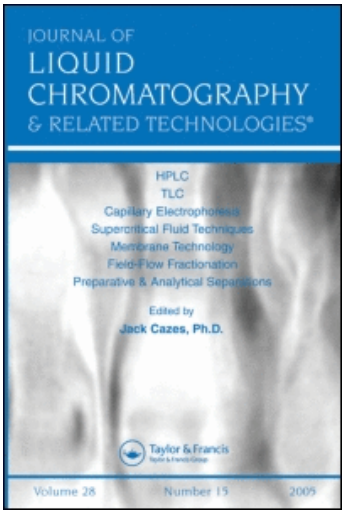

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Comparison of Silica-Based Strong Ion Exchangers and Low-Capacity Polymer-Based Strong Ion Exchangers For the Separation of Organic Analyte Ions Using Indirect Uv Detection

Thomas A. Walker[a]; Nisar Akbari[b]; Thanh V. Ho[b]
[a] Technical Development Marion Merrell Dow Inc., Kansas City, Missouri [b] Chemical Products Discovery the NutraSweet Company, Illinois

To cite this Article Walker, Thomas A. , Akbari, Nisar and Ho, Thanh V.(1991) 'Comparison of Silica-Based Strong Ion Exchangers and Low-Capacity Polymer-Based Strong Ion Exchangers For the Separation of Organic Analyte Ions Using Indirect Uv Detection', Journal of Liquid Chromatography & Related Technologies, 14: 4, 619 — 641
To link to this Article: DOI: 10.1080/01483919108049275
URL: http://dx.doi.org/10.1080/01483919108049275

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(4), 619-641 (1991)

COMPARISON OF SILICA-BASED STRONG ION EXCHANGERS AND LOW-CAPACITY POLYMER-BASED STRONG ION EXCHANGERS FOR THE SEPARATION OF ORGANIC ANALYTE IONS USING INDIRECT UV DETECTION

THOMAS A. WALKER*[1], NISAR AKBARI[2], AND THANH V. HO[2]

[1]*Technical Development*
Marion Merrell Dow Inc.
P.O. Box 9627
Kansas City, Missouri 64134
[2]*Chemical Products Discovery*
The NutraSweet Company
601 E. Kensington Road
Mt. Prospect, Illinois 60056

ABSTRACT

The retention and separation of organic analyte anions and cations was studied on two different types of columns; low-capacity polymer-based ion exchangers and silica-based strong ion exchangers. It was found that separations and elution orders were different between the two columns. The retention of an organic analyte ion on a particular stationary phase was dependant on the type of retention mechanism by which the analyte ion was retained: adsorption, ion exchange or a combination of the two. The low-capacity ion exchanger provides both adsorption sites and ion exchange sites with which the organic analyte ions may interact. Organic analyte ions are retained on the silica-based ion exchangers predominantly by ion exchange. The mobile phase variables that

were found to affect analyte ion retention were: ionic strength, concentration of UV-active counterion, mobile phase pH, and concentration of organic modifier. A comparison is done on the retention data and elution orders that were found for the organic analyte ions on the silica-based strong ion exchangers and the low-capacity ion exchangers.

INTRODUCTION

The routine analysis and determination of ionic analytes in different sample matrixes such as aqueous environments, biological fluids, and waste streams, has become very important. Until Small, et al. (1) published their work on ion chromatography (IC), the separation and detection of ionic analytes was quite difficult. This paper was of significance since it reported for the first time the rapid separation and determination of common anions, and the alkali and alkaline earth metals. This method used an ion exchange column (separator) with a second ion exchange column (suppressor) placed in series. The first column was used to separate the analyte ions of interest and the second column was used to decrease the background conductance so that sensitive detection could be done.

In 1979 and 1980, Fritz and co-workers (2,3) reported the separation of anions and cations using low-capacity ion exchangers and low-conducting mobile phases followed by conductivity detection. This system did not require the use of a suppressor column and was called "single column IC". This method provided separations and detection limits that were similar to that of dual column IC.

Two types of packings are commonly used for ion chromatography: silica-based and polymer-based ion exchangers (4). The polymer-based ion exchangers typically contain a polystyrenedivinylbenzene (PSDVB) backbone while the silica-based ion exchangers use a porous silica bead. The PSDVB is either lightly sulfonated (cation exchanger) or lightly aminated

(anion exchanger). The silica-based packings are chemically prepared to form the anion or cation exchanger. Silica-based ion exchangers retain analyte ions by ion exchange interactions while the PSDVB ion exchangers can retain analyte ions by a reversed-phase interactions, ion exchange, or a combination of the two (5-7). The organic analyte ions must contain a fixed charge site and a hydrophobic center in order for the dual retention mechanism to take place.

This paper compares the separation of organic analyte cations and anions on silica-based strong ion exchangers and low-capacity polymer-based ion exchangers. The mobile phase variables and the effect that these variables had on the separation of the analyte ions on each column is discussed. Several differences were observed for the separations of organic analyte ions when the two different types of stationary phases are compared. These differences include; elution orders, selectivities, and retention mechanisms.

EXPERIMENTAL

Apparatus

The liquid chromatographic system used in this study consisted of a Varian Vista 5500 HPLC, Varian UV-200 detector, and Brookfield strip chart recorder or Hewlett-Packard Model 1090M HPLC system. The stationary phases used in this study were: a 4.1 x 150 mm PRP-1 poly-(styrenedivinylbenzene) copolymer, a 4.1 x 150 mm PRP-X100 low-capacity anion exchange column, and a 4.1 x 150 mm PRP-X200 low-capacity cation exchange column (available from the Hamilton Company Reno, NV, U.S.A.), a 4.6 x 150 mm Spherex 10 SAX silica-based anion exchange column and a 4.6 x 150 mm Spherex 10 SCX silica-based cation exchange column (available from Phenomenex Torrance, CA, USA). The PRP-X100 column is a spherical, 10 µm poly(styrenedivinylbenzene)-based anion exchange column

with an anion exchange capacity of 200 μEq/g, while the PRP-X200 is a spherical, 10μm poly(styrenedivinylbenzene)-based cation exchange column with a cation exchange capacity of 35 μEq/g. Flow rates of 2.0 mL/min were used unless noted. Analyte samples of approximately 1 mg/mL were used with injection volumes of 10-50 μL. Inlet pressures of 500-600 psi were observed.

Chemicals

HPLC grade acetonitrile was obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.). Potassium hydrogen phthalate, inorganic salts, mono- and di-carboxylic acids, benzyltrimethylammonium chloride, tetraalkylammonium salts, and alkylamines were purchased from The Aldrich Chemical Company, Milwaukee, WI. Glacial acetic acid and concentrated phosphoric acid were obtained from Mallinckrodt, Paris, KY. All chemicals were reagent grade. HPLC grade water was obtained by passing de-ionized water through a Nanopure water purification unit.

RESULTS AND DISCUSSION

Differences in the retention mechanism of organic analyte ions have been shown to exist when comparing silica-based strong ion exchangers and low-capacity polymer-based ion exchangers. Silica-based ion exchangers retain charged analyte ions by ion exchange interactions (4,8) whereas polymer-based ion exchangers retain analyte ions by ion exchange, reversed-phase interactions, or a combination of the two (5-7). In order for the analyte ion to be retained by ion exchange and reversed-phase interactions on the polymer-based ion exchanger, the analyte ion must contain a fixed-charge site and a hydrophobic center (5,6,9,10).

The silica-based strong acid and base ion exchangers are composed of a silica backbone and contain charge bearing

functional groups. Strong cation exchangers are based on sulfonate groups while strong anion exchangers are based on trialkylammonium groups. The most common retention mechanism is simple ion exchange of sample ions X^- and mobile phase ions Y^- with the charged groups R^+ of the stationary phase (4,8). This mechanism is shown in equation 1 for an anion exchanger:

$$X^- + R^+Y^- \rightleftharpoons Y^- + R^+X^- \qquad (1)$$

Ion exchange chromatography has been used for the separation of many different types of analytes, including: inorganic ions (4,11-15), organic acids and bases (4,11-15), carbohydrates (16,17), amino acids (18-20), peptides (21-23) and proteins (24-26). The mobile phase parameters that are important to ion exchange include (4,14): 1) selectivity of the resin for various ions, 2) the particular ionic form (counterion) of the resin at the start of the analysis, 3) the concentration of the counterion in the mobile phase, and 4) the pH of the mobile phase. Samples that interact weakly with the ion exchanger will be retained weakly on the column and will elute early while samples that interact strongly with the ion exchanger will be retained more strongly and elute later. Analytes that are weak acids or bases will be retained on the ion exchanger as a function of ionization; an analyte that is charged will be retained much longer than an uncharged analyte. The extent of ionization is controlled by the mobile phase pH. As the pH of the mobile phase is increased, retention of weak acids will increase whereas the retention of weak bases will decrease.

Low-capacity polymer-based ion exchangers have been shown to have a dual retention mechanism of ion exchange and adsorption for analyte ions that contain both a hydrophobic center and a fixed charged site (5-7,9). In order for this dual retention mechanism to be present, the stationary phase must be non-polar, have a high surface area and provide relatively few

ion exchange sites. The electrical potential of the stationary phase depends on the number of ion exchange sites present. Adsorption will also depend on the number of ion exchange sites on the stationary phase; the more ion exchange sites that are present on the stationary phase, the fewer adsorption sites that will exist. The two mechanisms that are present, ion exchange (IE) and adsorption (Ads) can be represented by equations 2 and 3. In this case, an anion exchanger is represented:

$$A\text{-}NMe_3^+C^- + R\text{-}X^- + M^+ \underset{}{\overset{IE}{\rightleftharpoons}} A\text{-}NMe_3^+\,^-X\text{-}R + C^- + M^+ \quad (2)$$

$$A\text{-}NMe_3^+C^- + R\text{-}X^- + M^+ \underset{}{\overset{Ads}{\rightleftharpoons}} M^+\,^-X\text{-}R\cdots A\text{-}NMe_3^+C^- \quad (3)$$

where A represents the copolymeric matrix, C^- is the counteranion (or UV-active counteranion), $R\text{-}X^-$ is an analyte with an anionic site X^- and a hydrophobic center R, and M^+ is the mobile phase countercation.

Previous studies have shown what mobile phase parameters are involved with the separation of organic analyte ions on the polymer-based ion exchangers and what affect each parameter will have on their retention and separation (7,27-29). Organic analyte ions that are retained predominantly by ion exchange will be influenced by mobile phase ionic strength, concentration of counterion (UV-active counterion), mobile phase pH, and the number of ion exchange sites present on the stationary phase. As the mobile phase ionic strength is increased (by the addition of inert electrolytes, increased concentration of buffers and/or UV-active counterion to the mobile phase), the retention of the analyte ions will decrease. The mobile phase pH will affect the ionization of weak acids and bases and their retention. The UV-active counterion is a weak acid or base and will also be affected by the mobile phase pH. If the counterion is unionized, it will not compete for the ion exchange sites and the analyte ions that are retained by ion exchange will be highly retained.

Adsorption of the organic analyte ions onto the polymeric backbone will be affected by the following: the mobile phase concentration of organic modifier, mobile phase ionic strength, mobile phase pH, hydrophobicity of the organic analyte, and the hydrophobicity of the stationary phase. Retention of the organic analyte ions will decrease as the amount of organic modifier added to the mobile phase increases. Changes in the ionic strength will produce a change in analyte retention. The ionic strength is affected by the concentration of added inert electrolytes, buffers and UV-active counterions. The mobile phase pH will affect the charge of the organic analyte and its hydrophobicity. Retention of an organic analyte by adsorption will decrease as the analyte becomes ionized. Adsorption of an organic analyte will decrease as the number of fixed ion exchange sites increases.

In this study, indirect UV detection was used to visualize the organic analyte ions of interest. Indirect Photometric (UV) or "vacancy" chromatography (IPC) was introduced as an extension of ion chromatography where detection was done by UV absorbance rather than by conductance (30). IPC employs a mobile phase that contains a UV-active counterion which competes with the analyte ions for the ion exchange sites. Typically weak organic acids (potassium hydrogen phthalate, KHP) or bases (benzyltrimethylammonium chloride) are used as the mobile phase UV-active counterion. The UV-active counterion must be charged in order to obtain a satisfactory separation of the analyte ions of interest and also to participate in the detection of the UV-transparent analyte ions. The UV-active counterion may be used to control the mobile phase ionic strength and pH. However, the concentration of the UV-active counterion is critical since too high of a concentration may overload the detector and cause it to be out of the optimal working range.

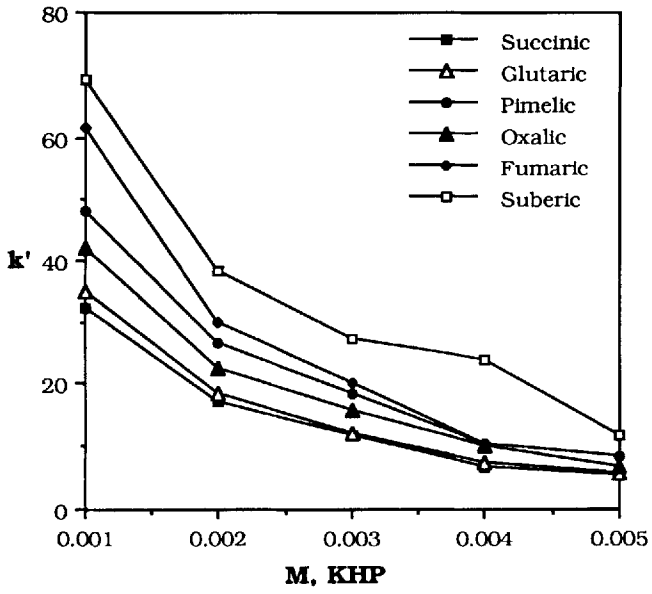

Fig 1 Effect of UV-active counteranion concentration on organic analyte anion retention on a Spherex SAX column. Eluent- KHP, pH 6.5, 5:95 CH_3CN/H_2O.

Anion Exchange

The separation of mono- and di-carboxylic acids on a silica-based strong anion exchange column (SAX) and a low-capacity polymer-based anion exchanger (PRP-X100) were studied to determine how retention may differ. Several mobile phase variables were identified as having an effect on the retention and separation of the organic analyte anions. These variables were studied on a SAX column. A similar study was previously done on a low-capacity anion exchange column to determine what affect the mobile phase variables had on the separation and retention of organic analyte anions (29).

Figure 1 shows the effect of ionic strength (concentration of KHP) on the retention of several di-carboxylic acids on the SAX

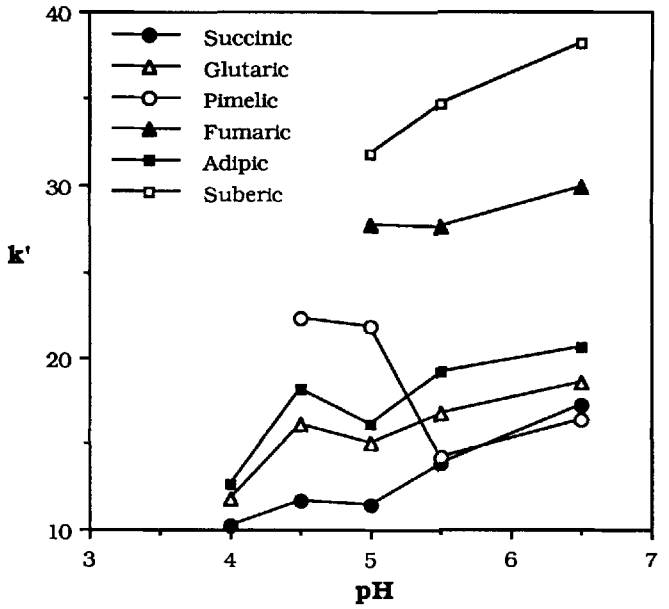

Fig. 2 Effect of mobile phase pH on organic analyte anion retention on a Spherex SAX column. Eluent- 0.005 M KHP, 5:95 CH_3CN/H_2O.

column. The retention of the di-carboxylic acids decreased as the concentration of KHP was increased and follows what would be expected. According to equation 1, the equilibrium will be shifted to the left as the concentration of KHP is increased and a corresponding decrease in the retention of the organic analyte anions will be observed. Similar results were also found for the organic analyte anions on the PRP-X100 (29).

In order for an organic analyte to be retained by ion exchange it must be ionized. If the analyte is unionized, it will have little or no retention on a silica-based anion exchanger. The effect of the mobile phase pH is shown in Figure 2. As the mobile phase pH was raised, retention of the di-carboxylic acids were found to increase. This would be expected since the di-

carboxylic acids are going from being unionized to mono-anionic to di-anionic. For all of the di-carboxylic acids studied, except pimelic acid, retention increased as the pH was increased. Pimelic acid, which has a pK_a of 4.75, is almost completely ionized at pH 4.5 and would, therefore, not significantly increase in retention as the mobile phase pH is increased. The other di-carboxylic acids studied, however, interacted more strongly with the fixed ion exchange sites as the mobile phase pH was raised due to increased ionization.

Similar results were not observed on the PRP-X100 column (29). As the mobile phase pH was raised, the retention of the di-carboxylic acids was found to decrease for the longer chain acids while the shorter-chain acids showed almost no change. This is attributed to the longer chain acids being retained predominantly by adsorption at low mobile phase pH (pH 4.0) where the acids are not ionized or only slightly ionized. Ionization of the organic acids took place when the mobile phase pH was increased and retention changed from that of adsorption to anion exchange or a combination of anion exchange/adsorption. The long-chain acids interact more strongly with the adsorption sites than with the ion exchange sites and therefore decreased in retention. The shorter chain di-carboxylic acids are retained predominantly by anion exchange and showed little or no change in retention.

The third mobile phase variable studied on the silica-based anion exchanger was the concentration of organic modifier. The retention of the di-carboxylic acids were found to decrease as the amount of acetonitrile added to the mobile phase increased (Figure 3). Research has shown that an organic modifier will have an effect on the retention of ions on an ion exchange column (8,31,32). The organic modifier may lead to an increase or decrease in analyte ion retention, depending on the chemical nature of the analyte ions.

The results that were found on the PRP-X100 column for the acids were different than that observed on the SAX column.

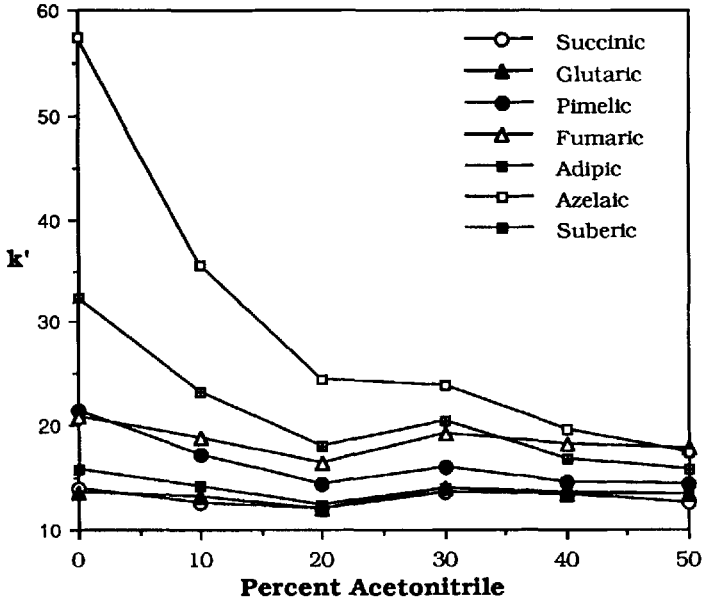

Fig. 3 Effect of organic modifier concentration on organic analyte anion retention on a Spherex SAX column. Eluent- 0.005 M KHP, pH 6.5, CH_3CN/H_2O.

In general, the shorter-chain organic acids (i.e., acetic, malonic, fumaric) increased in retention on the PRP-X100 column as the concentration of acetonitrile was increased. The longer chain di-carboxylic acids (i.e., pimelic acid), however, showed a significant decrease in retention with increasing acetonitrile concentrations. The longer chain acids are retained predominantly by adsorption and showed a decrease in retention with increasing acetonitrile concentration. The shorter chain acids, whose retention is due to anion exchange, increased in retention. These differences can be explained by looking at the relative polarities of the mobile and stationary phases. As the mobile phase becomes more nonpolar (increasing concentration

of organic modifier), the stationary phase with respect to the mobile phase becomes more polar. Therefore, analytes that are ionic and have a small hydrophobic center will tend to be attracted toward the phase that is more polar and will, therefore, be more highly retained on the ion-exchange sites as the concentration of organic modifier increases. The longer chain acids that are retained by adsorption will tend to decrease in retention (29).

A separation of the di-carboxylic acids on the Spherex SAX column is shown in Figure 4A, while Figure 4B shows the separation on the PRP-X100 column. The mobile phase conditions were optimized for this separation on both columns. Several differences were observed in elution orders between the two columns. On the SAX column, oxalic acid (H) and fumaric acid (G) did not co-elute whereas this pair was difficult to resolve on the PRP-X100 column. Succinic acid (A) and malonic acid (C), as well as glutaric acid (B) and maleic acid (E) co-eluted on the SAX column but not on the PRP-X100 column. These differences may be attributed to the type of interactions that each analyte has toward the particular stationary phase: ion exchange on the SAX versus ion exchange/adsorption on the PRP-X100. Overall, it was found that the PRP-X100 is a better choice for the separation of the dicarboxylic acids.

Cation Exchange

A similar study was done on a silica-based strong cation exchanger (SCX) and a low-capacity polymer-based strong cation exchanger. The SCX column used in this study was a Spherex 10 SCX while the low-capacity cation exchanger was a Hamilton PRP-X200. Differences in cation retention and separations were observed for the two different types of columns.

The effect that the concentration of UV-active countercation had on organic analyte cation retention using the SCX column is shown in Figure 5. It was found that retention of the cations decreased as the concentration of UV-active countercation was

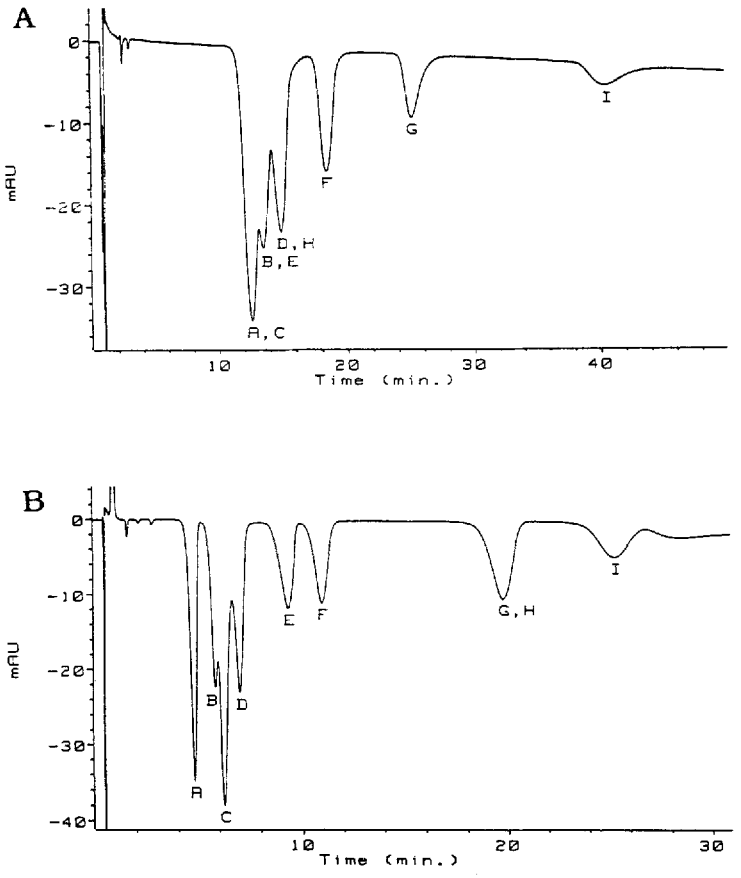

Fig. 4 Separation of di-carboxylic acids on A) Spherex SAX and B) PRP-X100. A) Eluent- 0.002 M KHP, pH 6.5, 5:95 $CH_3CN:H_2O$, B) Eluent-0.001 M KHP, pH 4.5, 5:95 $CH_3CN:H_2O$. A) succinic acid, B) glutaric acid, C) malonic acid, D) adipic acid, E) maleic acid, F) pimelic acid, G) fumaric acid, H) oxalic acid, I) suberic acid.

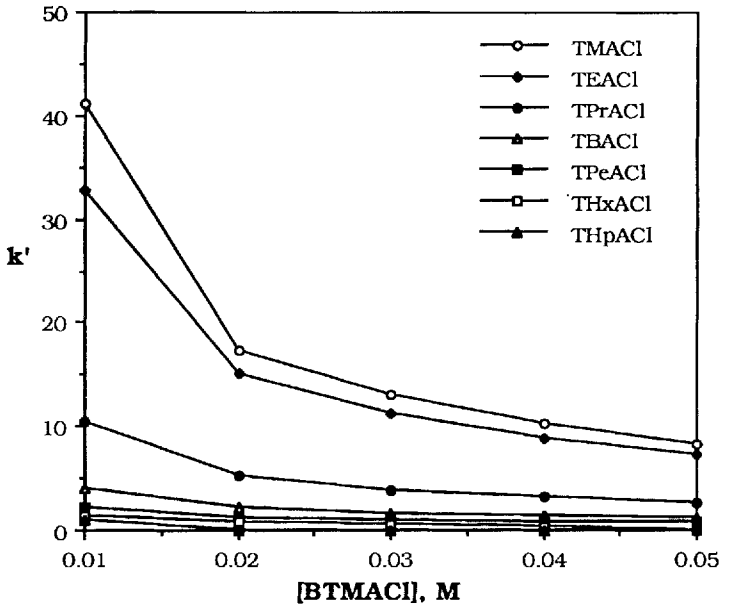

Fig 5 Effect of UV-active countercation on TAA^+ salt retention on a Spherex SCX column. Eluent- $BTMA^+Cl^-$, 0.01 M Acetic acid, 70:30 $CH_3CN:H_2O$.

increased (eq. 1 shifts to the left). This would be expected due to increased competition for the cation exchange sites from the higher concentration of benzyltrimethylammonium chloride ($BTMA^+Cl^-$). Similar results were also observed on the low-capacity polymer-based cation exchanger (PRP-X200).

Figure 6 shows the effect that the concentration of organic modifier had on organic analyte retention on the SCX column Tetramethylammonium chloride (TMA^+Cl^-) and tetraethylammonium chloride (TEA^+Cl^-) increased in retention with increasing organic modifier concentration. The longer chain tetraalkylammonium ions (TAA^+), however, decreased in retention. Increasing the mobile phase concentration of organic

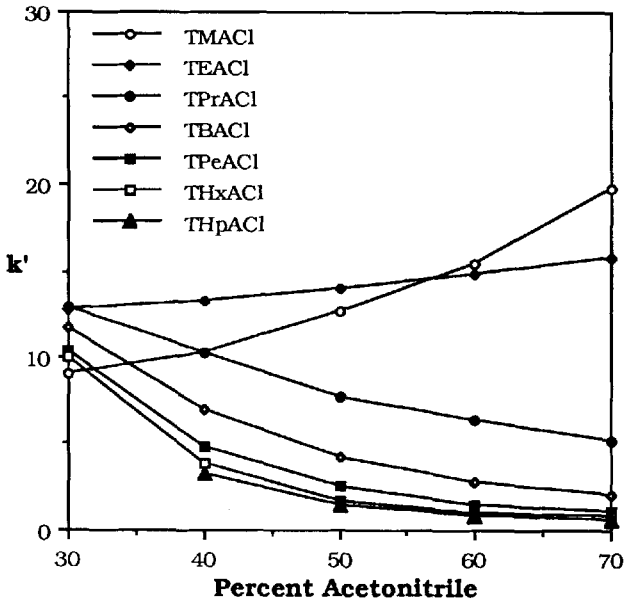

Fig. 6 Effect of organic modifier concentration on TAA^+ salt retention on a Spherex SCX column. Eluent- 0.02 M $BTMA^+Cl^-$, 0.01 M Acetic acid, $CH_3CN:H_2O$.

modifier leads to a change in the relative polarity of the mobile and stationary phases. As the mobile phase becomes more nonpolar (increasing the organic modifier concentration), the stationary phase, with respect to the mobile phase becomes more polar. Analytes that are cationic and are retained predominantly by cation exchange will be attracted to the phase that is more polar. This leads to an increase in retention for the analytes retained by cation exchange. The longer chain TAA^+ salts are retained by adsorption or a combination of adsorption/cation exchange and showed a decrease in retention.

The same organic modifier study was also done with several alkylamines. The results that were found are shown in Figure 7.

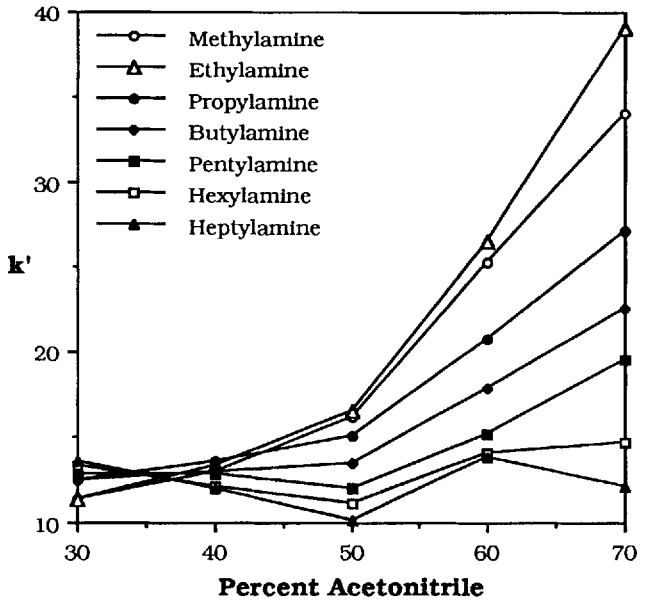

Fig. 7 Effect of organic modifier concentration on alkylamine retention on a Spherex SCX column. Eluent- 0.01 M $BTMA^+Cl^-$, 0.01 M Acetic acid, $CH_3CN:H_2O$.

The alkylamines that interact most strongly with the cation exchange sites had the highest increase in retention. Methylamine and ethylamine showed the greatest increase in retention whereas heptylamine and hexylamine showed only a slight change in retention.

Elution orders were found to be different for the organic cations studied on the SCX and the PRP-X200 columns. Figure 8 compares the separation of TAA^+ salts on the SCX column (8A) and on the PRP-X200 column (8B). The SCX column had an elution order of $THpA^+Cl^- < THxA^+Cl^- < TPeA^+Cl^- < TBA^+Cl^- < TPrA^+Cl^- < TEA^+Cl^- < TMA^+Cl^-$ whereas the elution order on the PRP-X200 was $TPrA^+Cl^- < TBA^+Cl^- < TPeA^+Cl^- < TEA^+Cl^- < TMA^+Cl^- < THxA^+Cl^- < THpA^+Cl^-$. The differences in the

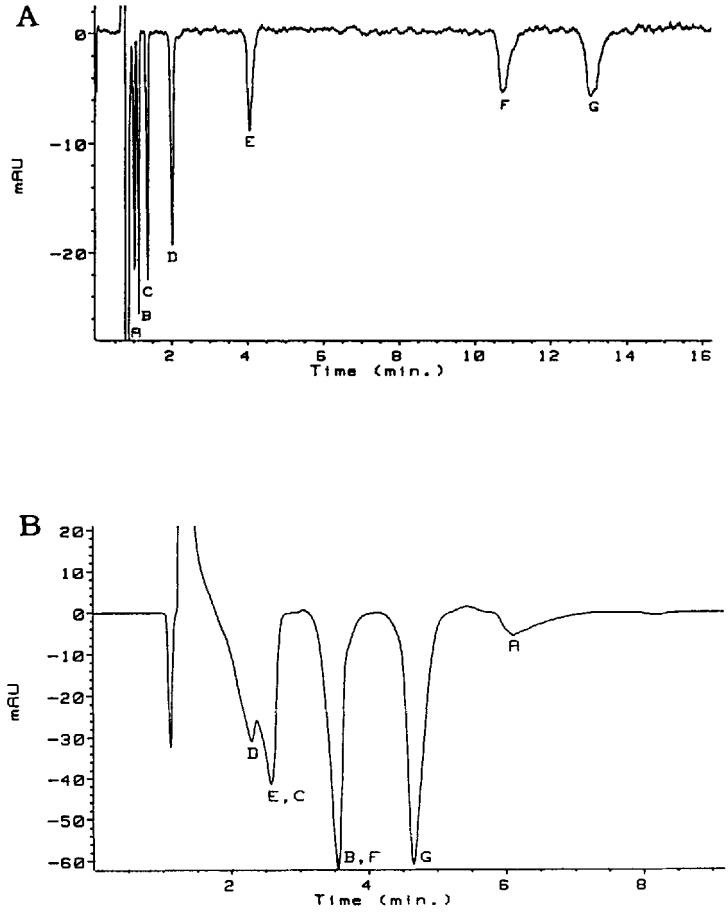

Fig. 8 Separation of TAA^+ salts on A) Spherex SCX column and B) PRP-X200 column. A) Eluent- 0.02 M $BTMA^+Cl^-$, 0.01 M Acetic acid, 70:30 $CH_3CN:H_2O$, B) 0.003 M $BTMA^+Cl^-$, 0.01 M Acetic acid, 70:30 $CH_3CN:H_2O$. A) $THpA^+Cl^-$, B) $THxA^+Cl^-$, C) $TPeA^+Cl^-$, D) TBA^+Cl^-, E) $TPrA^+Cl^-$, F) TEA^+Cl^-, G) TMA^+Cl^-.

elution order can be explained by the retention mechanism(s) that take place on each packing. The SCX stationary phase retains cations predominantly by cation exchange whereas the PRP-X200 has a dual retention mechanism of cation exchange and adsorption. When the TAA^+ salts are separated on a PRP-1 column, which is the same packing as the PRP-X200 column except for the fixed sulfonate charge sites, the TAA^+ were found to elute according to the chain length of the alkyl groups; the longer the alkyl chain the greater its retention. The elution order on the PRP-1 column was as follows: $TMA^+Cl^- < TEA^+Cl^- < TPrA^+Cl^- < TBA^+Cl^- < TPeA^+Cl^- < THxA^+Cl^- < THpA^+Cl^-$. This is the opposite of the elution order found on the SCX column (17,30). Therefore, when both adsorption and cation exchange sites are present (PRP-X200), differences in elution orders will be found when compared to either cation exchanger (SCX) or a reversed-phase packing (PRP-1). The data from both cation exchangers and the reversed-phase packing helps to distinguish between the retention mechanism for different organic analyte cations. The longer chain TAA^+ salts ($TPeA^+Cl^-$, $THxA^+Cl^-$, $THpA^+Cl^-$) are retained on the PRP-X200 predominantly by adsorption whereas the smaller chain TAA^+ salts (TMA^+Cl^-, TEA^+Cl^-) are retained by cation exchange. The other TAA^+ salts ($TPrA^+Cl^-$, TBA^+Cl^-) are retained by a mixed mechanism of ion exchange and adsorption.

Figure 9 compares the separation of alkylamines on the SCX column (9A) and the PRP-X200 column (9B). The elution orders are almost reversed on the two columns. On the SCX column the elution order was found to be: heptylamine < hexylamine < pentylamine < butylamine < propylamine < methylamine < ethylamine, while the elution order on the PRP-X200 column was found to be: methylamine < ethylamine < propylamine < butylamine < pentylamine < hexylamine < heptylamine. The retention of the alkylamines on the SCX column is due to cation exchange. The alkylamines that have shorter alkyl chains have a more densely packed charge and are more highly retained. On

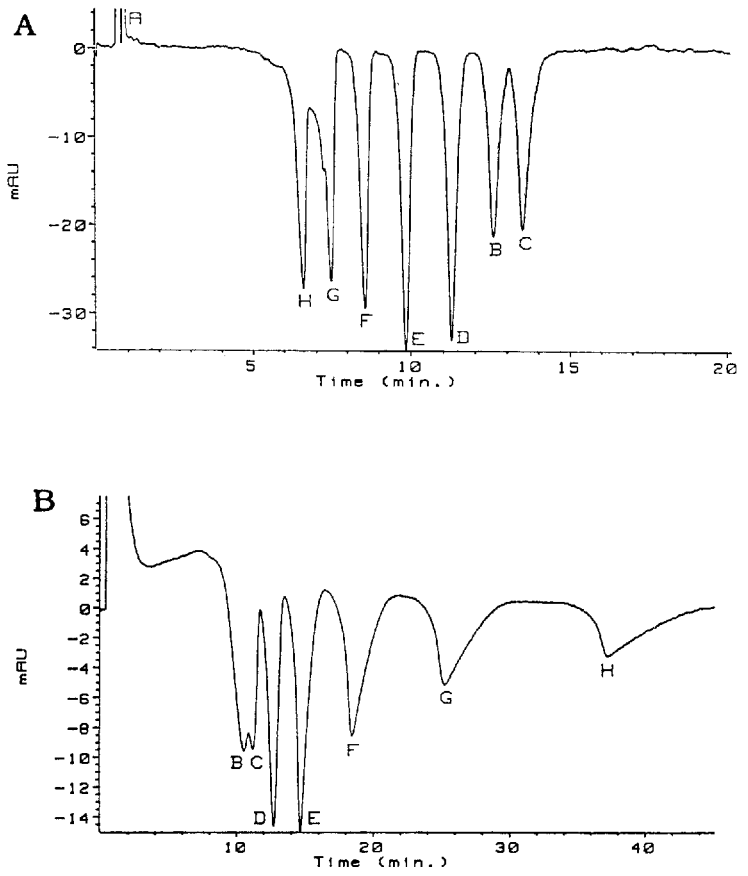

Fig. 9 Separation of alkylamines on A) Spherex SCX column and B) PRP-X200 column. A) Eluent- 0.01 M $BTMA^+Cl^-$, 0.01 M Acetic acid, 60:40 $CH_3CN:H_2O$, B) 0.0008 M $BTMA^+Cl^-$, 35:65 $CH_3CN:H_2O$. A) Systems Peak, B) methylamine, C) ethylamine, D) propylamine, E) butylamine, F) pentylamine, G) hexylamine, H) heptylamine.

the PRP-X200 column, the alkylamines are retained by cation exchange (short chain length), adsorption (long chain length) or a combination of the two (moderate chain length).

CONCLUSIONS

A comparison of the retention of organic analyte ions on low-capacity polymer-based ion exchangers and silica-based strong ion exchangers showed that several differences exist. The differences that were found include: elution orders, selectivities and retention times. Analytes that are not retained or slightly retained on the SCX or SAX column typically had higher retentions on the PRP-X200 or PRP-X100 column. This higher retention on the low-capacity ion exchanger can be attributed to the presence of adsorption sites with which the longer chain analytes may interact.

REFERENCES

1. Small, H., Stevens, T.C. and Bauman, W.C., Novel Ion Exchange Chromatographic Method Using Conductiometric Detection, Anal. Chem., 47, 1801, 1975.

2. Gjerde, D.T., Fritz, J.S. and Schmuckler, G., Anion Chromatography with Low-Conductivity Eluents, J. Chromatogr., 186, 509, 1979.

3. Fritz, J.S., Gjerde, D.T. and Becker, R.M., Cation Chromatography with a Conductivity Detector, Anal. Chem., 52, 1519, 1980.

4. Walton, H.F. and Rocklin, R.D., Ion Exchange in Analytical Chemistry, CRC Press, Boca Raton, FL., 1990.

5. Afrashtefar, S. and Cantwell, F.F., Chromatographic Retention Mechanism of Organic Ions on a Low-Capacity Ion Exchange Adsorbent, Anal. Chem., 54, 2422, 1982.

6. Hux, R.A. and Cantwell, F.F., Surface Adsorption and Ion Exchange in Chromatographic Retention of Ions on Low-Capacity Cation Exchangers, Anal. Chem., 56, 1258, 1984.

7. Pietrzyk, D.J., Iskandarani, Z. and Schmitt, G.L., Anion Exchange on Low Capacity Anion Exchangers: Separation of Organic Acids, Amino Acids, Small Chain Peptides, J. Liq. Chromatogr., 9, 2633, 1986.

8. Snyder, L.R. and Kirkland, J.J., "Introduction to Modern Liquid Chromatography", 2nd ed. John Wiley and Sons, New York, 410, 1980.

9. Cantwell, F.F., "Ion Exchange and Solvent Extraction", vol. 9, J.A. Marinsky and Y. Marcus eds., M. Dekker, Inc., 339, 1985.

10. Hux, R.A. and Cantwell, F.F, Anal. Chem., 56, 1258, 1984.

11. Skelly, N.E., Separation of Inorganic and Organic Anions on Reversed-Phase Liquid Chromatography Columns, Anal. Chem., 54, 712, 1982.

12. Jones, W.R., Jandik, P. and Swartz, M.T., Automated Column Coupled System for Simultaneous Determination of Carboxylic Acids and Inorganic Anions, J. Chromatogr., 473, 171, 1989.

13. Cheam, V., Determination of Organic and Inorganic Acids in Precipitation Samples, J. Chromatogr., 482, 381, 1989.

14. Miller, J.M., "Chromatography: Concepts and Contrasts", John-Wiley and Sons, New York, 171, 1988.

15. Haddad, P.R., Jackson, P.E. and Heckenberg, A.L., Performance Characteristics of Some Commercially Available Low-Capacity Anion-Exchange Columns Suitable for Non-Suppressed Ion Chromatography, J. Chromatogr., 346, 139, 1985.

16. Hardy, M.R. and Townsend, R.R., Separation of Positional Isomers of Oligosaccharides and Glycoproteins by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection, Proc. Natl. Acad. Sci. U.S.A., 85, 3289, 1988.

17. Lee, Y.C., High-Performance Anion-Exchange Chromatography for Carbohydrate Analysis, Anal. Biochem., 189, 151, 1990.

18. Moore, S. and Stein, W.H., Chromatography of Amino Acids on Sulfonated Polystyrene Resins, J. Biol. Chem., 192, 663, 1951.

19. Pfeiffer, R.F. and Hill, D.W., HPLC of Amino Acids; Ion Exchange and Reversed-Phase Strategies, Adv. Chromatogr., 22, 44, 1983.

20. Polta, J.A. and Johnson, D.C., The Direct Electrochemical Detection of Amino Acids at a Platinum Electrode in an Alkaline Chromatographic Effluent, J. Liq. Chromatogr., 6, 1727, 1983.

21. Regnier, F.E., High-Performance Ion-Exchange Chromatography, Methods Enzymol., 104, 170, 1984.

22. Hearn, M.T.W., Hodder, A.N., Stanton, P.G. and Aguilar, M.I., High-Performance Liquid Chromatography of Amino Acid, Peptides and Proteins. LXXXIII. Evaluation of Retention and Bandwidth Relationships for Proteins Separated by Isocratic Anion-Exchange Chromatography, Chromatographia, 24, 769, 1987.

23. Bradshaw, R.A., Bates, O.J. and Benson, J.R., Peptide Separations on Substituted Polystyrene Resin: Effect of Crosslinkage, J. Chromatogr., 187, 27, 1980.

24. Regnier, F.E. and Noel, R., Glycerolpropylsilane Bonded Phases in the Steric Exclusion Chromatography of Biological Macromolecules, J. Chromatogr. Sci., 14, 316, 1976.

25. Change, S.H., Noel, R. and Regnier, F.E., High-Speed Ion-Exchange Chromatography of Proteins, Anal. Chem., 48, 1839, 1976.

26. Regnier, F.E. and Gooding, K.M., Review: HPLC of Proteins, Anal. Biochem., 103, 1, 1980.

27. Walker, T., The Separation of Organic Analyte Cations on a Low-Capacity Cation Exchange Column Using Indirect UV Detection, J. Liq. Chromatogr., 11, 1513, 1988.

28. Walker, T.A., Separation of Simple Guanidines on Cation-Exchange Columns Using Indirect Ultraviolet Detection, J. Chromatogr., 466, 438, 1989.

29. Walker, T.A., Ho, T.V. and Akbari, N., Indirect UV Detection of Organic Analyte Anions Using a Low-Capacity Anion Exchange Column, J. Liq. Chromatogr., 12, 1213, 1989.

30. Small, H. and Miller, T.E., Jr., Indirect Photometric Chromatography, Anal. Chem., 54, 462, 1982.

31. Eksteen, R., Kraak, J.C. and Linssen, P., Conditions for Rapid Separations of Nucleobases and Nucleosides by High-Pressure Anion-Exchange Chromatography, J. Chromatogr., 148, 413, 1978.

32. Haddad, P.R. and Cowie, C.E., Computer-Assisted Optimisation of Eluent Concentration and pH in Ion Chromatography, J. Chromatogr., 303, 321, 1984.