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

II. WEAK AND STRONG CATION EXCHANGERS

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SUMMARY

Surface reactions of chlorodimethyl [4-(4-chloromethylphenyl)butyl] silane and chlorodimethyl (4-phenylbutyl) silane with small-particle silica, followed by simple chemical modifications, lead to the preparation of weak and strong cation-exchange packing materials for high-performance liquid chromatography. Retention of various solutes on such bonded phases was investigated under different conditions of pH, ionic strength, and mobile-phase composition. Results of these studies are compared with those previously obtained on classical cation-exchange materials under comparable operating conditions.

INTRODUCTION

Continuous interest in the modern technology of chromatographic columns in which organic stationary phases are permanently bonded to the surfaces of various siliceous materials[1] has also led to consideration of the well-established ion-exchange principles in this direction[2–6]. Since both good sample capacity and high column efficiencies are expected with small porous particles ($<15\ \mu m$), various reactions leading to introduction of ion-exchange moieties onto the siliceous surface are worth exploring. Recently, weak and strong anion exchangers based on small-particle column technology were described as quite effective in separations of nucleotides[6–8] and certain pharmaceuticals[3,7].

In order to predict optimum systems for separations of various compounds on chemically bonded stationary phases, studies of the chromatographic behavior of model solutes are desirable. An example of such column characterization within ion-exchange bonded phases is given in a recent work of Knox and Pryde[7]. Their analysis of the kinetic performance of column packings using reduced plate height *vs.* reduced velocity plots have shown that ion-exchange columns can be prepared with efficiencies comparable to those obtained with underivatized adsorbents (silica and alumina). When new ion-exchange materials become available, retention studies are also needed to evaluate potential uses of such chromatographic media.

As an extension of earlier work of this laboratory on polar chemically bonded phases[9,10], three ion-exchange packings were prepared through the reaction of surface silanol groups of porous silica with chlorodimethyl [4-(4-chloromethylphenyl)butyl] silane and chlorodimethyl (4-phenylbutyl) silane and their subsequent modifications. The reactions involved are shown in Fig. 1. Whereas the preparation and chromatographic evaluation of a strong anion exchanger was reported in the previous publication[11], this communication is dealing with a weak and a strong cation exchanger prepared by a similar technology. Just as in the evaluation of the strong anion exchanger, various classes of standard solutes were investigated here under different chromatographic conditions. The effects of pH, ionic strength, and the mobile-phase composition were studied to elucidate the retention mechanisms involved and for predicting potential applications of such column packing materials.

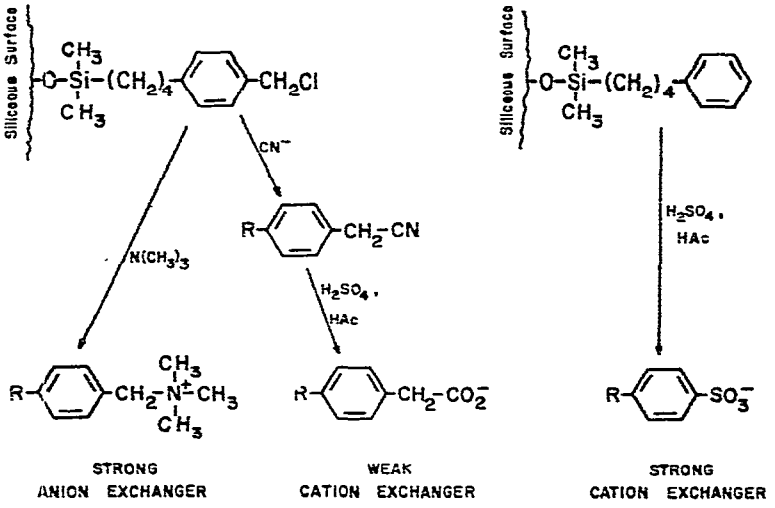

Fig. 1. Reaction sequences for the preparation of the various ion-exchange materials studied.

EXPERIMENTAL

Preparation of ion exchangers

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. Chlorodimethyl [4-(4-chloromethylphenyl)butyl] silane was prepared by the previously described method[12,13]. Chlorodimethyl (4-phenylbutyl) silane was obtained through a simple catalytic hydrosilylation of 4-phenyl-1-butene (using platinum chloric acid as a catalyst).

Chlorodimethyl [4-(4-chloromethylphenyl)butyl] silane was reacted with Silica A using a modification of the previously described method[14]. The bonded material was exhaustively extracted with a series of solvents in a soxhlet apparatus and dried.

A cyano derivative was made by reacting this bonded phase with a solution of potassium cyanide (2 g/l) in ethanol–water (2:1) at room temperature for 6 h. The

product was thoroughly washed with water and dried. The nitrile group was converted to a carboxylic acid by reaction in conc. sulfuric acid–glacial acetic acid (1:1) at 80° for 10 h. This final product was washed with water, exhaustively extracted in a soxhlet apparatus with a series of solvents and dried.

Chlorodimethyl (4-phenylbutyl) silane was reacted with Silica A and extracted as above to form a partition material previously referred[11] to as "the phenyl phase". While this phase (when used for comparative retention studies) was not further modified, the strong cation exchanger was prepared from it by sulfonation of the aromatic group in a solution of conc. sulfuric acid–glacial acetic acid (1:1) at 90° for 5 h. After filtering off the sulfonating mixture, the product was washed with deionized water, acetone and chloroform. The final material was then extracted for 24 h in a soxhlet apparatus with benzene and dried.

Organic carbon content of these materials (as determined by elemental analyses) was 6.1% for the phenyl phase, 2.1% for the strong cation exchanger and 7.0% for the weak cation exchanger. Ion-exchange capacity was determined by potentiometric titration with 0.01 *N* KOH to be 95 μequiv./g and 130 μequiv./g for the strong and weak cation exchangers, respectively. This corresponds to 0.2 and 0.3 ionic groups per 100 Å^2 of the modified surface.

Chromatographic equipment

The liquid chromatograph used for all measurements was a Waters Assoc. Model ALC/GPC 202 instrument with a UV detector and a modified injection system. The columns were maintained at 25° with a thermostatically controlled circulating bath.

The chemically bonded stationary-phase packings were dried and packed into stainless steel tubes by an isodensity slurry packing procedure[15]. Columns, 300 × 2.1 mm I.D., were terminated by 5-μm stainless steel frits (Crawford Fitting Co., Cleveland, Ohio, U.S.A.).

RESULTS AND DISCUSSION

Retention of model compounds

Basic substances with positive charge that have pK_a values close to the pH of a mobile phase should exhibit appreciable retention on the cation-exchange columns. Table I compares k values obtained with selected bases and nucleosides measured for the phenyl (uncharged) phase and both the weak and strong cation exchangers. Although these data are not comparable in absolute sense (somewhat different buffers were used for each type of packing), both cation exchangers show selectivity for stronger bases, indicating that the ion-exchange process is involved. Because of a smaller total amount of stationary phase on the strong cation exchanger, the cation-exchange capacity was lower and a less concentrated mobile phase had to be used. As shown in our previous work on the anion-exchange packing[11], the phenyl phase exhibits the "matrix effect" and causes a small increase in retention of studied compounds as compared to the underivatized silica. It is most likely that such phenomenon occurs also with both cation exchangers. As will be shown below, only small differences in retention are observed when ammonium phosphate buffer is substituted in the mobile phase for potassium phosphate buffer of the same concentration. Thus, any signifi-

TABLE I

COMPARISON OF COLUMN SELECTIVITIES OF PHENYL PHASE, WEAK AND STRONG CATION EXCHANGERS AT pH 3.0

Solute	*k Value*		
	Phenyl phase (0.2 M KH_2PO_4)	*Weak cation-exchanger (0.2 M $NH_4H_2PO_4$)*	*Strong cation-exchanger (0.05 M $NH_4H_2PO_4$)*
Adenine	0.51	8.10	3.32
Cytosine	0.19	2.04	2.02
Uracil	0.25	0.41	0.45
Thymine	0.38	0.78	0.74
Xanthine	0.64	0.87	0.49
Guanine	—	0.54	0.52
Hypoxanthine	0.69	1.12	0.74
Theophylline	4.76	8.17	1.32
Adenosine	0.45	6.91	1.73
Uridine	0.21	0.32	0.30

cant increase in retention of these bases on the cation exchangers appears to be primarily due to their selective interaction with the charged surface.

As expected, there are some selectivity differences between the weak and the strong cation-exchange packings (Table I). Except for minor differences, the same order of elution of these studied solutes is found in this work compared to the results of others[16–18] obtained with classical and pellicular ion exchangers. Cytosine and adenine are more cationic at the low pH of this experiment than uracil and thymine[19]. The somewhat larger retention of thymine than uracil (structures differing only by a methyl group) is consistent with the earlier observation[17] and indicates some importance of the interaction between the aromatic matrix and these solutes.

The three aromatic amino acids, tyrosine, phenylalanine and tryptophan were well retained on the weak cation exchanger, and their order of elution (tyrosine< phenylalanine<tryptophan) which was not changed in the pH range 3.0–6.0 and in solutions of different ionic strength, is similar to the results of previous work[20–22] on the classical ion-exchange materials. Some participation of the aromatic matrix in the separation process[22] is quite likely.

Retention of several biogenic amines was investigated on both types of ion-

TABLE II

COMPARISON OF COLUMN SELECTIVITIES FOR BIOLOGICAL AMINES AT pH 3.0

Solute	*k Value*	
	Weak cation-exchanger (0.2 M $NH_4H_2PO_4$)	*Strong cation-exchanger (0.05 M $NH_4H_2PO_4$)*
Norepinephrine	0.85	0.96
Octopamine	1.57	1.16
Epinephrine	2.14	1.51
Dopamine	3.35	1.46
Tyramine	5.67	2.00

exchange packings. As shown in Table II, both materials retain these compounds. Lesser retention on the strong cation exchanger is again the result of the lower stationary-phase content of this packing. The retention order is similar to previous work done on the classical weak cation-exchange resins[22–24], except that we observe the elution of epinephrine at higher k than norepinephrine and octopamine. It appears that the addition of the carboxylic acid group to the packing material acts to selectively decrease the retention of epinephrine, thus explaining the reversed order of elution of dopamine and epinephrine on the weak and strong cation exchangers studied here.

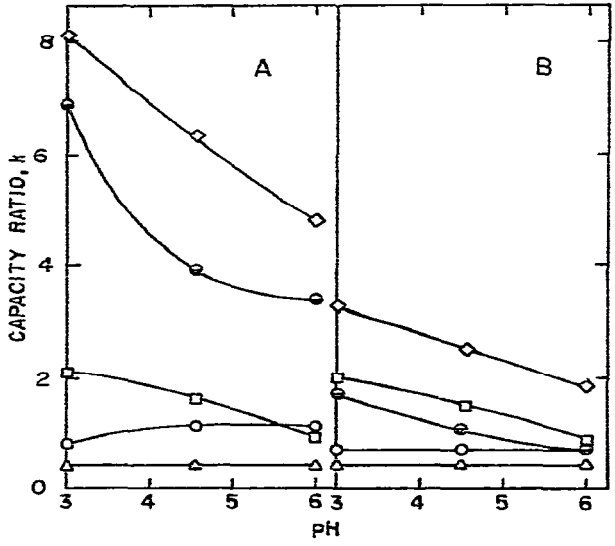

Fig. 2. Dependence of capacity ratio, k, of adenosine and nitrogen bases on the pH of the mobile phase. Conditions: A, weak cation-exchange packing, 0.2 M $(NH_4)H_2PO_4$; B, strong cation-exchange packing, 0.05 M $(NH_4)H_2PO_4$; temperature, 25°; mobile phase flow-rate, 1.2 ml/min. ◇, Adenine; ◒, adenosine; □, cytosine; ○, thymine; △, uracil.

Effect of pH

For the bases that have their pK_a values within the studied pH range, a significant drop of retention is expected with increasing pH as these solutes become less cationic. Fig. 2 shows that k values of adenine, adenosine, and cytosine decrease at higher pH on both types of cation exchangers studied in this work. This behavior is in agreement with their pK_a values (around 4.5). Since the pK_a values of the remaining solutes are outside of the studied range, no changes are anticipated in their retention behavior. The shapes of these k *vs.* pH curves show exactly the opposite trend of those observed with the same compounds on the strong anion exchanger described previously[11]. Similar phenomena were also observed with other basic solutes, such as pyridine and quinoline. Thus, cationic interaction is strongly suggested.

No variations in retention of the biogenic amines used in this study were observed on either cation exchanger within the pH range of 3–6 due to very high pK_a values of these solutes.

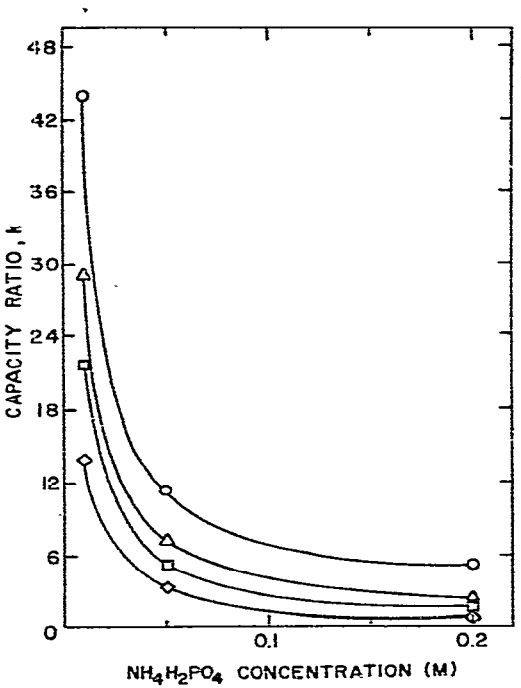

Fig. 3. Dependence of capacity ratio, k, of biogenic amines on ionic strength of the phosphate buffer mobile phase. Conditions: weak cation-exchange packing; pH 6.0; temperature, 25°; mobile phase flow-rate, 1.2 ml/min. ○, Dopamine; △, epinephrine; □, octopamine; ◇, norepinephrine.

Effect of ionic strength

While adenine, adenosine, and cytosine showed only a moderate decrease in their k values on both cation exchangers with increased ionic strength of the mobile phase, a dramatic effect was noticed with the biogenic amines at pH 6.00. This retention behavior shown in Fig. 3, is very consistent with the occurrence of ion-exchange phenomena. As with our previously reported work[11], the less understood "salting-in" and "salting-out" phenomena were observed here with some other classes of compounds. A particularly interesting case was noticed where some ionizable solutes, though not ionized at this pH, followed the same dependence of k on ionic strength as non-electrolytes (aromatic hydrocarbons) when chromatographed on the strong cation exchanger (Fig. 4).

Effect of mobile-phase composition

Addition of ethanol to the aqueous mobile phase resulted in a sharp decrease of retention with miscellaneous compounds due to primarily their increased solubility in the mobile phase. However, as with anionic solutes (such as nucleotides and organic acids) on the strong anion-exchange column[11], a competition between the solubility effects and the ion-exchange mechanism could be observed during chromatography of charged bases on the strong cation exchanger (Fig. 5). This is demonstrated by noting that cytosine shows greater retention than adenine in aqueous ethanol, while in an aqueous buffer solution the opposite is found.

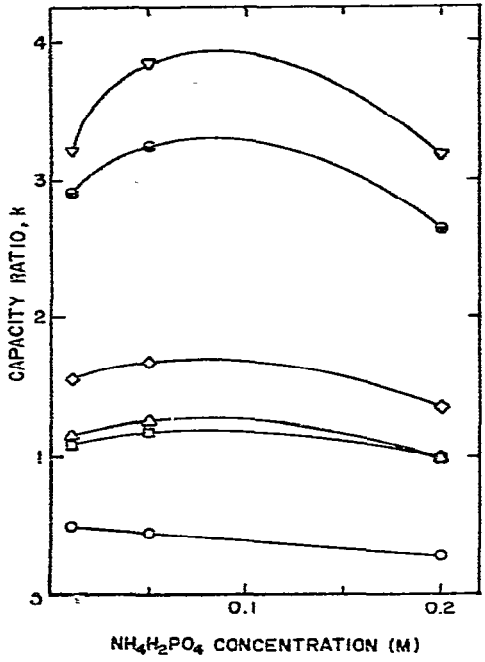

Fig. 4. Dependence of capacity ratio, k, of aromatic hydrocarbons and weak bases on the ionic strength of the phosphate buffer mobile phase. Conditions: strong cation-exchange packing; pH 6.0; temperature, 25°; mobile phase flow-rate, 1.2 ml/min. ▽, *o*-Xylene; ◒, caffeine; ◊, toluene; △, benzene; □, theophylline; ○, xanthine.

The monophosphate nucleotides and acids were found to be less retained than sampled water in aqueous ethanol mobile phase. This is probably due to ion exclusion, where the sulfonated surface repels the negatively charged phosphates and acids. The retention of water was measured by timing the first refractive index "artifact peak" after injection of pure deionized water.

The effect of different mobile phase cations on solute retention was observed previously with a classical strong cation exchanger by Murgia *et al.*[25]. When the sodium, potassium, and ammonium phosphate buffers of the same concentration were compared as mobile phases (Table III), the effect of the counter ion on solute retention was found to be quite small for the whole cross-section of various species. Very basic substances (*i.e.*, adenine, cytosine, and biogenic amines) display the greatest effect. These compounds are most retained in the sodium buffer, least in the potassium buffer, and intermediate retention was observed in the ammonium buffer. This behavior is consistent with the selectivity order found for these ions in classical cation-exchange resins[26]. The overall effect is, however, small. Nevertheless, the differences in retention times between the mentioned bases and other studied compounds (such as aromatic hydrocarbons, phenacetin, and the weak nitrogen bases) indicate that cation exchange is involved in retention of the former, and predominantly matrix affinity in the latter group.

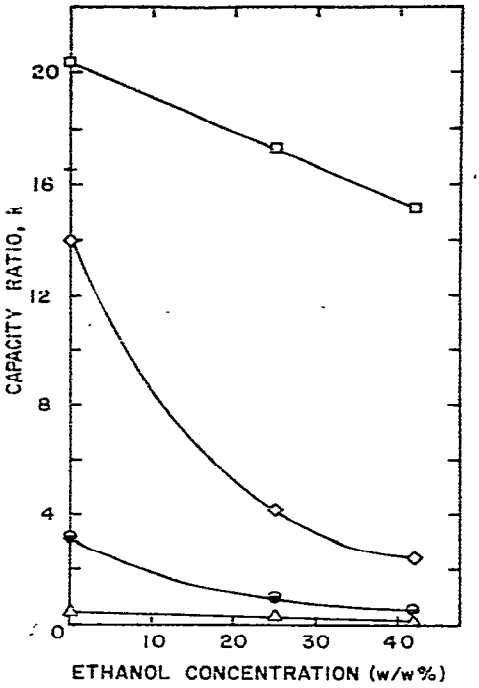

Fig. 5. Dependence of capacity ratio, k, of nitrogen bases on the concentration of ethanol in aqueous mobile phase. Conditions: strong cation-exchange packing; temperature, 25°; mobile phase flow-rate, 1.2 ml/min. □, Cytosine; ◇, adenine; ◒, adenosine; △, uracil.

TABLE III

EFFECT OF MOBILE-PHASE COUNTER ION ON RETENTION BEHAVIOR

Solute	k_{K^+}	$k_{NH_4^+}$	k_{Na^+}	$k_{NH_4^+}/k_{K^+}$	k_{Na^+}/k_{K^+}
Cytosine	1.67	1.82	2.06	1.09	1.23
Uracil	0.35	0.34	0.35	0.97	1.00
Adenine	2.83	3.10	3.49	1.10	1.23
Xanthine	0.42	0.42	0.39	1.00	0.93
Hypoxanthine	0.67	0.69	0.70	1.03	1.06
Theophylline	1.21	1.20	1.20	0.99	0.99
Caffeine	3.39	3.38	3.35	1.00	0.99
Uridine	0.21	0.22	0.21	1.05	1.00
Adenosine	1.60	1.73	1.91	1.08	1.19
Norepinephrine	0.85	0.91	1.01	1.11	1.19
Epinephrine	1.36	1.49	1.65	1.10	1.21
Dopamine	1.31	1.44	1.61	1.10	1.23
Tyramine	1.80	1.98	2.23	1.10	1.24
Phenacetin	4.08	4.07	3.98	1.00	0.98
Aspirin	0.91	0.89	0.85	0.98	0.93
Benzene	1.12	1.12	1.10	1.00	0.98
Toluene	1.74	1.76	1.68	1.01	0.97
Xylene	4.02	4.02	3.89	1.00	0.97

CONCLUSIONS

This and a previous publication[11] show three selected cases where a series of surface reactions can lead to introduction of permanently bonded charged moieties onto the siliceous surfaces. Other modifications are theoretically possible. With an increased popularity of various siliceous supports in biochemical separations, it is likely that such approaches will receive increased attention. It is, therefore, necessary that a proper understanding of their chromatographic properties be obtained.

The work presented in this and the preceding paper[11] indicates that whereas the primary retention mechanism is ion exchange, and many parallels between chemically bonded and classical ion exchangers can be observed, there are certain properties of the bonded phases which are somewhat unusual. While changes in a mobile-phase property, such as pH, ionic strength, amount of organic solvent, etc., can be used to primarily affect solute retention as based on the ion-exchange principle, the contribution of organic matrix and/or residual surface silanol groups can be quite significant under certain circumstances.

Whereas the ion-exchange bonded materials studied here as well as those prepared by others[3,5–8] would appear to cover the whole range of weak and strong ion exchangers, further developments leading to both new materials and optimum column technology for each individual case are highly desirable.

ACKNOWLEDGEMENT

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