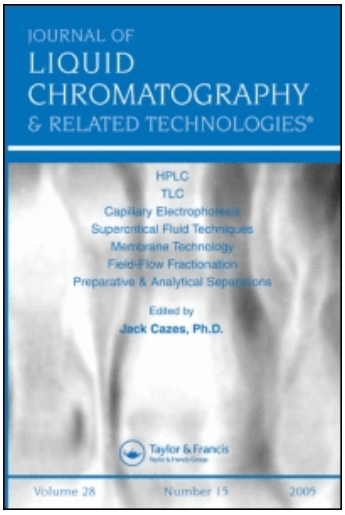

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DEVELOPMENT OF CHEMICALLY STABLE ION-EXCHANGERS BASED ON SILICA GELS

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DEVELOPMENT OF CHEMICALLY STABLE ION-EXCHANGERS BASED ON SILICA GELS

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ABSTRACT

Strong cation and anion-exchangers were synthesized from phenylhexyl-bonded silica gel. These ion-exchange silica gels were stable after continuous flushing with over 45,000 column volumes of 25 mM disodiumhydrogen phosphate in 50% methanol solution. Their chromatographic specificities were studied for separation of saccharides, and drugs.

INTRODUCTION

Amino acid analyzers,[1-3] which comprise one element of automated high performance liquid chromatography systems are automated ion-exchange liquid chromatography systems.

Automated chromatographic systems for analysis of nucleosides and nucleotides[4] and physiological fluids[5] have been developed using ion-exchange liquid chromatography. Saccharides[6] and proteins[7] have also been separated on ion-exchange resins. The typical method of ion-exchange liquid chromatography (so-called "ion-chromatography")[8] was developed using resins the ion-exchange groups of which bonded only the surface of packing materials.[9] Such packing materials permit high-speed separation of ions in water and beverages. An ion-chromatograph constructed with such ion-exchange resin columns and a conductive detector was used for separation of sugarphosphates.[10]

In the early stages of the development of modern liquid chromatography methods, pellicular ion-exchangers using silica gel as the matrix were introduced,[11] and used for analysis of nucleotides[12] and fluorescence compounds in urine.[13] Adenine nucleotides were separated by ion-exchange liquid chromatography using an anion-exchanger made from porous silica gel.[14] The advantage of silica gels is that they are a good matrix in which to synthesize designed bonded-phases such as amino acid bonded-phases.[15] Many applications of ion-exchange liquid chromatography were later replaced by ion-pair liquid chromatography[16] because of the lack of reproducibility of liquid chromatographic data due to the instability of the ion-exchangers made from silica gels and the stable operation of reversed-phase ion-pair liquid chromatography. The instability of bonded silica ion-exchangers is due to slow dissociation of the silica gel in buffer solutions. However, ion-exchange liquid chromatography is suitable for the separation of biologically important compounds under mild conditions as it requires less organic solvent than reversed-phase liquid chromatography.

When choosing a packing material, one of the most important parameters to consider is stability. Organic polymer-based products are stable to salts and over a wide range of pH values. They can also be cleaned with organic solvents without harming the products. Organic polymer-based packing materials have a distinct advantage over the bonded-silica ion-exchangers in their ion-exchange capacity, which is up to 25-fold greater. The disadvantage is the longer separations and equilibration times necessary with the totally porous resinous ion-exchangers, which take at least 2 to 4 times longer for separation than bonded silica ion-exchangers.[17] Polymer coatings were applied to synthesize chemically stable ion-exchangers from silica. Alkaline and alkaline earth cations were separated on the weak cation-exchanger acrylic acid.[18] A cation-exchanger with high electrostatic binding capacity (1.485 $\mu mol/m^2$) was synthesized for chromatography of proteins.[19] If ion-exchangers with properties of both silica based- and organic polymer- based packing materials are developed, more selective separations could be achieved in liquid chromatography.

In reversed-phase liquid chromatography, the separation selectivity of packing materials is dependent mainly on hydrophobicity and the π-electron of bonded-phases, and aromatic compounds are strongly retained on phenyl

bonded-phases.[20, 21] Aliphatic compounds are strongly retained on alkyl-chain bonded phases. The ionization of analytes decreases their retention. However, all types of compounds are retained on ion-exchangers made from a hydrophobic matrix. The ionization of analytes increases their retention on ion-exchangers with counter ions. The latter selectivity should be used for the selective separation of ionized compounds. Such selective separation can be understood from the retention behavior of benzoic acid (log P = 1.94, pKa = 4.19) and mandelic acids (log P = 1.26, pKa = 3.42).[13]

More benzoic acid than mandelic acid was retained on the hydrophobic phase in low-pH eluent due to its hydrophobicity (related to log P values), and more mandelic acid than benzoic acid was retained on an anion-exchanger due to the strong acidity related to the dissociation constant (pKa). The values of log P are predicted or measured as partition coefficients between octanol and water. Acidic compounds are strongly retained on the hydrophobic phase in low-pH eluent due to their hydrophobicity, and are strongly retained on an ion-exchanger phase in high-pH eluent due to their ion-ion interactions. Therefore, the selective separation of ionized acids can be achieved at high pH using an anion-exchanger if the bonded phase is stable in high-pH solutions.

The prediction of retention times on ion-exchange liquid chromatography is still difficult compared to that on reversed-phase liquid chromatography where the retention times can be predicted from log P and pKa values of analytes. The chromatographic behavior of aromatic acids is dependent on the ion-exchanger used. Especially, the dissociation constants showed marked variations according to the type of ion-exchanger used.[22] The variation of dissociation constants and the matrix effect on ionic strength are disadvantageous for the optimization of ion-exchange liquid chromatography. It is still very difficult to predict the variations of dissociation constants and matrix effect.

The handling of packing materials made from silica gels is easier than handling of those made from organic polymers. However, silica gel-based packing materials have the disadvantage of chemical instability. Ion-exchangers based on silica gel are therefore not commonly used as packing materials. The recent development of bonding technology using pure silica gels improved the chemical stability of alkyl and phenyl-bonded silica gels. The usable pH range was expanded to between 1.5 and 10.0 from 2.0 and 7.5, and the lifetime is guaranteed for continuous usage over 1,500 hours.[21, 23] A phenyl-bonded silica gel was therefore used in the present study to develop chemically stable ion-exchangers. The phenyl group was converted to a sulfonyl phenyl group as a strong cation exchanger using chlorosulfonic acid, and also converted to a triethylphenylammonium group using chloromethylmethylether and triethylamine as a strong anion exchanger for chromatography of small molecules.[24] The stability and selectivity of these newly developed ion-exchangers were examined.

EXPERIMENTAL

The properties of the newly developed ion-exchangers were studied by assessing the chromatographic behavior of saccharides and drugs. The liquid chromatographic systems used were a Hewlett Packard model 1190 and Shimadzu model LC10 system including a model SIL-10AXL auto-injector and a model SPD-10AV detector. The chemicals were purchased from Aldrich Chemicals, Fisher Scientific, and Wako Chemicals.

Synthesis of Strong Anion-Exchanger

A strong anion-exchanger was synthesized according to the method described previously.[24] Briefly, phenylhexyl-bonded silica gel (2.5 g) from Phenomenex (Torrance, CA, USA) was mixed with chloromethylmethylether (33 mL) and refluxed for one hour, then added to 2.5 g of anhydrous zinc chloride in 17 mL of chloromethylmethylether and further refluxed for one hour. The solution was filtrated and the particles were washed with 1,4-dioxane and ether. The dried particles were placed in 80 mL of 50 % triethylamine in 1,4-dioxane, then stored at 0°C for one week. The particles were washed with 1,4-dioxane and acetone and then dried.

Synthesis of Strong Cation-Exchangers

A strong cation-exchanger was also synthesized from phenylhexyl-bonded silica gel using the method described previously.[24] Phenylhexyl-bonded silica gel (5 g) was added to a mixture of chlorosulfonic acid and acetone (33 mL) and stirred for 30 minutes. The solution was filtrated, and the particles were washed with acetone and dried.

RESULTS AND DISCUSSION

The ion-exchange capacity of the triethylphenylammonium phase was 0.13 meq/g, while that of the sulfophenyl phase varied from 20-1000 μeq/g by titration. The chemical stability of the new ion-exchangers was examined in a buffer containing 50 % methanol because the anion- and cation-exchangers were not readily miscible in water. The strong anion-exchanger was stable to continuous flushing with over 46,000 column volumes of a mixture of 25 mM disodium hydrogen phosphate (pH 8.6) and methanol (1 + 1) at ambient temperature (Figure 1). The stability was equivalent to that of original phenylhexyl-phase. A chromatogram of standard mixtures measured after flushing with over 46,000 column volumes in shown in Figure 2, where the peak shapes of four compounds, pyridine, aniline, phenol, and toluene, demonstrated the stability of the column.

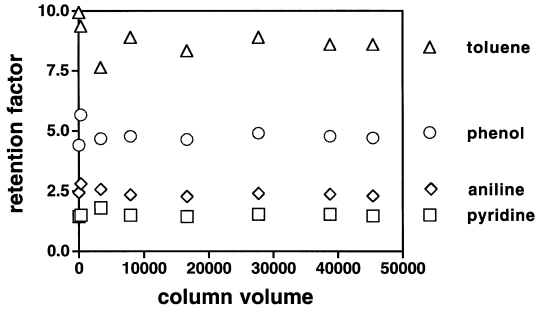

Figure 1. Stability of an anion-exchanger; Column size: 50 x 4.6 mm I.D.; Eluent: 50 mM disodiumphosphate in 50 % methanol; Flow rate: 0.5 mL/min at ambient temp.

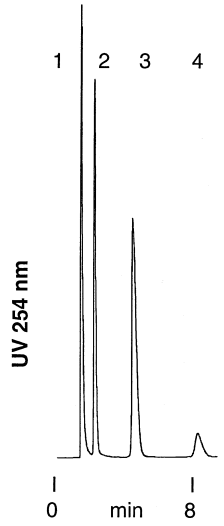

Figure 2. Chromatogram of standard compounds after 64,000 column-volume continuous flushing of 25 mM disodiumphosphate in 50% methanol; Column: anion exchanger 50 x 4.6 mm I.D.; Eluent: 50% methanol; Flow rate: 0.5 mL/min at ambient; Peak 1: pyridine, 2: aniline, 3: phenol, 4: toluene.

The stability was varied by the ion-exchange capacity of the strong cation-exchanger. The stable cation-exchanger was synthesized according to the previously described method,[24] but the ion-exchange capacity was only 20 μeq/g. The ion-exchange capacity was not sufficient for practical use as an ion-exchanger compared to those of ion-exchangers made from organic polymers.

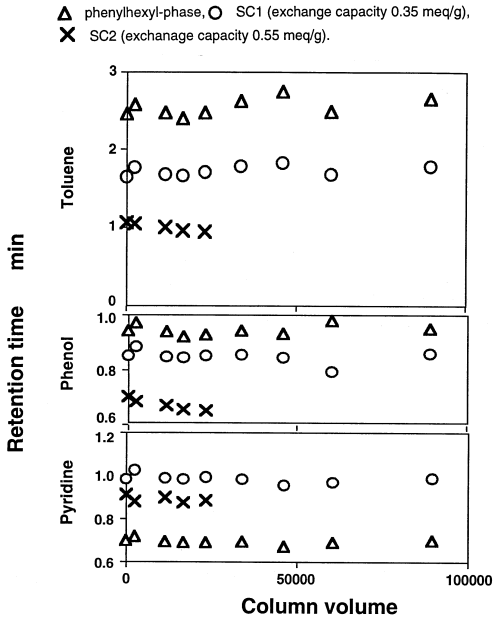

Figure 3. Stability of cation-exchangers; Column size: 50 x 4.6 mm I.D.; Eluent: 20 mM disodiumphosphate in 50 % aqueous acetonitrile; Flow rate: 0.5 mL/min at ambient; Δ: phenylhexyl-phase, O: SC1 (ion-exchange capacity 0.35 meq/g), X: SC2 (ion-exchange capacity 0.55 meq/g).

A strong cation- exchanger with an ion-exchange capacity of 1 meq/g could be synthesized using only chlorosulfonic acid. However, it was unstable in buffer solution, but it could be used within the limited pH range. The reaction conditions were too aggressive to obtain a reproducible product with the same ion-exchange capacity. The reaction products using 50 v% chlorosulfonic acid in acetone were stable to continuous flushing with over 90,000 column volumes (1061 hours) of a mixture of 25 mM disodium hydrogen phosphate (pH 8.6) and methanol (1 + 1) at ambient temperature, and the ion-exchange capacity was 0.35 meq/g (SC1) (Figure 3).

The stability of a cation-exchanger SC1 was equivalent of that of the phenylhexyl bonded-phase. A cation-exchanger with an ion-exchange capacity of 0.55 meq/g (SC2) was not stable under these conditions. Pyridine showed greater retention than phenol on these cation-exchangers (Figure 3) due to ion-ion interactions. The retention factors of toluene measured on SC1 and SC2 were 42 and 15 % of those measured on the original phenylhexyl-phase, respectively.

Table 1

Retention Index of Saccharides on Both Anion and Cation Exchangers in Aqueous Acetonitrile

Saccharides	Retention Factor k (1)	k (2)
Rhamnose	0.46	0.95
Ribose	0.46	1.06
Lyxose	0.62	1.26
Fucose	0.57	1.77*
Xylose	0.69	1.31
Arabinose	0.69	1.92*
Fructose	0.82	2.03
Altrose	0.85	1.52
Sorbose	0.90	1.77
Mannose	1.00	2.38*
Sorbitol	1.15	3.06
Glucose	1.21*	2.18
Galactose	1.26*	2.38
Mannitol	1.31	3.06
Lactulose	2.15	5.62
Sucrose	2.38	4.23
Maltose	2.54*	4.85
Cellobiose	2.62*	4.85
Lactose	2.72*	6.38
Trehalose	3.13	7.57
Milibiose	3.36*	7.57*
Melezitose	4.72	8.69
Maltotriose	5.11*	10.08
Raffinose	5.95	12.18

Retention factor k (1) on anion exchange column (50 x 4.6 mm I.D.), k (2) on cation exchange column (50 x 4.6 mm I.D.), Eluent: 85 v% aqueous acetonitrile at ambient. *Retention factor of the larger of two peaks.

The properties of the newly developed ion-exchangers were analyzed based on the chromatographic behavior of saccharides and drugs. Saccharides were separated on both the cation-exchanger with the ion-exchange capacity of 1 meq/g. and the anion-exchangers in aqueous acetonitrile. The retention factors of saccharides are summarized in Table 1.

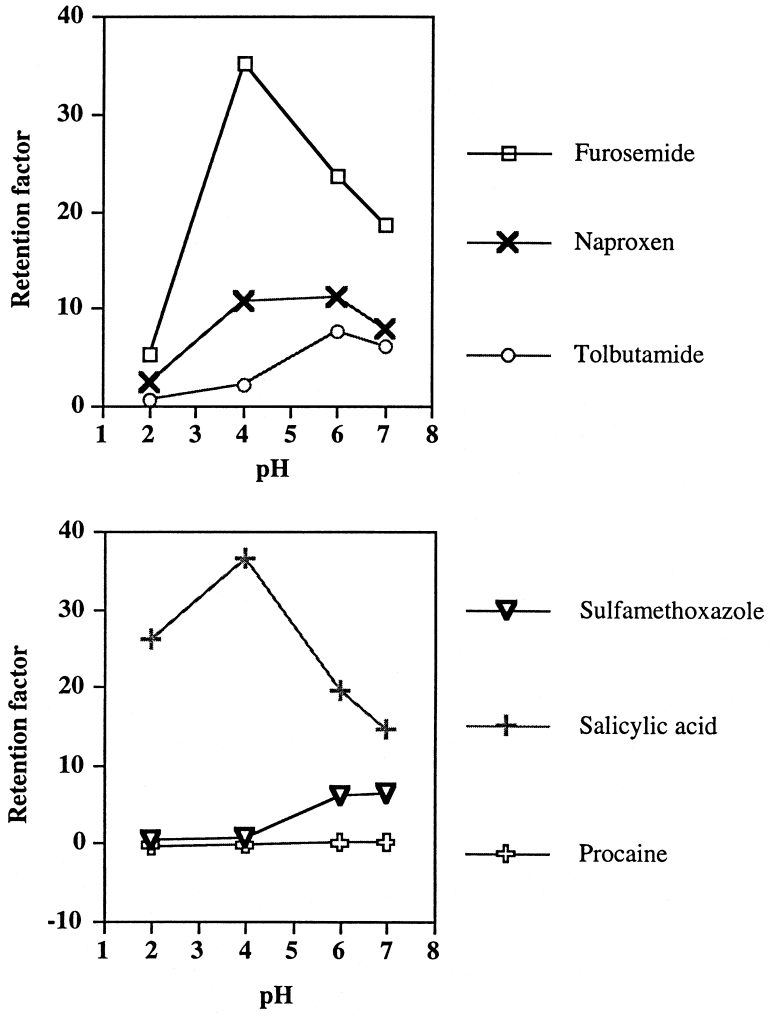

Figure 4. Retention factor of drugs on a strong anion exchanger; Column size: 50 x 4.6 mm I.D.; Eluent: 25mM sodium phosphate buffer in 70% methanol; Flow rate : 1.0 mL/min at 37°C.

Following injection of aqueous sample solutions, several saccharides showed two peaks. However, no such double peaks were observed when sample solutions were prepared using the eluent. The retention factors of major peaks are shown in Table 1, where the asterisk (*) indicates two peaks.

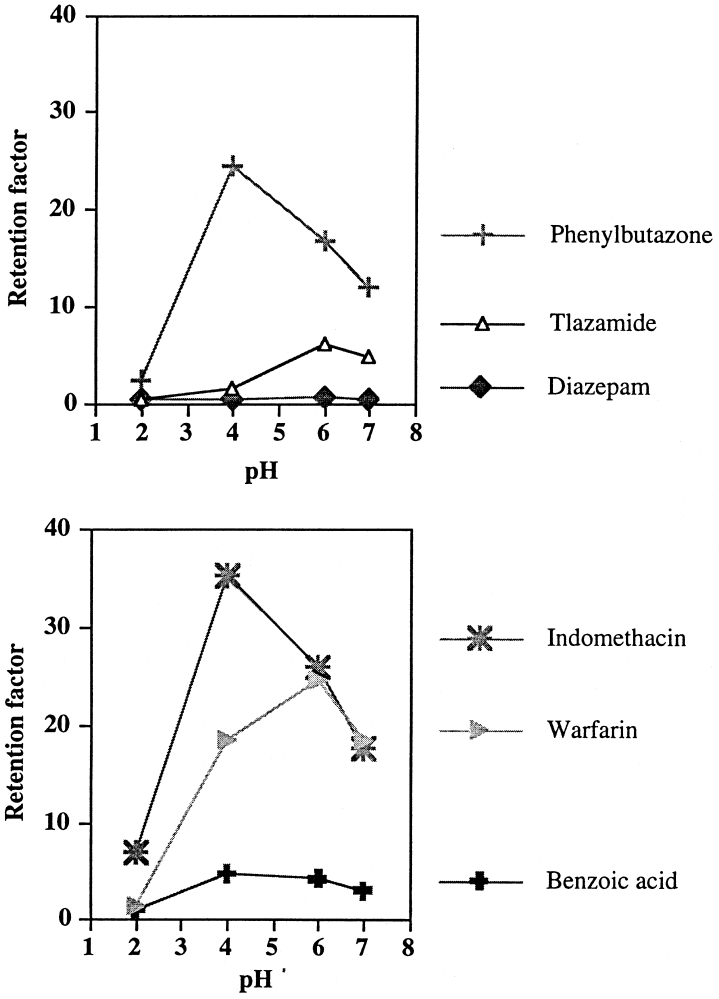

Figure 5. Retention factor of drugs on a strong anion exchanger; Column size: 50 x 4.6 mm I.D.; Eluent: 25mM sodiumphosphate buffer in 70% methanol; Flow rate: 1.0 mL/min at 37°C.

Their quantitative recovery could not be achieved from propylamine-bonded phases due to the glycosylation of saccharides with the primary amino group, but saccharides were quantitatively recovered from these cations and anion-exchangers as demonstrated with a guanidino-bonded phase.[25]

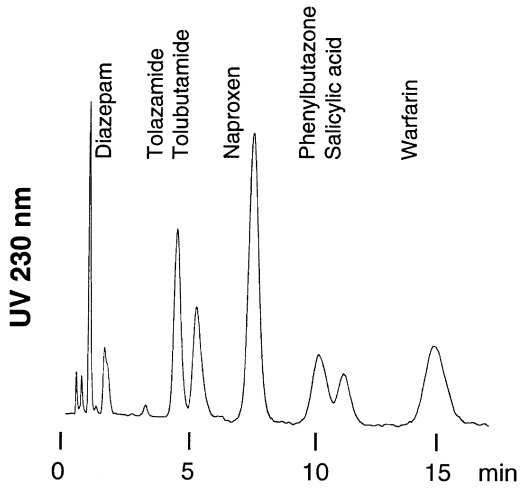

Figure 6. Separation of drugs on an anion-exchanger; Column: 50 x 4.6 mm I.D.; Eluent: 25 mM sodium phosphate buffer (pH 6.5) in 70% methanol; Flow rate: 1.0 mL/min at 37°C, 39 MPa.

With increasing ion-exchange capacity, the retention times of these acids increased, and the lifetime of the cation-exchanger was shortened (Figure 3). Chromatography of acidic drugs was performed on the anion-exchanger, and the effects of pH on their retention are summarized in Figures 4 and 5. A representative chromatogram is shown in Figure 6. The effects of pH on the retention of basic drugs were measured on the cation-exchanger with an ion-exchange capacity of 1 meq/g.

The results are summarized in Figure 7. The retention times of these drugs were very long at low pH even when a small column (50 x 4.6 mm I.D.) was used.

The retention capacities of these ion-exchangers were quite high, and addition of an organic modifier helped to shorten the retention times of the drugs. Organic compounds should be retained by hydrophobic and ion-ion interactions.

The ionized strong acidic compounds were strongly retained on the anion-exchanger and weakly retained on the hydrophobic phases such as octadecyl-bonded silica gels. In contrast, the weak acidic compounds were relatively strongly retained on both the anion exchanger and the hydrophobic phases. These ion-exchangers can be used for the separation of drugs without ion-pair reagents.

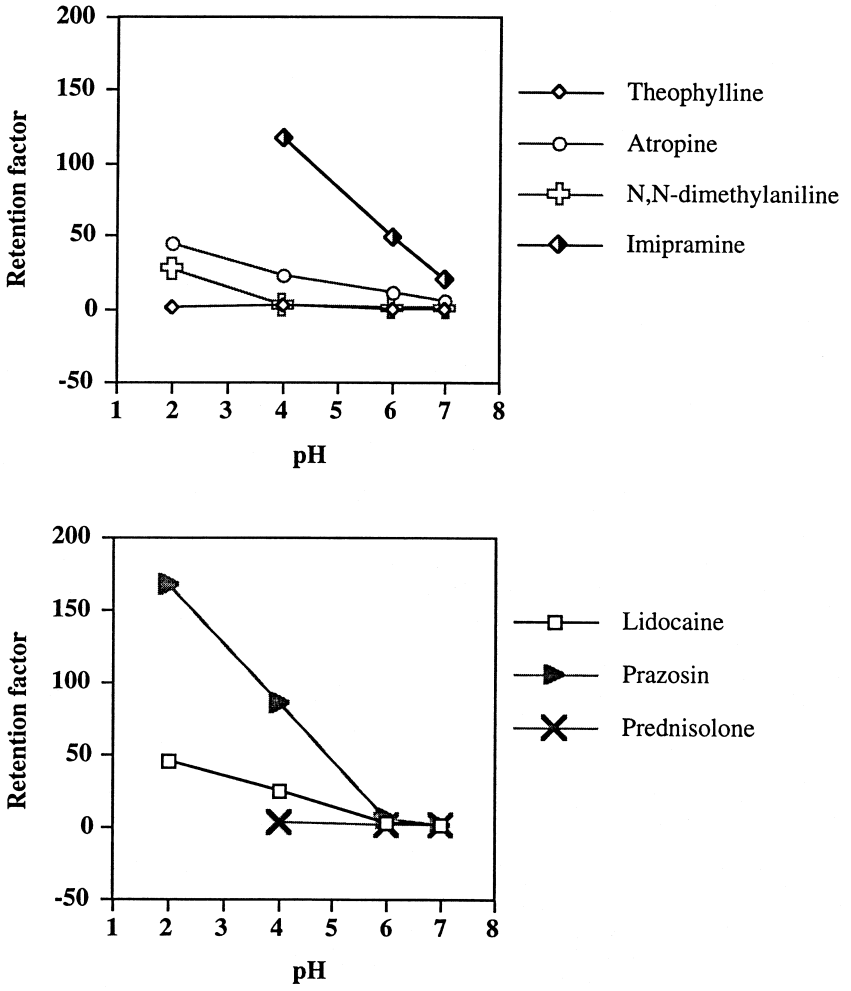

Figure 7. Retention factors of drugs on a strong cation exchanger; Column size: 100 x 2.1 mm I.D.; Eluent: 25 mM sodium phosphate buffer in 70 % methanol; Flow rate: 0.2 mL/min at 37°C (ion-exchange capacity 1 meq/g).

The new anion- and cation-exchangers were quite stable in buffer solution compared to ordinary ion-exchangers made from silica gels due to their hydrophobic matrix. Their use may expand the capability of ion-exchange liquid chromatography for a variety of applications commonly analyzed using reversed-phase ion-pair liquid chromatography.

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