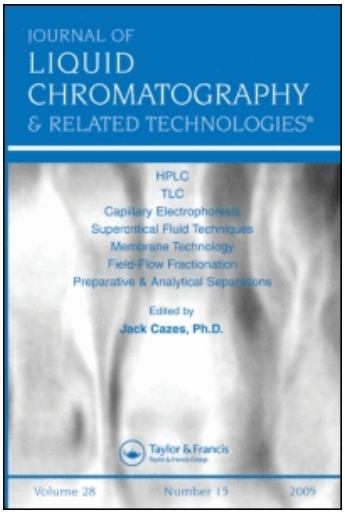

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INDIRECT UV DETECTION OF ORGANIC ANALYTE ANIONS USING A LOW-CAPACITY ANION EXCHANGE COLUMN

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ABSTRACT

Organic analyte anion retention on a low-capacity anion exchange column using indirect UV detection was studied. A combination of anion exchange/reversed-phase interactions were found to influence the retention of organic analyte anions provided the analytes have both an anionic charge site and a hydrophobic center. Organic analyte anion retention was found to be influenced by the following: concentration of organic modifier, concentration of UV absorbing analyte, pH, and mobile phase ionic strength. Correlation coefficients of better than 0.999 were found for several of the organic and inorganic analytes studied. Detection limits of 0.5 to 1.0 ppm were observed.

INTRODUCTION

The separation and detection of inorganic and organic analyte ions has been studied over the past several years. In 1975 Small, Stevens and Bauman (1) reported on a novel method for separating inorganic and organic anions followed by conductivity detection. This method was called ion chromatography and required the use of two columns in series. The first column, called the separator column, was a strong anion exchange column used for separating the analytes of interest. The second column, called a suppressor column, consisted of a strong cation exchanger that was used to neutralize or suppress the background conductance.

In 1979 and 1980, Fritz and co-workers (2-4) developed an ion chromatographic system that did not require the use of a suppressor column. A low-capacity ion exchange column was used for separating the analyte ions while the mobile phase was composed of a very dilute eluent that had a low background conductance. This nonsuppressed system provided separations and detection limits similar to that of the suppressed system

A major limitation in the early development of ion chromatography was the lack of sensitive and reliable detection methods. Therefore, new detectors and detection methods have been studied and reported on. These detection methods include: amperometric, potentiometric, UV absorbance, refractive index, fluorescence, atomic absorption and atomic emission (5).

One area of ion chromatography that has received considerable attention is indirect photometric or

vacancy chromatography (IPC). IPC is an analytical method where analyte ions are separated on an ion exchange column and are then detected through a photometric process (6-9). In IPC, an UV-active counterion is added to the mobile phase and competes with UV-transparent, injected analyte ions for the ion exchange sites. As the UV-transparent analyte ion elutes off the column, it replaces the UV-active counterion in the effluent. This leads to a decrease in absorbance at the detector and produces a negative peak. IPC provides the advantages of using conventional HPLC instrumentation and columns with greater sensitivity when compared to refractive index and conductometric detection (8,9). IPC allows standardless quantitation (8,10,11), and it is versatile.

Typically, IPC methods have used strong ion exchange columns for the separation of inorganic and organic UV-transparent analyte ions (11-16). However, reversed-phase chromatography has also been used with indirect UV-detection (17-19) as well as ion-interaction chromatographic separations (20-25). Takeuchi and co-workers (26) have reported on the separation of inorganic cations and anions on an alumina column using indirect UV detection. Recently, several publications have reported on the use of low-capacity polymer-based ion exchange columns in conjunction with indirect UV-detection (27,28). These ion exchange packings are composed of a high surface area, macroporous polystyrenedivinylbenzene copolymer. Several advantages are apparent with these packings: both ion exchange and reversed-phase sites are present, they are stable from pH 1 to 13, and mobile phases with

high organic concentrations may be used. Retention of an organic analyte ion on a low-capacity ion exchange column has been shown to be due primarily to two interactions (29-31); 1) adsorption of the organic analyte ion onto the nonpolar polymeric backbone, provided that the organic analyte has a hydrophobic center, and 2) ion exchange of the organic analyte ion in the diffuse part of the electrical layer resulting from the ion exchange site and its counterion. These types of ion exchangers have been used for the separation of both inorganic and organic analytes (29-33).

This paper describes the mobile phase parameters that affect the separation, indirect UV detection, and quantitation of inorganic anions, and mono- and di-carboxylic acids on a low-capacity polymeric-based anion exchange column.

EXPERIMENTAL

Apparatus

The liquid chromatographic apparatus used consisted of a WISP Model 710B autosampler, Waters Model 590 HPLC pump, Kratos Model 783 variable wavelength UV detector, Linear Model 500 strip chart recorder. The column used in this study was a 4.1 x 150 mm Hamilton PRP-X100 low-capacity anion exchange column available from Hamilton Company (Reno, NV, U.S.A.). The PRP-X100 column is a spherical, 10 µm poly(styrenedivinylbenzene)-based anion exchange column with an anion exchange capacity of 200 µEq/g. Flow rates of 1.0 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

Boric acid and HPLC grade acetonitrile were obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.). Potassium hydrogen phthalate, inorganic salts, mono- and di-carboxylic acids were purchased from The Aldrich Chemical Company (Milwaukee, WI, U.S.A.). All chemicals were reagent grade. HPLC grade water was obtained by passing de-ionized water through a Millipore water purification unit.

RESULTS AND DISCUSSION

Cantwell and co-workers (29-31,34) have shown that low-capacity ion exchange columns have a dual retention mechanism of ion exchange and adsorption for organic analyte ions that contain both a fixed charge site and a hydrophobic center. For this dual mechanism to be present, the stationary phase must be nonpolar, have a high surface area, and provide relatively few ion exchange sites. Adsorption of an organic analyte ion is dependent on the electrical potential of the surface and is affected by the number of ion exchange sites present while ion exchange is independent of electrical potential. The two mechanisms, ion exchange (IE) and adsorption (Ads), can be represented by the following equations:

$$A\text{-}NMe_3^+C^- + R\text{-}X^- + M^+ \underset{}{\overset{IE}{\rightleftharpoons}} A\text{-}NMe_3^+ {}^-X\text{-}R + C^- + M^+ \quad (1)$$

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

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

Organic analyte retention that is attributed to an ion exchange process will be influenced by mobile phase ionic strength, the concentration of counteranion in the mobile phase, mobile phase pH, and the number of anion exchange sites present on the stationary phase. As the mobile phase ionic strength is increased, retention of an analyte anion will decrease due to increased competition for the anion exchange sites. The pH of the mobile phase will affect the ionization of a weak acid. Therefore, retention due to anion exchange will be dependent on its degree of ionization. In this study both the buffer and the UV-active counteranion will influence both the mobile phase ionic strength and pH. The effect that the number of anion exchange sites has on organic analyte anion retention was not studied here but has been reported elsewhere (29-34).

An organic analyte that is retained by an adsorption process is affected by the concentration of organic modifier added to the mobile phase, the mobile phase ionic strength, mobile phase pH, the hydrophobicity of the organic analyte and the hydrophobicity of the stationary phase. Organic analyte retention will decrease as the concentration of the organic modifier that is added to the mobile phase is increased. Adjusting the ionic strength will produce a change in analyte retention. The pH of the mobile phase will affect the charge of the analyte and its hydrophobicity. As the number of anion exchange sites are increased, adsorption of an organic analyte will decrease. The effect of the number of anion exchange sites present was not studied here but has been reported elsewhere (29-34).

In Indirect Photometric Chromatography (IPC), the UV-active counteranion has the dual role of displacing an analyte anion from the anion exchange column and the detection of an UV-transparent analyte anion as a dip or trough in the baseline absorbance. When a low-capacity anion exchange column is used for separating organic analyte anions, the UV-active counteranion will be involved in both the detection of the organic analyte anion and in competing for the anionic exchange sites. If the UV-transparent analyte is charged, the UV-active counteranion will compete with the charged analyte for the anion exchange sites and will also participate in the indirect detection of the analyte anion. However, if the UV-transparent analyte is retained predominantly by adsorption, then the UV-active counteranions major role is in the indirect detection of the analyte.

An important mobile phase parameter that will influence the retention of an organic analyte anion is pH. For the analyte to be retained by anion exchange interactions, the mobile phase pH must be sufficiently high to insure that the analytes are ionized. Figure 1 shows the effect of the mobile phase pH on analyte retention. At pH 4, the organic analytes are sufficiently ionized so that they are retained. In this case, the analytes are retained by anion exchange or a combination of anion exchange/adsorption. If the pH was lowered to 2, then retention of the analytes, if un-ionized, would have been retained exclusively by adsorption (32). Fumaric acid is more highly retained at pH 4 than at pH 6.2. At pH 4, fumaric acid is mono-anionic and is retained by a dual anion exchange/adsorption mechanism. At pH 6.2, fumaric acid

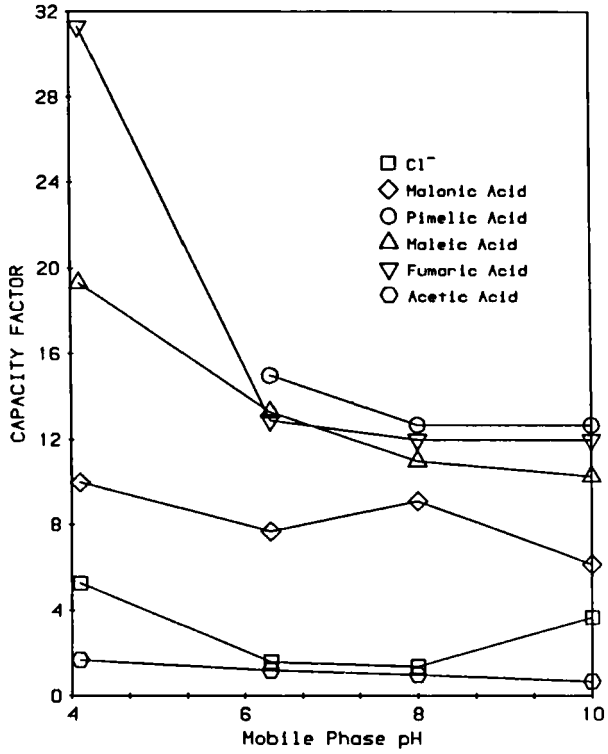

FIGURE 1

The Effect of Mobile Phase pH on Organic Acid Retention.

A 0.001 M KHP, 0.01 M H_3BO_3, 5:95 $CH_3CN:H_2O$ mobile phase.

is a di-anion and is retained predominantly by anion exchange. The retention of acetic acid, which is either partially or totally ionized over this pH range studied, did not change. This indicates that it was retained predominantly by an anion exchange mechanism. The other di-carboxylic acids follow a similar retention mode as did fumaric acid.

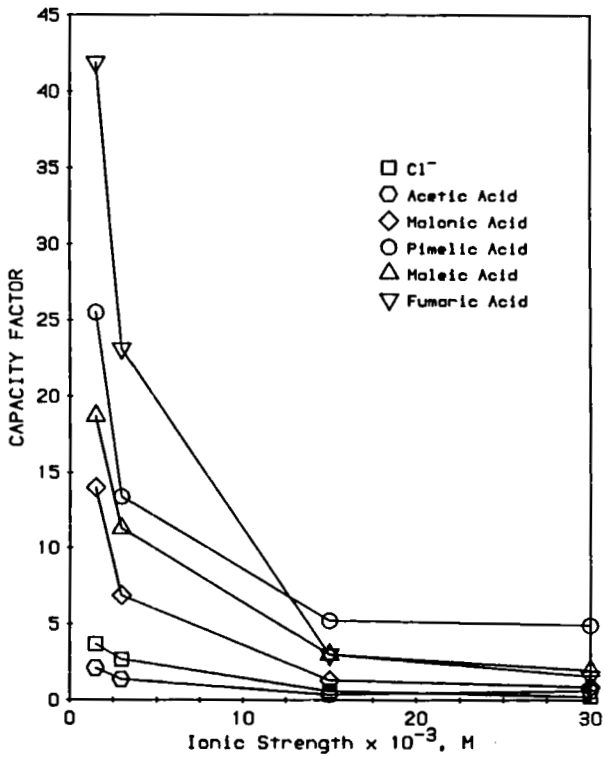

FIGURE 2

The Effect of Mobile Phase Ionic Strength on Organic Acid Retention.

A 0.001 M KHP, H_3BO_3, 5:95 $CH_3CN:H_2O$ mobile phase.

A second mobile phase parameter that will effect analyte retention is ionic strength. As the mobile phase ionic strength is increased, analyte retention will decrease due to increasing competition for the anion exchange sites (equation 1 will shift to the left). Figure 2 shows the effect of ionic strength on analyte retention. Just a slight increase in ionic

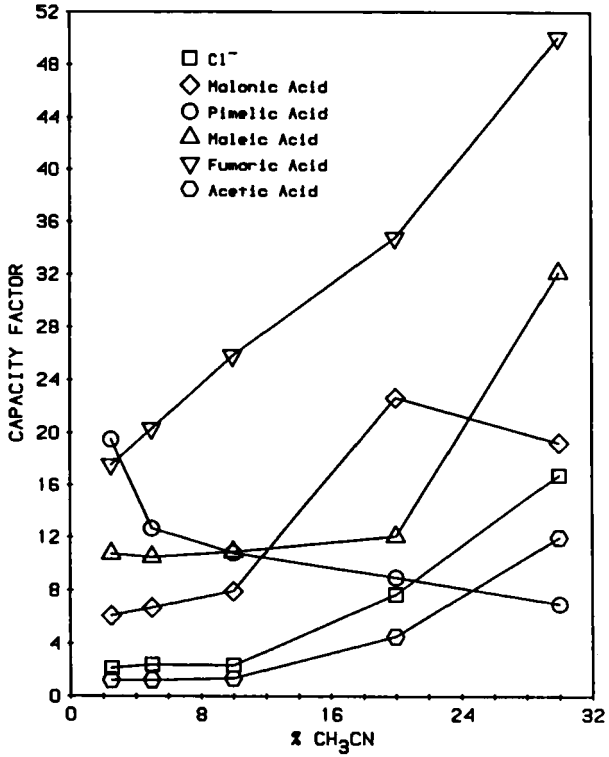

FIGURE 3

The Effect of Mobile Phase Organic Modifier on Organic Acid Retention.

A 0.001 M KHP, 0.01 M H_3BO_3, CH_3CN:H_2O mobile phase.

strength from 0.0005 M to 0.0010 M, resulted in analyte retention being decreased by nearly one-half. In this study, the concentration of both the UV-active counteranion and the buffer will affect the mobile phase ionic strength. Since the PRP-X100 is a low-capacity anion exchanger, it is more easily overloaded than a typical silica-based strong anion exchange

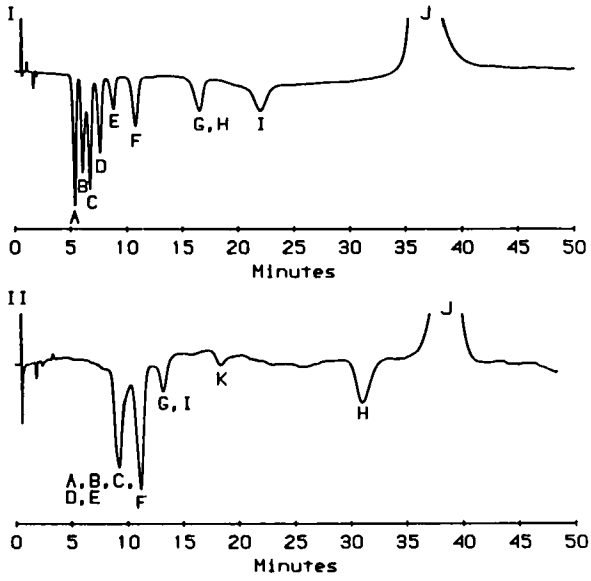

FIGURE 4

The Separation of 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, J) System Peak, K) Azelaic Acid, at Different Concentrations of Acetonitrile.

I) A 0.001 M KHP, 0.01 M H_3BO_3, 5:95 $CH_3CN:H_2O$ mobile phase.
II) A 0.001 M KHP, 0.01 M H_3BO_3, 20:80 $CH_3CN:H_2O$ mobile phase.

column. Therefore, the mobile phase ionic strength is critical in the separation of the analytes studied.

Figure 3 shows the effect that organic modifier has on the retention of several organic analytes. In general, the organic analytes that are retained predominantly by anion exchange (malonic, maleic, fumaric and acetic acid) increased in retention as the concentration of organic modifier increased whereas the

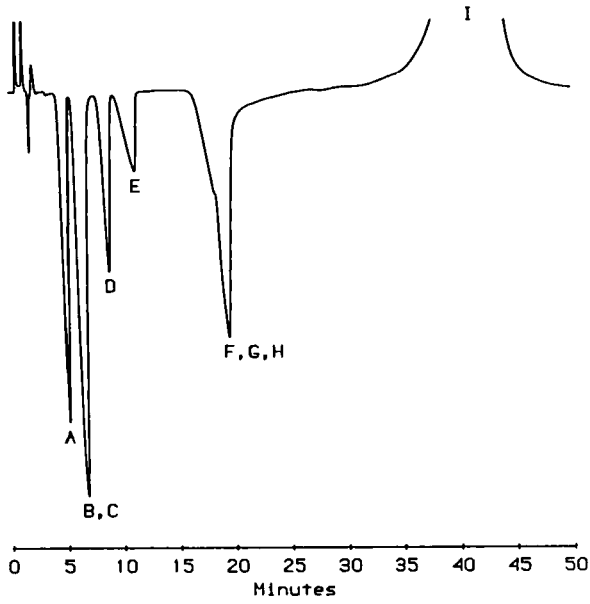

FIGURE 5

The Separation of 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) System Peak.

A 0.001 M KHP, 0.01 M H_3BO_3, pH 4.5, 2.5:97.5 $CH_3CN:H_2O$ mobile phase.

analytes that are retained predominantly by adsorption decreased in retention (pimelic acid). Increasing the mobile phase concentration of acetonitrile leads to a change in the relative polarity of the mobile and stationary phases. As the mobile phase becomes more nonpolar, the stationary phase, with respect to the mobile phase, increases in polarity. Analytes that are anionic 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 anion

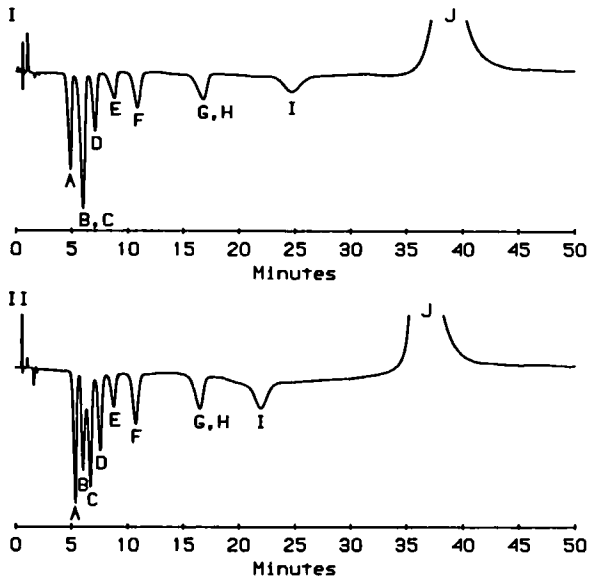

FIGURE 6

The Separation of 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, J) System Peak, at Different Ionic Strengths.

I) A 0.001 M KHP, 0.01 M H_3BO_3, pH 4.5, 5:95 $CH_3CN:H_2O$ mobile phase.
II) A 0.001 M KHP, pH 4.5, 5:95 $CH_3CN:H_2O$ mobile phase.

exchange sites as the concentration of acetonitrile increases. Figure 4 compares the separation of several di-carboxylic acids at two different concentrations of acetonitrile. Mobile phases consisting of five (I) and twenty percent (II) acetonitrile are shown. Even though retention times have increased for the more polar analytes at twenty percent acetonitrile, selectivity and resolution have decreased. The same separation is shown in Figure 5 except a mobile phase

of 2.5% acetonitrile was used, but it did not provide a better separation.

The effect of increasing buffer concentration was also studied. In this case, a borate buffer at pH 4.5 was used for the separation. Figure 6 shows the separation of several di-carboxylic acids where (I) 0.01 M H_3BO_3 and (II) no added H_3BO_3 were used. In the case where 0.01 M H_3BO_3 was added to the mobile phase (I), all of the analytes were resolved except, glutaric acid-malonic acid, and fumaric acid-oxalic acid. In the separation where no borate buffer was added to the mobile phase (II), all of the analytes were resolved except for fumaric and oxalic acid.

The separation and indirect detection of monocarboxylic acids and inorganic anions were also studied. Figure 7 shows the separation of acetic acid, formic acid and chloride. All three analytes are easily separated on the low-capacity anion exchange column. In order to separate mono- versus di-carboxylic acids, a mobile phase of lower ionic strength must be used. In this separation, the borate buffer was not added to the mobile phase. When the mobile phase contained a higher concentration of UV-active counteranion, added inert salts and/or borate buffer, the monocarboxylic acids and inorganic mono-anions were only slightly retained indicating that the retention mechanism of these analytes is anion exchange.

Standards were prepared for chloride, acetic and formic acids. Linear calibration curves of peak area versus ppm of analyte were obtained for a 50-μL injection over the range of 2.0 to 200 ppm. The correlation coefficients obtained were greater than 0.999 for all three standards.

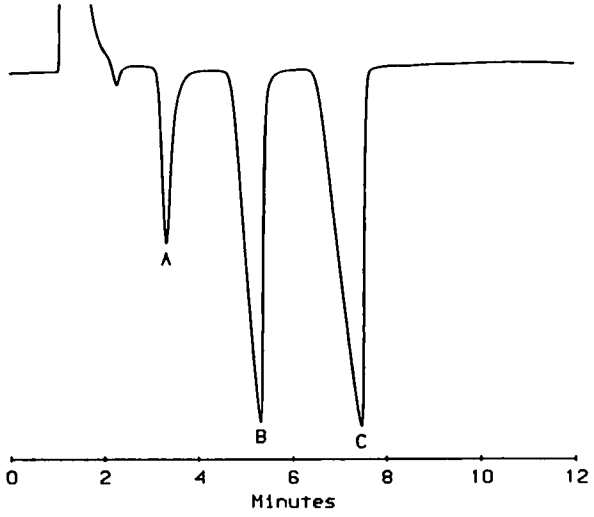

FIGURE 7

The Separation of A) Acetic Acid, B) Formic Acid, C) Chloride.

A 0.001 M KHP, pH 4.5, 5:95 $CH_3CN:H_2O$ mobile phase.

CONCLUSIONS

The separation of UV-transparent organic analyte anions on a low-capacity anion exchange column using indirect UV detection was studied. This separation and detection method has been successfully applied to several organic analyte anions. The effect that each mobile phase variable had on organic analyte anion retention and selectivity was studied. Calibration curves were found to be linear down to 2.0 ppm.

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