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PREPARATION AND CHROMATOGRAPHIC EVALUATION OF CHEMICALLY BONDED ION-EXCHANGE STATIONARY PHASES

I. STRONG ANION-EXCHANGER

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SUMMARY

An anion-exchanger packing for high-pressure liquid chromatography was synthesized by reacting chlorodimethyl[4-(4-chloromethylphenyl)butyl]silane with small-particle silica material and further modification of the bonded chloromethyl residues with trimethylamine.

The separation mechanism of this chromatographic packing was investigated using various ionic compounds under different conditions of pH, ionic strength, and mobile-phase composition.

Additional retention effects due to the organic matrix and residual silanol groups were investigated using columns packed with dimethyl(4-phenylbutyl)silyl bonded phase and underivatized silica. Although the ionic mechanism appears to be predominant, the matrix effect may give rise to useful separations, and the residual surface silanol groups can still cause substantial increase in retention of organic bases.

INTRODUCTION

Ion-exchange chromatography is one of the most widely used methods for separation of various charged species in aqueous media. The most frequently used stationary phases in ion-exchange chromatography are polymeric beads that have been chemically modified to introduce charged functional groups on their surface. Chromatographic separations obtained with such materials are then primarily dependent on the ionic interaction between the solutes chromatographed and positively or negatively charged groups located throughout the polymer backbone.

However, additional retentive effects may often be encountered which modify chromatographic characteristics of the commonly used ion-exchangers, namely, significant effects of organic matrices[1,2], salting-out[3], ligand-exchange phenomena[4] and ion exclusion[5,6] have been reported. Whereas these secondary effects may form the basis for effective separation in many instances[2], they present problems of their own in terms of data interpretation. As appropriately expressed by Horvath and Lipsky[7], "...this complex nature of ion-exchange chromatography, which is invaluable for

achieving the high selectivity characteristic for this technique, also makes data interpretation and prediction of solute behavior difficult, and optimum separating conditions must often be found by trial and error." Consequently, an improved understanding of solute–sorbent interactions in such separations is desirable.

The last decade's emphasis on the optimization of liquid chromatography and the subsequent use of high pressures has considerably affected the technology of ion-exchange columns and has led to the utilization of highly crosslinked materials[8,9], as well as to the development of pellicular packings[10,11]. More recently, advances in small-particle column technology and chemically bonded stationary phases have stimulated further interest in the preparation of highly efficient ion-exchange columns. With a suitable surface treatment method, ionic groups can be bonded or chemically generated on various siliceous matrices.

Surface silylation provides the most reliable method for the attachment of organic moieties to the mechanically stable silica column materials; only extremes of pH may result in cleavage of the bonded material from such surface. Although ion-exchange bonded phases have not yet been sufficiently developed and broadly characterized, columns of this type have been reported by Brust *et al.*[12] and Unger and Nyamah[13]. Cation-exchange packings can also be prepared by introducing aromatic moieties on to the surface by Grignard reaction and subsequent sulfonation[14–16]. More recently, certain siliceous ion-exchange packings have become commercially available[17,18].

Earlier work in this laboratory on polar chemically bonded phases[19,20] has led to consideration of the preparation of ion-exchange packings based on similar technology. Since only monomolecular surface coverage with ionic groups is expected through the reaction with chlorodimethyl[4-(4-chloromethylphenyl)butyl]silane[20] and its subsequent modification, retention behavior on such an ion exchanger could be more easily interpreted than with polymer-bonded packings prepared from differently substituted silanes. As of this date, a strong anion exchanger, a weak cation exchanger, and a strong cation exchanger have been prepared in our laboratory with chlorodimethyl[4-(4-chloromethylphenyl)butyl]silane and chlorodimethyl(4-phenylbutyl)-silane reagents and their subsequent modifications after bonding. This report describes only the preparation and chromatographic evaluation of the anion exchanger which has a quarternary ammonium group as the active moiety.

Various classes of standard solutes were employed to evaluate retention properties of this anion exchanger under different mobile-phase conditions. The effects of pH, ionic strength of mobile phase and solvent composition on solute retention were studied. In order to investigate possible matrix affinity, comparative studies were carried out with underivatized silica and the bonded phase prepared through the reaction of silica with chlorodimethyl(4-phenylbutyl)silane.

EXPERIMENTAL

Chlorodimethyl[4-(4-chloromethylphenyl)butyl]silane was prepared by the previously described method[21,22]. Chlorodimethyl(4-phenylbutyl)silane was obtained through a simple catalytic hydrosilylation of 4-phenyl-1-butene (using platinum chloric acid as a catalyst). Silica A (average particle size 14 μm) was obtained from Perkin-Elmer (Norwalk, Conn., U.S.A.), and dried prior to use at 200° for 4 h. Both

silanes were reacted with Silica A under the previously described conditions[23]. The bonded materials were exhaustively extracted in a Soxhlet apparatus with a series of solvents and dried. Organic carbon content of these materials (as determined by elemental analyses) ranges typically from 6 to 8%. The columns used in this study had 6.1% and 7.2% carbon for the phenyl phase and the anion exchanger, respectively.

While the phenyl phase (used only for comparative purposes) was not further modified after the bonding process and extractions, the chloromethylated material was further reacted with a 1:1 mixture of trimethylamine and methanol at 0° for one week, excessively washed with distilled water, and dried. Its ion-exchange capacity was determined by potentiometric titration with 0.015 *N* HCl. The prepared packings had capacities of about 200 μequiv./g; the material used for these measurements had 180 μequiv./g, corresponding to 0.4 ionic groups per 100 Å^2.

The chemically bonded stationary phases or silica were packed into 300 × 2.1 mm I.D. columns using a slurry packing procedure[24], and sufficiently equilibrated prior to their use.

All measurements were performed with a Model 202 Waters Assoc. liquid chromatograph provided with a UV monitor and a modified injection system.

RESULTS AND DISCUSSION

In order to investigate the nature of solute–sorbent interactions with the synthesized anion-exchange material, several classes of standard solutes of different

TABLE I

COMPARISON OF COLUMN SELECTIVITIES FOR VARIOUS CLASSES OF COMPOUNDS WITH 0.05 *M* KH_2PO_4 BUFFER (pH 3.0) AS THE MOBILE PHASE

Compound	*Capacity ratio k*		
	Silica	*Phenyl phase*	*Anion exchanger*
Cytidylic acid	0.15	0.07	0.40
Uridylic acid	0.11	0.12	2.30
Adenylic acid	0.20	0.49	6.11
Guanylic acid	0.13	0.42	3.50
Cytosine	0.25	0.19	0.00
Uracil	0.22	0.34	0.48
Xanthine	0.18	0.99	1.06
Hypoxanthine	0.29	1.05	1.09
Adenine	0.32	0.82	0.37
Uric acid	0.09	0.62	0.90
Adenosine	0.19	0.81	0.55
Uridine	0.12	0.22	1.08
Theophylline	0.52	6.99	4.53
Caffeine	1.55	21.0	11.2
Benzoic acid	0.57	1.19	3.51
Phthalic acid	0.30	0.50	8.86
Terephthalic acid	0.10	1.14	9.56
Acetylsalicylic acid	0.65	1.48	4.48
Dinitrophenol	1.97	2.03	18.0

chemical types were chromatographed under the conditions where the anionic interaction is expected (Table I). The retention characteristics (capacity ratios k) measured for the anion exchanger, underivatized silica and the bonded phenyl phase, are compared in Table I.

Two classes of compounds, ribonucleoside phosphates and aromatic acids, that are traditionally analyzed with the classical or pellicular anion-exchange resins exhibit good affinity for the bonded anion exchanger. Thus, the results included in Table I for these compounds suggest that their interaction with this packing is primarily of ionic nature, whereas the unreacted silanol groups and/or the organic matrix appear to play a minor role. However, noteworthy is a small degree of retention of the purine nucleotides due to the aromatic matrix of the phenyl phase. Some aromatic acids also have affinity for the aromatic surface monolayer, but their retention on the anion exchanger is considerably stronger.

Since the only part of a nucleotide molecule responsible for the ionic attraction to the stationary phase at pH 3 is the (negatively charged) phosphate, retention of nucleosides and bases is expected to be small. As can be seen from Table I, retention decreases here in the order of increasing molecular weight on silica and the phenyl phase, *i.e.*, base > nucleoside > nucleotide, while the opposite is observed for the anion exchanger. Although selectivity of the anion-exchange stationary phase is relatively low for bases and nucleosides, the effect of the matrix may still result in some analytically useful separations (Fig. 1).

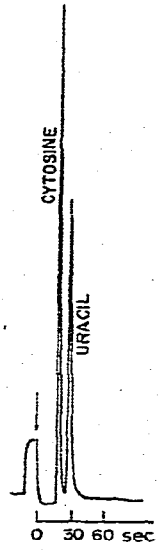

Fig. 1. Resolution of cytosine and uracil with the anion-exchange bonded phase. Conditions: 0.05 *M* KH_2PO_4 (pH 3.0); temperature, 25°; flow-rate, 0.8 ml/min.

Chromatographic behavior of many identical compounds on a resin with similar surface ionic groups was described by Katz and Burtis[25]. While a direct comparison between our and their results is difficult because of somewhat different experimental conditions, the column selectivity appears markedly different in a few

TABLE II

DEPENDENCE OF CAPACITY RATIOS *k* OF MISCELLANEOUS SUBSTANCES ON pH OF THE MOBILE PHASE (0.05 *M* PHOSPHATE BUFFER)

Stationary phase	*Compound*	*k Value*		
		pH 3.0	*pH 5.0*	*pH 6.0*
Anion exchanger	Acetylsalicylic acid	4.48	4.80	1.48
	Benzoic acid	3.51	4.48	1.44
	Phthalic acid	8.86	6.43	1.26
	Terephthalic acid	9.56	13.5	2.16
	Caffeine	11.2	8.90	7.31
	Theophylline	4.53	5.13	3.12
	Cytidylic acid	0.40	1.61	0.94
	Uridylic acid	2.30	2.54	1.18
	Guanylic acid	6.11	7.61	3.58
	Adenylic acid	3.50	17.8	7.08
	Adenosine	0.55	2.39	1.73
	Uridine	1.08	0.30	0.18
	Cytosine	0.00	0.50	0.49
	Uracil	0.48	0.38	0.25
	Thymine	0.89	0.71	0.52
	Adenine	0.36	3.65	3.45
	Benzene	1.87	1.61	1.25
	Toluene	3.75	2.85	2.27
	o-Xylene	6.65	5.13	3.27
	Naphthalene	17.9	12.3	11.0
	Pyridine	0.54	4.87	7.23
	Quinoline	2.06	11.9	13.4
	Nicotine	1.22	7.91	19.8
Phenyl phase	Pyridine	1.50	4.25	8.20
	Quinoline	4.40	10.9	16.2
	Nicotine	2.55	16.5	25.4
Silica	Pyridine	2.50	6.16	10.3
	Quinoline	6.43	7.16	7.06
	Nicotine	5.80	26.0	46.5

cases. For example, an explanation for a large retention of theophylline and caffeine, and a small retention of xanthine and uric acid cannot readily be suggested.

Retention of the selected solutes was further observed as a function of pH (Table II). As expected, retention of aromatic acids is somewhat increased in the vicinity of their pK_a values (*ca.* 3–4), suggesting again the ion-exchange mechanism. A sharp, rather regular decrease of retention at higher pH is likely to be due to increased negative charge in the mobile-phase ions which subsequently compete for the sorbent sites.

Chromatographic behavior of solutes with more than one ionizable group is considerably more difficult to predict. In some cases, the net charge of a molecule at a given pH can be correlated with its chromatographic mobility. However, other factors can also have a profound influence such as, for example, differences among solubility of individual compounds. Thus, different retention behavior between the classes of purine and pyrimidine derivatives observed in this work can be partially explained by their solubilities as was hypothesized by Cohn[26].

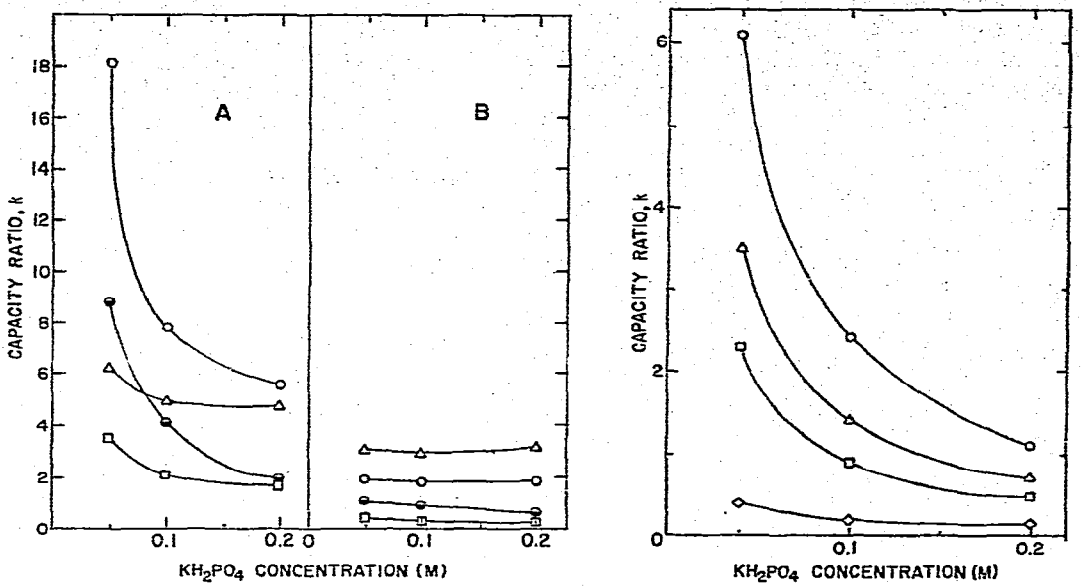

Fig. 2. Dependence of capacity ratio k of aromatic acids on the ionic strength of the phosphate-buffer mobile phase. Conditions: pH 3.0; temperature, 25°; mobile-phase flow-rate, 0.8 ml/min. A, anion-exchange packing; B, phenyl phase packing. ○, 2,4-Dinitrophenol; ◒, phthalic acid; △, dinitrobenzene; □, benzoic acid.

Fig. 3. Dependence of capacity ratio k of nucleotides on the ionic strength of the phosphate-buffer mobile phase. Conditions: anion-exchange column; pH 3.0; temperature, 25°; mobile-phase flow-rate, 1.2 ml/min. ○, Guanylic acid; △, adenylic acid; □, uridylic acid; ◇, cytidylic acid.

Since increased retention of some nitrogen-containing species was observed with the anion-exchange packing at higher pH, k values for pyridine, quinoline, and nicotine were also measured on silica and the phenyl phase (see Table II). With the significant increase in retention on the phenyl phase (in this particular case, the packing with less organic content than the ion exchanger), and even more dramatic increase on the silica column, there is little doubt the residual surface silanol groups are involved in retaining some nitrogen compounds on the anion-exchange column.

Further evidence for the ion-exchange mechanism is presented in Fig. 2, where the retention of aromatic acids and a phenol is plotted as a function of ionic strength. In contrast to the measurements carried out on the phenyl phase (Fig. 2B), k values of these solutes on the ion exchanger sharply decrease (Fig. 2A) as competition for exchange sites becomes greater. A similar trend is observed for nucleotides (Fig. 3).

Effects of ionic strength that are much less understandable were also observed with other classes of ionizable compounds (bases, nucleosides, and nitrogen drugs) and non-electrolytes (aromatic hydrocarbons). "Salting-in" and "salting-out" phenomena must be strongly considered in such cases, even though these effects appear less important.

In view of previous studies using organic solvents as mobile phases in columns of ionic resins[2,27–29], it was of interest to investigate the retention behavior of solutes in organic (ethanol) mobile phase on ion exchangers bonded to silica. Fig. 4 shows that the retention of solutes which are expected to be retained only due to the bonded

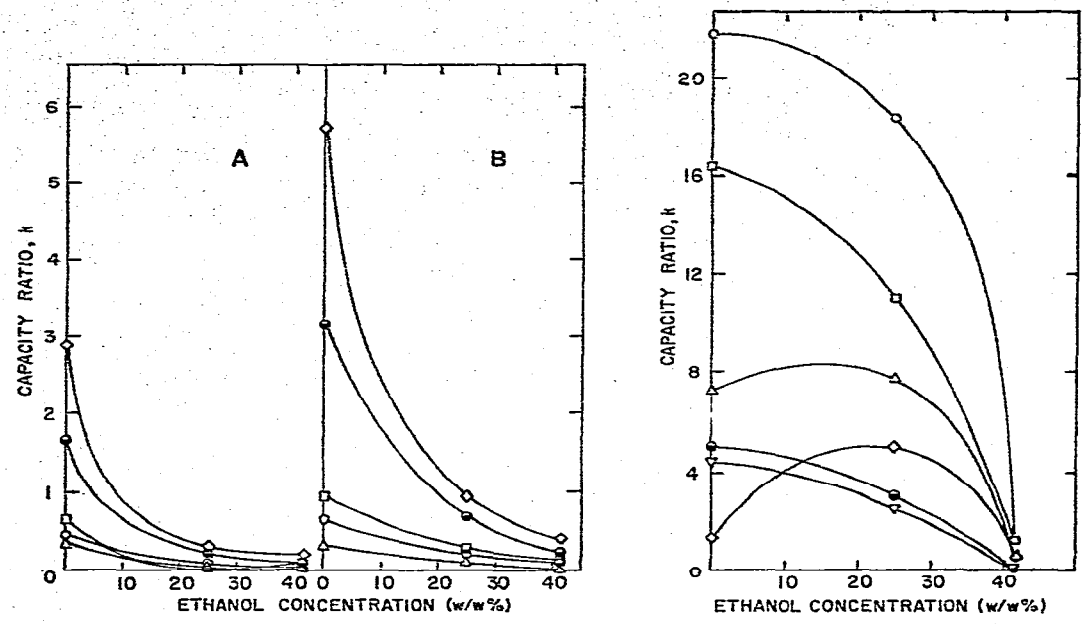

Fig. 4. Dependence of capacity ratio k of nucleosides and bases on the percentage of ethanol in the aqueous mobile phase. Conditions: temperature, 25°; mobile-phase flow-rate, 1.2 ml/min; A, anion-exchange packing; B, phenyl phase packing. ◇, Adenine; ◒, adenosine; □, cytosine; ○, thymine; △, uracil.

Fig. 5. Dependence of capacity ratio k of nucleotides and aromatic acids on the percentage of ethanol in the aqueous mobile phase. Conditions: anion-exchange column; temperature, 25°; mobile-phase flow-rate, 1.2 ml/min. ○, Adenylic acid; □, guanylic acid; △, uridylic acid; ◇, cytidylic acid; ◒, uric acid; ▽, acetylsalicylic acid.

organic matrix exponentially decreases with increased ethanol concentration in the mobile phase. The curves obtained with solutes that contain acidic groups also show a decreasing retention with addition of ethanol (Fig. 5), but the effect is considerably less dramatic here. While the decrease in retention of the solutes shown in Fig. 4 is attributed to their increased solubility in the mobile phase, a competition between the solubility effects and the ion-exchange mechanism is suggested for acids and nucleotides (Fig. 5).

It is interesting to note that retention of most of these bases and nucleosides

TABLE III

RETENTION OF AROMATIC HYDROCARBONS ON THE PHENYL AND ANION-EXCHANGE STATIONARY PHASES IN DIFFERENT SOLVENT SYSTEMS

Compound	*k Value*			
	25% Ethanol aqueous solution		*0.05 M KH_2PO_4 (pH 5.0)*	
	Phenyl phase	*Anion exchanger*	*Phenyl phase*	*Anion exchanger*
Benzene	1.20	0.45	1.35	1.61
Toluene	2.05	0.70	2.10	2.85
o-Xylene	4.70	0.90	4.60	5.13

on the phenyl phase (Fig. 4B) at the lowest ethanol concentration used in this experiment is approximately twice as great as it is on the anion exchanger (Fig. 4A), in spite of the fact that the amount of bonded material in both cases was roughly the same. Electrostatic repulsion may account for this phenomenon.

Quaternary ammonium groups of the ion-exchanger may also be responsible for hindering relatively non-polar solutes from interaction with the matrix. The results of an experiment supporting this hypothesis are shown in Table III. The retention behavior of aromatic hydrocarbons was studied in both the ethanol–water system and the normal aqueous phosphate buffer. While their retention is very similar in both solvent systems on the phenyl phase, the values obtained with the ethanol–water mobile phase on the anion exchanger are abnormally low. A lower wettability and solvation of the ionic surface with the organic solvent, is a likely explanation.

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