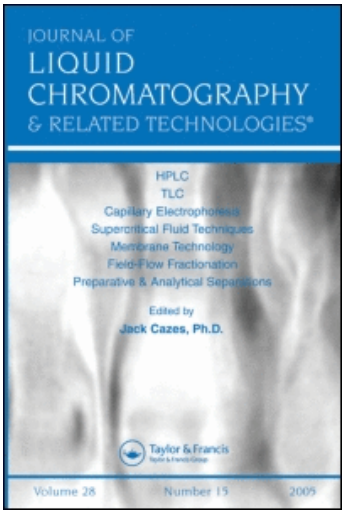


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Comparison of Reversed Stationary Phases for the Chromatographic Separation of Inorganic Analytes Using Hydrophobic Ion Mobile Phase Additives

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**To cite this Article** Smith, Ronald L. , Iskandarani, Ziad and Pietrzyk, Donald J.(1984) 'Comparison of Reversed Stationary Phases for the Chromatographic Separation of Inorganic Analytes Using Hydrophobic Ion Mobile Phase Additives', Journal of Liquid Chromatography & Related Technologies, 7: 10, 1935 — 1959
**To link to this Article: DOI:** 10.1080/01483918408068847
**URL:** http://dx.doi.org/10.1080/01483918408068847

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(10), 1935-1959 (1984)

# COMPARISON OF REVERSED STATIONARY PHASES FOR THE CHROMATOGRAPHIC SEPARATION OF INORGANIC ANALYTES USING HYDROPHOBIC ION MOBILE PHASE ADDITIVES

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## ABSTRACT

Alkyl-modified silica (RSi) and polystyrenedivinylbenzene (PRP-1) stationary phases are compared for the chromatographic separation of inorganic analyte anions and cations using hydrophobic ions of opposite charge as mobile phase additives. Tetraalkylammonium salts were used for anion separations and alkyl sulfonate salts for cation separations. Two major equilibria influence the retention of analyte ions on PRP-1. These are: retention of the hydrophobic ion on PRP-1 and an ion exchange selectivity between the hydrophobic counterion and the analyte ion. When using RSi retention is also influenced by ion exchange at residual silanol groups, which act as weak cation exchange sites. Mobile and stationary phase variables that influence analyte retention are identified. Optimization of these provides favorable eluting conditions for the separation of inorganic ionic analytes. Of particular interest is the potential use of PRP-1 and RSi columns for the separation of inorganic cations; conditions for the separation of alkali metals and alkaline earths are discussed.

## INTRODUCTION

A useful reversed phase liquid chromatographic (RPLC) technique for the separation of charged organic analytes, often called ion pair chromatography (IPC), is to add a hydrophobic ion of opposite charge to the predominately aqueous mobile phase and



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take advantage of an enhanced analyte retention. Although many different hydrophobic ions can be used, most applications employ alkylsulfonate ($RSO_3^-$) salts or tetraalkylammonium ($R_4N^+$) salts for the separation of organic analyte cations and anions, respectively. Recent studies have demonstrated that this approach can also be used for the separation of inorganic cations (1-4) and anions (1,5-9) and should compliment the inorganic ion exchange LC procedures known as ion chromatography (10).

Several views concerning the importance of the interactions between the organic analyte ion, hydrophobic ion, and the stationary and mobile phase have emerged as IPC has been developed. A recent review that focuses on $R_4N^+$ salts as mobile phase additives documents these different views (11). Clearly, one model does not fit all possible experimental situations. For predominately aqueous mobile phases and low concentrations of hydrophobic ions of modest hydrophobicity one major view suggests that ion pairs form between the hydrophobic ion and analyte ion prior to retention onto the stationary phase. The other suggests that the hydrophobic ion is first retained by the stationary phase and ion pairing or ion exchange takes place between the analyte ion and the charged site provided by the retained hydrophobic ion. These models, their variations, and evidence supporting them are discussed in detail elsewhere (11,12-20).

Recent studies of inorganic analyte anion and cation retention using $R_4N^+$ (7,19) and $RSO_3^-$ (20) salts, respectively, and a polystyrenedivinylbenzene (PSDB) copolymeric nonpolar adsorbent as the stationary phase suggest that the enhanced analyte retention is the result of the contribution of two key equilibria. One describes retention of the hydrophobic ion onto the stationary phase surface while the second describes an ion exchange selectivity between the analyte ion and the counterion

accompanying the hydrophobic ion. This mode of interaction is similar to the ion interaction (dynamic ion exchange) model suggested to account for analyte ion retention onto an alkyl-modified silica (RSi) stationary phase from an aqueous-organic modifier mobile phase containing a hydrophobic ion additive (11,14,17). Applying this model to organic analyte ion retention must be done with caution since the hydrophobic nature of organic ions can vary widely. Conductance studies (see 7, 15, and references within) have suggested that association between hydrophobic and organic analyte ions can be appreciable and depends on the hydrophobicity of both ions. For example, association constants for the more polar catecholammonium octylsulfonate salts are reported to be about $18M^{-1}$ while for the less polar octylammonium octylsulfonate salt the constant is $500M^{-1}$ (15). Thus, at some point contribution of ion association (ion pairing) in the mobile phase must also be considered as the hydrophobicity of either or both the organic analyte ion and hydrophobic ion mobile phase additive increases. In contrast conductance data (7,21) strongly suggest that association between certain inorganic ions and hydrophobic ions of opposite charge is negligible particularly if the R groups in the hydrophobic salts are of modest hydrophobicity, hydrophobic salt concentrations are well below critical micelle formation, and mobile phase solvent mixtures are predominately aqueous.

There are several intrinsic differences between RSi and PSDB stationary phases even though both are reversed phases. This report focuses on a comparative study of these two stationary phases using $R_4N^+$ and $RSO_3^-$ salts as mobile phase additives and inorganic anions and cations, respectively, as analytes. These analytes were used for two major reaons. First, equilibria involving inorganic analytes should be less complex than with organic analytes because: 1) association equilibria between inorganic analyte ions and hydrophobic ions should be

minimal; 2) for many inorganic analytes dissociation via pH is unnecessary; and 3) these analytes should not be retained by the stationary phases in the absence of the hydrophobic ions. Second, from a practical viewpoint, this single column procedure has many potential applications in the separation of inorganic ions and should be a viable alternative to ion chromatography (10).

## MATERIALS AND METHODS

### Materials

Analytical reagent grade inorganic salts, acids, and bases, tetrapentylammonium ($TPeA^+Br^-$) bromide and sodium octane sulfonate ($C_8SO_3^-Na^+$) were obtained from Aldrich, Eastman Kodak, or Sigma Chemical Co. TPeAF was prepared as previously described (19). $C_8SO_3^-Li^+$ was prepared by passing the Na salt through a strong acid cation exchanger in a $H^+$ form and subsequently titrating $C_8SO_3^-H^+$ with a standard LiOH solution. MeOH and $CH_3CN$ were obtained as LC quality from MCB Manufacturing Co. LC quality water was prepared with a Sybron/Bronstead water purification unit.

Prepacked columns were obtained from Hamilton Co. (PRP-1) and DuPont (Zorbax $C_1$, $C_8$, $C_{18}$). The PRP-1 column is a PSDB, 10 μm, spherical particle while the Zorbax columns are 6 μm, spherical, alkyl-modified silica particles where the alkyl groups are methyl, octyl, or octadecyl, respectively. The columns are 150 mm x 4.6 mm i.d. except PRP-1 which is 4.2 mm i.d.

### Instrumentation

A Waters 202 and Altex 421 LC were used with a Beckman 160 selectable wavelength, a Spectra Physics 770 variable wavelength, or a Wescan 213 or 213A conductivity detector.

## Procedures

Procedures for column conditioning, changing of counterion form, and determination of breakthrough volumes are described elsewhere (7,19,20). HCl, NaOH, and phosphate salts, except where noted, were used to adjust mobile phase pH. Ionic strength was fixed when desired by the addition of known amounts of inorganic electrolyte. All solvent mixtures are per cent by volume and column temperature (25 ± 1°C) was ambient.

Analyte solutions, prepared by dissolving 1 to 5 mg per 5 mL of $H_2O$, were stored in closed containers and refrigerated when not in use. Sample aliquots were 1 to 5 µl. Column inlet pressures ranged from 500 to 3000 psi depending on the column, mobile phase, and flow rate (usually 1 or 2 mL/min). Detection was at 254 nm or by conductance. Capacity factors were calculated in the usual way where the column void volume was determined by using several samples that were known to have no retention at the mobile conditions being tested.



## RESULTS AND DISCUSSION

Mobile and Stationary Phase Variables. Two equilibria which appear to be the major factors influencing the enhanced retention of inorganic analyte anions or cations on PRP-1 from a mobile phase containing a hydrophobic ion (7,20) are given by eq. 1 and 2

$$A + R_4N^+ + C^- \underset{}{\overset{K_1}{\rightleftharpoons}} A\cdots R_4N^+C^- \quad (1a)$$

$$A\cdots R_4N^+C^- + X^- \overset{K_2}{\rightleftharpoons} A\cdots R_4N^+X^- + C^- \quad (1b)$$

(1)

$$A + RSO_3^- + C^+ \overset{K_1}{\rightleftharpoons} A\cdots RSO_3^-C^+ \quad (2a)$$

$$A\cdots RSO_3^-C^+ + X^+ \overset{K_2}{\rightleftharpoons} A\cdots RSO_3^-X^+ + C^+ \quad (2b)$$

(2)

where A is the stationary phase, $R_4N^+$ and $RSO_3^-$ are the mobile phase additives, X is the analyte ion, and C is the counterion. Equations 1a and 2a describe the retention of the hydrophobic ion on PRP-1 while eqs. 1b and 2b describe an ion exchange selectivity between the analyte ion and any counterions that are part of the mobile phase due to the presence of hydrophobic, ionic strength, and buffer salts. Both experimental evidence and control of experimental conditions are consistent with this view and are discussed elsewhere (7,19,20).

The relationship between retention of the analyte, the mobile phase variables, and the equilibrium constants defining the equilibria (7,19,20) is given by

$$1/k'_X = \frac{1}{q\,K_o}\left[[X]_m + \frac{1}{K_1[L]_m} + \frac{[C]_m}{K_2}\right] \qquad (3)$$

where $k'_X$ is the capacity factor for the retention of the analyte ion, q is the ratio of stationary phase volume to mobile phase volume, $K_o$ is the sorption capacity for PRP-1, and m is the mobile phase. When a $R_4N^+$ salt is in the mobile phase X, L, and C, are the analyte anion, hydrophobic cation, and counteranion concentration, respectively, and $K_1$ and $K_2$ are equilibrium constants for retention of the $R_4N^+$ salt and for the ion exchange selectivity between the analyte anion and a given counteranion; for $RSO_3^-$ salts X, L, and C are analyte cation, hydrophobic anion, and countercation concentrations, respectively, and $K_1$ and $K_2$ are equilibrium constants for the retention of the $RSO_3^-$ salt and an ion exchange selectivity between the analyte cation and a given countercation, respectively. The significance of eq. 3 is that it focuses on the key equilibria and the controllable mobile phase variables that influence analyte retention. For PRP-1 and defined mobile phase conditions eq. 3 was consistent with retention data when using inorganic analyte anions and

cations and $R_4N^+$ (7) and $RSO_3^-$ (20) salts as mobile phase additives, respectively.

Preliminary experiments indicated that eqs. 1-2 also apply to RSi. When analytical samples of $R_4N^+$ and $RSO_3^-$ salts were used their retention on $C_1$, $C_8$, and $C_{18}$ at controlled mobile phase conditions: 1) increased as R group hydrophobicity increased; 2) increased as the mobile phase organic modifier: water ratio decreased; 3) was greater in MeOH:$H_2O$ over $CH_3CN$:$H_2O$ at identical solvent ratios; 4) increased as ionic strength increased; and 5) was dependent on the type of counter-ion accompanying the $R_4N^+$ or $RSO_3^-$ analyte salt where retention of the $R_4N^+C^-$ and $RSO_3^-C^+$ analytes for the counterion C followed the order

$$I^- > NO_3^- > Br^- > NO_2^- > Cl^- > F^- \quad (4)$$

and

$$M^{+2} > Cs^+ > Rb^+ > K^+ > Na^+ > Li^+ \quad (5)$$

These trends are similar to those found when using PRP-1 (7,19, 20).

A well-defined linear relationship was found on PRP-1 (7,19,20) when plotting retention, $1/k'$, of analytical samples of $R_4N^+$ and $RSO_3^-$ salts versus $1/\sqrt{\mu}$, where $\mu$ is the mobile phase ionic strength. This indicates analyte retention occurs in a double layer (22,23), where the hydrophobic ion occupies the primary layer at the PRP-1 surface and the counterion occupies a diffuse secondary layer. A similar result was found when using the $C_1$, $C_8$, and $C_{18}$ and several different $RSO_3^-$ salts as analytes. This is illustrated in Fig. 1 for $C_8SO_3^-Na^+$ as the analyte indicating $RSO_3^-$ salt retention occurs as a double layer. The mobile phase solvent for $C_1$ and PRP-1 was 1:9 $CH_3CN$:$H_2O$ while for

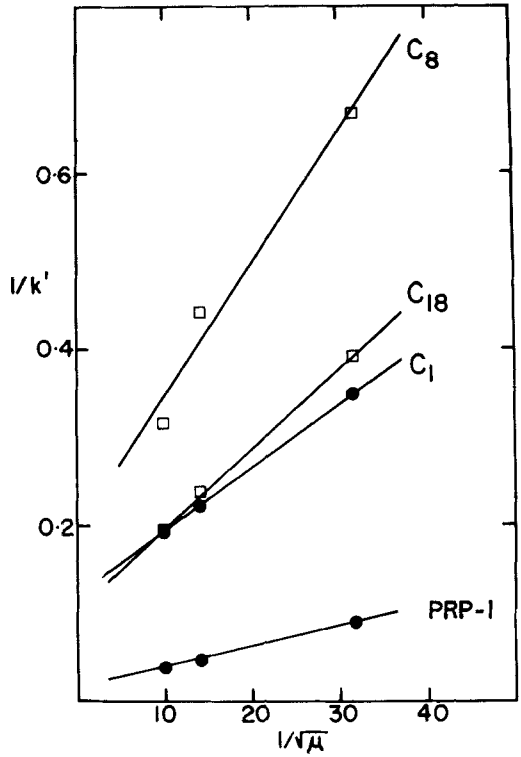



Figure 1 Relationship between analyte retention on RSi and PRP-1 and mobile phase ionic strength.



$C_8$ and $C_{18}$ it was 1:1 MeOH:$H_2O$; the ionic strength covered the range $1.0 \times 10^{-3}$ to $1.0 \times 10^{-2}$M NaCl.

Attempts to determine a similar relationship between 1/k' and $1/\sqrt{\mu}$ for retention of analytical samples of $R_4N^+$ salts on $C_1$, $C_8$, and $C_{18}$ were not successful. This was not the case when PRP-1 was used (20). It appeared that the $R_4N^+$ salts ($R > C_2$) were never eluted from the column even when 100% $CH_3CN$, a very strong eluent if retention is hydrophobic in nature, was used. The reason for this, which became clear in subsequent experiments, is due to a second type of interaction, namely, ion ex-

change between $R_4N^+$ and $H^+$ at the free -SiOH sites within the RSi stationary phase.

An inherent difficulty in comparing PRP-1 and RSi is their intrinsic difference in their ability to sorb hydrophobic ions, eqs. 1a and 2a. For a given mobile phase composition the amount of hydrophobic ion retained (moles/column) will vary for the four columns. Thus, the number of electrostatic interactions between the retained hydrophobic ion and the analyte ion of opposite charge (see exchange equilibria in eqs. 1b and 2b) will differ between the stationary phases. If an electrostatic interaction, as the data appear to indicate, is a major retention process, then a viable comparison can be made only when the number of such interactions are normalized. This requirement was satisfied by maintaining the hydrophobic ion concentration in the mobile phase at a constant concentration and varying the mobile phase solvent composition (organic solvent:$H_2O$ ratio) so that the amount of retained hydrophobic ion on the stationary phase was approximately the same.



Figure 2 illustrates the retention isotherms for the retention of $TPeA^+F^-$ and $C_8SO_3^-Na^+$ on PRP-1, $C_1$, $C_8$, and $C_{18}$ as a function of hydrophobic ion concentration in the mobile phase. The amount retained was calculated from breakthrough volumes obtained by passing a mobile phase of defined concentration through the column and monitoring the column effluent for appearance of the hydrophobic ion. Since appearance time and the mobile phase concentrations are known the amount retained can be calculated. Manipulating the $CH_3CN$:$H_2O$ ratio ($R_4N^+$ salts) or the MeOH:$H_2O$ ratio ($RSO_3^-$ salts) controls the amount of retained hydrophobic ion. Data for one set of solvent compositions where the isotherms are nearly equal are shown in Fig. 2. As hydrophobic ion concentration increases the amount retained (number of apparent ion exchange sites, or the

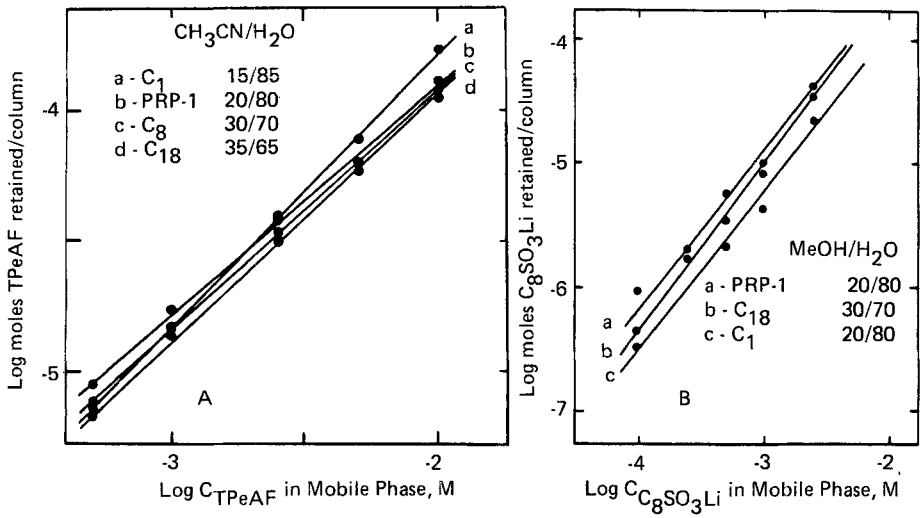



Figure 2 Sorption isotherms on RSi and PRP-1 at a solvent composition that provides the same number of retained hydrophobic ion sites.



apparent ion exchange capacity) increases. For TPeAF and the concentration range studied retention from a mobile phase containing an organic modifier follows the order $C_{18} > C_8 > PRP\text{-}1 > C_1$ while for $C_8SO_3Li$ retention the order is $C_{18} > PRP\text{-}1 > C_8 > C_1$. Thus, organic modifier was adjusted accordingly so that the amount of hydrophobic salt retained at a given mobile phase hydrophobic salt concentration would be the same for the four columns. Increasing mobile phase $R_4N^+$ or $RSO_3^-$ salt concentration or decreasing organic modifier increases the number of ion exchange sites.

According to eq. 3 analyte retention is indirectly proportional to analyte and counterion concentration and directly to hydrophobic ion concentration. These trends were followed for the retention of inorganic anion and cation analytes when using PRP-1 and $R_4N^+$ (7) or $RSO_3^-$ (20) salts, respectively.

Similarly, an indirect relationship between analyte retention and analyte or co-ion concentration was found when using RSi stationary phases (24). The dependence on analyte concentration occurs only at higher concentrations. At low analyte concentration the analyte term in eq. 3 becomes negligible in comparison to the other terms, and at these conditions retention is independent of analyte concentration. Subsequent column experiments and separations were carried out, in general, at these latter conditions. When different electrolytes were used to establish that analyte retention at a fixed hydrophobic ion concentration on RSi is indirectly related to the counterion concentration, analyte retention varied with the type of counterion used. This is consistent with an ion exchange like selectivity as shown in eqs. 1b and 2b. Thus, for different ionic strength salts inorganic analyte anion or cation retention changes, just like with PRP-1 (7,20), according to the selectivity order listed in eqs. 4 and 5, respectively. That is, analyte anion and cation retention is the highest for $F^-$ and $Li^+$ salt solutions, respectively, at constant hydrophobic ion concentration. However, when inorganic analyte retention was determined as a function of hydrophobic ion concentration (see Fig. 3), several differences between the PRP-1 and RSi were apparent. Since the number of ion exchange sites due to retained hydrophobic ion is approximately the same for the four columns, this factor is not responsible for the differences.

In a $R_4N^+$ salt mobile phase retention of inorganic analyte anions on PRP-1 increases with $TPeA^+$ salt concentration and passes through a well-defined maximum; only $NO_2^-$ retention is shown in Fig. 3a. The maximum is less defined on RSi and appears to require a higher $TPeA^+$ salt concentration. The major difference is a reduced retention on RSi compared to PRP-1. Similar results were found when using other monovalent inorganic analytes. The maximum is consistent with an ion exchange like

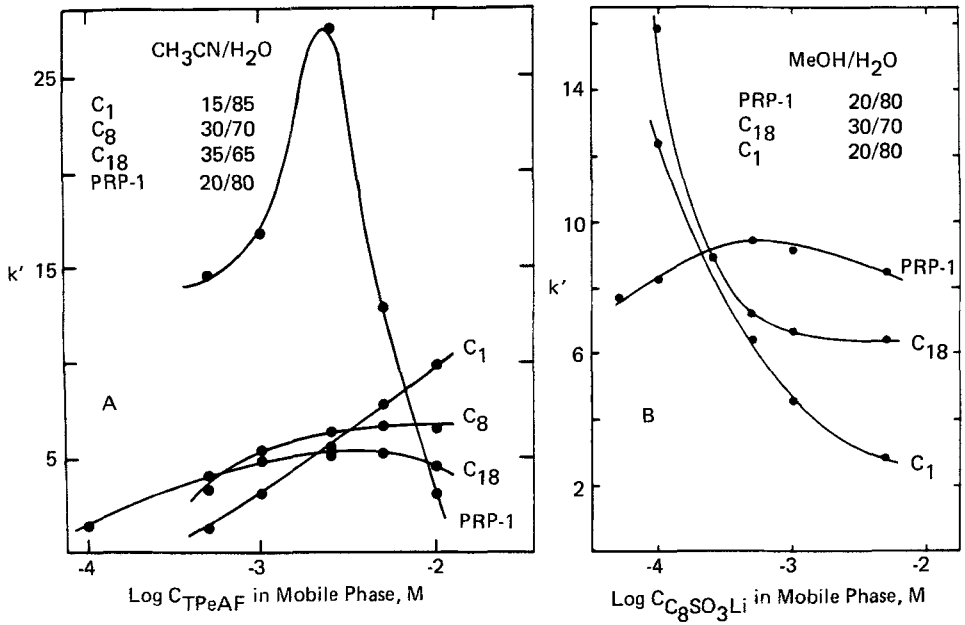



Figure 3 Retention of analyte ion on RSi and PRP-1 as a function of hydrophobic ion concentration. Analyte is $NO_2^-$ (A) and $K^+$ (B); mobile phase composition is adjusted to provide the same number of retained hydrophobic ion sites in (A) and (B).

selectivity, as shown in eq. 1b. As the $R_4N^+$ salt concentration increases, its retention, and subsequently the number of exchange sites, becomes larger causing increased analyte retention. Since the counteranion concentration also increases, it competes with the analyte anion for the charge site and causes analyte retention to decrease (analyte retention is inversely related to counteranion concentration as shown in eq. 3) due to mass action and the ion exchange selectivity constant for the exchange between the analyte anion and the counteranion; this competition is discussed in detail elsewhere (7,19,20). For a $RSO_3^-$ salt

and $K^+$ (or other inorganic cations) as the analyte (see Fig. 3B) retention on RSi differs significantly from that on PRP-1. On PRP-1 a well-defined maximum is observed and is consistent with the ion exchange like selectivity shown in eq. 2b and the influence of mass action and selectivity due to the counter-cation. In contrast, retention increases on RSi as the $RSO_3^-C^+$ concentration approaches zero and no maximum was found even when dilute $RSO_3^-C^+$ solutions were examined. The data further suggest that inorganic cations are retained by RSi in the absence of $RSO_3^-$ and that retention differs between these cations. This was verified in subsequent experiments (see Fig. 7).



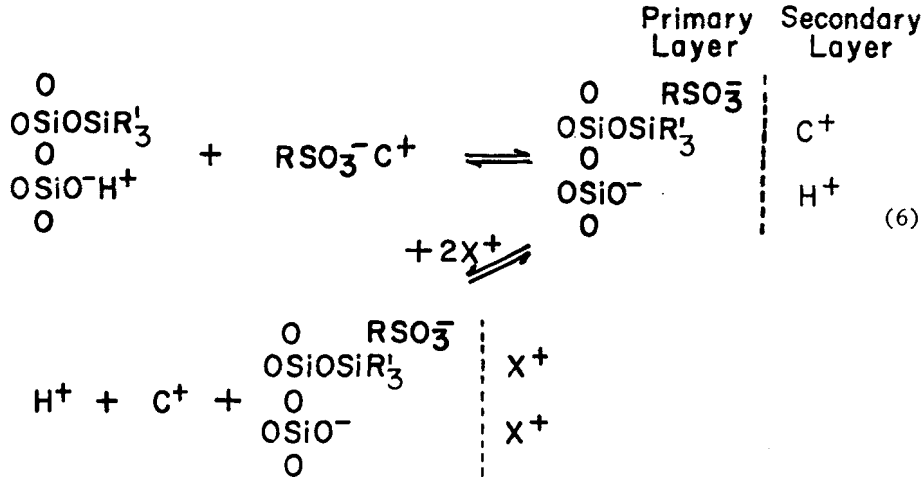
These trends on RSi are consistent with the presence of two types of cation exchange sites. One is provided by the retained hydrophobic ion while the other, we conclude, is provided by the residual -SiOH groups on the RSi stationary phase. When using a $R_4N^+$ salt as an analyte, its retention on the RSi, as indicated previously, is very high because of cation exchange at the -SiOH group. Similarly when $R_4N^+$ is used as a mobile phase additive it is partially consumed by the -SiOH cation exchange site. Thus, the available hydrophobic ion exchange sites indicated by the isotherms in Fig. 2A are less than that shown and analyte anion retention on RSi compared to PRP-1 (see Fig. 3A) is less. When $RSO_3^-$ salts are used, the retention of $K^+$ and other inorganic cations on RSi is high even at low $RSO_3^-$ salt concentration because of the availability of the -SiOH exchange sites. As the $RSO_3^-$ salt concentration increases, the RSi coverage increases probably making the -SiOH exchange sites less accessible and causes the exchange between the $RSO_3^-C^+$ sites and the analyte cation, see eq. 2, to become the more important interaction.

The presence of -SiOH exchange sites in RSi and their chromatographic effects have been noted by many workers (25-28). Several have suggested using short chain $R_4N^+$ cations to mask the

-SiOH groups via cation exchange (25-27). It is also likely that RSi stationary phases obtained from different manufacturers will differ in amounts of residual -SiOH exchange sites; only Zorbax RSi stationary phases were used in this study.

A typical silica is estimated to have about 7 $\mu mol/m^2$ of reactive, free silanol groups (29). Depending on the derivatization procedure, approximately half of these are available for conversion to the -SiOR group. Thus, a maximum mono-layer phase coverage of organic material of about 3.5 $\mu mol/m^2$ is obtained; it is not unusual for commercial R-Si stationary phases to be below this value. Because of steric properties the number and accessibility of the remaining -SiOH groups should differ between $C_1$, $C_8$, and $C_{18}$. Any other variable which influences accessibility will therefore also influence exchange capacity. Our experiments, based on breakthrough measurements indicated a cation exchange capacity of about 4 to 17 μmole/column for the three RSi columns; capacities of 10 to 15 μmole/column have been reported for a $C_8$ column (30). Although the data suggested a difference between the three RSi columns an accurate determination of the exchange capacity at these low levels is difficult because -SiOH is a weak acid and it shows a high exchange selectivity for $H^+$. Even the higher capacities reported for silica (0.2 to 1.0 mmole/g) are difficult to determine accurately (29).

The two electrostatic interactions that appear to be responsible for the retention of inorganic cation analytes, $X^+$, on RSi from mobile phases containing $RSO_3^-$ salts are schematically shown in eq. 6. The corresponding equilibria contributing to this retention are: 1) retention of the $RSO_3^-C^+$ on RSi (eq. 2b); 2) an ion exchange selectivity between the countercation accompanying the $RSO_3^-$ salt and the inorganic analyte cation; 3) dissociation of the weak acid -SiOH group; its $pK_a$ is estimated to be 4 to 7 (25,29); and




(6)

4) ion exchange selectivities between the countercations, $C^+$ and/or $H^+$ associated with the silanol sites, and the analyte cation.

If it is assumed that analyte cation retention at the accessible -SiOH exchange sites is electrostatic and is only by cation exchange, retention at this site, considering the cation exchange selectivities and the ionization of the -SiOH site, can be shown (24,30) to be given by

$$1/k'_{X^+} = \frac{1}{q\,K_o}\left[\frac{[H^+]_m(1 + K_a)}{K_3} + [X^+]_m + \frac{[C^+]_m}{K_4}\right] \qquad (7)$$

where $k'_{X^+}$ is the capacity factor for the retention of $X^+$ by cation exchange at the -SiOH site, q is the ratio of stationary phase volume to mobile phase volume, $K_o$ is the available exchange capacity, m is the mobile phase, $K_a$ is the ionization constant for the -SiOH site, and $K_3$ and $K_4$ are cation exchange selectivities according to eqs. 8 and 9, respectively. Thus, inorganic cation retention at the -SiOH sites is indirectly

$$-SiO^-H^+ + X^+ \underset{}{\overset{K_3}{\rightleftharpoons}} -SiO^-X^+ + H^+ \qquad (8)$$

$$-SiO^-C^+ + X^+ \underset{}{\overset{K_4}{\rightleftharpoons}} -SiO^-X^+ + C^+ \qquad (9)$$

proportional to $H^+$, analyte cation (at low concentration retention becomes independent of analyte concentration), and countercation concentration, to silanol $K_a$, and directly to the cation exchange selectivities between the analyte cation and mobile phase cations. Combing eqs. 3 and 7 to account for cation exchange at both the retained $RSO_3^-$ site and the -SiOH site (24) yields

$$1/k'_{X^+} = \frac{1}{q\,K'_o}\left[\frac{1}{K_1[RSO_3^-]_m} + \frac{(1+K_a)[H^+]_m}{K_3} + [X^+]_m + K_2K_4[C^+]_m\right] \qquad (10)$$

where $k'_{X^+}$ is now the capacity factor for retention at the two sites, $K'_o$ is total retention capacity due to the two exchange sites, $K_1$ is an equilibrium constant for the retention of the $RSO_3^-$ salt, $K_2$ is the cation exchange selectivity at the $RSO_3^-$ site for exchange between the analyte cation, $X^+$, and a mobile phase countercation, $C^+$, $K_3$ and $K_4$ are cation exchange selectivities for cation exchange at the -SiOH site according to eqs. 8 and 9, respectively, and $K_a$ is the ionization constant for the -SiOH group. If the analyte cation concentration is low enough then its retention is independent of concentration and $[X^+]$ is insignificant in eq. 10. A practical consequence of eq. 10 is that it identifies the key mobile phase parameters and equilibria and how they can be manipulated in order to optimize separation of inorganic analyte cations on RSi. For example, increasing $RSO_3^-$ salt concentration and decreasing $H^+$ and countercation con-

centration should increase analyte retention. However, the range over which these can be adjusted is not unlimited because of additive and/or competitive effects. Thus, using buffer salts to control pH and -SiOH ionization will also contribute to $C^+$ concentration. Similarly, increasing the $RSO_3^-C^+$ concentration will increase its countercation concentration. Adjustment of the type of countercation will also influence analyte cation retention because of the cation exchange selectivities and eluting power can be altered according to eq. 5. In general, this selectivity order is similar to that observed when using conventional strong acid cation exchangers (32).



Separations. Figure 4 shows the separation of a mixture of four inorganic anions on PRP-1, $C_1$, $C_8$, and $C_{18}$ using a TPeAF mobile phase. The exchange capacity due to the retained $TPeA^+$ salt is the same for the four columns (15 $\pm$ 1 μmole/column); this was accomplished by adjusting the solvent mixture according to the isotherms in Fig. 2. The retention order is the same on the four columns and is also identical to the order found for typical strong base anion exchangers (32). Resolution at these conditions is better on PRP-1 because of a higher retention and a more favorable selectivity even though efficiency, which favors the order $C_{18} > C_8 > C_1$, is more favorable with the RSi columns (part of this is due to smaller RSi particles and lower retention times). Increasing the ionic strength or using a counteranion of greater eluting power (see eq. 4) reduces analyte retention. The reasons for using TPeAF and the concentrations listed are provided elsewhere (7,19). In general, it would appear that the high efficiency offered by $C_{18}$ would be preferable, however, if a basic (pH > 8) mobile phase condition is required, as would be the case for anions derived from certain weak acids, only PRP-1 would be compatible with this condition.

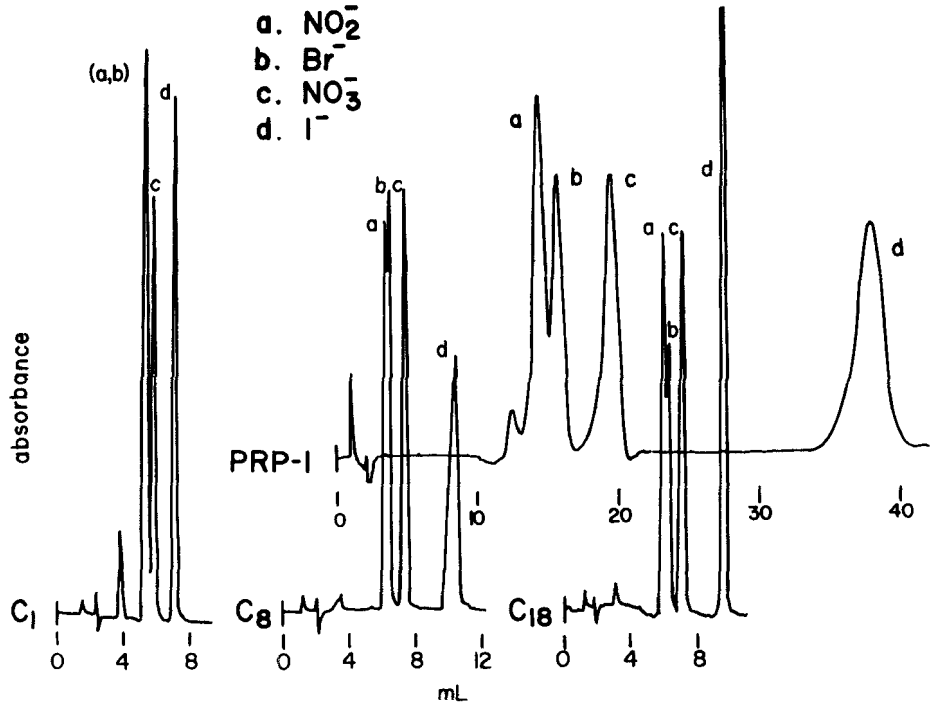



Figure 4 Separation of inorganic anions on PRP-1 and RSi columns using a $TPeA^+F^-$ mobile phase additive. PRP-1: A 22:78 $CH_3CN:H_2O$, 1.0 x $10^{-3}$M $TPeA^+F^-$ mobile phase; $C_1$: Same except 15:85 $CH_3CN:H_2O$; $C_8$: Same except 30:70 $CH_3CN:H_2O$; $C_{18}$: Same except 35:65 $CH_3CN$; at 1.0 mL/min flow rate.

Figures 5 to 7 focuses on the parameters that influence inorganic cation retention. Chromatograms for the separation of alkali metals are shown in Fig. 5. In Fig. 5, the MeOH:$H_2O$ ratio is adjusted to fix the exchange capacity of the retained $C_8SO_3^-$ salt at 30 μmole/column (see isotherms in Fig. 2B). When compared with PRP-1 with a similar number of sites (20), retention on the $C_8$ is higher apparently due to the contribution of the -SiOH exchange sites.

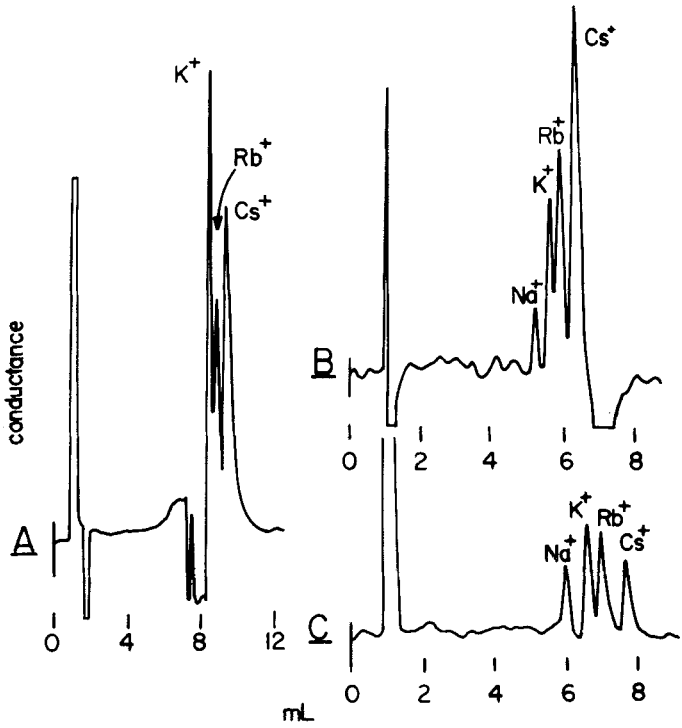



Figure 5 Separation of alkali metal cations on a Zorbax $C_8$ column as a function of mobile phase variables.

(A) A 27.5:72.5 MeOH:$H_2O$, $2.5 \times 10^{-3}$M $C_8SO_3^-Li^+$ mobile phase; (B) A 100% $H_2O$, $1.0 \times 10^{-3}$M $C_8SO_3^-Li^+$, $1.0 \times 10^{-2}$M LiCl, $1.0 \times 10^{-3}$M HCl (pH=2.9) mobile phase; (C) Same as B except $1.0 \times 10^{-5}$M HCl (pH=5.2) mobile phase; at a 1.0 mL/min flow rate.

The mobile phase solvent composition has opposing effects on retention. When the MeOH increases at low MeOH:$H_2O$ ratios retention drops because retention of the $RSO_3^-$ salt decreases. However, at higher MeOH ratios the MeOH influences the cation exchange selectivity. The former produces the more significant change. For example, k' for the retention of $Na^+$ on $C_{18}$ is 6.10

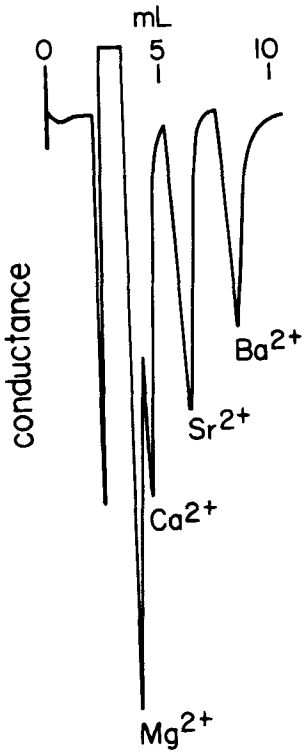



Figure 6 Separation of alkaline earth cations on a Zorbax $C_8$ column using a $C_8SO_3^-Li^+$ mobile phase additive. A 3:7 MeOH:$H_2O$, 5.0 x $10^{-4}$M $C_8SO_3^-Li^+$, 5.0 x $10^{-4}$M Na citrate (pH=7.0), 1.0 x $10^{-3}$M LiCl mobile phase at a 1.0 mL/min flow rate.

from a 1:10 $CH_3OH$:$H_2O$, 5.0 x $10^{-3}$M LiCl, 5.0 x $10^{-4}$M $C_8SO_3^-Li^+$ mobile phase. If the $C_8SO_3^-Li^+$ is omitted, the k' is 0.34 while at 9:1 MeOH:$H_2O$, 2.5 x $10^{-3}$M LiCl the k' is 1.29. These and similar data for other cation analytes suggest that the majority of the exchange sites at MeOH:$H_2O$ ratios, where the $RSO_3^-$ salt is retained (its k' > 3), are due to the $RSO_3^-$ salt and not the -SiOH sites.

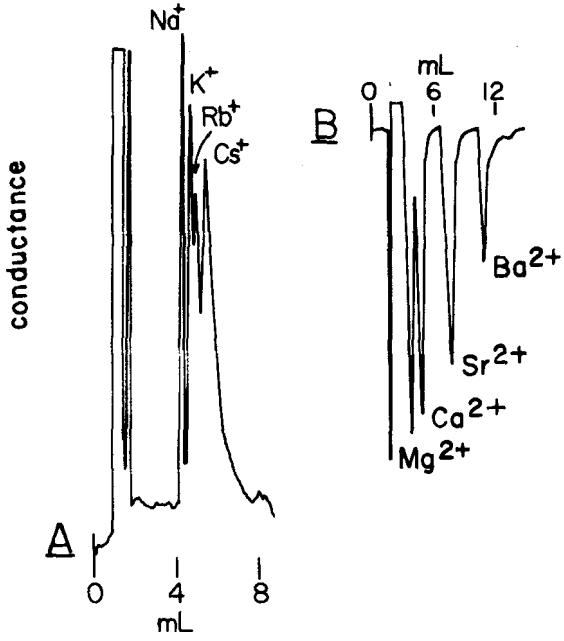



Figure 7 Separation of alkali metal (A) and alkaline earth (B) cations on a Zorbax $C_8$ column in the absence of a $RSO_3^-$ salt additive.

(A) A 3:7 MeOH:$H_2O$, 2.5 x $10^{-3}$M LiCl mobile phase and (B) A 3:7 MeOH:$H_2O$, 5 x $10^{-4}$M Na citrate (pH=7.0) mobile phase; at 1.0 mL/min flow rate.

Increasing the pH increases inorganic analyte retention due to ionization of the -SiOH sites, however, this effect is restricted by the upper pH limit (pH = 8) of RSi. In Fig. 5B retention and resolution is less favorable than at the higher pH used in Fig. 5C where retention is almost 15% greater. Increasing the pH also decreases the cation exchange selectivity due to $H^+$ but adds the selectivity effects of other cations if buffer salts are used. The location of the $Cs^+$ peak in Figure 5B is not well-defined because of a system peak caused by the $H^+$ and

detected by the conductivity detector. At a higher pH the system peak has much less effect on the detector response.

Cation retention follows the order shown in eq. 5 ($NH_4^+ \simeq Rb^+$) and is similar to that found on strong acid cation exchangers (32). This is also the order for eluent strength for countercations that accompany the $RSO_3^-$ salt or are introduced for ionic strength control or as buffer salts. Thus, $Li^+$ salts were usually used (Figures 5 to 7) since it provides the weakest eluting power. Switching to other countercations according to eq. 5 or increasing ionic strength will decrease retention.

Figure 6 illustrates the separation of alkaline earths on a $C_8$ column. Since divalent inorganic cations are more retained than monovalent ones the mobile phase eluting strength was increased to reduce analysis time. The complex mobile phase used and the effects of each component are predictable. 1) The $C_8SO_3^-$ salt provides many of the exchange sites. If its concentration is increased more sites are produced and retention is increased. However, eventually this is compensated for by increased eluting power due to higher countercation concentration. (Increased eluting power can be achieved by using a cation of greater selectivity.) 3) Since the divalent analytes are highly retained adding a ligand (citrate) sharply decreases retention due to analyte-ligand complex formation; increasing ligand concentration therefore decreases analyte retention. 4) The 3:7 MeOH:$H_2O$ ratio influences retention of the $RSO_3^-$ salt but has a larger effect on the formation constant for the analyte-ligand complex; increasing the MeOH decreases $RSO_3^-$ salt retention and increases the formation constant both of which contribute to reduced retention. 5) The mobile phase at pH = 7 provides a large number of dissociated -SiOH sites, thus, increasing analyte cation retention. 6) Adding LiCl improves the eluting power because of increased countercation concentration.

Figure 7 shows that even after omitting the $RSO_3^-$ salt from the mobile phase enough cation exchange capacity due to the residual -SiOH sites is available in the RSi column to effectively separate mixtures of alkali metal and alkaline earth cations. Since the total number of cation exchange sites is reduced due to the absence of retained $RSO_3^-$ salt, mobile phase eluting power is decreased and its pH is adjusted to favor -SiOH ionization. The alkyl group on RSi is not necessary for the separation of inorganic cations in the absence of the $RSO_3^-$ salt and its elimination should increase cation exchange capacity, retention times, and perhaps efficiency. Data illustrating the retention and separation of alkali and alkaline metal ions using ordinary silica columns are reported elsewhere (31).



## ACKNOWLEDGEMENTS

This work was presented at the 184th American Chemical Society Meeting, Kansas City, MO., Sept., 1982, Paper ANYL-29 and at the 1983 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March, 1983, Paper 946. The investigation was supported in part by a research grant awarded by the National Science Foundation.

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