N* KOH. This silylating solution was added to a quantity of inorganic support not exceeding 30 g and vacuum applied to remove air from the pores. This slurry was heated at 90° for 30 min with swirling every 5 min. The bonded phase support was filtered and washed with water and acetone. The support was then either dried *in vacuo* or washed with the solvent to be used in a subsequent synthesis. (Elemental analysis of 74–125 μm support of 250-Å pore diameter: C, 2.63%; H, 0.56%.)

Preparation of amine coupled supports. Solutions containing a primary or secondary amine in dimethylformamide were used to open the epoxide ring on the γ-glycidoxypropylsilyl support and effect an amine coupling of the stationary phase (P_s). The two amines used in this work were polyethyleneimine 230 and diethylamine. Amounts of 3–30 g of γ-glycidoxypropylsilyl support were added to the amine solution (10% polyethyleneimine 230 in dimethylformamide or 80% diethylamine in dimethylformamide). The suspension was swirled and left at room temperature for 24–48 h. The resulting PEI or DEA bonded support was then filtered, washed with water and methanol, and dried under vacuum. (Elemental analysis of 74–125 μm support of 250-Å pore diameter: PEI: C, 4.34; H, 0.67; DEA: C, 3.76%; H, 0.66%.)

Preparation of ether bond supports. Three to thirty grams of γ-glycidoxypropylsilyl support were added to 50 ml of dioxane containing 40 mmoles of the appropriate alcohol or polyethylene glycol and 1 ml of boron trifluoride etherate. After mixing, the suspension was maintained at room temperature for 5 min before heating at 90° for 30 min. The support was then filtered, washed with water and acetone, and dried under vacuum. The dimethylaminoethanol (DMAE), diethylaminoethanol (DEAE), carboxymethyl (CM), polyethyleneglycol (PEG), hydrophobic (HP), and phenylethanol (PE) supports in Table I were prepared by this procedure. (Elemental analysis of 74–125 μm DEAE support of 250-Å pore diameter: C, 4.28; H, 0.79.)

Preparation of ester bonded supports. Three grams of γ-glycidoxypropylsilyl support were added to a solution of 3 g of acid in 20 ml of dimethylformamide. After mixing, the suspension was left at room temperature for 48 h. The glass was then

filtered, washed with water and acetone, and dried under vacuum. The BA and CA supports in Table I were prepared by this procedure.

Preparation of glycerolpropyl support. Three grams of γ-glycidoxypropylsilyl support were treated with 100 ml of 10^{-3} *N* HCl for 1 h. After filtration, the support was washed with water and acetone, and dried *in vacuo*.

Preparation of carboxymethyl (CM) support. Ten grams of glycerolpropyl bonded support were treated with 200 ml of 8 m*M* sodium metaperiodate, 2m*M* potassium carbonate, and 0.134 m*M* potassium permanganate. Conversion of the diol to the carboxymethyl cation exchanger was achieved in 8 h at room temperature. Removal of oxidants by filtration was followed by a 500-ml wash with 1 *M* sodium bisulfite and water washes to neutrality. A final wash with acetone and vacuum drying completed the preparation of the support.

RESULTS AND DISCUSSION

Preparation of supports

γ-Glycidoxypropylsilyl supports were prepared according to reaction 1. An aqueous solution of the organosilane monomer

$$\text{CPG} + (CH_3O)_3Si(CH_2)_3OCH_2\underset{\diagup\ \diagdown}{\overset{O}{}}CH\text{–}CH_2 \xrightarrow[90°]{\text{pH 5.8}} \equiv Si(CH_2)_3OCH_2\underset{\diagup\ \diagdown}{\overset{O}{}}CH\text{–}CH_2 \qquad (1)$$

adjusted to pH 5.8 during preparation is added to the support and heated at 90°. Bonding is achieved in 30 min at this temperature and cannot be removed by 24-h Soxhlet extraction with methanol. Heating this solution for a longer period of time results in polymerization and substantial loss of oxirane on the support. After filtering the support and drying, nucleophilic stationary phases (P_s) were used to open the oxirane ring and complete the attachment of the stationary phase to the support according to reaction 2. Exact reaction conditions and catalysts required for addition

$$\equiv Si(CH_2)_3OCH_2\underset{\diagup\ \diagdown}{\overset{O}{}}CH\text{–}CH_2 + P_s \longrightarrow \equiv Si(CH_2)_3OCH_2\overset{OH}{\underset{|}{CH}}\text{–}CH_2P_s \qquad (2)$$

depend on the chemical nature of P_s and are described in Experimental. The chemical composition of a series of P_s groups will be seen in Table I.

Applications

One of the principle objectives in the preparation of these chromatographic supports was to provide column packings that would be useful in the high-speed analysis of many types of compounds ranging from hydrocarbons to biopolymers. To accomplish this the support must (1) have sufficient mechanical stability to withstand high mobile phase velocity, (2) have enough porosity to allow penetration and partitioning of macromolecules, (3) have sufficient hydrolytic stability to allow extended operation in aqueous systems, and (4) have partitioning capacities equivalent

TABLE I

STATIONARY PHASE (P_s) GROUPS BONDED TO SUPPORTS

No.	*Formula*	*P_s group*		
		*Chromatographic function**	*Structural unit*	*Abbreviation*
1	$-N(CH_2CH_3)_2$	WAX	diethylamine	DEA
2	$-OCH_2CH_2N(CH_3)_2$	WAX	dimethylaminoethanol	DMAE
3	$-OCH_2CH_2N(CH_2CH_3)_2$	WAX	diethylaminoethanol	DEAE
4	$-(NHCH_2CH_2)_nNH_2$	WAX	polyethyleneimine	PEI
5	$-OCH_2CO_2H$	WCX	carboxymethyl	CM
6	$-SO_3H$	SCX	sulfonic acid	SA
7	$-(OCH_2CH_2)_nOH$	hydrophobic	polyethylene glycol (n = 4–44)	PEG
8	$-O(CH_2)_nCH_3$	hydrophobic	C_4–C_{18} alkanols	HP
9	$-OCH_2CH_2-C_6H_5$	hydrophobic	phenylethanol	PE
10	$-\overset{O}{\overset{\|\|}{C}}-C_6H_5$	hydrophobic	benzoic acid	BA
11	$-\overset{O}{\overset{\|\|}{C}}-CH_2CN$	hydrophilic	cyanoacetic acid	CA
12	$-OH$	hydrophilic	hydroxyl	G

* WAX = weak anion exchanger; WCX = weak cation exchanger; SCX = strong cation exchanger.

to those of classical supports. These objectives were accomplished through the use of large-pore-diameter glass and silica supports. It was found that inorganic supports with pore diameters ranging up to 250 Å would give suitable separations on molecules with molecular weights up to 5×10^5 daltons. Columns could be operated a month or more without degradation of column efficiency from the loss of bonded phase.

Liquid–liquid partition chromatography

Liquid–liquid partition chromatography of proteins was achieved on 37–74 μm CPG of 100-Å pore diameter with a PEG 400, PEG 4000, or heptanol bonded phase. Samples were run isocratically using pH 7 sodium phosphate buffer. On the PEG 400 support, 0.1 and 0.01 *M* buffers gave good though different separations of a pancreatin mixture while 0.05 *M* buffer gave poor resolution. Examination of fifteen proteins indicated that one third behaved hydrophobically on these columns. The capacity factors (k') for several proteins on hydrophobic columns are shown in Table II. $k' = (t_r - t_0)/t_0$, where t_r and t_0 are the respective retention times of the solute and an unretained solute. It appears that the ionic strength of the mobile phase (P_m) influences the hydrophobic association of proteins with supports. The partition coefficients for hydrophobic proteins tend to increase with ionic strength. These results are in general agreement with recent observations made on carbohydrate supports[5]. Shanbhag and Johansson[6] have found that polyethylene glycol–water systems will partition proteins while Hjertén *et al.*[7] have found the hydrophobic association of a protein with an aliphatic stationary phase to reach a maximum with

TABLE II

PARTITIONING OF PROTEINS WITH REVERSED-PHASE SUPPORTS

pH 7.0 buffer with 4% T-20 Dextran was used in all cases to elute the column. The linear flow-rate was 3.0 mm/sec.

Compound	*Capacity factor, k'*		
	PEG 400 0.1 M phosphate buffer	*PEG 400 0.04 M phosphate buffer*	*PEG 4000 0.04 M phosphate buffer*
Albumin	0.33	0.04	0.14
Chymotrypsin	1.23	0.47	1.24
Hemoglobin	1.27	0.22	0.21
Chymotrypsinogen A	—	0.8	1.21

high ionic strength mobile phases. Many proteins are known to have hydrophobic sites that bind hydrocarbons and detergents[8].

None of the proteins examined were totally adsorbed on the PEG bonded supports under any experimental conditions while total adsorption of the metalloproteins hemoglobin, myoglobin, and amino acid oxidase was observed on the heptanol bonded phase.

Resolution and polarity of various bonded supports with organic mobile phases were evaluated using a hexane P_m and a series of aromatic hydrocarbons, aldehydes, ketones, esters, and alcohols as solutes (*e.g.* Fig. 1). Capacity factors (k') were used to record retention data with benzene being the unretained solute.

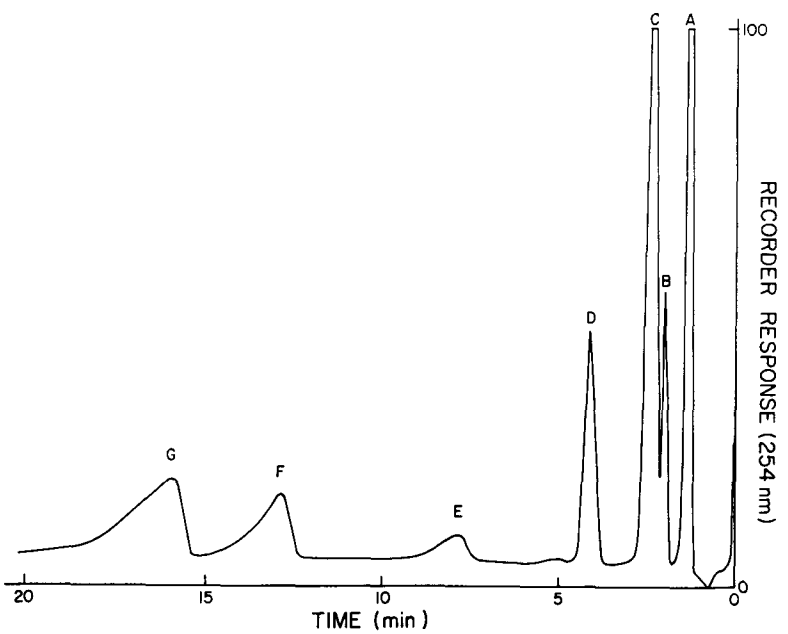

Fig. 1. Liquid–liquid partition chromatography of a series of aromatic compounds on a glycerolpropyl bonded phase support (37–74 μm particle size; 100-Å pore diameter). Column, 1 m × 2 mm I.D. stainless steel; solvent, hexane; pressure, 200 p.s.i.g.; flow-rate, 1.3 cm/sec. A = Benzene; B = benzaldehyde and benzophenone; C = acetophenone; D = nitrophenyl acetate; E = 1-phenylethanol; F = 2-phenylethanol; G = benzyl alcohol.

The γ-aminopropyl support described by Lynn[9] was used as a reference for a typical polar commercial support. Capacity factors of compounds on 37–74 μm CPG supports of 100-Å pore diameter with γ-aminopropyl, PEI 230, glycerolpropyl, DEA, PEG 400 and PEG 4000 stationary phase groups are shown in Table III. It is seen that the alkylamine bonded phase was considerably more polar than any of the others. As expected, the primary and secondary amino groups of PEI 230 yielded a polar support material. It will also be seen in Table III that a gradation of support polarities has been generated with these different stationary phases. By further varying the components of the mobile phase (P_m) and thus stationary phase (P_s) solvation, a continuous series of column polarities could be obtained. No attempt was made to optimize efficiency. Flow-rates of 125 ml/h (1.67 cm/sec linear velocity) gave HETP values of approximately 4 mm for alcohols. These supports were hydrolytically stable and may be used with mobile phases ranging from hexane to water.

TABLE III

CAPACITY FACTORS (k') OF COMPOUNDS ON VARIOUS BONDED PHASE SUPPORTS*

Compound	*Bonded phase*						
	PEG 400	*PEG 4000*	*DEA*	*Glycerol-propyl*	*PEI 230*	*γ-Amino-propyl**	*γ-Amino-propyl***
Benzene	0	0	0	0	0	0	0
Benzaldehyde	0.3	0.5	0.4	0.4	0.3	—	—
Acetophenone	0.5	0.6	0.5	0.8	0.6	3.0	0.4
Benzophenone	0.5	0.6	0.7	0.5	0.7	2.7	0.4
Nitrophenyl acetate	2.2	2.5	2.5	1.9	0.3	4.2	0.9
Diethyl phthalate	1.0	1.1	1.6	—	2.0		1.2
Phthalic anhydride	3.7	4.5	—	7.9	9.1		4.4
1-Phenylethanol	4.1	4.9	5.8	10.4	11.8		4.9
2-Phenylethanol	5.3	6.7	7.4	—	13.4		9.2
Benzyl alcohol	7.1	8.8	9.6	11.9	22.7		10.1

* Mobile phase: hexane.
** Mobile phase: hexane–chloroform (80:20).

Ion-exchange chromatography

Separations of biological compounds were carried out on 250 Å pore diameter CPG supports with either a DEA, DEAE, PEI, or CM bonded stationary phase. The resolution of components in a human serum sample by gradient elution on the PEI anion-exchange support is shown in Fig. 2. The components eluting early in the chromatogram are immunoglobulins while the major component is serum albumin. The use of the DEA support in the resolution of a commercial trypsin sample is shown in Fig. 3. The multiple components in this sample are indicative of the problems encountered in analyzing proteolytic enzymes. It is probable that the enzyme auto-digested before or during analysis. Resolution of a rat liver homogenate (10,000 *g* supernatant) on the DEAE support is shown in Fig. 4.

Ion-exchange capacities of supports may be reported in several ways such as: (1) milliequivalents of ion-exchange species per unit weight of support, (2) milli-equivalents of ion-exchange species per unit volume of support, (3) amount of an

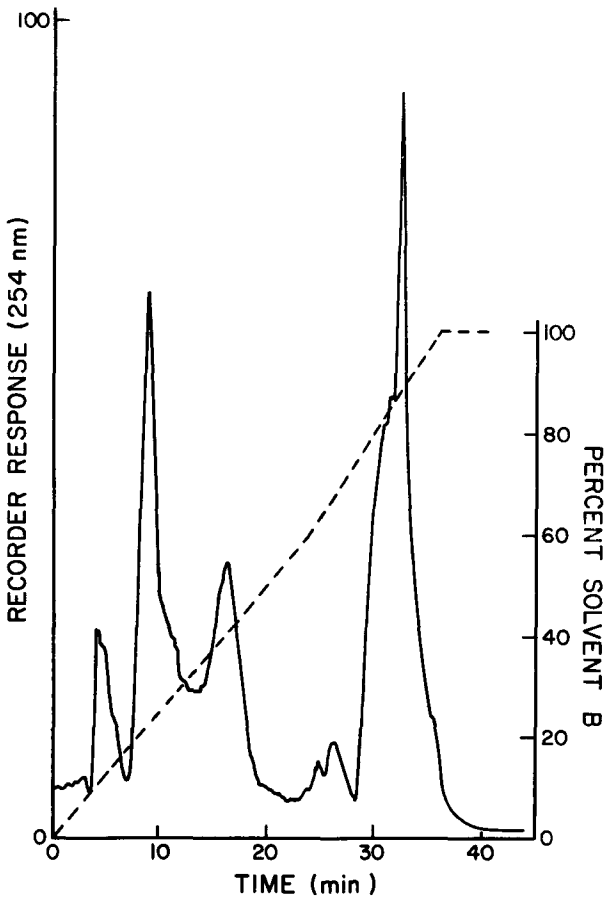

Fig. 2. Resolution of human serum by gradient elution analysis on a PEI bonded phase support (37–74 μm particle size; 250-Å pore diameter). Column, 0.6 m × 5 mm I.D.; solvents, (A) 0.05 *M* Tris (pH 7.5); (B) 0.05 *M* Tris (pH 7.0) + 0.3 *N* NaCl; pressure, 100 p.s.i.g.

ionic molecule that is ion exchanged per weight of support, or (4) amount of an ionic molecule that is ion exchanged per volume of support. In the recent literature, dealing with protein separations, it is becoming common practice to express the ion-exchange capacity of supports in terms of hemoglobin bound per unit volume of support. In this way gel, semi-rigid, and rigid support ion-exchange capacities may be compared.

Hemoglobin ion-exchange capacities of various ion-exchange supports are shown in Table IV. The ion-exchange capacity of the corresponding polydextran-based Sephadex C-25 and A-25 weak cation- and anion-exchange resins is about 60 mg of hemoglobin/ml of support[10]. It is seen that the ion-exchange capacity of a bonded phase inorganic support is a function of surface area. With the DEA support of 550-Å pore diameter, it may be calculated from Table IV that the support binds 10^{-6} moles of hemoglobin per 74 m^2 of surface area or a μmole/7.4 × 10^{21} $Å^2$. Since a single hemoglobin molecule occupies 6000 $Å^2$ of surface, it may be calculated that a μmole of hemoglobin occupies 3.6 × 10^{21} $Å^2$ of surface. This indicates that 49%

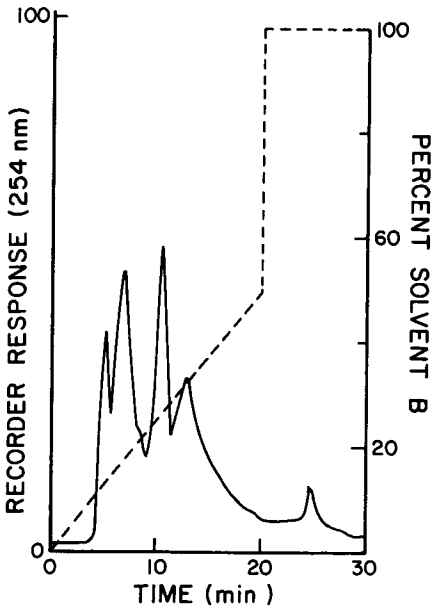

Fig. 3. Resolution of a commercial trypsin sample on a DEA bonded phase support (74–128 μm particle size; 250-Å pore diameter). Column, 0.6 m × 5 mm I.D.; solvents, (A) 0.05 *M* Tris (pH 8.0); (B) 0.05 *M* Tris (pH 8.0) + 0.3 *N* NaCl; pressure, 50 p.s.i.g.

of the surface is covered. In the case of the 250-Å pore diameter support, it may be calculated that 55% of the surface is covered with hemoglobin at maximum loading.

It is interesting to note in the above calculations that there are 100 μmoles of anion exchanger present on 1 g of support while only 1 μmole of a protein is bound. If the anion-exchange groups are uniformly distributed on the surface, an ion-exchange group will be located every 74 Å^2. It is obvious that a macromolecule occupying 6000 Å^2 of surface will cover multiple ion-exchange groups. These calculations suggest that the number of ion-exchange groups on the surface of a support could be reduced 10- to 100-fold without significantly changing the ion-exchange capacity of the support for macromolecules.

The relationship between plate height (HETP) and mobile phase velocity (v) with several different ion-exchange supports is shown in Fig. 5. Plate heights were determined using the protein myoglobin which had a capacity factor (k') of 2.4. The efficiencies of these supports are very similar to those of macromolecules on porous inorganic steric exclusion supports[11].

In comparing these inorganic ion-exchange supports to the carbohydrate gel type supports normally used for biopolymers, we have found that the inorganic supports may be operated at 10–20 times greater mobile phase velocity with comparable separation efficiencies. This may be particularly significant in clinical analyses. It has recently been demonstrated[12] that myocardial infarction may be diagnosed by the DEAE-cellulose analysis of creatine phosphokinase isoenzymes in human serum. Through the use of inorganic supports this analysis could be accomplished in 10–15 min.

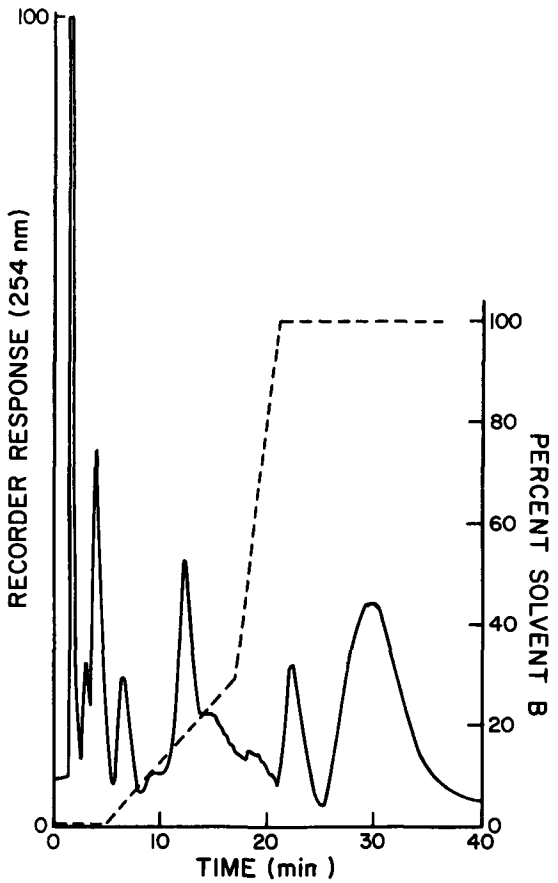

Fig. 4. Resolution of a rat liver homogenate (10,000 *g* supernatant) on a DEAE bonded phase support (37–74 μm particle size; 250-Å pore diameter). Column, 0.6 m × 5 mm I.D.; solvents, (A) 0.05 *M* Tris (pH 7.5); (B) 0.05 *M* Tris (pH 7.0) + 0.5 *M* NaCl; pressure, 100 p.s.i.g.

TABLE IV

ION-EXCHANGE CAPACITIES OF BONDED PHASE SUPPORTS

Bond phase	*Particle size (μm)*	*Pore diameter (Å)*	*Surface area (m^2/g)*	*Hemoglobin ion-exchange capacity (mg/ml)**
DEA	74–125	550	70	28
DEA	74–125	250	130	54
DEAE	74–125	550	70	26
DEAE	74–125	250	130	55
DEAE	74–125	100	170	21
PEI 230	74–125	550	70	30
PEI 230	74–125	250	130	60
CM	74–125	550	70	19
CM	74–125	250	130	38

* Procedures for determining ion-exchange capacities are described in Experimental.

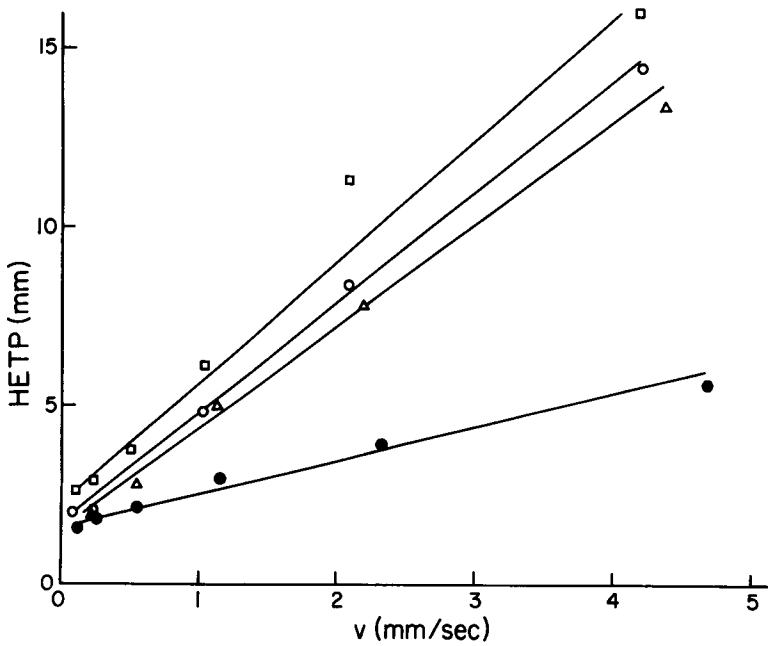

Fig. 5. Plate height curve for ion-exchange supports with myoglobin as solute. Column, 0.6 m × 5 mm I.D. □, DEA (74–128 μm); ○, DEAE (74–128 μm); △, PEI (74–128 μm); ●, DEAE (37–74 μm).

Gas chromatography

Ten bonded phases were compared gas chromatographically with respect to polarity, thermal stability, and efficiency. PEG 191 and PEG 750 were bonded to Porasil C while PEG's 400, 1540, 4000, and 20,000, CA, BA, and heptanol were bonded to 550 Å 74–238 μm CPG of 550-Å pore diameter. Using McReynold's ten probes[13], no differences in polarity were seen for these supports. Alcohols were generally adsorbed on the supports until the supports were silylated. It is probable that surface silanols contributed significantly in separations of polar materials. Karger and Sibley[14] also found unreacted silanols to be a partitioning factor. This could explain the difference between these results and those of Little *et al.*[15], who found higher-molecular-weight Carbowax bonded phase supports to be more polar than those of low molecular weight. Little *et al.*[15] bonded Carbowax to supports through silyl esterification, so it is probable that surface silanols increased with increasing molecular weight. However, in these studies, the supports were first silylated and then the Carbowaxes bonded. Thus the number of surface silanols remaining after the synthesis of the support is independent of the molecular weight of Carbowax.

The PEI 230 support, due to the presence of primary amines, reacted with ketones so no McReynolds' data were obtained on it. However, without additional derivatization, the PEI 230 support gave separations of free amines with little tailing.

All bonded phase supports separated homologous series well, giving McReynolds' *b* values of 0.26 to 0.31. A typical chromatogram is shown in Fig. 6. All supports were thermally stable to approximately 230°, at which temperature the bleed

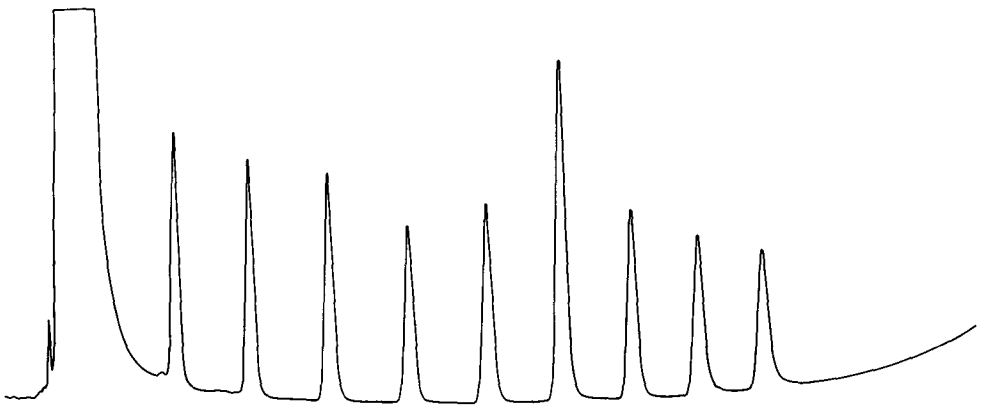

Fig. 6. Gas chromatographic separation of *n*-alkanes (octane–hexadecane) on 80–100 mesh Glycophase BA of 550 Å pore diameter. Column, 6 ft.; temperature programmed from 100–250° at a rate of 8°/min; sample size, 2 μl.

was equal to 170 ng/sec. Thermal stability was independent of the molecular weight of the bonded phase. The Van Deemter plots are typical of porous supports[16], having minima of 0.5–0.8 mm at linear velocities near 3 cm/sec.

CONCLUSIONS

A simple technique has been developed for bonding numerous types of stationary phases to silaceous inorganic supports. The resulting bonded phase supports are stable to 230° and will function in both organic solvents and water. These chromatography supports are sufficiently porous to allow the partitioning of biopolymers with loading capacities similar to tightly cross-linked carbohydrate gels. With these bonded phase inorganic supports it has been possible to achieve the separation of compounds ranging from 10^2–10^6 molecular weights by both liquid–liquid partition and ion-exchange chromatography. In the case of proteins, separations were achieved 10–20 times faster than with conventional carbohydrate supports.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. Nabila El Khodary and Mr. Timothy Weaver for their assistance in the synthesis and analysis of these supports.

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