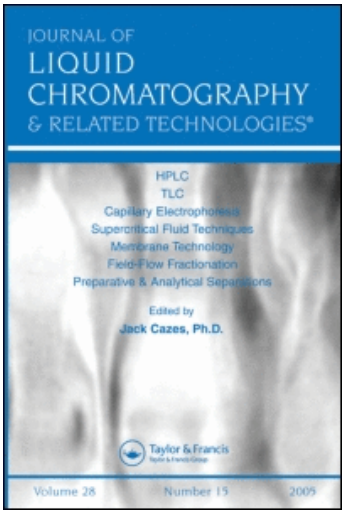

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Utilization of Weak Acid-Base Interactions to Improve Separations in Normal Phase Liquid Preparative Chromatography

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To cite this Article Zief, Morris , Crane, Laura J. and Horvath, Joseph(1982) 'Utilization of Weak Acid-Base Interactions to Improve Separations in Normal Phase Liquid Preparative Chromatography', Journal of Liquid Chromatography & Related Technologies, 5: 12, 2271 — 2292
To link to this Article: DOI: 10.1080/01483918208067635
URL: http://dx.doi.org/10.1080/01483918208067635

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(12), 2271-2292 (1982)

UTILIZATION OF WEAK ACID-BASE INTERACTIONS TO IMPROVE SEPARATIONS IN NORMAL PHASE LIQUID PREPARATIVE CHROMATOGRAPHY

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ABSTRACT

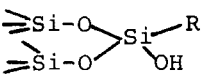
Three 40 μm derivatized silicas, two aminoalkyl and one carboxyalkyl, have been compared to 40 μm silica gel for performance in normal phase preparative liquid chromatography. The three derivatives showed higher selectivity for compounds capable of hydrogen-bond formation or acid-base interactions. Retention times were related to the basicity of the amine bonded derivatives or the acidity of the carboxylic acid derivative and the pK values of the solutes. The advantages for each of the four sorbents for neutral, acidic and basic compounds have been described.

INTRODUCTION

In aqueous systems hydrogen bonding capabilities and acid-base interactions between organic moieties are minimized because of the polarity of the aqueous medium and the strong hydrogen bonding properties of the water molecules themselves. In organic solvents or organic solvent-water mixtures, however, these properties become important and can be used to chromatographically resolve compounds with interacting functional groups.

We have explored the capabilities of three siloxane bonded polar phases in normal phase chromatography, wishing to exploit the enhancement of weak acid-base interactions in solvents most often utilized by the preparative chemist. We have limited ourselves to isocratic elution, relying for resolution enhancement upon solvent composition and the nature of the bonded phase.

The three bonded phases were siloxanes in which R



was 3-aminopropyl,3-(2-aminoethylamino)propyl or 2-carboxyethyl. All derivatives were bonded to silica of 40 μm average particle size. These bonded phases have recently become available commercially as bulk packing and seemed to offer good possibilities for selective retention and purification of compounds possessing weakly basic or weakly acidic sites for hydrogen bonding.

The hydrogen bonding capabilities of the aminopropyl bonded phase have been exploited extensively in analytical HPLC (in the so-called "carbohydrate column") to separate mono, di and trisaccharides (1). The resulting complexes formed between the amine groups of the bonded phase and the very weakly acidic hydroxyl groups of the carbohydrates result in different reten-

tion times in an acetonitrile-water mobile phase and, therefore, resolve the carbohydrates.

The other end of the scale of acid-base interactions in organic media with this bonded phase is the use by Pirkle of the stable ionic bond between R-N-3,5-dinitrobenzoylphenylglycine and the amine group to create an immobilized chiral phase to chromatographically separate optical isomers (2).

We have limited our exploration of the preparative capabilities of the amine bonded phases primarily to the intermediate range of interactions between these two extremes, though a brief examination of carbohydrate separation is also included.

EXPERIMENTAL

MATERIALS

Ethyl acetate, hexane, acetonitrile, and methylene chloride were 'Baker Analyzed'® solvents. The solutes were Baker organic reagents.

The silica and the three bonded phase packings were obtained from Baker as "flash chromatography" grade silica and Baker amino (NH_2), Baker 1°,2°-amino (NH_2-NH) and Baker carboxyl (COOH) bonded phases for preparative liquid chromatography. The 3-aminopropyl bonded phase contained 2.45% N; 1.75 meq N/g; loss on drying at 110°C was 6.3%; loss on ignition at 600°C

after drying at 110°C was 10.72%. The diamine bonded phase contained 2.86% N; 2.04 meq N/g; loss on ignition at 600°C after drying at 110°C was 16.06%. The carboxyl bonded phase contained 4.6% C; 1.0 meq acid/g groups by titration.

The weight of the support loading was determined by heating 1 g of the siloxane derivative in a crucible at 600°C in an oven for 3 hours.

$$\% \text{ loading} = \frac{\text{wt of loss at } 600° \times 100}{\text{wt of phase after heating at } 110°C}$$

In order to show that the weight loss above 110°C is due primarily to the support loading and not to the elimination of water from vicinal hydroxyl groups, silica itself was first ignited at 600°C. From 100-600°C a loss of 3.3% took place. We can conclude, therefore, that the weight loss due to water formation is below 3.3% in the amine bonded derivatives because of the reduced content of unreacted hydroxyls.

Support loadings of 10.72% and 16.06% for the monoamine and diamine derivatives suggest a small amount of polymer formation for both derivatives (1).

APPARATUS

An Altex glass chromatography column, 9 mm x 250 mm, equipped with a stainless steel plunger fitted with Teflon® tubing was used in all experiments. An Altex Tefzel slider injection valve with a 0.5 mL loop was

connected directly to the plunger. A laboratory pump (1/4" piston, flow rate 0-19 mL/min, Fluid Metering Company, Oyster Bay, NY), was the pressure source. A pulse dampener (Fluid Metering Company) was inserted between the pump and the injection valve. The eluate from the column was monitored by a Waters 403 refractive index detector which, in turn, was connected to a Hewlett-Packard strip chart recorder.

PROCEDURE

Column packing: The irregular-shaped particles had an average diameter of 40 μm with a narrow distribution of 30-65 μm. Columns were dry packed by first pouring enough adsorbent into the column to fill a 30 mm height. The column was then carefully "bounced" on the table top to settle the adsorbent. Repetitive additions of similar small aliquots were added and the columns were "bounced" or tapped until a 200 mm height of settled adsorbent was added. The plunger was then inserted and adjusted carefully to contact the surface of the adsorbent.

Chromatographic Evaluation of Phases

Capacity factors (k') were determined by injecting 100 μL samples of solutions containing 1 g of solute per 100 mL of mobile phase. The flow rate of mobile phase was 7.5 mL/min.

DISCUSSION

Since no analytical TLC plates of the three bonded phases exist, chromatography on a small column is the best method available for optimizing mobile phase composition for later scale-up separations of a variety of solutes. When larger preparative columns are packed with the 40 μm adsorbents used in this study, similar capacity factors are routinely obtained (results reported elsewhere).

Monoamine Bonded Phase

The chromatographic medium most commonly utilized for normal phase chromatographic purification of organic compounds is silica. Therefore, for purposes of providing a baseline for better understanding the chromatographic properties of the relatively new bonded phase packings, it seemed useful to do comparative studies under identical conditions. Table I illustrates that with a mobile phase of low solvent strength (3) silica exhibits relatively non-specific interactions with acidic materials having a wide range of pK values. Specific interactions occasionally credited to silica can probably be ascribed to trace impurities such as sodium, aluminum or sulfate; these impurities impart to silica acidic or basic properties as evidenced by the 2-9 pH range of various silicas in water

(4). The silica used in these experiments gave a neutral pH when slurried in water.

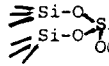
With the monoamine phase the range of retention times with the same mobile phase used for silica increases considerably for the series of alcohols, with retention times of 4-5 minutes for very weak aliphatic alcohols having pKa's of 19, increasing to retention times of greater than 20 minutes for the substituted phenols having pKa's of 7 or less. It is reasonable to assume that for this series of alcohols the acid-base interaction between the hydroxyl hydrogen of the solute and the amine nitrogen of the solid phase determines retention time.

Even with alcohols such as chloroethanol and phenethyl alcohol showing high pKa values of 18 or greater, the results are consistent with those expected from electronic effects; for example, the increased retention of chloroethanol over ethanol or benzyl alcohol over phenethyl alcohol can be predicted on relative acidities, even through actual pKa values do not appear in the literature.

It is interesting to note, however, that retention times for both mixtures are reversed on silica. With these alcohols (pKa about 18) the major interactions with silica occur between the silanol hydrogen and the hydroxyl oxygen (5). In the case of benzyl and phen-

TABLE 1

Separation of Alcohols and Amines on Silica and 3-Aminopropyl-Derivatized Silica



$Si(CH_2)_3NH_2$ Bonded Phase**

Adsorbent		Silica*								
wt g:		5.6				6.6				
Mobile Phase		Hexane/Ethyl Acetate 3/1		Hexane Ethyl Acetate (3/1)		Hexane Ethyl Acetate (3/7)		Acetonitrile Water (7/3)		Acetonitrile 0.05M Aqueous Sodium Bicarb. (7/3)
Flow Rate 7/5 mL/min										
E^{O}***		0.23		0.23		0.34		>0.75		>0.75
Solute	pKa	Retention Time (min)	k'	Retention Time (min)	k'	Retention Time (min)	k'	Retention Time (min)	k'	Retention Time (min)
benzene	37	1.5	--	1.4	--	1.3	--			
benzyl alcohol	~18	5.0	2.3	10.4	6.3	3.4	1.6	1.3	--	
1-decanol	19	3.6	1.4	4.4	2.1	2.4	0.7			
hexanol	19	4.2	1.8	4.8	2.4	2.5	0.7			
ethanol	18	8.8	4.9			3.0	1.3			
chloroethanol	<18	7.5	4.0			4.2	2.2			

phenethyl alcohol	>18	5.8	3.1	9.0	5.4	2.8	1.2			
eugenol	10	2.4	0.6	13.0	8.3	4.5	2.5			
phenol	9.9	2.2	0.5			7.5	4.8	1.3	--	
p-nitrophenol	7.2	4.8	2.2	>20		>20		11.0	7.4	2.2
2,4-dinitro-phenol	4.0	>20		>20		>20		>20		1.6
aniline****	4.6	4.8	2.2	3.5	1.5	2.0	0.5			
o-nitroaniline	0.5	3.5	1.3	6.5	3.6	2.5	0.8			
m-nitroaniline	2.5	6.8	3.5	9.8	6.0	2.7	1.1			
p-nitroaniline	1.0	10.8	9.8	6.2		4.7	2.6			
N-methylaniline	4.84					1.7	0.3			
N-dimethylaniline	5.07					1.5	0.15			

*J. T. Baker Catalog No. 7030-0. **J. T. Baker Catalog No. 7034-0. ***Solvent strength determined on silica; see ref. 3.
****For aniline and its derivatives the pKa represents the dissociation of the conjugate acid BH+ of the listed base B.
Experimental Conditions: Altex glass column (9 mm x 25 cm) containing an adjustable plunger; FMI laboratory pump; FMI pulse dampener; Altex loop injector; Waters refractive index detector R403; Hewlett-Packard strip chart recorder.

ethyl alcohols the greater basicity of the oxygen in phenethyl alcohol is responsible for a stronger hydrogen bond with the silica silanol group. The concomitant decreased acidity of the phenethyl alcohol hydroxyl hydrogen affords a weaker hydrogen bond with the nitrogen of the amino bonded phase.

Even though the relative retention times of both aromatic and aliphatic alcohols on the amine phase are a function of the acidity of the hydroxyl hydrogen, the absolute retention times are not a function of aqueous pKa alone. The electronic effects of the aromatic ring in the organic mobile phase are far stronger than in the primarily aqueous solutions used for pKa determinations. Hence the aromatic alcohols as a group bind more tightly to the amine bonded phase than the aliphatic alcohols.

As the acidity of the solute increases, solvents of ever-increasing solvent strength are required to achieve reasonable elution times from the amine phase. The strongest acid eluted with an aqueous organic mix was 2,4-dinitrophenol with a pKa of 4; in this case elution was achieved only by including sodium bicarbonate in the aqueous component of the mobile phase to form the dinitrophenolate ion. The inclusion of 0.05M sodium bicarbonate (pH 8) has a greater effect on the elution time of 2,4-dinitrophenol than on the less

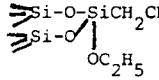
acidic p-nitrophenol because the former ionizes more readily. Such control of elution times by mobile phase composition is highly desirable in preparative chromatography, since purification in preparative liquid chromatography is best achieved when the desired compound is eluted after the removal of impurities. The amine bonded phase can be used to advantage for early separation of materials incapable of hydrogen bonding and subsequent controlled elution of acidic materials.

Diamine Bonded Phase

The dominant interacting amine group of the diamine bonded phase is the terminal primary amine which is less basic than the comparable amine group in the monoamine derivative; a pKb of 7.0 for the diamine phase was determined by acid titration. The lower basicity explains the lower retention times of the alcohols in Table II; for example, retention times of 7.5 and 4.5 minutes for phenol and eugenol on the monoamine phase are reduced to 4.1 and 2.6 minutes, respectively, on the diamine phase. The differences in retention time increase as the pKa of the interacting aromatic or aliphatic alcohol decreases. Eugenol is a somewhat special case since its methoxy group can intramolecularly hydrogen bond with the adjacent hydroxyl group, thereby competing with the bonded

TABLE II

Separation of Alcohols and Amines on 3-(2-Aminoethylamine)propyl-Derivatized Silica



Bonded Phase+

Adsorbent wt g: 6.5 g

Mobile Phase:	Hexane / Ethyl Acetate (3/1)		Hexane / Ethyl Acetate (3/7)		Methylene Chloride / Acetonitrile (2/8)	
E°	0.23		0.34		0.5	
	Retention Time (min)	k^1	Retention Time (min)	k^1	Retention Time (min)	k^1
Solute						
benzene	1.4	--	1.3	--	1.2	--
benzyl alcohol	9.6	5.9	3.0	1.3	1.8	0.5
1-decanol			1.9	0.5		
hexanol	4.0	1.9	2.1	0.6		
ethanol			2.6	1.0		

chloroethanol			3.4	1.6		
phenethyl alcohol	7.8	4.6	2.6	1.0		
eugenol	7.6	4.4	2.6	1.0		
phenol			4.1	2.2	2.6	1.2
p-nitrophenol	>20		>20		>20	
2,4-dinitrophenol	>20		>20		>20	
aniline	4.8	2.4	2.0	0.5		
o-nitroaniline	8.3	4.9	2.6	1.0		
m-nitroaniline	14.5	9.4	3.2	1.5		
p-nitroaniline	>28.0	>19.0	5.7	3.4	2.1	0.8
N-methylaniline			1.6	0.2		
N-dimethylaniline			1.4	0.1		

*J. T. Baker Catalog No. 7042-o.
Experimental Conditions: See Table I.

phases for hydrogen-bond interactions. Thus, the retention times for eugenol on both phases are significantly less than expected by considerations of pKa alone.

Recently Becker and Unger (6) related the capacity factors of various phenols to their pKa values on a similar diamine bonded silica. Their results clearly showed that an increased binding of the phenols with the polar support paralleled increased acidity of the solute. Our data are in agreement with this conclusion, but the binding capacity of the two diamine derivatives are strikingly different in a manner that correlates directly with the nitrogen contents of the bonded phases of 2.32 and 2.86%, respectively. Our bonded phase gave higher k' values with the same solvent system (dichlormethane/acetonitrile 20/80; E = 0.50); for example, phenol showed a k^1 value of 1.2 rather than 0.2. More importantly, 4-nitrophenol and 2,4-dinitrophenol could only be eluted from our column with a much more polar mobile phase. The higher nitrogen content and increased capacity of our derivative reflects a more highly polymerized coating on silica.

Comparison of the Amine Bonded Phases

It is clear that retention on both derivatives increases as pKa decreases. Hydrogen bonding falls off

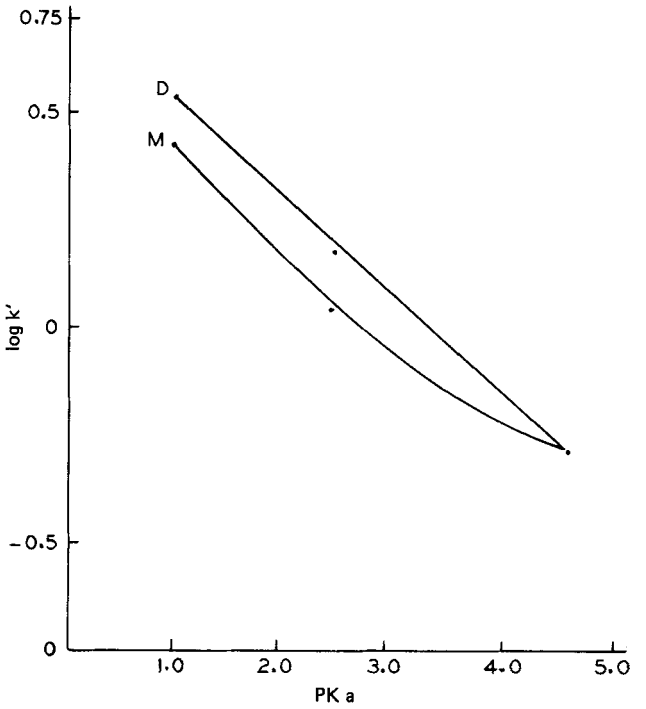


Figure 1. Plot of Log k^1 Versus pKa for Weak Metallic Bases

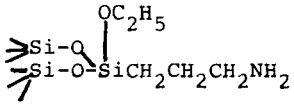
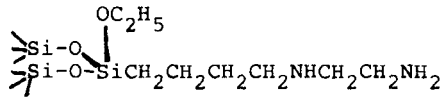
Chromatographic Conditions: Column, M = Monoamine Bonded Phase; D = Diamine Bonded Phase; Mobile Phase, Ethyl Acetate-Hexane (7/2); Flow Rate, 7.5 mL/min

so sharply at pKa 18 that no difference between hexanol and decanol can be detected. Although the monoamine is more retentive for alcohols, the diamine binds more tightly to weak aromatic bases (Figure 1) that have two hydrogen atoms available for interaction with the amine nitrogens of the adsorbent. No difference is seen with bases having pKb's less than 2 pK units different from the adsorbents; aniline and methylated anilines behave

similarly with both adsorbents, but differences become apparent with the more acidic m- and p-nitroanilines. The intramolecular hydrogen bonding in o-nitroaniline precludes consideration in this plot (Figure 1).

Since 5 and 10 μm aminopropyl silica are extensively used analytically for HPLC analysis of carbohydrates, it seemed useful to test the two 40 μm bonded phases for preparative separation capabilities of these compounds. A closely related series of carbohydrates was resolved on both amine bonded phases (Table III). As the relative basicity of the two bonded phases predicts, the aminopropyl silica binds more strongly to carbohydrates than the diamino derivative. Arabinose, for example, elutes in 1.8 minutes from the diamino column with an acetonitrile-water (4/1) mobile phase. Under the same conditions arabinose was still retained on the aminopropyl column after 18 minutes. When the mobile phase was changed to acetonitrile-water (3/1), however, arabinose eluted in 4.5 minutes; the k^1 values for all the solutes then approximate the data obtained on the diamine column with the solvent mixture of lower solvent strength. The incorporation of water in the mobile phase substantially increased the pressure in the analytical columns (65-80 psi), but analysis of carbohydrate systems with both 40 μm bonded derivatives continued to

TABLE III
Separation of Carbohydrates

Adsorbent		
A. Analytical Column 9 mm x 20 cm		
Adsorbent wt g:	6.6	6.5
Mobile Phase	Acetonitrile/Water 3/1	Acetonitrile/Water 4/1
Flow Rate: mL/min	7.2	7.6
Pressure: psi	65	80
	Retention Time (min) / k^1	Retention Time (min) / k^1
Solute		
Solvent front	1.3 / --	1.2 / --
thymidine	2.6 / 1.0	1.8 / 0.5
arabinose	4.5 / 2.4	3.8 / 2.2
fructose	5.0 / 2.8	4.4 / 2.6
glucose	6.2 / 3.7	5.2 / 3.3
sucrose	9.0 / 5.9	8.0 / 5.7
maltose	11.6 / 7.9	9.6 / 7.0
lactose	12.2 / 8.3	11.0 / 8.2

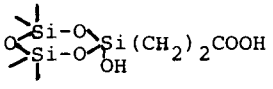
be an unusually simple procedure. Acetonitrile-water mobile phases with carbohydrate solutes must be categorized as normal phase chromatography, since in this case water is used to expedite elution.

The acid-base properties of amino bonded derivatives can exhibit wide variations, possibly dependent on the nitrogen content and the amount of cross-linked aminosilane coating on the silica. Whereas our monoamine bonded silica is clearly more basic than the diamine derivative, Jesorek et al (7) described products with quite different behavior. For example, the pH of an aqueous slurry of their mono and diamino derivatives is 8-9 and 9-10, respectively; our comparable pH values are 8.2 and 6.9. Further, they reported the apparent (pKb) of the mono and diamino bonded phases to be 7.0 and 6.4; we found 6.2 and 7.0 after a very slow attainment of equilibrium in a titration with 0.1N HCl. The relative retentivities of our two amine phases correlated with our pK measurements.

2-Carboxyethyl Bonded Phase

The pH of a suspension of the bonded phase in water was 4.1. Table IV shows the behavior of this polar bonded phase under the conditions reported with the amine-bonded derivatives. Again the dominant mechanism appears to be an acid-base interaction, in this case between the carboxylic acid of the solid

TABLE IV
Separation of Amines on Silica and
2-Carboxyethyl-Derivatized Silica

Adsorbent		Silica (5.6 g)				(6.8 g)	
Mobile Phase Flow Rate		A 7.5 mL/min		A 7.5 mL/min		B 7.5 mL/min	
	pKb	Retention Time (min)	k^1	Retention Time (min)	k^1	Retention Time (min)	k^1
Solute							
benzene		1.4	--	1.4	--	1.3	--
aniline	9.42	2.4	0.71	1.5	0.07		
N-N-dimethyl-aniline	8.94	1.6	0.14	1.4	--		
pyridine	8.77	6.1	3.35	2.4	0.71		
n-butylamine	3.39	>20	--	>20	--	2.5	0.9
diethylamine	2.9	>20	--	>20	--	2.8	1.15
piperidine	2.8	>20	--	>20	--	3.6	1.77

Mobile Phase

A = Ethyl Acetate/Hexane (7/3)
B = Acetonitrile/Methanol/Acetic Acid 9/1/0.12

phase and the basic group of the solute. As would be predicted, retention times increase with a decrease in the pKb of the solute, but the interaction becomes important only with moderately strong bases. A weak base such as aniline is practically unretained; pyridine (pKb = 8.77) binds more tightly and is easily separated from aniline (pKb = 9.42). With the increased basicity of aliphatic amines, however, strong interaction with the carboxyl groups of the sorbent occurs; retention times beyond 20 minutes are observed.

A mobile phase of substantially stronger elution strength (acetonitrile/methanol/acetic acid 9/1/0.12) removes n-butylamine, diethylamine and piperidine at 2.5, 2.8 and 3.6 minutes, respectively. These amines elute in order of their basicities; the α value of 1.97 for piperidine and n-butylamine represents a fairly easy separation.

As Table IV shows, this carboxylic acid bonded phase is less retentive than free silica for weakly basic compounds. For example, under comparable conditions, aniline and pyridine are eluted from silica at 2.4 and 6.1 minutes, but in 1.5 and 2.4 minutes from the acid derivative. Since a suspension of silica in water gave a neutral pH, its bonding properties can be attributed to the polar properties of the free silanol groups rather than the acidic nature of silica.

As one inspects all the retention data for the three bonded phases plus silica, it becomes clear that collectively they offer the preparative chromatographer a new dimension in optimizing practical separations on a preparative scale. By selecting the appropriate solid phase for his compound, he can maintain the convenience of isocratic elution with readily volatile solvents. For example, ethyl acetate/hexane mobile phases resolve moderately acidic compounds on the diamine phase, weakly acidic on the monoamine phase and neutral and weakly basic compounds on silica. Moderately to strongly basic compounds can be resolved on the carboxylic acid bonded phase with a volatile mobile phase of strong solvent strength. Ultimately, the limit to this approach is solubility. Strongly acidic and basic materials usually require aqueous systems and the advantage of a volatile solvent is lost.

ACKNOWLEDGEMENT

We thank Dr. A. J. Barnard, Jr. for the aqueous titrations of the two amino bonded phases.

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