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Indirect Photometric Chromatography of Inorganic and Organic Anions Using Benzoate as an Eluent

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INDIRECT PHOTOMETRIC CHROMATOGRAPHY OF INORGANIC AND ORGANIC ANIONS USING BENZOATE AS AN ELUENT

(TITLE)

BY

Shahab A Shamsi

THESIS

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INDIRECT PHOTOMETRIC CHROMATOGRAPHY OF INORGANIC AND ORGANIC ANIONS USING BENZOATE AS AN ELUENT.

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Shahab A Shamsi

ABSTRACT

For many years the determination of common inorganic and organic anions was hindered by the lack of adequately sensitive analytical techniques. The determination of these anions was laborious and time consuming (e.g. titration, precipitaion, gravimetric analysis etc.). Because of the development of high-performance liquid chromatography, the anion exchange separation of simple inorganic and organic anions is now possible. Now, there are a broad and ever-increasing array of chromatographic methods supplanting these older chemical methods. However, detection is still a problem, but indirect detection provides a practical route for the detection of non-chromophoric inorganic and organic anions.

This dissertation describes the technique of Indirect Photometric Chromatography (IPC) for the determination of common inorganic anions and short-chain carboxylic and sulfonic acids. In this study, inverted peaks were observed in an elevated baseline as the transparent sample ions were selectively displaced from the anion exchange column by the UV absorbing mobile phase ion sodium

benzoate. A Hamilton PRP-X100 (styrene-divinyl benzene) anion exchange column which possessed a low capacity and a wide working pH range (1-13) was employed. The influence of various mobile phase parameters such as benzoate concentration, pH, and percent organic content was investigated. A 2.0 mM concentration of sodium benzoate provided maximum eluting ability for the separation of common inorganic anions (F^-, Cl^-, Br^-, NO_2^- NO_3^-, and $H_2PO_4^-$) within eight minutes with UV detection at 260 nm. At high pH, the competition between hydroxide and benzoate ions in the elution process decreases the detection sensitivity. Multicomponent mixtures of straight-chain alkyl carboxylic and sulfonic acids in the anionic form were also separated upon the addition of acetonitrile to the mobile phase. Combined anion exchange/reversed phase interactions were found to influence the retention of these organic acids.

The application of the benzoate eluent with UV detection method to the determination of anions was demonstrated via the analysis of chloride, EDTA and sorbate in a commercial saline solution. The limits of detection for injected chloride and EDTA were 1.5 ppm, while sorbate was detected at 0.5 ppm.

DEDICATION

To my Professor Dr. Blair. E. Miller for his tremendous contribution in providing me guidance, inspiration, and assistance time and again. Today, whatever knowledge I have in the area of chromatography would not have been possible without his generous time and suggestions in helping me to build the concept in this area.

ACKNOWLEDGEMENTS

I wish to express my sincere apppreciation to the faculty and staff of the Chemistry Department at Eastern Illinois University especially Dr.David Buchanan and Dr. Mark Mcguire for their active interest and help.

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CHAPTER I

SECTION A : A BRIEF REVIEW OF SEPARATION AND DETECTION METHODS IN ION EXCHANGE HPLC

In this disssertation, a promising mode of ion-exchange chromatography (IEC) monitored by indirect ultraviolet detection is described. The method allows IEC to be carried out with commercially available HPLC equipment. The analytes of interest have been a variety of inorganic and organic anions. The analyses of these ionic species in aqueous solutions are becoming increasingly important. Thus ion-exchange chromatography provides the needed specificity for these ionic compounds.

In an ion-exchange process, counter-ions are removed from the ion-exchanger upon injection of a sample solution. The retention of a sample anion on a typical anion-exchange column can be represented by the equation:

$$\text{Resin--}N^{+}(CH_3)_3E^{-} + A^{-} \rightleftharpoons \text{Resin--}N^{+}(CH_3)_3A^{-} + E^{-} \quad \text{---(1)}$$

where E^{-} and A^{-} are the eluent and solute anions, respectively, and the strongly basic (aminated) ion-exchange resin is designated as Resin----$N^{+}(CH_3)_3$ [1]. According to the principles of electroneutrality and equivalence of exchange [2-6], when a solute anion elutes from the anion exchange column, it displaces an equivalent number of

eluent charges from the mobile phase. The detection of an eluted ion merely requires that there is some difference in the property being measured between the analyte and the eluent anions.

In modern ion exchange chromatography, the capacity of a column is usually measured from the retention volume (V) or retention time (t) of the eluted ion. The capacity factor (k') is simply the amount of solute ion retained in the stationary phase compared to the amount in the mobile phase. One of the following equations [7] can be used to calculate the capacity factor.

$$k' = \frac{V - V_0}{V_0} \qquad (2)$$

$$k' = \frac{t - t_0}{t_0} \qquad (3)$$

where V_0 and t_0 are the void volume and the void time of an unretained substance injected onto the column. If the mobile phase contains a single eluent anion , the equilibrium that exists between the two ionic species of similar charge type on a strong anion exchange resin can have the following form:

$$xA_m^{y-} + yE_s^{x-} \rightleftharpoons xA_s^{y-} + yE_m^{x-} \qquad (4)$$

where E^{x-} and A^{y-} represent the eluent and solute anions, respec-

tively. The superscripts x and y are the absolute values of their associated charges, whereas the subscripts m and s refer to the mobile and stationary phases, respectively. The selectivity coefficient(K_E^A) for this expression is shown in equation 5.

$$K_E^A = \frac{[E^{x-}]_m^y \; [A^{y-}]_s^x}{[A^{y-}]_m^x \; [E^{x-}]_s^y} \qquad (5)$$

If the sample size is small, the quantity of the eluent ion on the equilibrated stationary phase $[E^{x-}]_s^y$ is essentially constant and equal to the column capacity Q. The ratio of the amount of the solute anions on the resin to that of the solution phase is called the distribution ratio or partition coefficient and is expressed by the following equation.

$$D = \frac{[A^{y-}]_s^x}{[A^{y-}]_m^x} \qquad (6)$$

Substituting D and Q into equation 5 yields equation 7.

$$K_E^A = \frac{D \; ([E^{x-}]_m^y)}{Q} \qquad (7)$$

In chromatography it is convenient to use the capacity factor instead of the distribution ratio. Capacity factor is related to the distribution ratio by the following equation [3]:

$$D = k' R^x \qquad (8)$$

where R is the column packing characteristics (ratio of mL of solution in the column to gram of resin in the column). Making this substitution in equation 7 and solving for k'produces equation 9.

$$k' = \frac{K_E^A Q}{([E^{x-}]_m^y) R^x} \qquad (9)$$

As noted above in equation 9, the capacity factor k' depends on the column packing characteristics R, eluent concentration $[E^{x-}]_m^y$, selectivity coefficient K_E^A, and resin capacity Q. The selectivity coefficient for any specific ion will vary according to the eluent used and the particular resin. By rearranging and taking the log on both sides of equation 9 yields the following relationship.

$$x \log k'R = \log K_E^A + \log Q - y \log [E^{x-}]_m \qquad (10)$$

If the capacity and the packing characteristics of the column are constant and the selectivity coefficient is independent of the mobile

phase composition, then equation 10 is simplified to the following form:

$$\log k' = \text{constant} - \frac{y}{x} \log [E^{x-}]_m \qquad (11)$$

which is consistent with the Haddad [8] and Gjerde [9] derivation. This equation 11, which is basic for ion chromatography, predicts a linear dependence of log k' on the -log of the eluent concentration with a negative slope equal to the ratio of the charges on the solute and on the eluent anions.

Two major separation and detection methods for the determination of inorganic and organic species have been developed depending on the capacity of the ion exchange material of the chromatographic column. These methods can be broadly divided into suppressed conductivity detection [10-12] and non-suppressed or single column ion-exchange chromatography using either conductivity [13-15] or indirect detection [16-18].

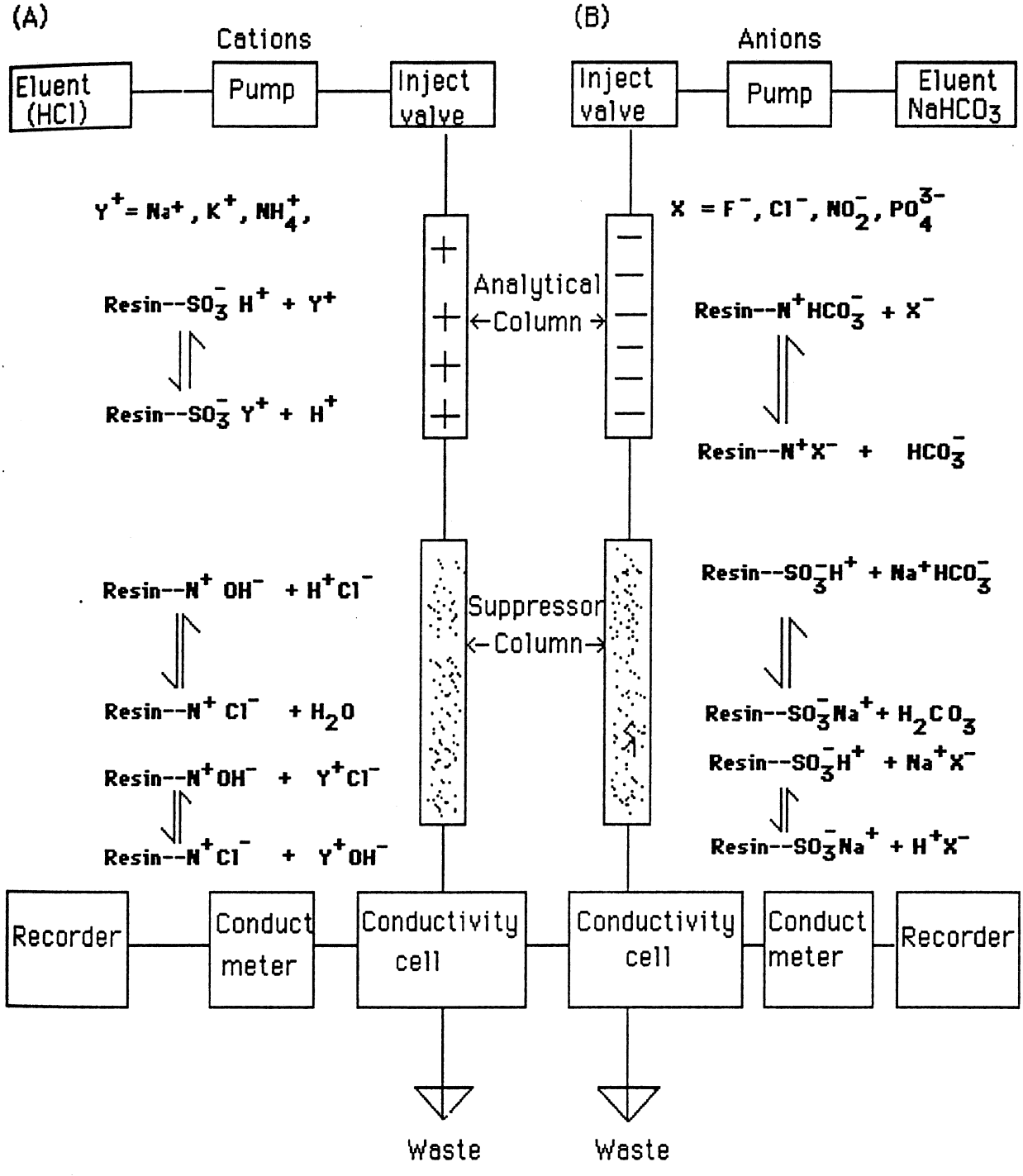
Suppressed conductivity detection commonly known as ion chromatography employs an ion exchange separator column, which separates the solute ions, and a second suppressor column which decreases the background conductivity of the eluent. The key to the suppression reaction is that the counterions associated with active

eluent species are replaced in the mobile phase by either a hydrogen or a hydroxide ion to form water through an ion exchange process. The net result is the neutralization of the active eluent species. This nonionized eluent is poorly conducting. The principles of both anion and cation analysis are shown schematically in Figure 1(a) and 1(b), respectively. Except for the suppressor column, each case mimics the standard HPLC system set-up with a pump, injector, separator column and detector. Although this method has been successful, it is often time consuming, due to the necessity of regeneration. The use of a second column and extra column tubing results in some band broadening which decreases the overall efficiency of the analysis and leads to certain drawbacks especially in quantitation. In addition, the detector response to some ions is dependent on the life-time of the suppressor column. A number of reviews [19-21] and applications [22-25] of suppressed systems have been reported.

Non-suppressed ion chromatography was originally developed by Fritz and others [26]. This technique involves the use of an ion exchange resin of very low capacity employed with eluents of low ionic strength. In the case of anion separations, an eluent such as sodium phthalate or sodium benzoate is the typical eluent used for direct conductivity. These eluent anions have lower equivalent conductances than sample anions such as chloride, nitrate, sulfate and

Figure 1 : (A) Illustration of suppressed cation exchange and (B) anion exchange chromatographic principles. *Note that suppression products H_2O and H_2CO_3 are non-conductive (Reprint from Mulik, J.D.; Sawicki, E. *Environ. Sci Technol.* **1979,** 13, 490.)

Ion chromatographic principle:



others. Thus when a sample anion is eluted from the column, there is an increase in conductance, and a positive peak is observed for the anion on the chromatogram. Interestingly, phthalate and benzoate salts also possess strong UV absorption characteristics and have considerable refractive indices in aqueous solution.

Indirect photometric chromatography (IPC) is a non-suppressed analytical technique for the separation of ions on a single ion-exchange column, following which detection of analytes is accomplished through a photometric process. The theoretical principle of IPC, alternatively referred to as "Vacancy " chromatography, can be qualitatively described as follows. IPC involves measuring the decrease in the absorbance signal as the transparent analyte ion displaces the ultraviolet and/or visible absorbing counter-ion of the mobile phase. In general IPC is simply a charge displacement process. IPC is possible whenever there is a competition between the light absorbing mobile phase ion and a UV transparent analyte ion for the ion exchange sites in the stationary phase. Thus, the UV detector responds to the presence of analyte by producing a negative peak in the chromatogram's baseline absorbance. The retention of the analyte peaks on the ion-exchange column vary with the size and charge of the analyte ion and the concentration of the eluent ion and its size and charge. In addition, the area under the peak is proportional to the

amount of solute injected.

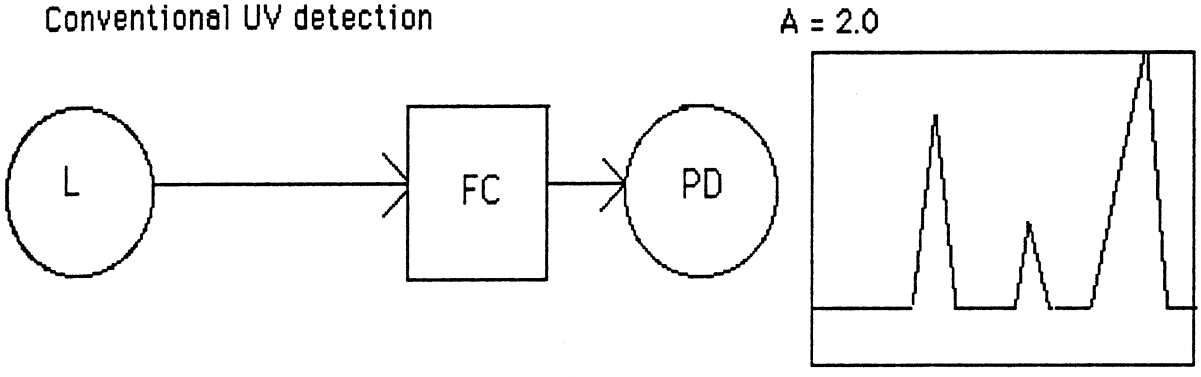
The analysis of UV transparent organic [27-29] and inorganic [30-32] anions by IPC has been gaining more and more prominence since it was first developed by Small and Miller in 1982 [17]. There are many ions of interest,for example fluoride, sulfate, lactate etc, which do not display any optical absorption of significant utility. The analysis of these weak UV absorbing and non-absorbing species has presented a significant problem to the ion chromatographer, specifically with regard to the detection at trace levels. The advent of indirect ultraviolet detection has overcome many of these problems.

Although non-suppressed ion separation techniques employing conductivity and indirect detection may have slightly higher detection limits than the suppressed approach, it provides a simple, inexpensive, and efficient method for separating and detecting ionic or ionizable (acids or bases) analytes. The direct UV detection generally refers to a situation where the eluent anions have a lower value of the measured property whereas indirect detection refers to reverse condition. These two situations are shown schematically in Figure 2.

Figure 2 : (A) Illustration of a conventional UV detection. and (B) indirect detection. The notations are, L = lamp, FC = flow cell, PD = photodiode, A = absorbance.(Reprint from Gaffney, M. H.;Cooke, M. *Anal. Proced.* **1985,** 22 ,25).

(A)

Conventional UV detection

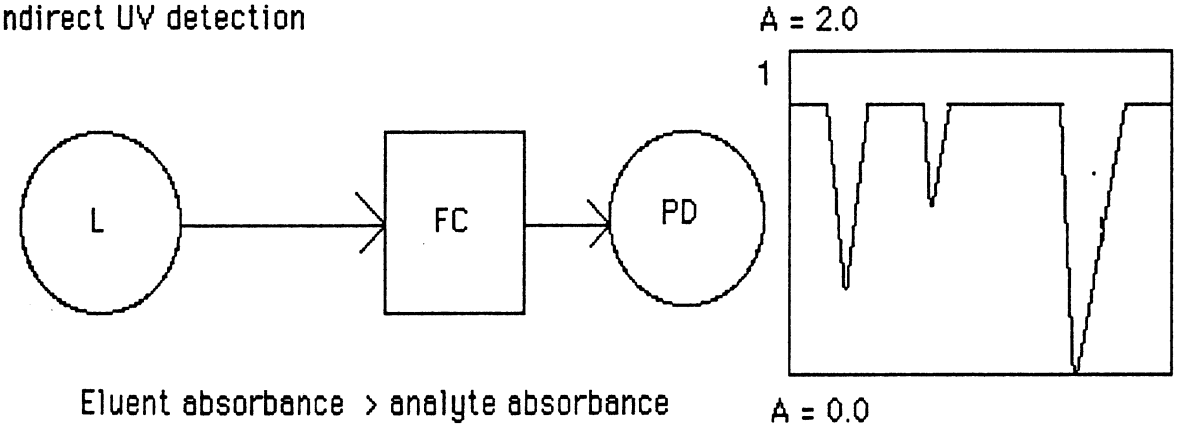


Analyte absorbance > eluent absorbance

Positive peaks

(B)

Indirect UV detection



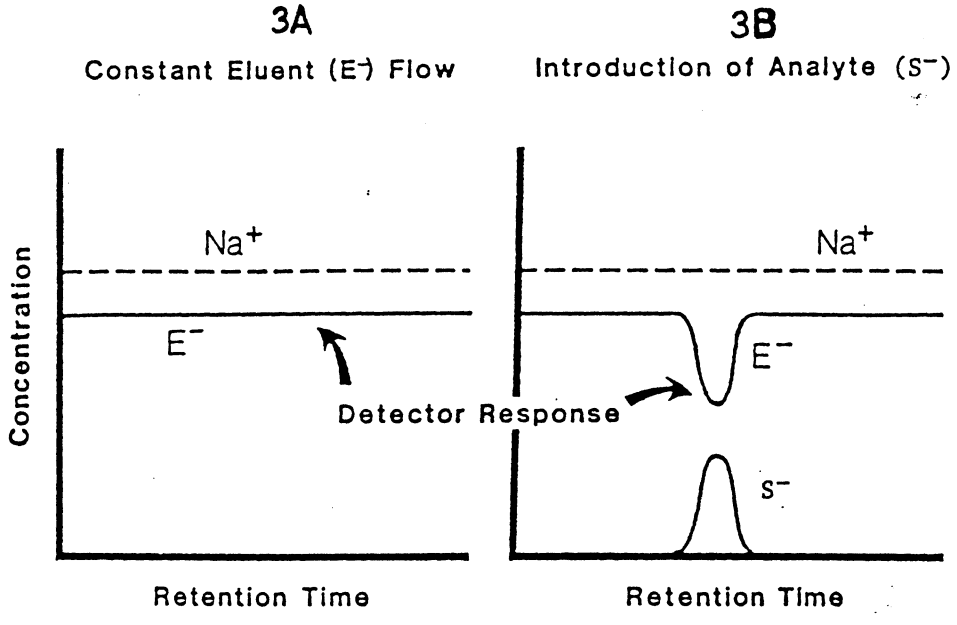
Eluent absorbance > analyte absorbance

Negative peaks

SECTION B : PRINCIPLE OF INDIRECT DETECTION

Indirect photometric chromatography (IPC) is a unique yet simple technique that has found increased use in the literature in just the last five years. Indirect detection methods are based on the principle that the effluent ionic concentration remains fixed at all times. In an anion exchange situation, consider a salt denoted by Na^+E^- which has been equilibrated with the HPLC column. The anion exchange sites in the column are occupied by the eluent anions E^-. If the concentration of the eluent remains constant, a steady level of Na^+ and E^- is usually observed (Figure 3A), by the concentration monitor. When a sample denoted by Na^+S^- is injected, the sample anion S^- will be retained in the anion exchange column for a certain period of time and will elute at a characteristic retention time. The concentration monitor will display the rise in the concentration of S^- and fall in a similar fashion as it leaves the column (Figure 3B). Thus, when the sample ion S^- elutes from the column, the concentration of the eluent ion E^- drops concomitantly by an equivalent amount. For example, assume that 4 mM Na^+E^- is being used as the eluent ion; then when the sample ion S^- appears, there must be an equivalent

Figure 3 An illustration of indirect photometric chromatography at (A) a constant concentration of the eluent and (B) an injection of a sample anion. The notations are Na^+ = Sodium ions, E^- = eluent anions. S^- = sample anions.(Reprint from Small, H.; Miller, T. E. *Anal.Chem.* **1987,** 59, 490.)



Principle of indirect photometric chromatography.

change in E^-. If at the peak of the elution band, the concentration of the sample ion is 1 mM, then the effluent at that point has a composition of 3 mM Na^+E^- and 1 mM Na^+S^-; therefore, it can be concluded that both the sample and effluent total ionic concentration remains fixed and is not altered. With respect to IPC, it is the absorbance of the counter-ion that the detector actually monitors, and it follows that the emergence of non-absorbing ion is indicated by a dip or trough in the baseline.

Using the anion exchange system described earlier with IPC, it is helpful to develop a detector equation [8]. The background absorbance of the eluent (A_E) is given by the following equation.

$$A_E = [\varepsilon_E - C_E I_E + \varepsilon_{HE} C_E (1 - I_E)] \times l \quad \text{——————(12)}$$

where ε_{E^-} and ε_{HE} are the molar absorptivities of the eluent in the ionized (E-) and the neutral (HE) forms, respectively. The total eluent concentration is given by C_E, I_E is the degree of ionization of the eluent, and l is the path length of the detector cell. It is assumed in equation 12 that the eluent cation does not absorb any UV radiation at the detection wavelength used. If C_S is the concentration of a completely dissociated sample anion S- eluting off the column, the concentration of the eluent anion in the detector cell is ($C_E I_E - C_{S^-}$). The

absorbance signal measured by the detector during sample elution is given by equation 13.

$$A_S = [\varepsilon_{E^-} (C_E l_E - C_{S^-}) + \varepsilon_{HE} C_E (1-l_E) + \varepsilon_{S^-} C_{S^-}] \times l \quad \text{---(13)}$$

where ε_S is the molar absorptivity of the solute anion. The change in absorbance can be obtained by subtracting the absorbance of eluent anion A_E from the sample anion A_S to give equation 14 which shows that the magnitude of this inverse peak is directly related to the

$$\Delta A = A_S - A_E = [(\varepsilon_{S^-} - \varepsilon_{E^-}) C_S] \times l \quad \text{----------(14)}$$

difference in concentration and molar absorptivity between the analyte and the eluent species. Thus, the detector response is dependent on the solute concentration, the detector cell path length and the difference in molar absorptivities between the solute and eluent anions. For a transparent analyte, $\varepsilon_S = 0$ and thus, the signal response is described by equation 15 which shows that the instrumental response R is independent of the nature of the solute. In most

$$R = -C_S \varepsilon_E \quad \text{----------(15)}$$

cases, the ε_E constant can be used to determine the molar concentration of any other transparent analyte irrespective of its identity.

In IPC, noise (N) is usually larger in the presence of large background signal. The signal (S) as a fraction of baseline absorbance can be shown by the following equation.

$$S = \frac{C_S\ (A_S - A_E)}{C_E\ A_E} \qquad (16)$$

From equation 16 it follows that, the below proportionality constant occurs.

$$\frac{S}{N} \propto \frac{C_S\ (A_S - A_E)}{N\ C_E\ A_E} \qquad (17)$$

For a transparent ion, A_S is zero, equation 17 can be simplified as follows.

$$\frac{S}{N} \propto \frac{C_S}{N\ C_E} \qquad (18)$$

Equation 18 [17] relates the signal to noise ratio to the eluent concentration and illustrates that the lower the concentration of eluent employed, the better the sensitivity.

In a similar fashion, Yeung [33] has shown that in IPC the limit of detection can be calculated by the following equation.

$$C_{lim} = \frac{C_M}{D_R \times T_R} \qquad (19)$$

Here, C_{lim} is the limit of detection, and C_M is the concentration of the corresponding mobile phase component. The transfer ratio, T_R explains the displacement process which can be either displacement by charge or displacement by volume. In IPC the more efficient the charge displacement process (larger T_R), the smaller the fractional change in the baseline absorbance can be detected. In anion-exchange, a T_R of 2 is obtained when the analyte anion, A^{2-}, is replaced by the eluent anion, E^-. In indirect detection a large background signal is a necessity. D_R represents the ratio of background signal to background noise. Briefly, if the background signal is large with a low noise level, a better limit of detection is achievable. In conclusion, detection limits are highly related to low eluent concentrations and low noise levels. This can be achieved in IPC with an eluent which possesses a high molar absorptivity, good eluent strength, and an absorbance level below 1.O A.

SECTION C: DETERMINATION OF OPTIMUM CONDITIONS TO EFFECT THE CHROMATOGRAPHIC SEPARATION BY IPC

"Optimizing the separation " is an important part of any methods development project involving high-performance liquid chromatography (HPLC). Many factors influence the separation and detection of anions in IPC, and it is necessary for a chromatographer to understand the role played by each factor.

The capacity of an ion exchanger is a quantitative measure of its ability to take up exchangeable counter-ions and is therefore of major importance. Many workers have reported the separations of organic and inorganic anions employing low capacity anion exchange columns [8,34-36]. In terms of HPLC separations, low capacity ion exchange columns have fewer active sites and are very mechanically stable due to high crosslinking, unlike earlier exchange resins used in column chromatography. In addition, HPLC ion-exchange columns based on small polymeric or silica particles have more theoretical plates than older columns which means that they provide successful separations even when there are only small differences in retention times of analyte ions. The commercially available anion exchange columns used in IPC are typically small in size (10-25 cm) and have low ion-exchange capacities (5-300 μequiv/g) [35]. With these new low capacity ion exchangers, the use of dilute aromatic eluents with

high background absorbances is viable. Naish [37] examined the use of high and low capacity anion exchange columns in IPC. Simple anions like nitrite and bromide were poorly resolved on high capacity columns. A low capacity anion exchanger decreased the elution time, provided better resolution and increased the sensitivity. Computer simulation of the factors affecting ion-exchange chromatography with indirect detection has confirmed that the use of low capacity ion-exchange column is essential for sensitive detection [38].

Generally, in IPC a highly absorbing eluent with a large molar absorptivity will give the greatest sensitivity, but the background absorbance of the detector is then large which may result in baseline noise and drift. However, the detector saturation can be prevented by using a low concentration of the eluent or by selecting a wavelength that combines maximum sensitivity within a reasonable absorbance range. Photometric error should be taken into consideration. According to Beer's law, the linear relationship between the concentration and the absorbance is observed within approximate absorbance range of (0.2-0.8) [39]. It is therefore necessary to select an appropriate wavelength of detection which is adjusted so that the background absorbance of the eluent is not greater than 0.8. Variable wavelength detectors are usually employed in IPC due to the fact that if the eluent concentration is fixed, the eluent absorbance can be adjusted

by changing the detector wavelength.

Experimentally controllable parameters used to govern the retention and detection of organic and inorganic ions include the structure and concentration of the eluent, the polarity and concentration of the organic modifier, and the pH of the mobile phase. Several eluents have been studied for use in anion exchange IPC. Examples include phthalate [34-37], nitrate [17], sulfobenzoate [40], iodide [17], and benzene tricarboxylate [41]. In general, a good eluent is an aromatic organic anion which provides a balance between UV absorptivity and elution ability. From a practical stand point, the salt, completely ionized form of an aromatic eluent is more sensitive in IPC as shown by Sato [38] who separated five different anions using Tiron (1,2-dihydroxybenzene-3,5-disulfonic acid, sodium salt form). These results are in sharp contrast to the conductivity work by Gjerde et.al [26] who found that if the pH of the eluent is maintained in the acidic region (3-4) where the eluent is partially ionized the sensitivity of conductivity detection increases. Basically, the lower the percent ionization of the eluent, the lower the background conductance (G_B); thus the sensitivity of conductivity detection is improved by increasing the detector response ΔG, as ($G_S - G_B = \Delta G$). As mentioned earlier both IPC and direct conductivity are non-suppressed techniques.

The selectivity coefficient K_E^A of the eluent is also an

important factor which needs to be considered when choosing the concentration of the eluent. When values of K_E^A are relatively large, the eluting power of the eluent ion is considered to be strong. Different ions vary widely in their displacing power. Small et. al.[17] studied a large variety of candidate eluent ions with a wide range of ion exchange affinities. It was concluded that the polyvalent anions are much stronger eluents than monovalent anions. Fritz et. al.[42] reported that a greater interaction (adsorption) of the eluent anion with resin structure predicts the effectiveness of an eluent to elute sample anions. Usually the eluents which adsorb strongly on the resin phase have long equilibration times.

Eluent concentration plays a crucial role in ion-exchange separations, especially in IPC. Not only do retention times of the analyte ions gradually decrease as the concentration of the eluent ion increases, but in addition, the role of the detector background signal must be considered in IPC. Low capacity anion exchangers have a tendency to swell when salts of high ionic strength are used as mobile phase. Jenke [43] observed in the study of ion exchange resins that as the resin swells within the confined space of the column, some sites may become unavailable for interaction with either the eluent or the sample species. This type of swelling causes the

column capacity to decrease. On the other hand, using an eluent that is too dilute results in extremely long retention for anions which can decrease the sensitivity of detection due to band broadening.

The percentage of organic modifier in the eluent is an additional factor in IPC, especially in the case of organic analytes. First, adjustment of the percentage of the organic modifier is the most convenient method for the prevention of adsorption of non-polar sample components to the column packing material. Recent studies have shown that small amounts of acetonitrile were required for selectivity and resolution of organic and inorganic anions whereas methanol or ethanol tended to produce broader peaks [36,44]. The poor chromatography in purely aqueous solvents has been attributed in part to the considerable degree of reverse phase characteristic that the packing has. There are appreciable areas on the polymeric based column which are non-polar and unfunctionalized. Addition of an organic modifier to the mobile phase helps to negate the adsorption of non-polar solutes and improves the resolution of organic analytes.

The ionic strength of the mobile phase is also controlled by the pH. The pH at which the eluent, for example phthalate, is used controls the position of its dissociation equilibrium and hence, the proportion of the relative concentration of monovalent and divalent ions. The eluent charge is in turn inversely proportional to the retention

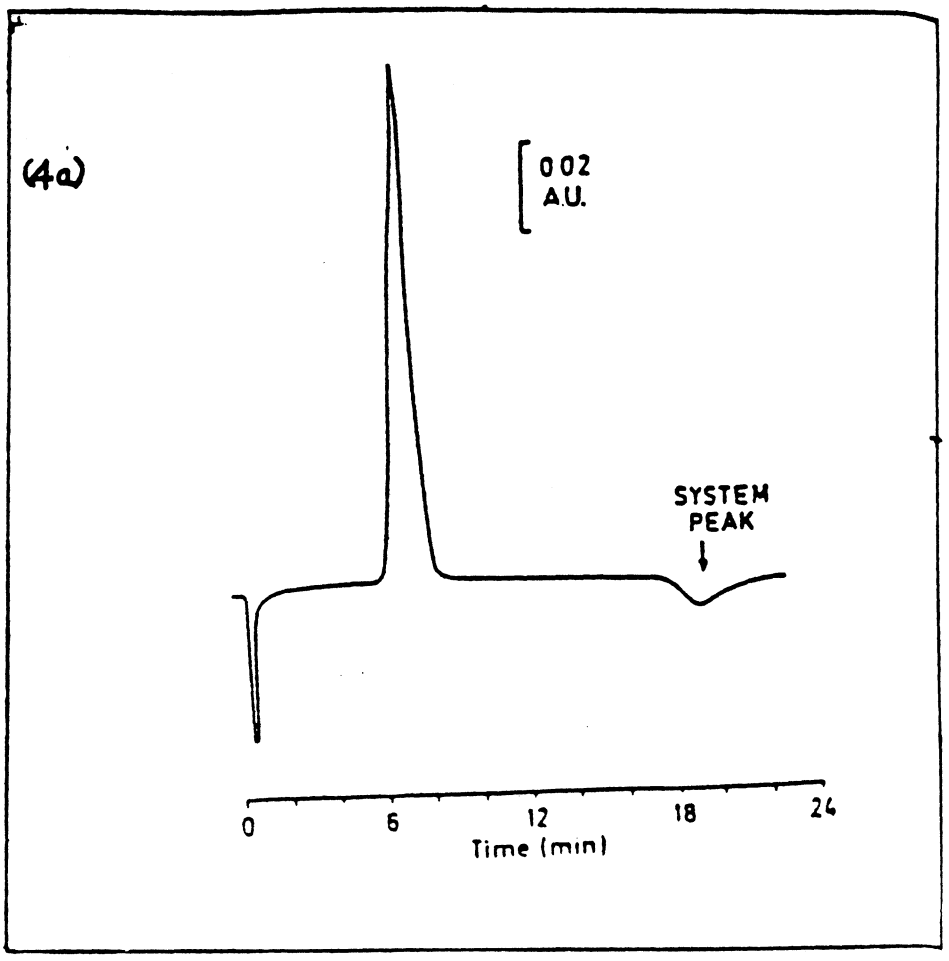
times of the analyte ions. A 2^- ion in the eluent will be taken up more strongly by the resin than a 1^- ion. A single negative charged eluent such as benzoate works well for the separation of simple anions, and a doubly negative eluent such as phthalate or p-hydoxy-benzoate, can be used to separate late eluting monovalent and diva-lent anions. However, the very early eluting monovalent anions are not retained by the resin when divalent anions are used as mobile phase [3].

The eluent pH is an important consideration that will influence the retention time by determining not only the eluent charge but the charge of the sample ion. If a sample ion is not ionized, it will not exchange with the resin and will pass quickly through the column. Sample ions such as phosphate can exist as 1^- or 2^- or 3^- charges depending upon the eluent pH and move at different rates through the column for the same eluent concentration. To ensure complete disso-ciation of the mobile phase, a rule of thumb for the starting pH is to take the highest pKa value of the eluent and add 1.5. However, it is advisable to maintain the pH of the eluent in the vicinity of the pKa of the eluent acid, so that eluent is well buffered. An eluent that is buffered produces more reproducible ion-exchange behavior. On the other hand, if the pH of the eluent is less than the pKa of an acid, the

eluent is not completely ionized. Thus, the molecular form of the eluent is sorbed by the resin matrix, and system peaks are observed [45-48]. These peaks can complicate chromatograms by causing separation interferences and time delays. Figure 4 (a) illustrates that for nitrite (NO_2^-) a system peak was obtained after 20 minutes, causing an increase in analysis time. The co-elution of late eluting anions such as sulfate (SO_4^{2-}) is shown in Figure 4 (b).

However, if the eluent pH is raised to a level where the eluent is completely ionized, the system peak can be eliminated. On a practical level, great care should be exercised in preparing and storing basic eluents free from carbon dioxide contamination. Very recently, it has been reported that IPC could detect a carbonate system peak if the eluent pH $\geq$ 7 was used [49]. Absorption of carbon dioxide from the atmosphere was considered to be the cause. For the sensitive and accurate determination of analyte ions, it is necessary to remove this interference. This removal can be accomplished by purging the eluent with an inert gas like nitrogen or helium. Ascarite traps can also be useful while working at high pH. One of the primary conclusions resulting from this review of IPC is that, if this technique is used, then compromises must be made between sensitivity, retention time, optimum UV absorption and the properties of the mobile phase. Of

Figure 4. Chromatogram of (a) nitrite with a late eluting system peak and (b) coelution of the system peak with sulfate. Conditions: eluent, 2.0 mM potassium hydrogen phthalate, pH 4.2; indirect UV at 265 nm. (Reprint from Haddad, P.R.; Jackson, P. E. *J. Chromatogr.* **,1985,** 312, 381.)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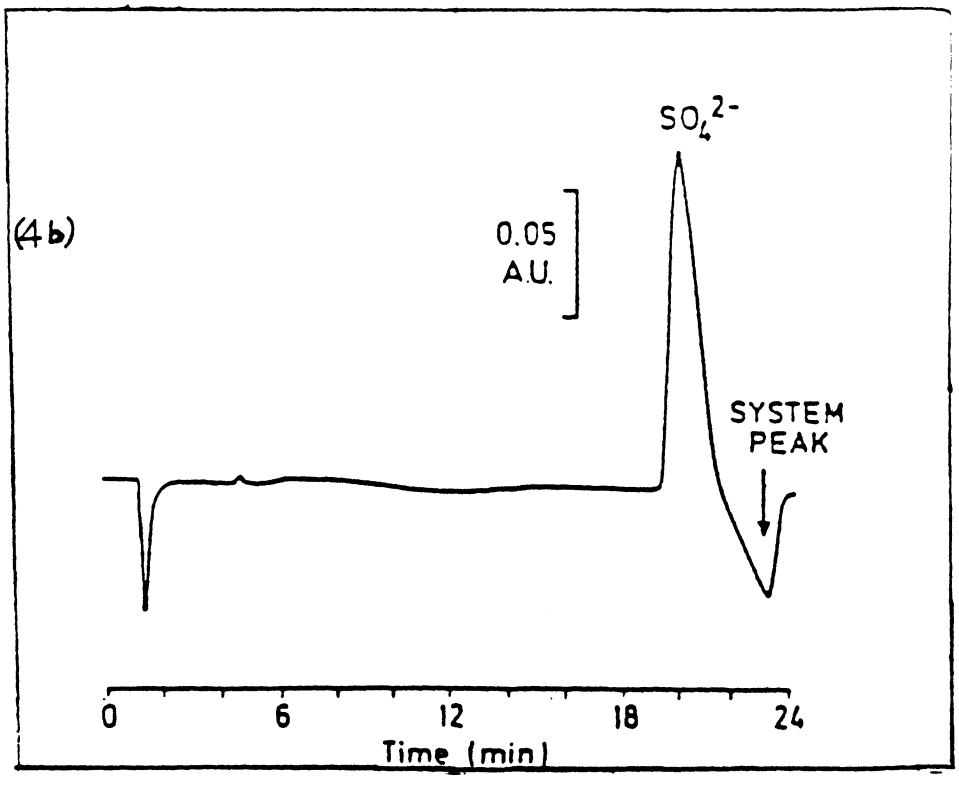
24
Time (min)


(4b)
SO_4^{2-}
0.05
A.U.
SYSTEM
PEAK
0
6
12
18
24
Time (min)

course, these compromises are no different than those found in other separation techniques.

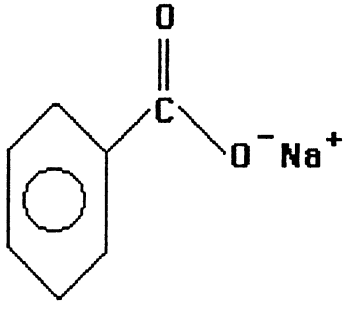
SECTION D : BENZOATE : A MOBILE PHASE FOR IPC

In the search for more suitable eluents for anion exchange IPC, sodium benzoate is considered a good candidate for the following reasons :

a) Sodium benzoate is sufficiently water soluble and works very well in aqueous solution.

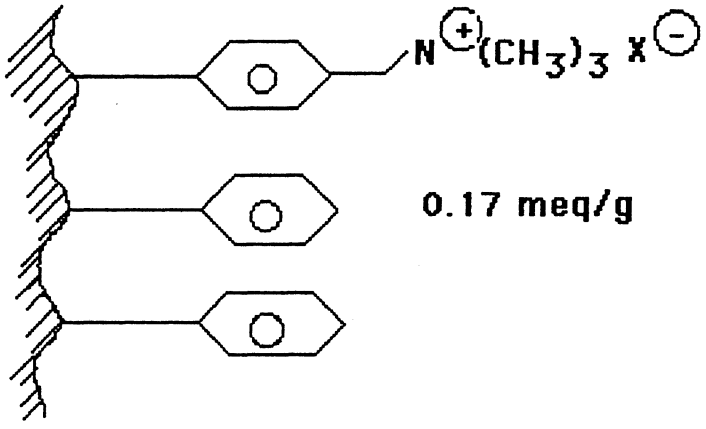
b) The acid dissociation constant for benzoic acid (pKa =4.18) is such that the buffering range of the eluent is in the acidic region.(pH 4-6) [50]. At low pH the contribution of OH^- to analyte elution is small.

c) Sodium benzoate being aromatic has a relatively good molar absorptivity. Its structure is shown below:



d) The structure is quite similar to the resin matrix (qualitatively the resin is a low capacity strong base anion exchanger derived from polystyrene divinyl benzene) [15]. A greater interaction of the benzoate anion with the anion exchanger enhances

the selectivity of an eluent and therefore its effectiveness to elute sample anions. A qualitative picture of the resin is shown as :



e) It should provide a satisfactory balance between UV absorptivity and elution ability.

f) It would be expected to work well for separating all monovalent anions especially very early eluting anions like fluoride, acetate and formate.

g) It is inexpensive and easily available in appropriate purity.

h) It is usable at high pH without degradation or system peak occurrence.

SECTION E :IPC : A PROMISING APPROACH TO UNIVERSAL DETECTION

IPC has many potential advantages over other ion chromatographic methods of detection. One of the major advantages of the indirect detection technique is that it provides essentially constant molar response for all ions. Unless the analyte ion absorbs at the specific wavelength, the decrease in signal will be the same regardless of the exact nature of the sample. In addition, even UV-absorbing ions can be monitored under IPC conditions. Thus, these properties make indirect detection a universal approach. The importance of universal detectors in the development of analytical methods becomes more broadly applicable when lack of characteristic spectroscopic properties, or lack of detector active functional groups hamper the detection of an analyte ion. Another analytical technique for measuring non detectable analytes is chemical derivatization. However, this technique is time consuming, destructive, and limited by the quantity of the analyte.

IPC allows the analyst greater control over instrumental sensitivity. This control is obtained by selecting an appropriate wavelength to take advantage of changes in the molar absorptivity of the eluent species. The potential of this technique is even more pronounced when using very concentrated eluents where either the back-

ground conductance may be sufficiently high to prevent monitoring of the baseline response or where the high eluent concentration results in rapid depletion of the suppressor column. The former situation can be prevented in IPC by selecting a wavelength at which the eluent has a high molar absorptivity. In the latter case, the detector saturation is prevented by choosing a wavelength at which the absorptivity is low. In a few instances, flexibility is provided by the variation of wavelength in indirect UV absorption which can be used to eliminate matrix effects [51]. For example if a sample matrix contains high levels of nitrate (produced when the sample is digested with nitric acid), then it would be advantageous if nitrate ions were not detectable in the chromatogram. The procedure is somewhat difficult to follow, but it can be accomplished by selecting a wavelength at which the molar absorptivities of the eluent and the solute ions (nitrate) are equal, that is ($\varepsilon_S = \varepsilon_E$). When the molar absorptivity of the eluent and the sample ions are equal, no detector signal will result for a particular solute.

The method of indirect detection is applicable to many situations in which relatively few species (less than 10) are to be determined and where only simple instrumentation is available. The technique does not require expensive hardware. Jenke [52] has presen-

ted data further verifying the validity and utility of universal detection in indirect optical detection. Quantitation is performed by constructing the calibration curves from standard injections. The technique is more sensitive than non-suppressed conductivity using the same eluents [38], but is generally less sensitive than suppressed conductivity detection. Sub-parts-per million level of detection (LOD) were claimed for a variety of organic and inorganic common ions [53].

The technique of indirect detection is also applicable to various detectors such as refractive index [53,54] and fluorescence [55,56]. A refractive index detector is very insensitive and is also subject to temperature effects. However, in this detection mode the background refractive index of the eluent is not restricted because the measurements are conveniently made by comparison of the column effluent with a pure eluent contained in the reference cell. Sensitivity is therefore limited by the performance of the detector used which does, however, impose a practical limitation. Most commercially available refractive index detectors are designed to operate with large solute concentration and are generally not optimized for high sensitivity applications.

The use of a fluorescent eluent ion for indirect detection has also been devised but has not been explored widely. Haddad [8] reported

the use of sodium salicylate as a fluorescent eluting ion for anion exchange chromatography. A decrease in the background fluorescence was used to detect the solute anions. The detection sensitivity achieved is comparable to indirect UV detection and conductivity detection methods; however, the attainable LOD is limited by baseline noise rather than by sensitivity.

A strange feature usually observed in indirect detection is the appearance of system peak which is not directly attributed to any injected ion [45-48]. The appearance of system peaks represents a major interference to the quantitation of later eluting solute ions. It therefore imposes a serious limitation of IPC technique in ion chromatography.

To conclude the discussion of indirect detection it should be noted that IPC as a whole has the potential advantages of wide spread applicability with good sensitivity and selectivity. The instrumental simplicity and ready adaptibility to conventional HPLC equipment obviate the need for any specialized equipment.

CHAPTER II

ION EXCHANGE CHROMATOGRAPHY OF SOME COMMON INORGANIC AND ORGANIC ANIONS WITH INDIRECT PHOTOMETRIC DETECTION USING SODIUM BENZOATE

SECTION A :INTRODUCTION

Procedures for determining cations have received great attention in analytical chemistry. Reported methods include atomic absorption or emission spectroscopy, polarography, and others [39]. On the other hand, the situation is different where anion determinations are concerned. There exist various polarographic methods, but these approaches fail when the anions are to be determined in mixtures with various organic compounds [57]. In such a case only separation methods, especially those based on chromatographic principles, may be of help. However, the chromatographic detection of common organic and inorganic anions is a key problem. The direct photometric (UV/visible) methods of detection are unsuited to the measurement of many inorganic anions as they are not strong chromophores. In 1979 new instrumentation and techniques permitted anion chromatography without suppressor technology [9] . This new approach was termed non-suppressed or single column ion chromatography. The method employed a low-capacity ion exchanger (for example, less than 200 μ.eq/g) for the separation. Since the exchange capacity is low, an

effective eluent is one that has a low mobile phase counterion concentration. This fact in turn yields a low background conductivity which permits a favorable detection of analyte ions by conductivity. This approach which is reviewed elsewhere [26,42] is used widely and has benefitted from marked improvements in mobile and stationary phase technology and conductivity detector design.

In the present chapter, a single approach to the separation of common inorganic and organic anions using ion-exchange chromatography and indirect photometric detection is described. Special instrumentation is not necessary. All that is required is a standard liquid chromatograph with a multiple wavelength UV detector. The species detected include inorganic anions (fluoride, chloride, bromide, nitrate, nitrite, phospate, sulfate, and iodide), oxoanions (bromate, chlorate, cyanate, and iodate), and organic anions (acetate, and formate). The eluent used in the study is sodium benzoate. Detection by the indirect photometric method is based on monitoring the benzoate species in solution and relating it to the analyte using the principle of ion-exchange.

SECTION B :EXPERIMENTAL

Section B.1 : Chemicals

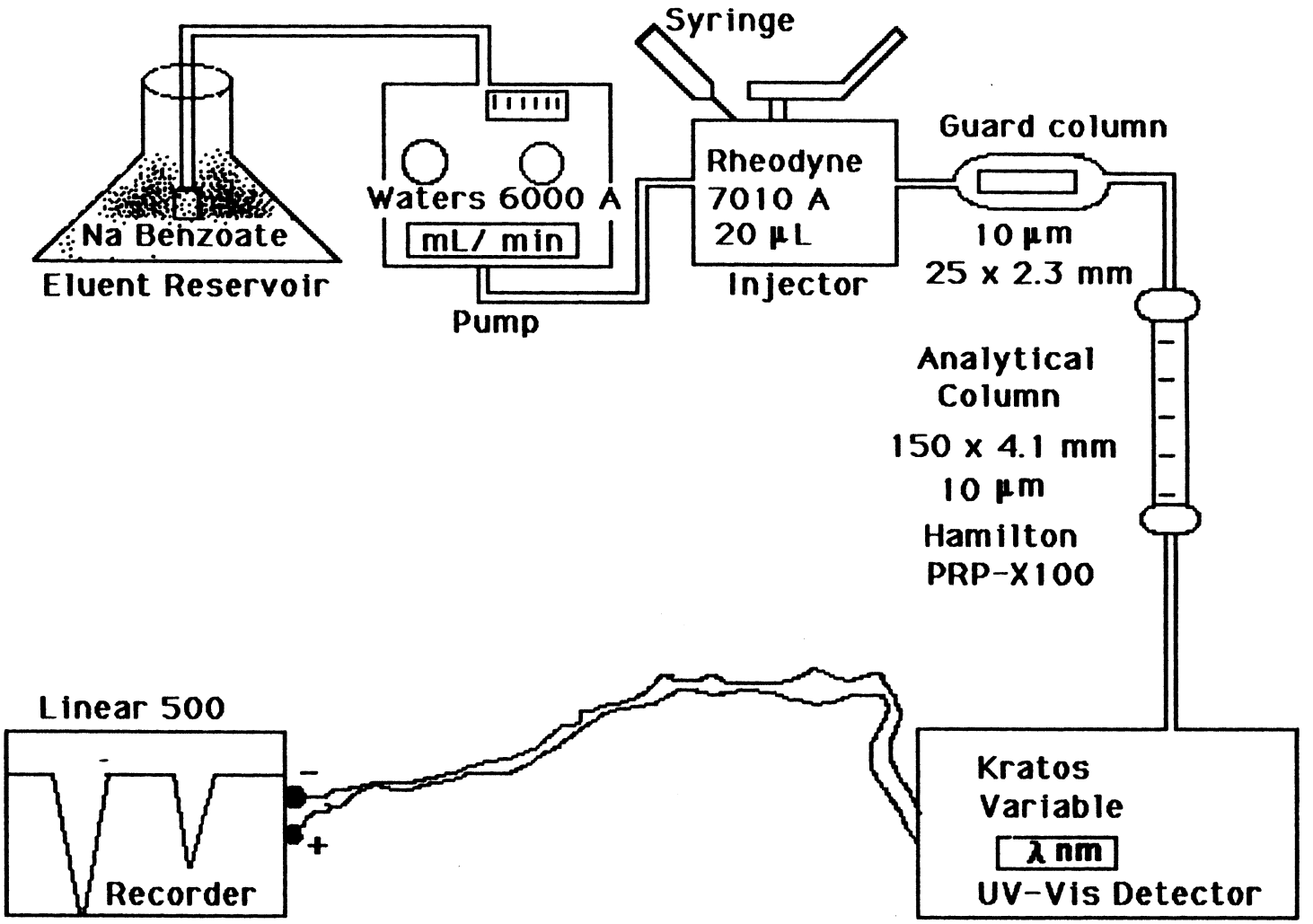
All the sodium and potassium salts used were of Analytical Reagent Grade from Fisher Scientific Co., (Fairlawn, NJ) and Aldrich Chemicals (Milwaukee, WI). Stock solutions (250 ppm) of the analytes of interest were prepared by dissolution of their sodium or potassium salts in doubly deionized water from the Milli-Q water purification system (Millipore, Bedford, MA) and stored in Pyrex and Kimax glassware. The sodium hydroxide (Aldrich Chemicals) used in the preparation of basic eluents was 99.99% pure. The Ascarite trap was purchased from Anspec (Ann Arbor, MI).

Section B.2 : Equipment

The basic instrumental requirements are an isocratic pump, an injector, a guard column located between the pump and the injector to filter out all the mobile phase contaminants which can irreversibly bind to the analytical column, and an ion exchange separator column, a variable wavelength UV detector, and a recorder. Figure 5 depicts the instrumentation used. Separations were carried out on a Hamilton PRP-X100 (Reno, NV) (150 x4.1mm), low capacity (200 μeq/g) anion exchange column and corresponding guard column. Solvent was delivered by a Waters Model 6000A pump (Water Associates, Inc., Milford, MA). Samples were introduced with a Rheodyne Model 7010

Figure 5 : Instrumental requirements for indirect photometric ion-exchange chromatography.

INSTRUMENTAL REQUIREMENT FOR IPC



loop injector (Berkeley, CA) fitted with a 20μL loop. Indirect ultraviolet detection was made with a Kratos-Schoeffel Spectro Flow Model SF 770 multiple wavelength ultraviolet-visible detector (Kratos Schoeffel Instruments, Westwood, NJ). The chromatograms were recorded on a Linear Recorder Series 500 recorder (Linear Instruments Corporation, Reno, NV). The pH values of the eluent used were measured by using Orion Research Model 701 digital pH meter (Orion Research, Cambridge, MA). The UV absorption spectrum of sodium benzoate was measured by Schimadzu UV 160U UV-Visible recording spectrophotometer (Schimadzu Scientific Instruments, Inc., Columbia, MD) .

Section B .3 : Operating Procedure

Inorganic and organic ions were determined by the following procedure:

1) The system was flushed with several column volumes of Millipore doubly deionized water.

2) Mobile phase preparation:

   a) 200 mM sodium benzoate was prepared in Millipore H_2O (28.5 g/1.0L).

   b) Mobile phase was degassed by helium purge for ten minutes.

3) Column preparation:

   a) 200-300 Column volumes (400-600 mL) of 200 mM sodium

benzoate were pumped through the column to fill the active sites, and the column was flushed again with several column volumes of Millipore doubly deionized water.

b) The system was equilibrated with a low concentration (0.5 mM -2.0 mM) of sodium benzoate. The equilibration time at a flow rate of 1.2 mL/min was approximately 2 hr.

4) Analysis:

a) The detector and the recorder were set at approximately an 80% full scale setting and a chloride sample was run to check the system performance.

b) If additional standards were to be run the same week, the mobile phase (0.25 mM) sodium benzoate was kept running through the system at a low flow rate, until the time of analysis.

c) The system storage procedure involved flushing the column with at least 100 mL of Millipore H_2O to insure the complete removal of salt.

Preparation of basic eluents :-

Special care is necessary for the preparation of a carbonate free mobile phase at high pH. Basic solutions of sodium benzoate were prepared by the following steps:-

1) A 50% w/v concentrated solution of sodium hydroxide was

prepared by weighing out 30 g of sodium hydroxide pellets (99.9% pure) and adding an equal weight, 30 g of boiled and doubly deionized Millipore water. After the pellets dissolved, 2.0 g of barium hydroxide was added to precipitate carbonate in the form of barium carbonate, and the solution was allowed to stand overnight in a polyethylene bottle.

2) The pH of the eluent was adjusted with a clean filtrate (centrifuged concentrated sodium hydroxide) using a pH meter.

3) The mobile phase was degassed by a helium purge. The helium line was equipped with an ascarite trap. Eluents were kept carbonate free by setting an ascarite trap on the eluent reservoir.

4) The column was equilibrated with at least 200 mL of the mobile phase at a flow rate of 1.2 mL/min. Column regeneration was needed after a certain period of time, approximately after 30 injections of chloride, due to carbonate build up.

Section C :RESULTS AND DISCUSSION

Choice of detection wavelength:

The UV spectrum of 2.0 mM sodium benzoate (H_2O), found in Figure 6, showed very high absorption at or below 250 nm. At the concentration of sodium benzoate eluent needed for optimum chromatographic results, it was necessary to select a wavelength to reduce the total absorbance of the eluent. The detection wavelength used throughout this study was 260 nm due to high noise obtained below 260 nm. The molar absorptivity at 260 nm was calculated to be 596 L $cm^{-1} moL^{-1}$. The background absorbance at this wavelength was 1.192 resulting in a reasonably stable baseline. Although, this absorbance value was outside the Beer's law range, it still was considered to be appropriate. Figure 7 illustrates the dependence of the chromatographic signal of sulfate anion upon detection wavelength. A very smooth baseline was obtained at 270 nm, but the sensitivity of detection decreased. It was concluded that for the separation and detection of anions, 260 nm was considered a suitable wavelength of detection. The baseline noise was quite reasonable at this wavelength.

Effect of the eluent concentration on the retention and separation of anions:

To determine the optimum mobile phase (benzoate) concen-

FIGURE 6: UV ABSORBTION SPECTRUM OF 2.0 mM SODIUM BENZOATE

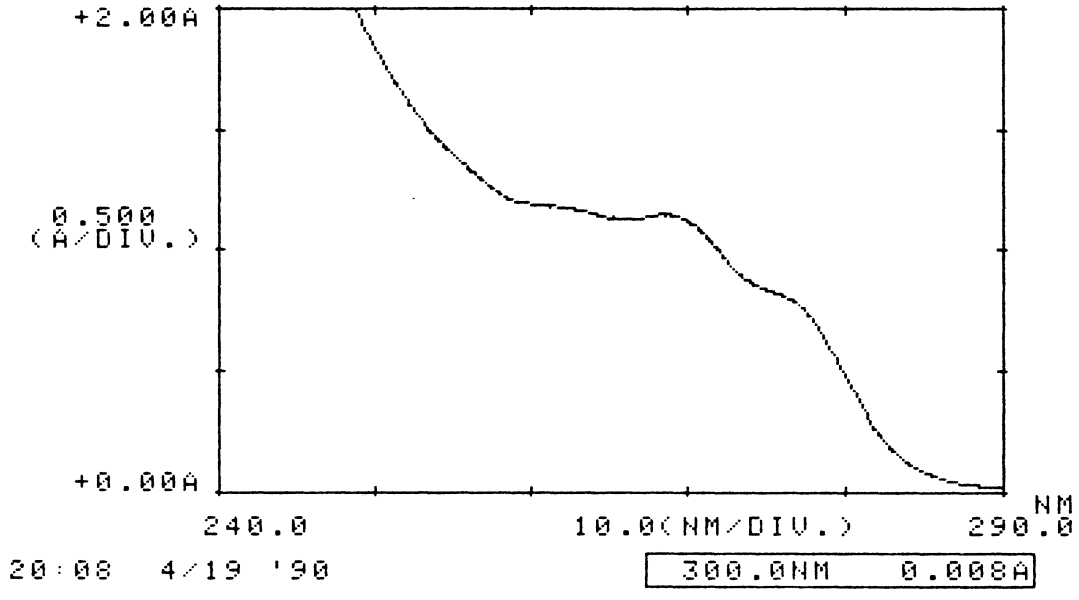
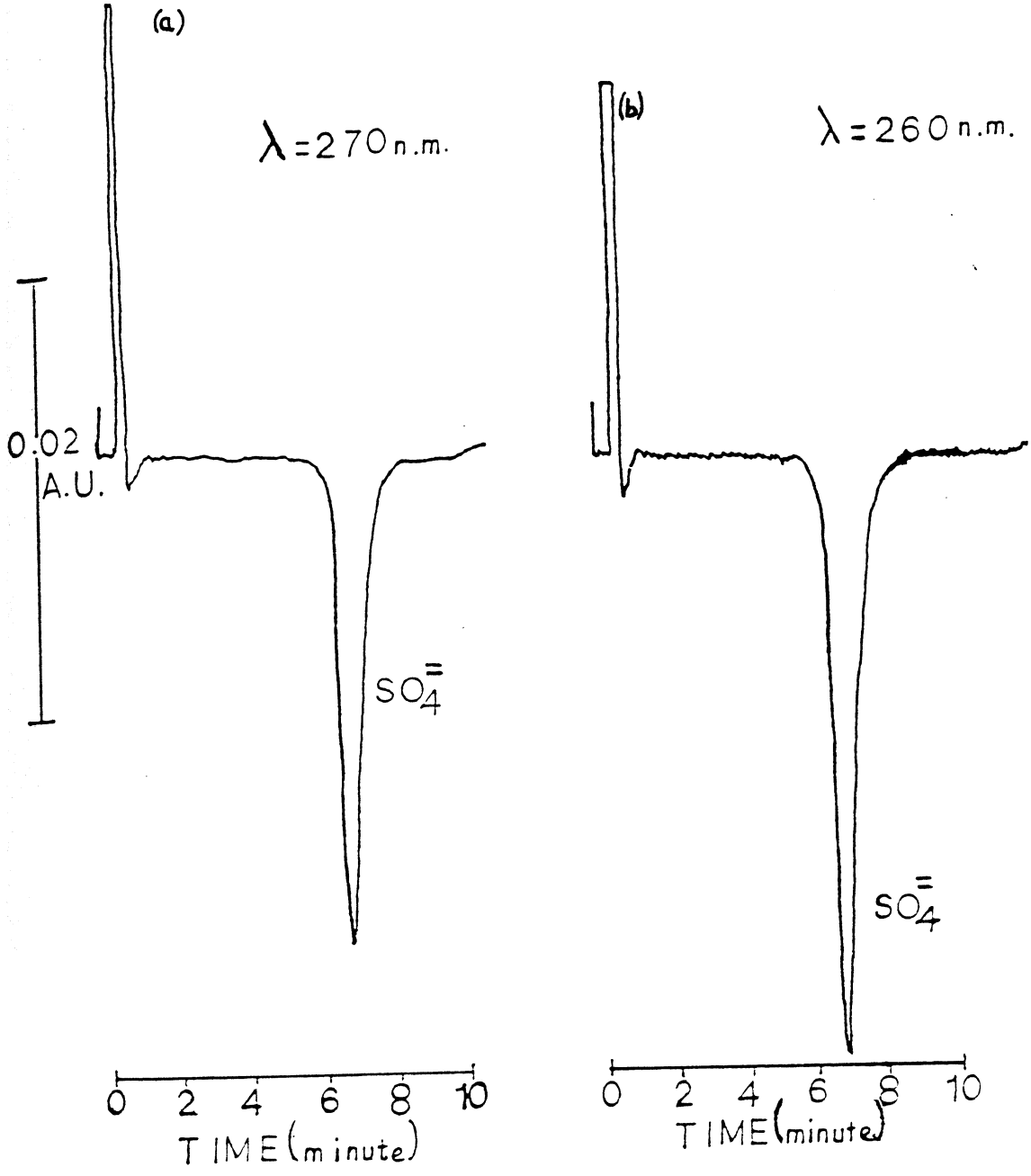


Figure 7 : Chromatogram of sulfate (125ppm) at two different wavelengths.(a) and (b). Conditions 4 mM sodium benzoate (pH 6.2), 2.O mL/min.


(a)
λ = 270 n.m.
0.02 A.U.
$SO_4^=$
0 2 4 6 8 10
TIME (minute)
(b)
λ = 260 n.m.
$SO_4^=$
0 2 4 6 8 10
TIME (minute)

tration for separating five common inorganic anions (F^-, Cl^-, Br^-, NO_2^-, NO_3^-) and organic anions (CH_3COO^-, $HCOO^-$,) a standard mixture of these anions was injected at various concentration of the eluent.

Figure 8a shows the separation of a mixture of organic and inorganic anions at 2.0 mM sodium benzoate. Figure 8b shows the same separation of a mixture of organic and inorganic anions at 0.5 mM sodium benzoate with indirect ultraviolet detection. The conditions used were as indicated. Baseline resolution was obtained for all the anions except acetate, formate,and fluoride ions. According to the principles of IPC, as the eluent concentration in the mobile phase increases, sensitivity decreases [4]. But the results obtained in Figure 8 suggest that at a concentration of 0.5 mM the sensitivity of indirect UV detection has decreased. In fact, the decrease in the photometric signal was due to band broadening. Sodium benzoate at this low concentration was not a very effective eluent. Further comparison showed that even though acetate and formate were partly resolved, the bands were broad, and the chromatography was poor at such a low concentration of 0.5 mM. A higher benzoate concentration (2.0 mM) provided a shorter retention time and reduced baseline noise. This separation was consistent with other reported indirect detection results [30-33]. The dependence of the retention on the concen-

Figure 8 : Chromatograms of inorganic and organic anions with (a) 2.0 mM and (b) 0.5 mM sodium benzoate.

Conditions: detection at 260 nm, 1.2 mL/min, Injection volume =20μL.

Solutes A = fluoride (30 ppm), B = acetate (30 ppm) C = formate (30 ppm), D = chloride (35 ppm), E = nitrite (35 ppm), F = bromide (35 ppm), G = nitrate (35 ppm).

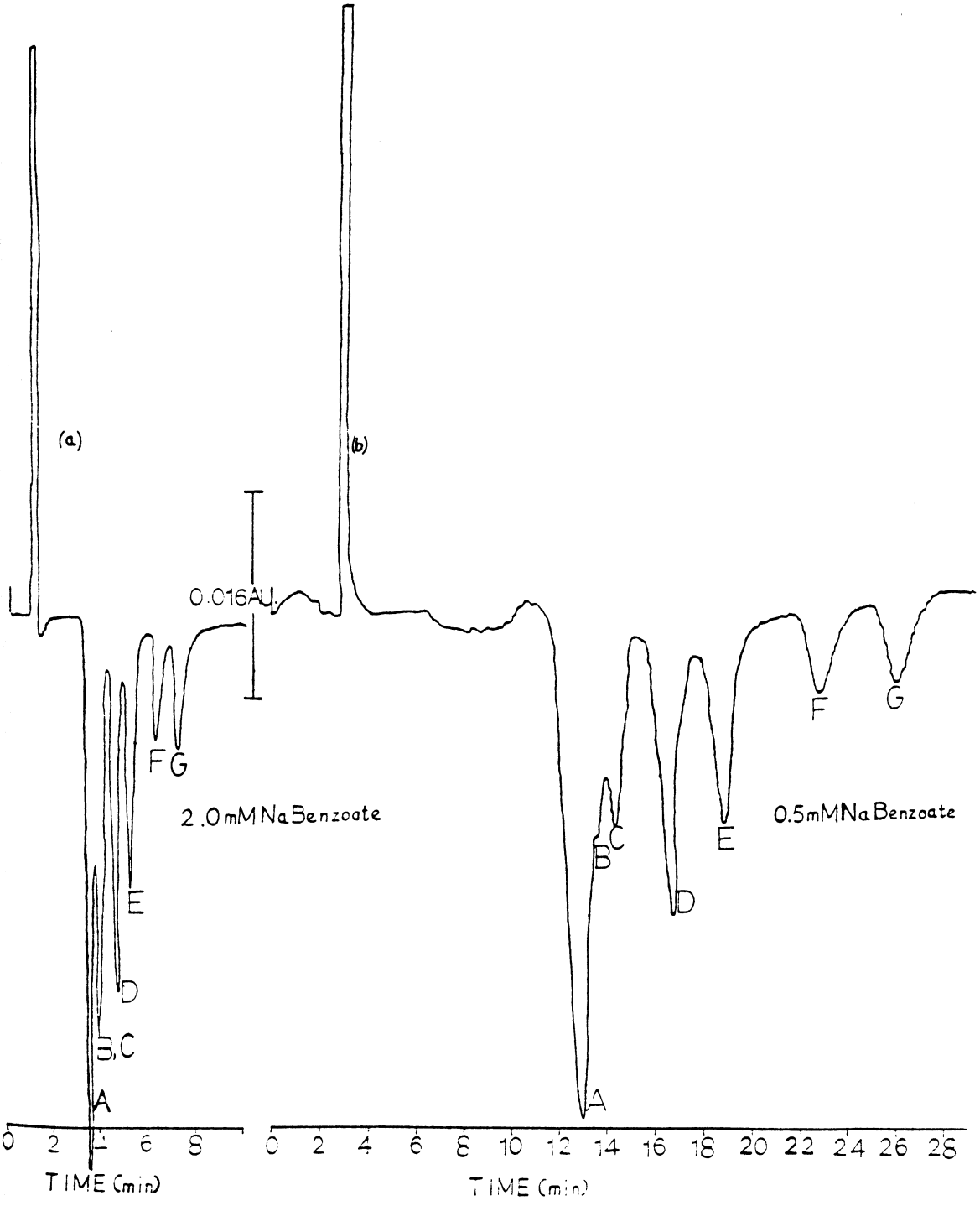

(a)
(b)
0.016AU
A
B,C
D
E
F G
2.0mM Na Benzoate
A
B
C
D
E
F
G
0.5mM Na Benzoate
0 2 4 6 8
TIME (min)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
TIME (min)

TABLE 1: ADJUSTED RETENTION TIME OF ANIONS WITH DIFFERENT CONCENTRATIONS OF SODIUM BENZOATE

Eluent — Adjusted retention time tr' (minute)

Sodium Benzoate (mM)	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	CH_3COO^-	$HCOO^-$
0.50	10.10	13.90	16.05	19.90	23.10	10.60	11.15
0.75	7.60	10.30	11.90	14.50	17.10	8.10	9.15
1.00	5.70	7.65	8.95	11.25	13.10	6.05	6.50
2.00	3.10	4.20	4.70	5.90	6.80	3.10	3.50

tration of sodium benzoate of six common inorganic anions is shown in Table 1. These results confirmed that for optimum chromatographic separation, a 2.0 mM sodium benzoate eluent provided appropriate separation selectivities, shorter retention times, and good detector sensitivity with a reasonably low baseline noise. However, shortly retained components such as acetate and formate would require a weaker mobile phase strength for separation.

Experimental data relating log k' versus log [eluent] were obtained and plotted. Figure 9 shows the results for various concentration of sodium benzoate eluents on the capacity factor (k') of various organic and inorganic anions. The slopes for the anions studied range from -0.85 to -0.89 which were close to the theoretical slope of -1, thus, indicating that they are all retained as monovalent anions. Correlation coefficients of better than 0.999 were found for most of the inorganic and organic anions. Thus, the instrumental response was acceptable over the desired concentration range. Figure 10 shows a separation by IPC of five common inorganic anions at a concentration of 5 ppm. At concentrations lower than 5 ppm, the peaks of the late eluting anions like nitrate (NO_3^-), and bromide (Br^-) became broad and mishaped. From Figure 10 it is evident that detection limits on the order of < 1 ppm are possible with benzoate.

A qualitative separation of six common inorganic anions is

Figure 9: Logarithm of capacity factor (k') of anions vs logarithm of different concentrations of sodium benzoate. Conditions flow-rate,1.2 mL/min, 20 µL injection volume, solute concentration (35 ppm), detection at 260 nm.

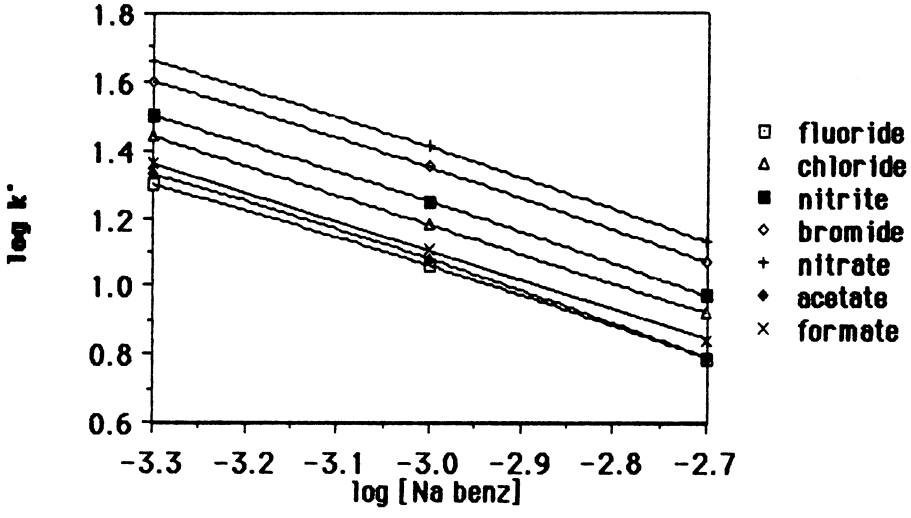

1.8
1.6
1.4
1.2
1.0
0.8
0.6
log k'
-3.3
-3.2
-3.1
-3.0
-2.9
-2.8
-2.7
log [Na benz]
fluoride
chloride
nitrite
bromide
nitrate
acetate
formate

Figure 10 : Separation of a standard mixture (5 ppm) of anions. Conditions: eluent 1.0 mM sodium benzoate, (pH 5.6), flow-rate 1.2 mL/min, detection at 260 nm. Attenuation : 0.04 A.U.F.S.

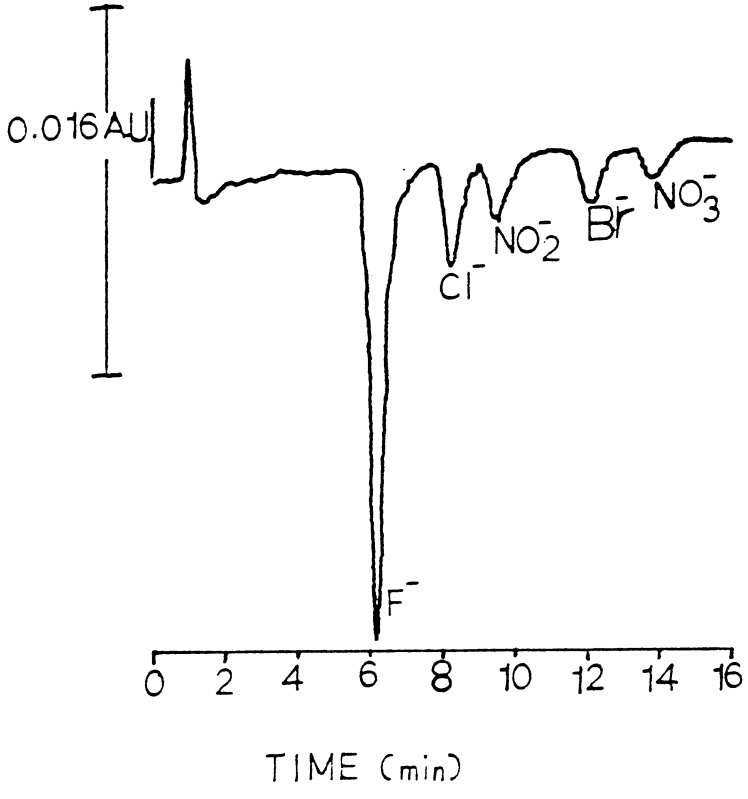
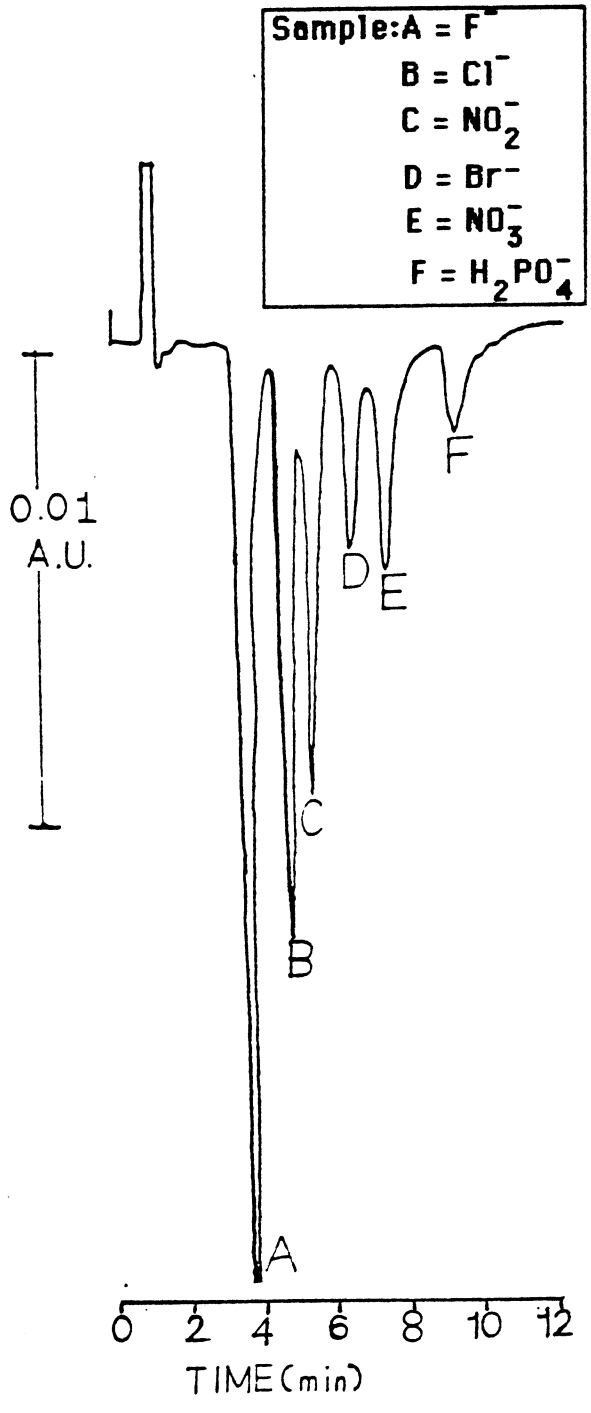

0.016 AU
F⁻
Cl⁻
NO₂⁻
Br⁻
NO₃⁻
0 2 4 6 8 10 12 14 16
TIME (min)

Figure 11 : Separation of six common inorganic anions Conditions: 2.0 mM sodium benzoate (pH 6.2), flow rate 1.2 mL/min at 260 nm, 20 μL sample, solutes concentration = (35 ppm).


Sample:A = F^-
B = Cl^-
C = NO_2^-
D = Br^-
E = NO_3^-
F = $H_2PO_4^-$
0.01
A.U.
A
B
C
D
E
F
0 2 4 6 8 10 12
TIME(min)

shown in Figure 11. The order of retention is $F^- < Cl^- < NO_2^- < Br^- < NO_3^- < H_2PO_4^-$. The order of elution of these anion was similar to that reported for conventional anion exchange resins [1,14-15] and was also consistent with the trend of hydration energies [58]. Bromide with its small hydration energy, large ionic radius, and higher polarizibility, eluted much later than fluoride and chloride and was baseline resolved. Nitrite and nitrate were somewhat displaced in their order of elution. If hydration and polarizibility were the sole considerations, nitrite should have eluted before chloride, but nitrate and nitrite are polyatomic anions stabilized by charge delocalization. Thus, they could not be directly compared with halide ions. Phosphate ($H_2PO_4^-$) eluted still later, after nitrate because of its low hydration energy, and higher polarizibility

Other separations :

By using 4.0 mM benzoate as the eluent, the large and highly charged anions such as iodide (I^-) and sulfate (SO_4^{2-}) were separated in under eight minutes (Figure 12). This separtion of multi-charged and large anions using sodium benzoate as an eluent has not been reported so far. However, the detection sensitivity for these anions decreased at such a high concentration of the eluent. This result was consistent with the principal of indirect detection (equation 19).

Figure 12 : Chromatogram for the separation of highly retained anions, iodide (125 ppm), sulfate (100 ppm), Conditions: eluent 4 mM sodium benzoate, pH 6.2, detection at 260 nm, flow rate 2.0 mL/min, 20 μL injection volume.

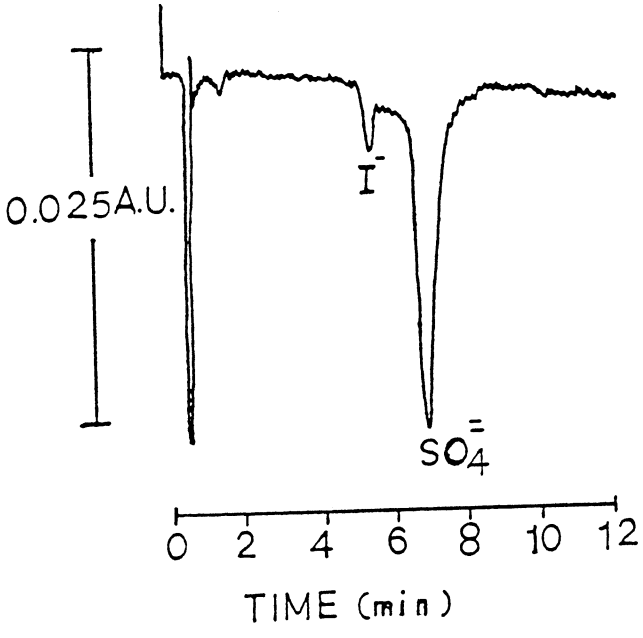
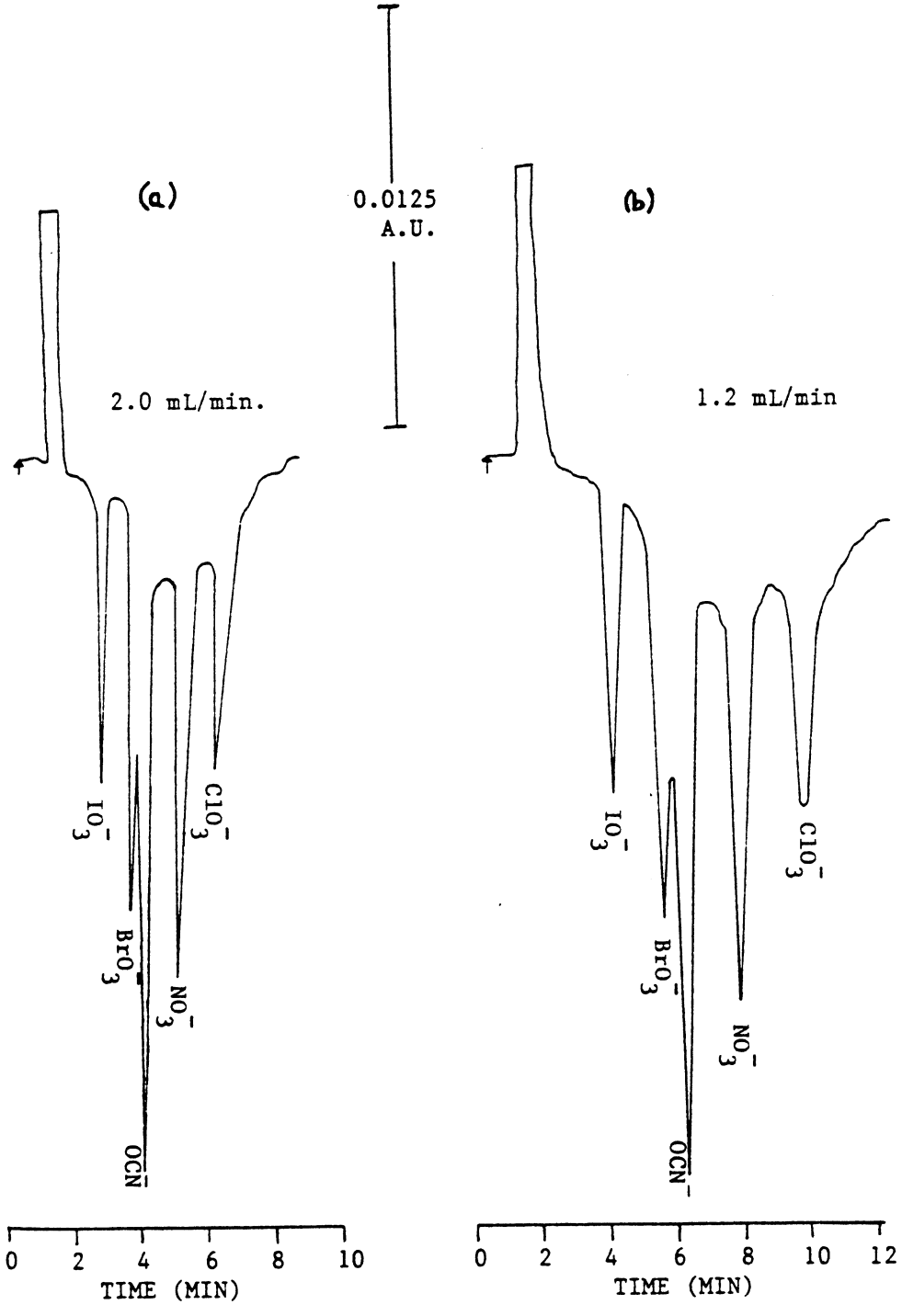

0.025A.U.
I⁻
SO4=
0 2 4 6 8 10 12
TIME (min)

Figure 13 : Chromatogram of oxo-anions.

Conditions: 2.0 mM sodium benzoate at a flow rate of (a) 2.0 mL/min and (b) 1.2 mL/min. Other conditions as in Figure 11. Peaks iodate (IO_3^-) = 35 ppm, bromate (BrO_3^-) = 35 ppm, cyanate (OCN^-) = 35 ppm, = nitrate (NO_3^-) = 40 ppm, chlorate (ClO_3^-) = 35 ppm.


(a)
0.0125
A.U.
(b)
2.0 mL/min.
1.2 mL/min
IO_3^-
BrO_3^-
NO_3^-
OCN^-
ClO_3^-
0 2 4 6 8 10
TIME (MIN)
0 2 4 6 8 10 12
TIME (MIN)

The separation of oxoanions is shown in Figure 13. It was quite interesting to compare the trends of retention for the separation of halates ($IO_3^- > BrO_3^- > ClO_3^-$), with halides. While the halogen ion X^- increases in size with atomic number of X, the corresponding halate ion XO_3^- decreases in size [58] . As Figure 13 shows, the oxoanions eluted according to size as theory predicted with the smaller anions eluting first. Cyanate is a linear anion and small in size. Nitrate is a planar anion which is larger in size and is expected to retain longer than cyanate on the anion exchange column.

Flow rates control the rate of ion exchange and hence the shape of the analyte peak front and the shape and sharpness of the peak (or trough) produced by the analyte ion. A comparison of flow rates (Fig.13) clearly showed that a flow rate of 2.0 mL/min provided a better separation by decreasing the analyte retentions and improving the peak shapes.

Separation of anions at high pH:

The eluents used so far in indirect photometric chromatography (IPC) have been effective for the separation of many inorganic and organic anions in solutions of pH below 7.0. However, their utilization for the separation and detection of anions of very weak acids, such as cyanide, borate, silicate, and arsenite which exist as anions only in basic solution has not been investigated with IPC. Separation

and detection of these anions are of great importance owing to their presence in pharmaceuticals, water, and food. For the separation of these weakly acidic anions, it is logical to use some kind of a basic solution as the eluent [59]. Although sodium hydroxide has been used as an eluent in indirect conductivity for the separation of cyanide, and other weakly acidic anions, the hydroxide is a rather weak eluent for the separation of many anions [3].

Presently, very little information is available for the separation of weakly acidic anions in IPC. Recently, Hawakawa[49] developed an IPC technique for the trace determination of carbonate using phthalate as an eluent. However, extensive measures were used to eliminate carbon dioxide contamination such as purging of the mobile phase continously at a low flow rate during the experiment. Despite some technical and chemical difficulties for the separation of weakly acidic anions at high pH, IPC is one of the most exciting new developments in ion exchange chromatography. The method is selective and efficient and could prove to be a viable route for the the detection of biological compounds such as sugars or other weakly acidic compounds that exist as anions only in basic solutions and lack UV chromophores.

Sodium benzoate has been investigated as an eluent at high pH (11.0) using a low capacity anion exchange column. Although the

column used is stable at high pH, the problem of absorption of atmospheric carbon-dioxide (CO_2) in the eluent can seriously limit the sensitive determination of anions. Glass solvent reservoirs must be avoided, since etching of silicate into the high pH mobile phase will occur and alter (shorten) the chromatographic retention time and response behaviour. Carbon dioxide, which becomes fixed in the basic mobile phase (pH >11.0) as carbonate, must be excluded with an ascarite trap or by an inert gas blanket. Carbonate (CO_3^{2-}) is a stronger eluent than benzoate and causes drifting baselines.

When Millipore water was injected into the IPC system using an eluent that was not treated correctly to exclude CO_2, a positive peak was observed. This peak showed an appropriate increase in size when an injection was spiked with 25 ppm of carbonate. Thus, the peak was confirmed as a"carbonate system peak". Figure 14 illustrates the effect of a carbonate system peak in IPC. Several attempts were made to remove this interference due to CO_2. An ascarite trap was set up on the mobile phase, and the eluent was purged with helium which was also connected to an ascarite trap to absorb the CO_2 from the gas tank. Initially, the attempts proved to be successful in minimizing the absorption of CO_2 from the atmosphere. However, the carbonate system peak developed in the mobile phase after several hours and grew in size with time upon injection of Millipore water.

Figure 14 : (a) Chromatogram of carbonate system peak with the injection of Millipore water. (b) Millipore water spiked with 25 ppm of carbonate.(CO_3^{2-}).

Conditions : 0.5 mM sodium benzoate, pH 5.6, 1.2mL/min, $\lambda = 260$ nm.

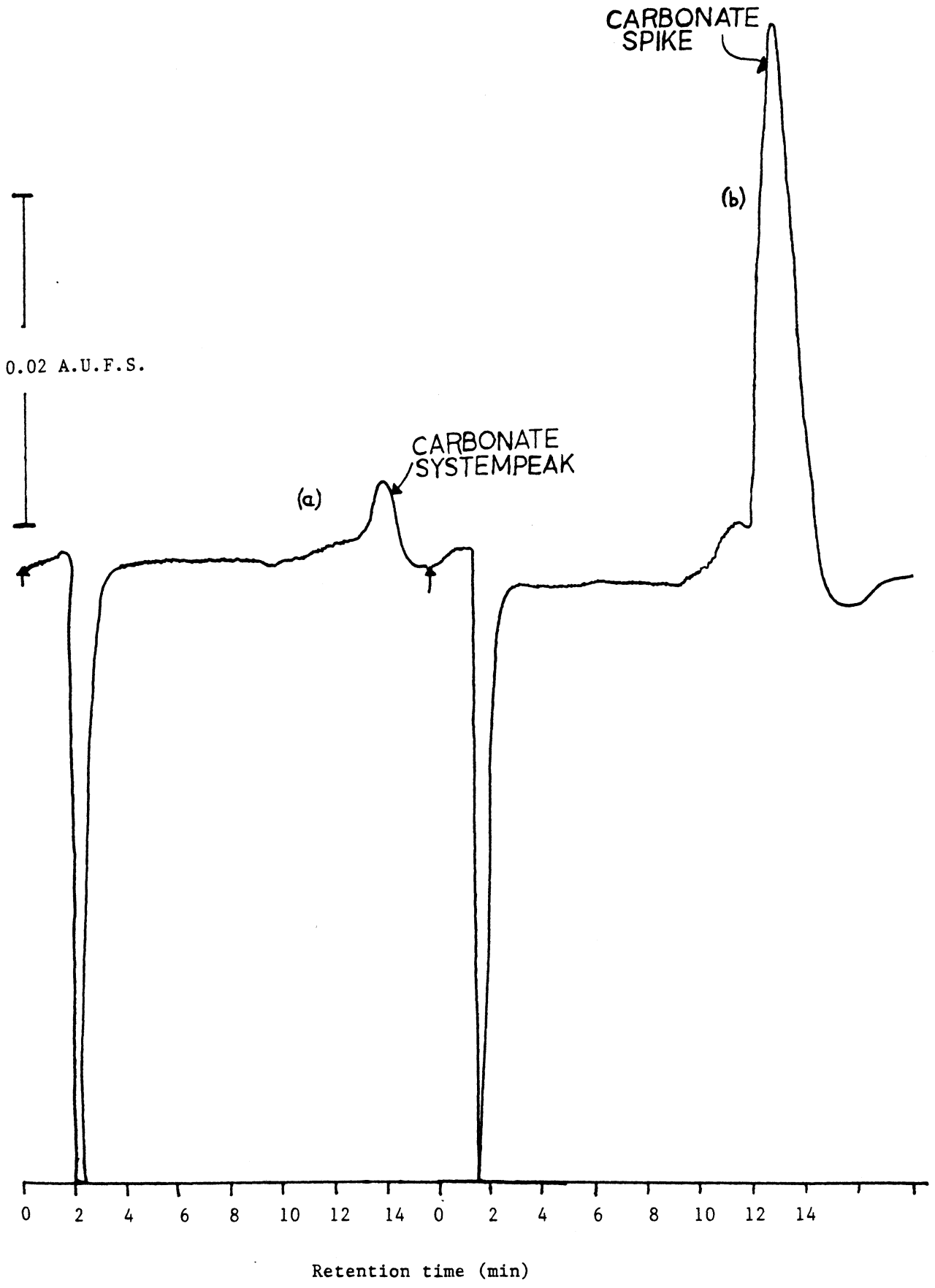
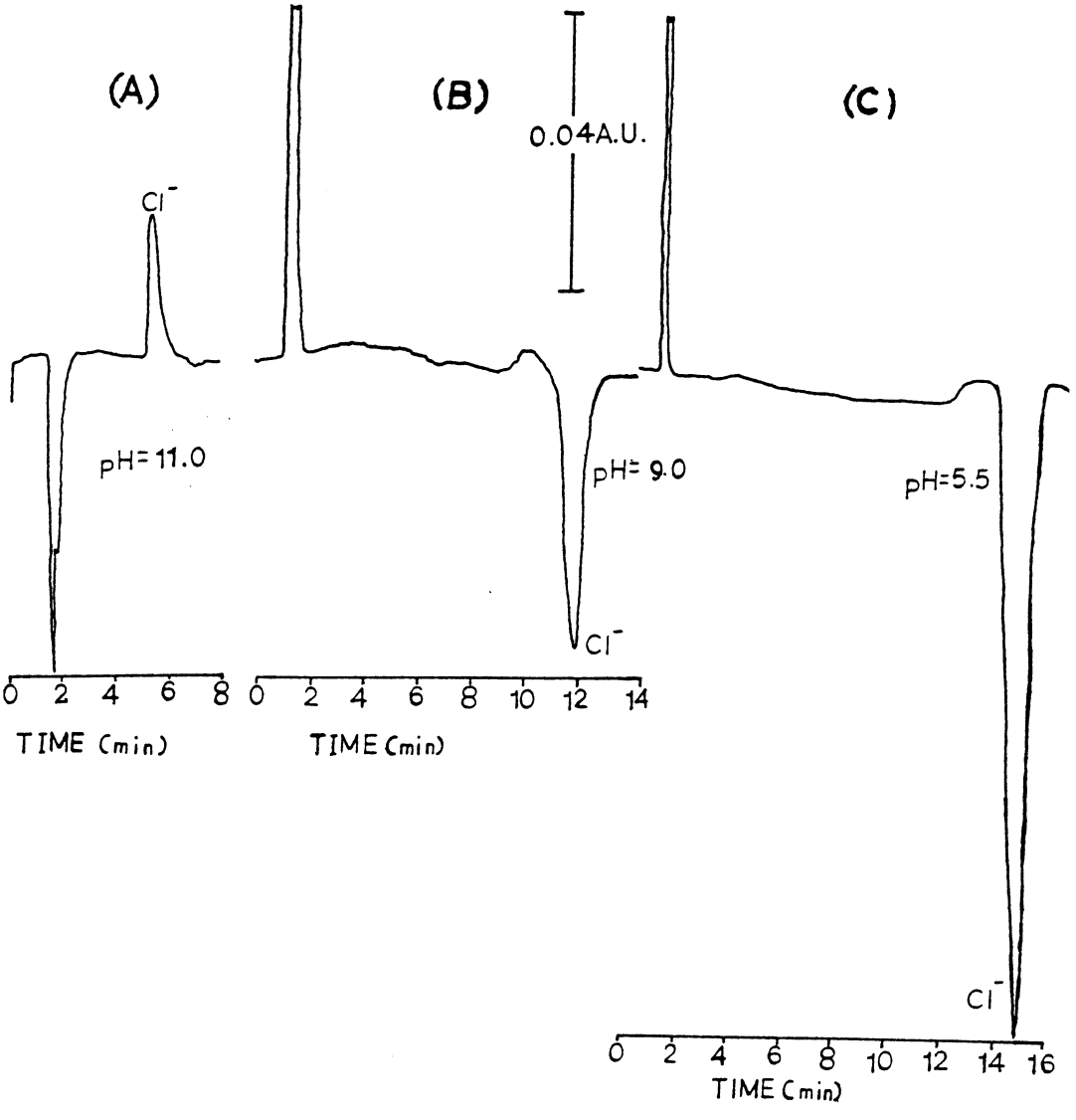

CARBONATE SPIKE
(b)
0.02 A.U.F.S.
CARBONATE SYSTEMPEAK
(a)
0 2 4 6 8 10 12 14 0 2 4 6 8 10 12 14
Retention time (min)

Figure 15 : Chromatogram of chloride ion (25 ppm) showing different response at various eluent pH. (A) pH = 11.0 (B) pH = 9.0 and (C) pH = 5.5, Conditions: 0.5 mM sodium benzoate, flow rate 1.0 mL/min λ =260 nm.


(A)
(B)
(C)
0.04A.U.
Cl⁻
pH= 11.0
pH= 9.0
pH=5.5
Cl⁻
Cl⁻
0 2 4 6 8
TIME (min)
0 2 4 6 8 10 12 14
TIME (min)
0 2 4 6 8 10 12 14 16
TIME (min)

The effect of eluent pH on the response of the chloride signal is illustrated in Figure 15. It can be seen that the sensitivity was reduced at a mobile phase of pH 9.0 compared to that at pH 5.5. The results clearly showed that both hydroxide and benzoate ions acted simultaneously as eluent anions. It followed that when both of these ions compete for the ion exchange sites, the indirect photometric signal was smaller than when elution was caused by one ion only. Furthermore, the retention time of chloride at pH 9.0 and pH 11.0 were considerably shorter than at pH 5.5. This decrease was indicative of the participation of hydroxide ions in the elution process. Interestingly, in the pH 11.0 mobile phase, the chloride signal became positive with hydroxide ions playing the dominant role in the particular case considered.

CHAPTER III.

CHROMATOGRAPHIC BEHAVIOR OF CARBOXYLIC ACIDS AND SULFONIC ACIDS ON A MACROPOROUS LOW CAPACITY ANION EXCHANGE COLUMN

Section A : Introduction

Short-chained mono-, di-, and polyprotic organic acids are biologically important metabolites of carbohydrate oxidation, amino acid degradation, and lipid breakdown. They are frequently studied in a variety of areas including metabolic disorders [60-61], food biochemistry [62], and others [63]. Methods of analyzing these organic acids were long and cumbersome (e.g. gas, paper and thin layer chromatography [64]) until the recent development of specialized, polymeric liquid chromatographic columns [65-67]. These columns can withstand extreme pH conditions and high percentages of organic solvents and can separate acids as organic anions using aromatic salts as mobile phases.

The retention of dicarboxylic acids as organic analyte ions on a low capacity anion exchange column has been discussed in the literature, and it was concluded that the hydrophobicity and the acid dissociation constants were significant factors in the separations [27]. As shown in Chapter 1 section D, the pH of the eluent is a very important

factor for successful IPC. In addition, bulky organic substituents such as long alkyl chains on the analyte ions are a probable reason for their high affinity on polymeric phases, so they are often termed as hydrophobic or hydroorganic ions. Elution of such ions from the ion-exchange resins commonly used requires an organic solvent as a modifier in the mobile phase. Two major interactions influence the retention of an organic analyte ion. First, the adsorption of the hydroorganic analyte ion on the non-polar surface of the resin must be considered. The second interaction is the ion-exchange selectivity between the eluent ion and the analyte ion. Both, empirical [68-70] and theoretical [13-15] approaches have been used to explain chromatographic retention on these types of ion-exchangers.

The need for a broad range of eluting conditions for the separation of a variety of carboxylic acids and sulfonic acids is important for several reasons. First, the carboxylic and sulfonic acids can vary in their structural features (e.g. side chain length). The mobile phase conditions must have a potential to distinguish between this major difference. Second, the mobile phase conditions must be compatible with sensitive detection devices if analytical HPLC is the goal. As these aliphatic carboxylic and sulfonic acids are essentially nonchromophoric, indirect detection is a viable route to facilitate their detection

In the present chapter, the chromatographic behavior of sulfonic acids and mono-carboxylic acids on a low capacity polymer-based anion exchange column, Hamilton PRP-X100, is described. The PRP-X100 anion columns are among the better known commercially available HPLC anion columns. They are stable from pH 1 to 13 and are compatible with mobile phases containing high organic content. The eluent used is a mixture of a sodium benzoate and acetonitrile (CH_3CN). The concentration effect of CH_3CN as an organic modifier is examined to provide controlled retention of these aliphatic acids on the ion-exchange resin. The analyte anions are detected indirectly with an UV detector. The application of the present chromatographic system to saline analysis is also described.

SECTION B :EXPERIMENTAL

SECTION B.1: CHEMICALS

HPLC grade acetonitrile and reagent grade ferric nitrate were obtained from Fisher Scientific Co. (Fairlawn, NJ). Sodium benzoate, sodium chloride, ethylenediamine tetraacetic acid disodium salt (Na_2EDTA) and mono-carboxylic acids were purchased from the Aldrich Chemical Co. (Milwaukee, WI.). Alkyl sulfonates were obtained from Eastman Kodak Chemical Co. (Rochester, NY). Millipore water was obtained from the Milli-Q water purification system (Millipore, Bedford, MA). The "commercial saline solution" was bought locally at Wal-Mart (Perrigo Co., Allegan, MI).

SECTION B.2: EQUIPMENT

The chromatographic system used in this work has been described in chapter II section B.2.(Figure 5).

SECTION. B.3: OPERATING PROCEDURE

Aqueous solutions of the alkyl sulfonic and monocarboxylic acids were prepared at about 250 ppm. In a typical procedure for mobile phase preparation, the weight of sodium benzoate necessary to produce the desired concentration was dissolved in a binary mixture of Millipore water/ acetonitrile of various vol %. The pH values of the eluents used, were measured after calibrating the pH meter against acetonitrile/water (1:1) buffers. The pH meter readouts

obtained are denoted by pH*. The pH of the aqueous solution used for the preparation of sodium benzoate eluent was approximately 1.1 pH units lower than the pH* measured in the eluent itself. The column was conditioned with the desired benzoate salt mobile phase by passing through the column at least 200 mL of the mobile phase (1.0 mL/min). The flow-rate was 1.2 mL/min unless otherwise specified, and the column inlet pressure was 2000-2500 psi, depending on the vol % of acetonitrile /water. Again, 20 μL injection volumes were used. Sulfonic acids and carboxylic acids are completely ionized and exist as anions at pH* 7.0. The capacity factor, k', was calculated in the usual way (equation 2), and the column void time was 0.5 min at the above flow-rate.

SECTION B.4: PROCEDURES USED FOR SALINE ANALYSIS

The mobile phase was prepared similar to that described above and had a benzoate concentration of 2mM with 20% acetonitrile. Chloride and sorbate (2,4 hexadienoic acid) stock solutions (250 ppm), were prepared with Millipore water. The Fe(III)-EDTA stock solution was prepared by mixing an appropriate amount of $Fe(III)(NO_3)_3$ containing 1000 ppm of Fe(III). to (500 ppm) of EDTA solution. This amount of Fe(III) was enough to mask the EDTA. The pH of the Fe(III)-EDTA standard was monitored to be about 3.6. At basic pH values Fe(III) precipitates. However, the larger formation

constant of the Fe (III)- EDTA complex permitted this pH of 3.6 without any precipitation. Fresh solutions of Fe(III)-EDTA should be prepared for analysis as precipitation of Fe (III) occured within 12 hours. The concentration of stock and standard solutions of Fe(III)-EDTA thus obtained are denoted by *EDTA. Eight standards solutions were prepared from the above stock solution for calibration study. These eight standard solutions each contained sorbate, chloride and *EDTA and ranged from 1.25 to 100 ppm for chloride and *EDTA and from 0.25 to 25 ppm for the sorbate. The saline sample was prepared as follows: 1 mL of saline solution was added to1 mL of Fe (III)(NO_3) containing 1000 ppm of Fe (III), shaken for 30 seconds and diluted to 50 mL with Millipore water. The standards and sample were injected into the system using a 20 μL injecting loop and at a rate of 1.20 mL/min. The pressure of the system averaged 1900- 2100 psi.

RESULT AND DISCUSSIONS

Cantwell and coworkers [69,72-73] have reported that organic acids that possess both an anionic charge and a hydroorganic center are retained by a combination of ion-exchange and adsorption processes. This combined mechanism is possible, providing that the stationary phase is non-polar, has a high surface area and contains a relatively low number of anion exchange sites. Two major equilibria influence the retention of organic acids on PRP X-100. For the ion-exchange (IE) and adsorption (Ads) processes, the retention can be viewed as follows:

$$P\text{--}N^+Me_3C^- + R\text{--}X^- + M^+ \underset{}{\overset{IE}{\rightleftharpoons}} P\text{--}N^+Me_3\,X^-\text{--}R + C^- + M^+ \quad (20)$$

$$P\text{--}N^+Me_3C^- + R\text{--}X^- + M^+ \underset{}{\overset{Ads}{\rightleftharpoons}} M^+X^-\text{--}R\cdots P\text{--}N^+Me_3C^- \quad (21)$$

where P is the copolymer matrix, C^- is a counter anion, R-X^- is an analyte with an anionic site X^- and a hydroorganic center R, and M^+ is the mobile phase cation.

The retention of an organic analyte that is retained by an anion exchange process is affected by the eluent's ionic strength, pH, and concentration and by the capacity of the stationary phase. These parameters were discussed earlier. The effect due to the concentration of an organic modifier is highly critical in the adsorption

process and will be discusssed.

Acetonitrile concentration effect

It is well known that when hydrophobic interactions are important and the solutes adsorb onto hydrophobic sites in the stationary phase an increase in the concentration of the organic modifier decreases the retention. Conversely, an increase of the organic modifier concentration increases the retention of solutes which are retained by the ion-exchange process. These effects were examined by plotting k' data for various organic anions versus organic modifier content of % acetonitrile (CH_3CN). Using an eluent at pH* 6.7-7.2.

As shown in Figure16, the retention of polar carboxylic acids, for which the ion-exchange process is predominantly strong, (acetate, propionate, butyrate) increased as the % CH_3CN was increased. Conversely, the retention times of hexanoate (caproic acid), and heptanoate (enanthic acid) which are retained by an adsorption process and have a high hydrophobicity were shortened by addition of CH_3CN. Thus even if hexanoic and heptanoic acid were completely ionized in this eluent, the hydrophobicity could not be neglected in discussing retention. The chromatographic behavior of other monocarboxylic acids like pentanoate (valeric acid) was found to be intermediate between these groups. This specific character of a

FIGURE 16

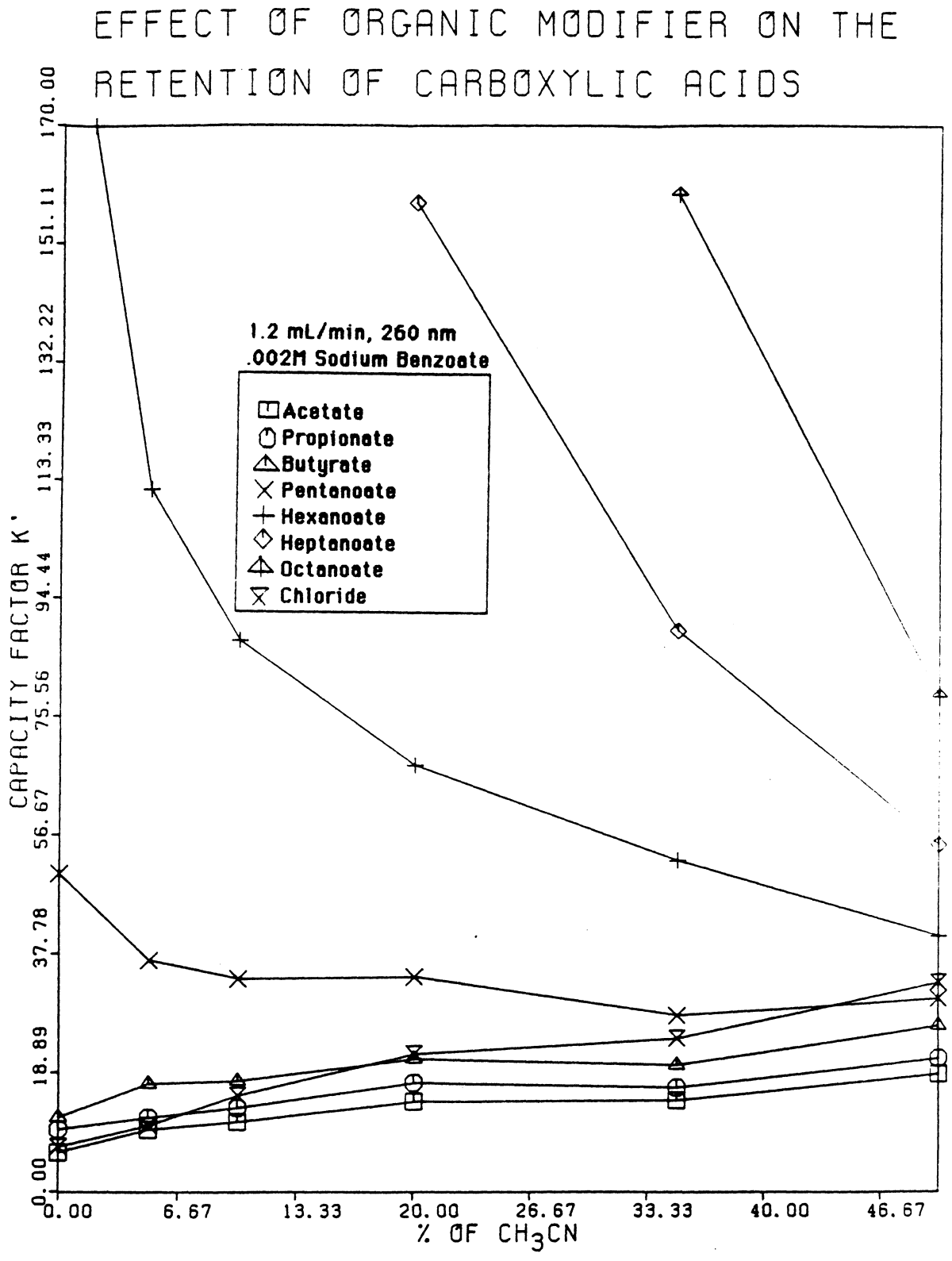
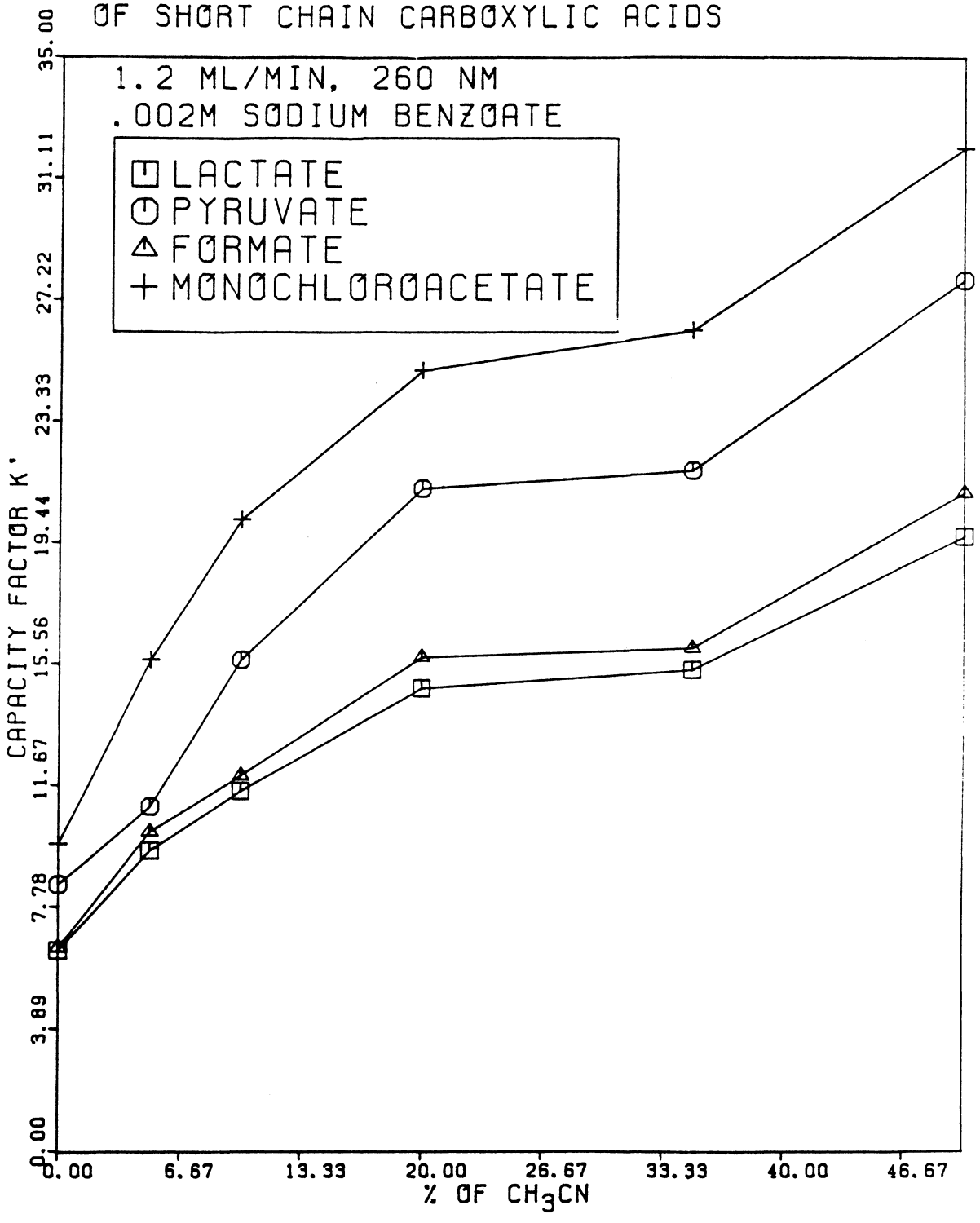

EFFECT OF ORGANIC MODIFIER ON THE RETENTION OF CARBOXYLIC ACIDS
1.2 mL/min, 260 nm
.002M Sodium Benzoate
Acetate
Propionate
Butyrate
Pentanoate
Hexanoate
Heptanoate
Octanoate
Chloride
CAPACITY FACTOR K'
0.00
18.89
37.78
56.67
75.56
94.44
113.33
132.22
151.11
170.00
0.00
6.67
13.33
20.00
26.67
33.33
40.00
46.67
% OF CH3CN

FIGURE 17

EFFECT OF ORGANIC MODIFIER ON THE RETENTION OF SHORT CHAIN CARBOXYLIC ACIDS



poly-styrene-divinyl benzene ion-exchange resin was expected, and would not be observed on ion-exchange cellulose which shows little matrix effect[3]. Figure 17 illustrates that the analytes that are very polar and have small hydrophobic centers (lactate, pyruvate, formate and mono-chloroacetate) increased considerably in retention with an increase in the concentration of CH_3CN. This phenomenon may be explained by the fact that, as the mobile phase becomes more non-polar, the resin phase, increases in relative polarity; thus, the analytes that are very polar are very highly retained on the anion exchange sites as the concentration of acetonitrile increases. The relationship between capacity ratio of short chain sulfonic acids and the concentration of CH_3CN is shown in Figure 18. Alkyl sulfonates (CSO_3^- —— $C_3SO_3^-$) showed an expected increase in retention with an increase in CH_3CN content; however, an interesting retention behavior was found for butane sulfonate ($C_4SO_3^-$). The retention of $C_4SO_3^-$ dropped with an increase in CH_3CN content, but there was a negligible effect on retention in going from 10 to 30% CH_3CN; however at 35% CH_3CN, it became more highly retained. Finally, the retention of $C_4SO_3^-$ was decreased at 50% CH_3CN.

In Table II, retention for the $RCOO^-$ and RSO_3^- analytes (except for acetate and formate) under a given condition increased as the

FIGURE 18

EFFECT OF ORGANIC MODIFIER ON THE RETENTION OF SULFONIC ACIDS

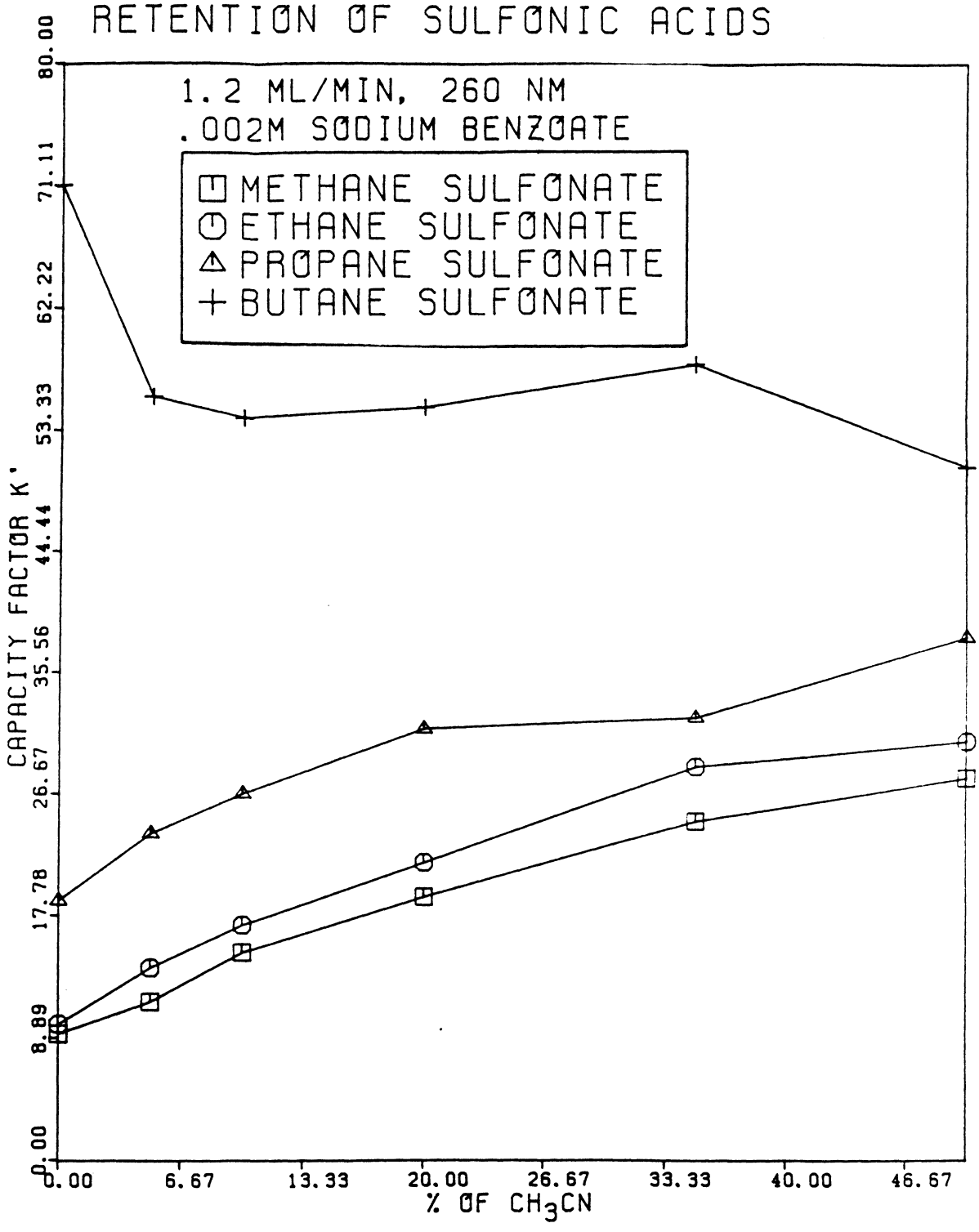


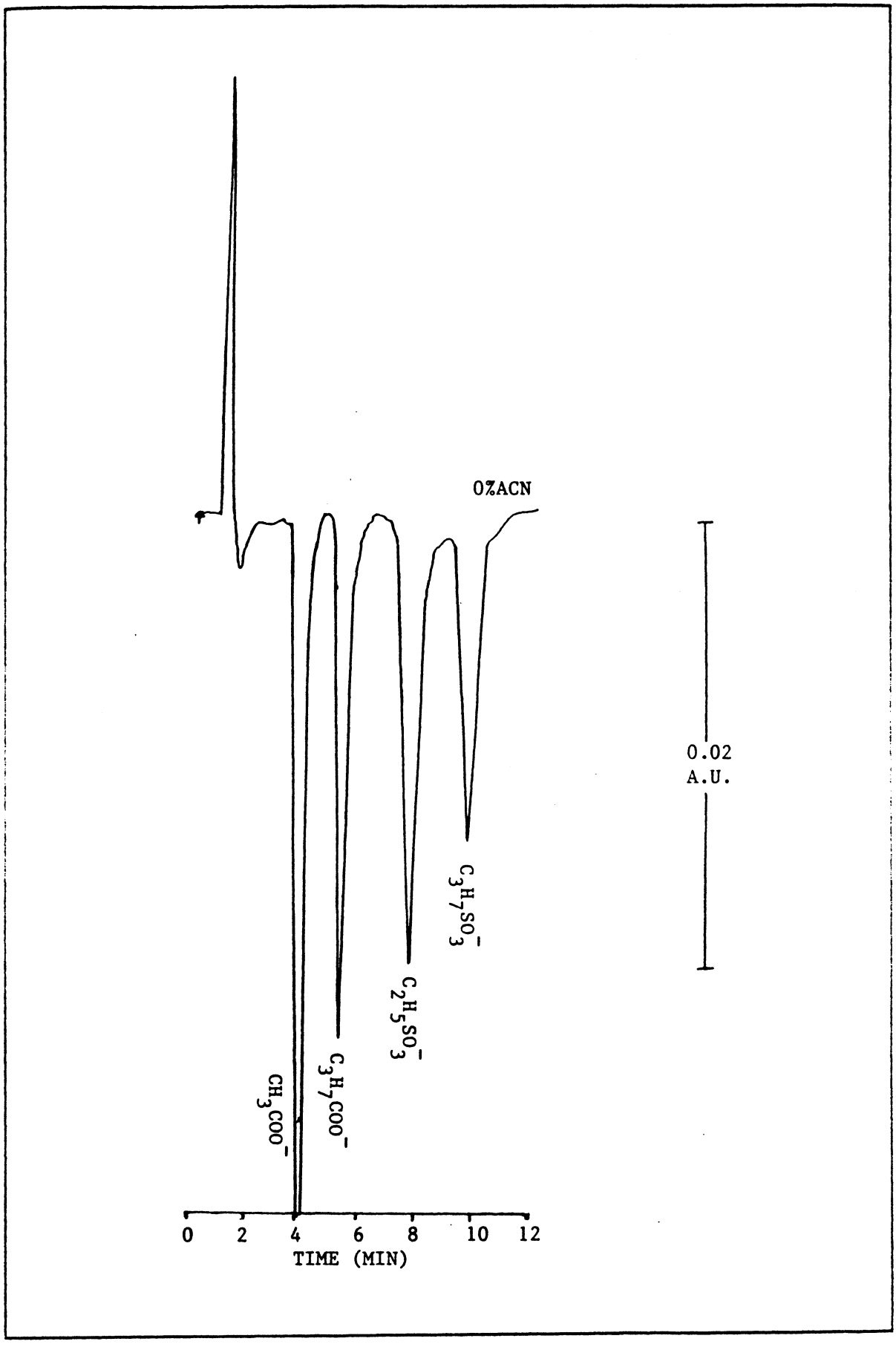
Table II. Effect of Organic Modifier on Alkyl Carboxylic and Alkyl Sulfonic Acids Retention

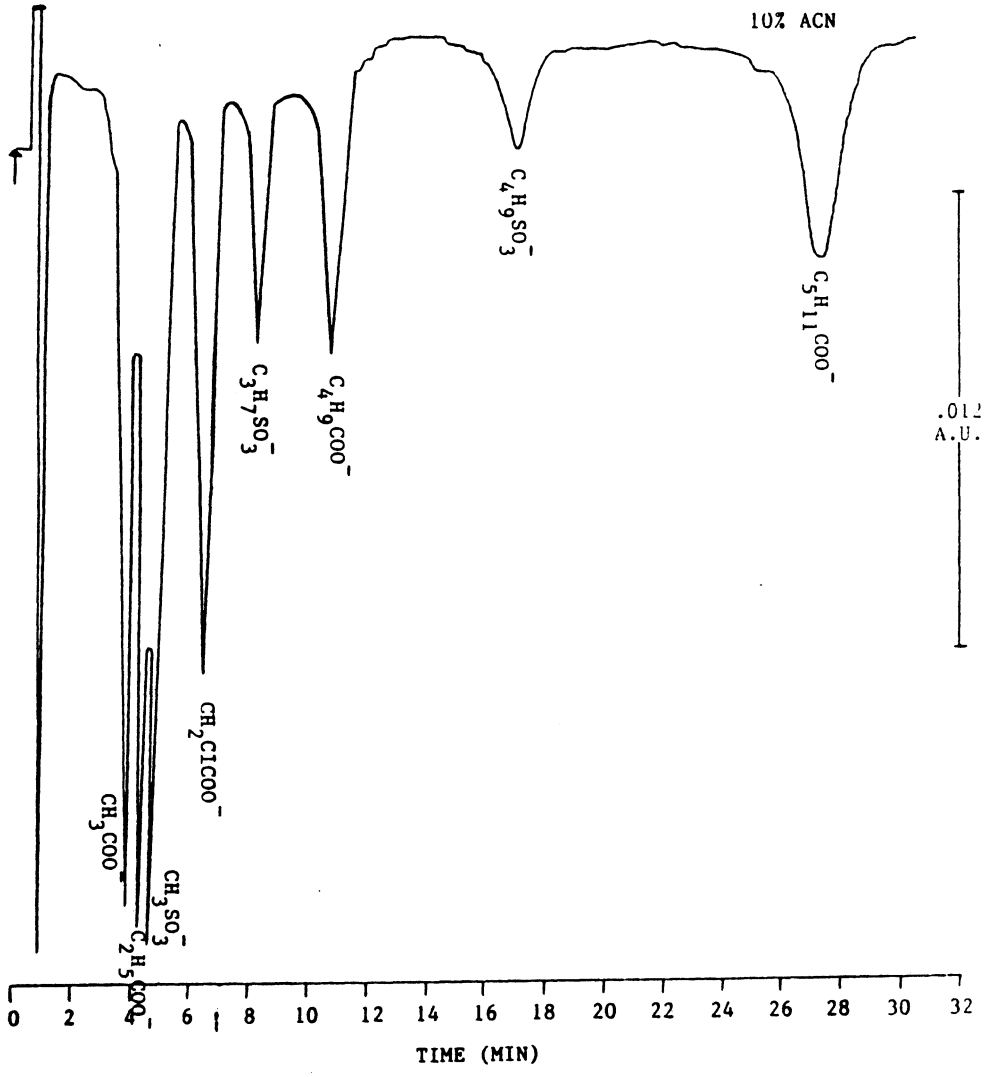
	Capacity factor k'					
	Percent CH_3CN					
†Analyte (ion)	0	5	10	20	35	50
Chloride (Cl^-)	7.2	10.6	15.4	22.0	24.6	33.5
Acetate (CH_3COO^-)	6.3	9.9	11.2	14.4	14.6	18.9
Formate ($HCOO^-$)	6.4	10.2	12.0	15.8	16.1	21.1
Propionate ($C_2H_5COO^-$)	8.2	11.8	13.4	17.4	16.6	21.4
Butyrate ($C_3H_7COO^-$)	11.8	17.2	17.7	21.2	20.2	26.6
Pentanoate ($C_4H_9COO^-$)	50.6	36.8	33.9	34.2	28.1	30.9
Hexanoate ($C_5H_{11}COO^-$)	**	111.8	87.8	67.8	52.7	40.8
Heptanoate ($C_6H_{13}COO^-$)	***	***	***	157.8	89.4	55.4
Octanoate ($C_7H_{15}COO^-$)	***	***	***	***	159.1	79.0
Methane—SO_3^-	9.2	11.5	15.1	19.2	24.7	27.9
Ethane—SO_3^-	9.9	14.0	17.1	21.7	28.7	30.6
Propane—SO_3^-	18.9	23.8	26.7	31.5	32.3	38.2
Butane—SO_3^-	**	55.8	54.2	55.0	58.2	50.6
Heptane—SO_3^-	***	***	***	***	***	212.1

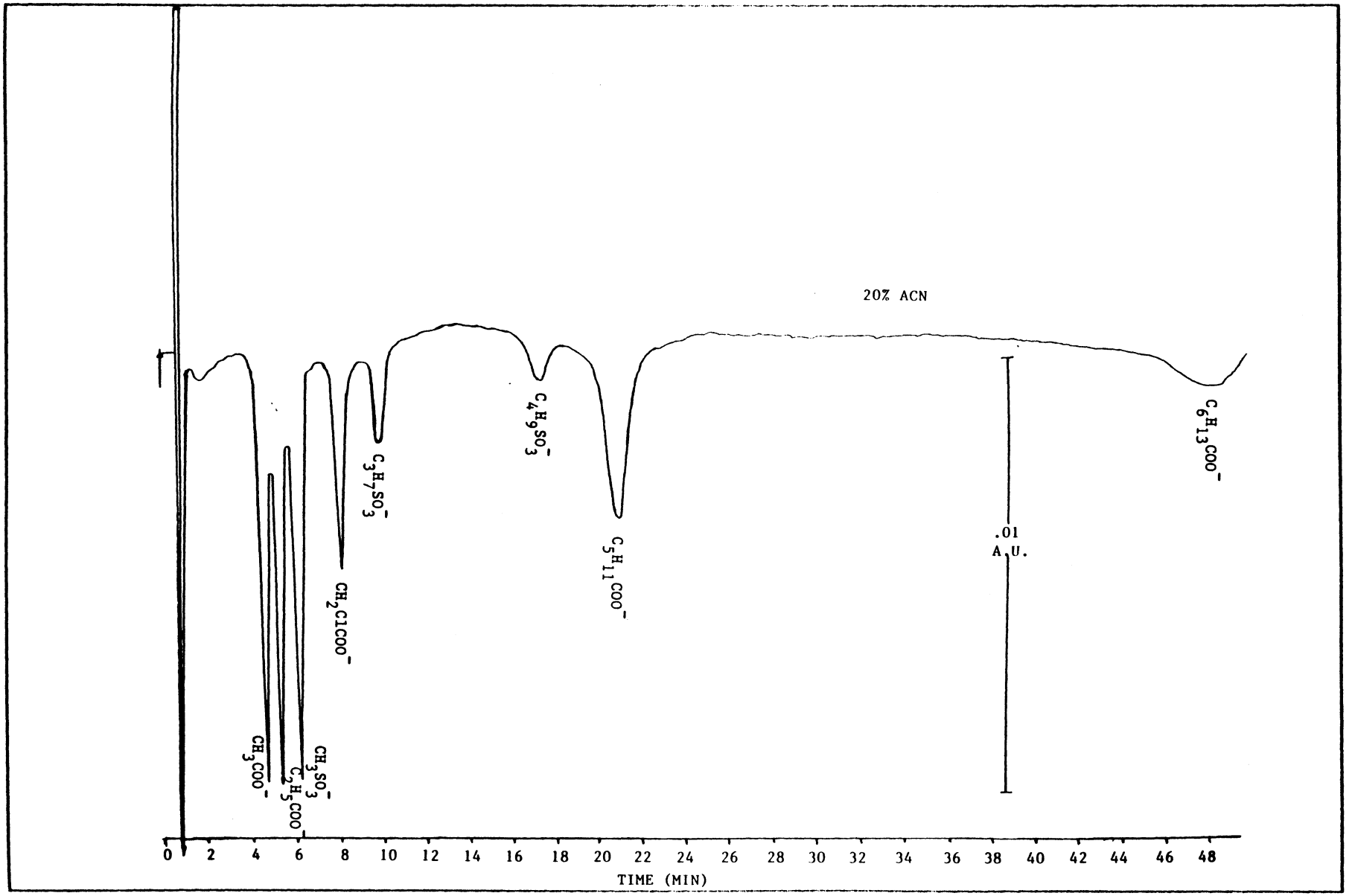
†Carboxylic and sulfonic acids exist as anions in 2.0 mM sodium benzoate *pH 7.2, CH_3CN : H_2O mobile phase. ** excessively long retention time
*** not studied.

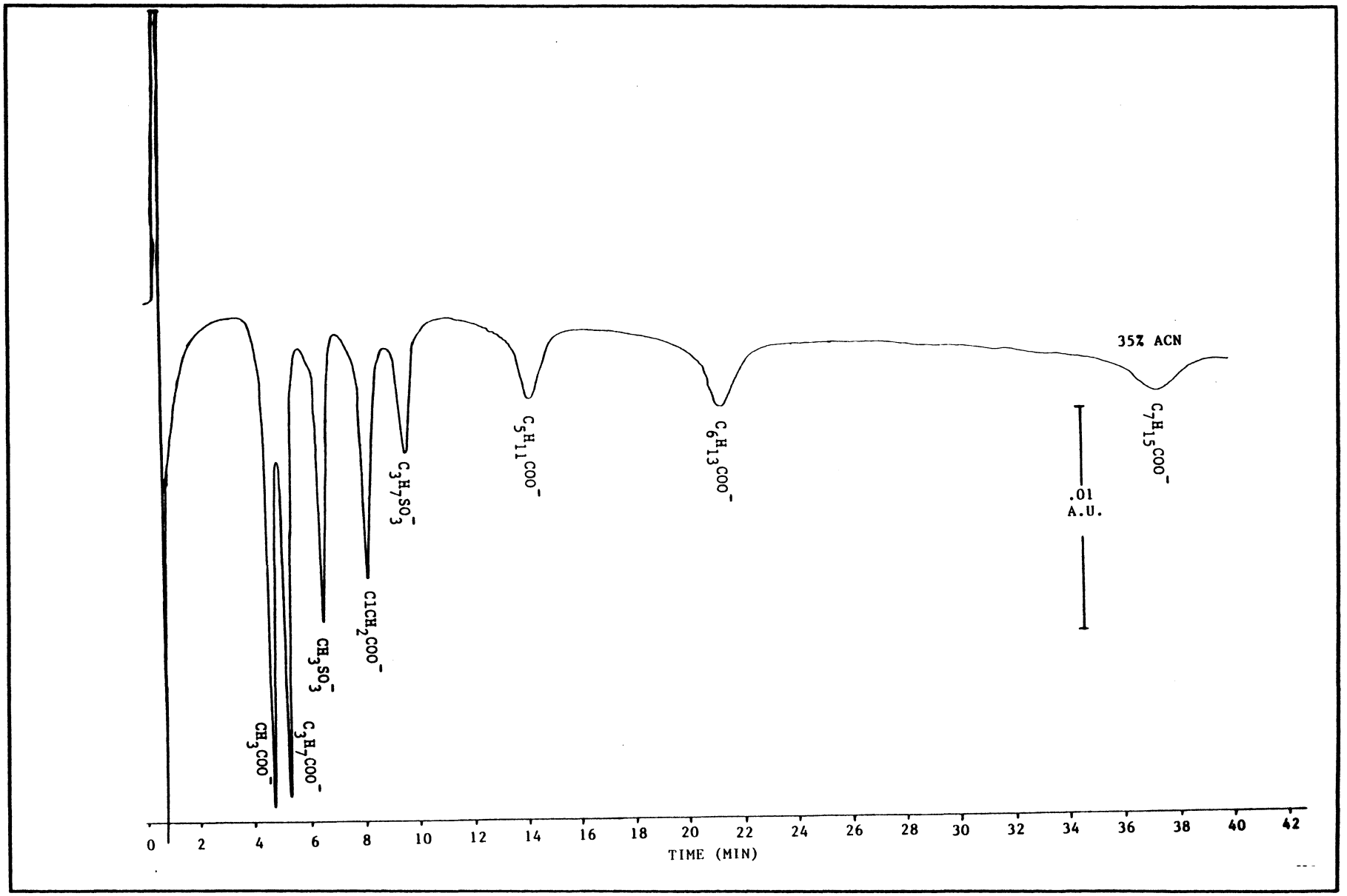
alkyl chain length increased. Thus, elution of the longer chain sulfonic acids and carboxylic acids in a reasonable time required, either a high CH_3CN concentration, a counter-anion of high anion exchange selectivity, a high concentration of counter-anion, or a combination of these factors. A second conclusion from Table II was that when comparing retention of alkyl sulfonic acids and carboxylic acids, the sulfonate had the higher retention when the carbon number for the alkyl group was the same. The data also suggested that at about C_6 or higher, reverse phase retention of $RCOO^-$ began to be competitively significant. Therefore, when carboxylic acids and sulfonic acids have short alkyl chain lengths, IPC of these analytes was the result of the effect of the counter anion selectivity and ionic strength on the equilibria described in equations (20) and (21). For the longer alkyl chain lengths, the competitive effect of the adsorption equilibria described by equation (21) became significant. Furthermore, the retention data in Table II suggested that the consecutive members of the homologous series of alkyl carboxylic and sulfonic acids were separable. However, the data also indicated that a multicomponent alkyl mixture cannot be readily separated in a reasonable analysis time by using an isocratic mobile phase. Thus, as the alkyl chain length in the sulfonate or carboxylate series increased in length, favorable elution was obtained by increasing the % CH_3CN in

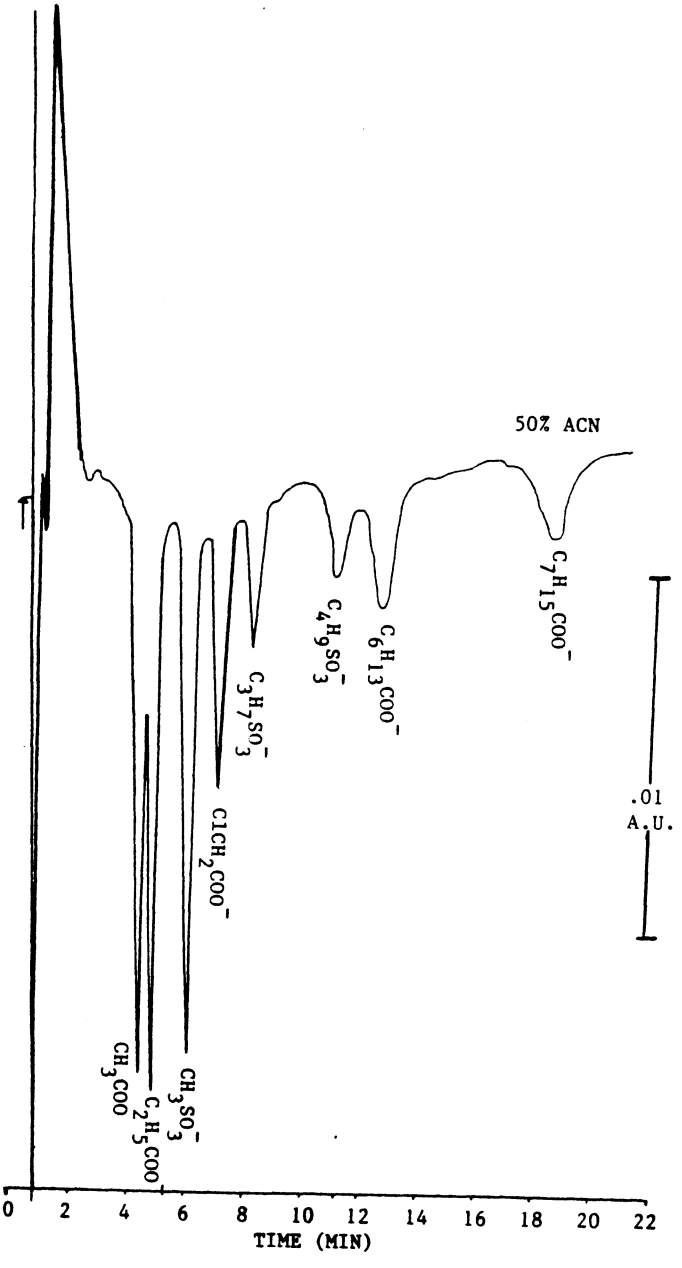
Figure 19: Separation of alkyl carboxylic and sulfonic acids isocratically.with various ratios of CH_3CN:H_2O A) 0% CH_3CN B) 10% CH_3CN C) 20% CH_3CN D) 35% CH_3CN E) 50% CH_3CN. Conditions : 2 mM sodium benzoate, pH* 6.7 - 7.2, 0.04 A.U.F.S., 2.0 mL/min, at 260 nm.


0%ACN
CH_3COO^-
$C_3H_7COO^-$
$C_2H_5SO_3^-$
$C_3H_7SO_3^-$
0.02
A.U.
0
2
4
6
8
10
12
TIME (MIN)


10% ACN
$C_4H_9SO_3^-$
$C_5H_{11}COO^-$
$C_3H_7SO_3^-$
$C_4H_9COO^-$
CH_2ClCOO^-
CH_3COO^-
$CH_3SO_3^-$
$C_2H_5COO^-$
.012
A.U.
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
TIME (MIN)


20% ACN
CH_3COO^-
$C_2H_5COO^-$
$CH_3SO_3^-$
CH_2ClCOO^-
$C_3H_7SO_3^-$
$C_4H_9SO_3^-$
$C_5H_{11}COO^-$
$C_6H_{13}COO^-$
.01
A.U.
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
TIME (MIN)


35% ACN
$C_7H_{15}COO^-$
$C_6H_{13}COO^-$
$C_5H_{11}COO^-$
$C_3H_7SO_3^-$
$ClCH_2COO^-$
$CH_3SO_3^-$
$C_3H_7COO^-$
CH_3COO^-
.01
A.U.
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42
TIME (MIN)


50% ACN
$C_7H_{15}COO^-$
$C_6H_{13}COO^-$
$C_4H_9SO_3^-$
$C_3H_7SO_3^-$
$ClCH_2COO^-$
$CH_3SO_3^-$
$C_2H_5COO^-$
CH_3COO^-
.01
A.U.
0
2
4
6
8
10
12
14
16
18
20
22
TIME (MIN)

the mobile phase. This effect is illustrated in Figure 19 (a to e), where mixtures of alkyl carboxylates and alkyl sulfonates of increasing chain length were separated under isocratic mobile phase conditions whereby the % CH_3CN was increased in the following steps: 0%, 10%, 20%, 35%, %50%. In all cases the nonchromophoric carboxylic acids and sulfonicacids were readily detected by IPC strategy. Analyte peaks were well defined and reproducible, and their peak heights were shown to correlate to the amount of analyte injected. Figure 20 illustrates that a standard mixture of alkyl carboxylic acids from C_2 to C_8 can be separated in a reasonable time of 22 minutes. At the lower concentration of CH_3CN, heptanoate and octanoate were eluted very late, and the peaks were broad. Therefore, 50% CH_3CN was considered to be the most appropriate concentration for the separation of the late eluting carboxylic acids. Short chain sulfonic acids(C_1 to C_3) exhibited typical anion exchange behavior, but butane sulfonate ($C_4SO_3^-$) was retained very long, about 36.0 min at 0% CH_3CN. However, 10% CH_3CN eluted all these alkyl sulfonates in a reasonable time of 17 minutes (Figure 21). A long chain alkyl sulfonate (heptane sulfonate) was retained excessively long even with 50 % CH_3CN, and the IPC peak was very broad.

Lactose fermentation can yield lactic, pyruvic, propionic and butyric acids. Separation of these acids as anions was achieved in

Figure 20 : Separation of alkyl carboxylic acids. Chromatographic conditions: 50% CH_3CN, 2mM sodium benzoate, *pH 7.2, flow rate = 2.0 mL/min, 260 nm solutes concentrations = 50 ppm.

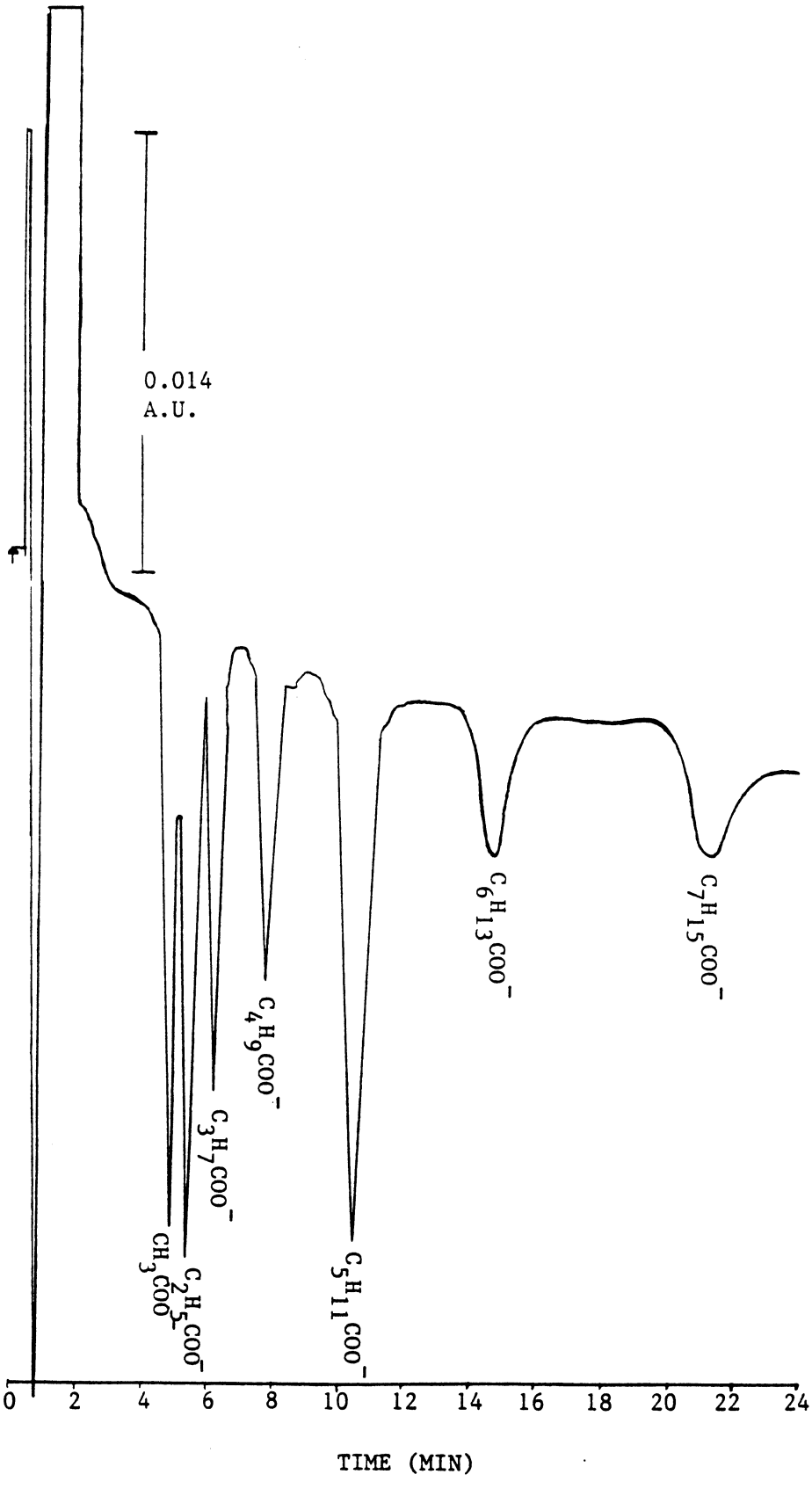

0.014
A.U.
CH_3COO^-
$C_2H_5COO^-$
$C_3H_7COO^-$
$C_4H_9COO^-$
$C_5H_{11}COO^-$
$C_6H_{13}COO^-$
$C_7H_{15}COO^-$
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (MIN)

Figure 21 : Separation of short chain sulfoni

Chromatographic conditions were the same a

Figure 20 except 10% CH_3CN was used.

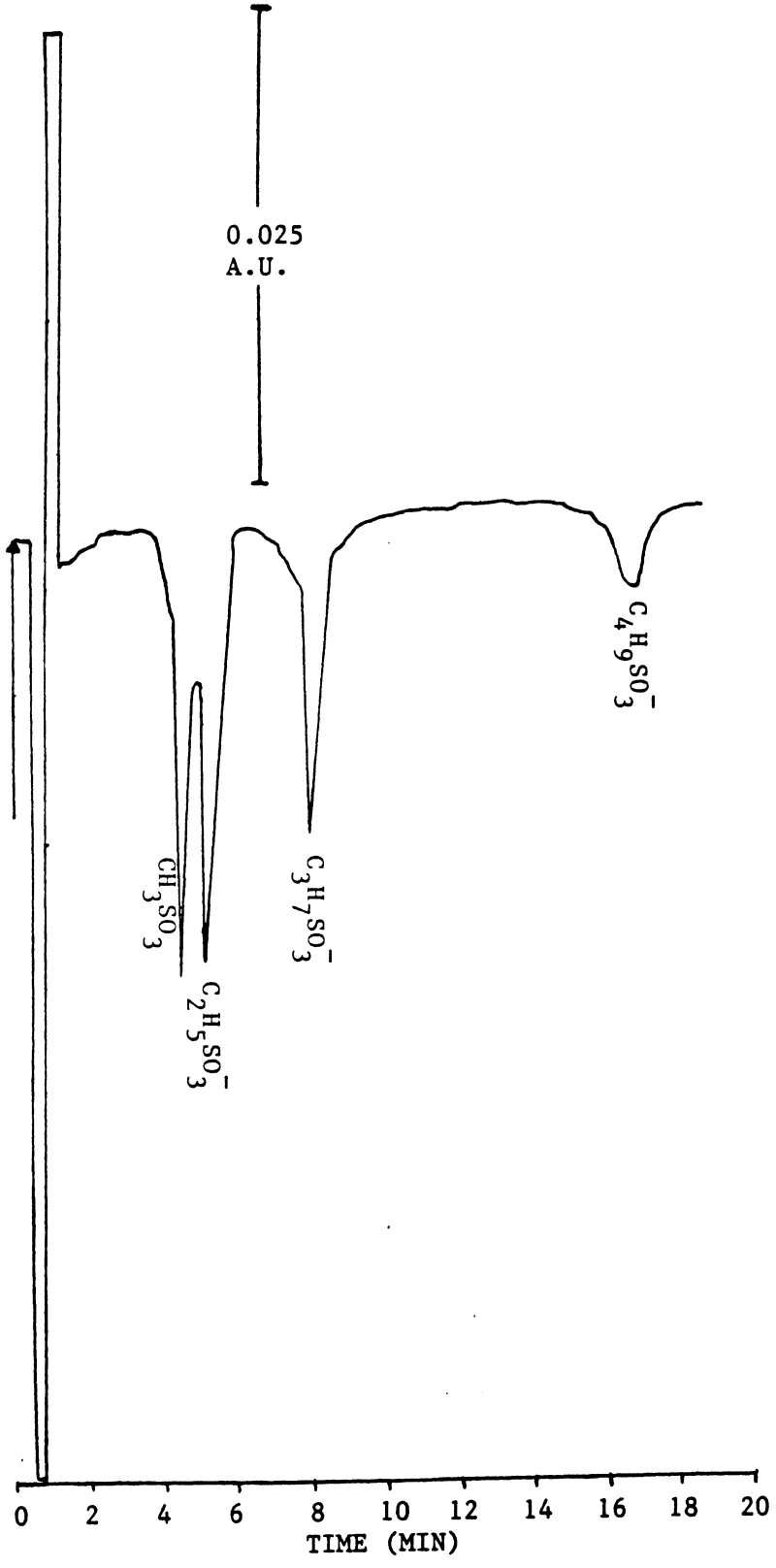

0.025
A.U.
$CH_3SO_3^-$
$C_2H_5SO_3^-$
$C_3H_7SO_3^-$
$C_4H_9SO_3^-$
0
2
4
6
8
10
12
14
16
18
20
TIME (MIN)

Figure 22 : Separation of short chain organic acids using 2.0 mM sodium benzoate, pH 6.2, 1.2 mL/min 260 nm. Solutes concentrations =35 ppm.

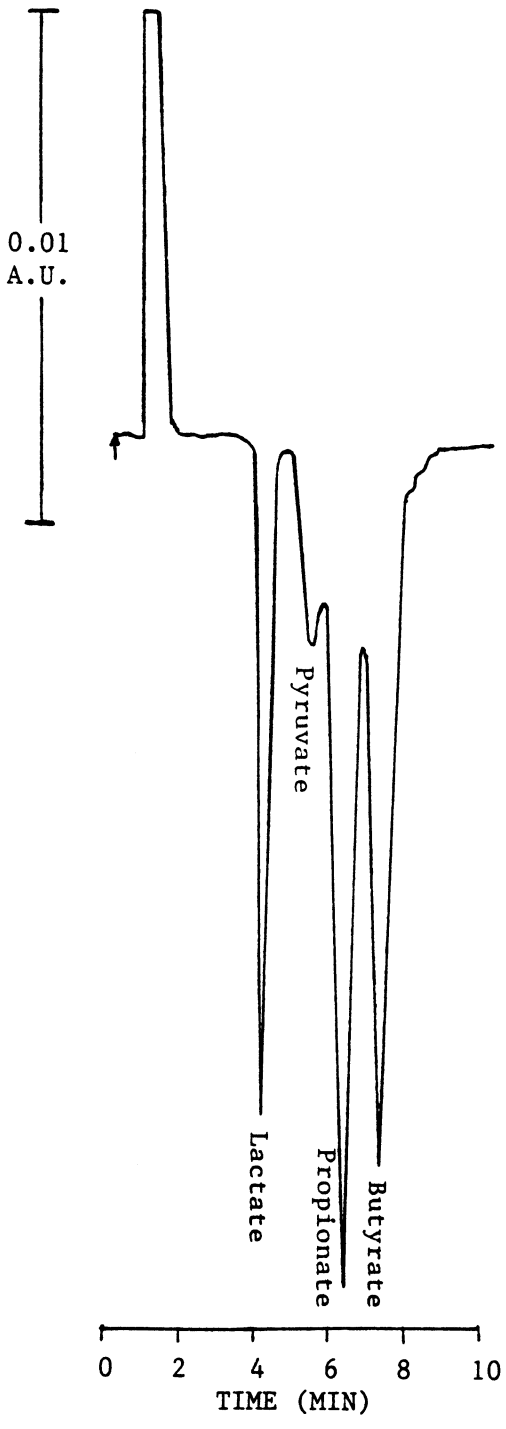
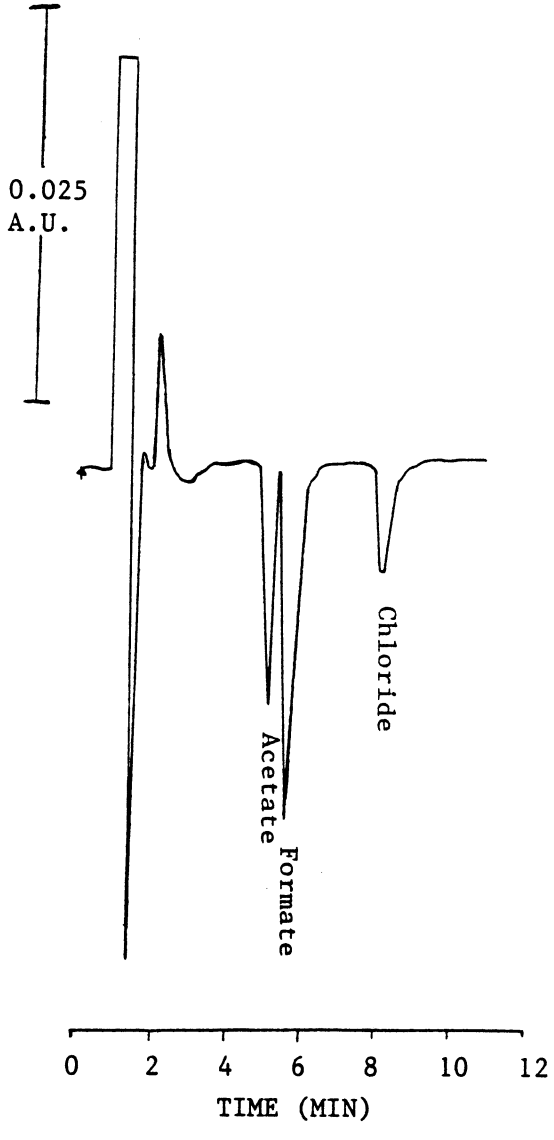

0.01
A.U.
Lactate
Pyruvate
Propionate
Butyrate
0
2
4
6
8
10
TIME (MIN)

Figure 23 :Separation of a standard mixture (50 ppm) of acetate, formate,and chloride. Chromatographic conditions: 2 mM sodium benzoate, 50% CH_3CN, *pH 7.2, 1.2 mL/min, 260 nm


0.025
A.U.
Acetate
Formate
Chloride
0
2
4
6
8
10
12
TIME (MIN)

under eight minutes (Figure 22). Since they were all short chain carboxylic acids, no organic modifier was required for their separation.

Acetate and formate are difficult to separate from each other by simple ion exchange chromatography unless acetonitrile is added to the eluent. Figure 23 shows the separation of acetate, formate, and chloride. Optimum resolution was obtained with 2.0 mM benzoate, (pH* 7.2), and 50% CH_3CN as the eluent.

In summary, hydrophobic interactions had a significant affect on the retention of carboxylic acids and sulfonic acids with increasing carbon number when chromatographed under anionic pH conditions employing a low capacity anion exchange column. Thus, it can be concluded that reversed-phase like sorption accompanies electrostatic attraction in the ion-exchange of organic anions at two sites. At one site, only reversed-phase interaction occurs. This interaction contributes little to the retention of small organic anions but has a significant contribution to hydrophobic anions. At the other site, the ion-exchange site, non-polarity of the mobile phase facilitates electrostatic interaction and makes a considerable contribution to the retention of organic anions that are retained by an ion-exchange process.

Saline analysis

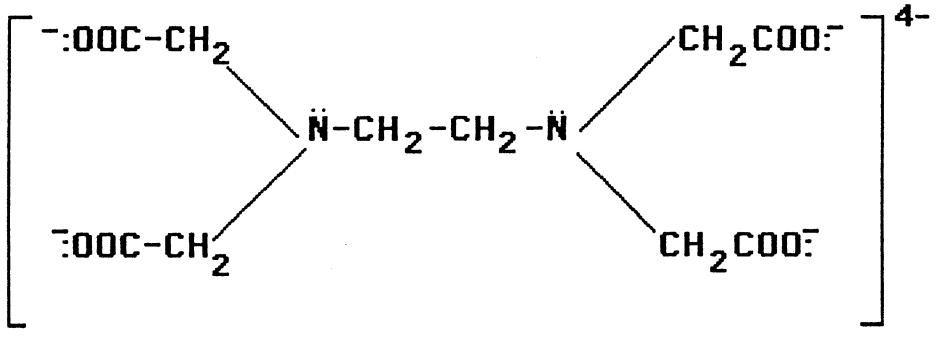
When both hydrophobic and hydrophilic anions are present in the same sample but the analyte of interest is in only one of the groups, the separation approach is simple. The situation is more complicated if we wish to determine both of these types of anions in one sample. A polar eluent with a reasonable ion exchange selectivity will elute the hydrophilic anions, but the ion-exchange column may still contain the hydrophobic anions. This may not pose any problem right away, but the hydrophobic anions may eventually co-elute with other anions in later injections or not at all. This problem can, however, cause significant errors in the determination of the hydrophilic anions. One solution to this problem is to use an organic modifier in the mobile phase.

To illustrate the application of ion-exchange chromatography, the chromatographic system described earlier in this chapter was used for the analysis of a commercial saline solution. The saline was a sterile, buffered, isotonic aqueous solution containing borate, chloride, EDTA and sorbate. Analysis of the saline solution was therefore limited by the requirement to separate these four very different species.

The chloride ion has been shown to be easily separated and detected by IPC. The other analytes require special mention with

respect to the nature and separation considerations. EDTA is a polyvalent anion and exists as various anions depending upon the pH. It poses a problem in eluting from the column because of its very high charge (-3) at neutral pH. A concentrated eluent would be necessary to dislodge it from the anion exchange resin. Sorbate is a hydrophobic anion and would be expected to have a very high affinity for the anion exchange resin. Borate is the anion of a very weak acid (pKa = 9.2) and is very weakly retained on an anion exchanger. In this section a method for separating these anions in the saline solution is presented.

EDTA is a strong chelating ion that forms stable complexes with transition metals. We can take advantage of this unique feature to facilitate its elution. The EDTA can be easily eluted from the anion exchange column by complexing it with a metal thereby reducing its charge density. Its structure is shown below.



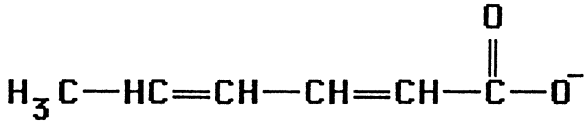
EDTA

For EDTA the acid dissociation constants are as follows: pKa_1 = 1.99, pKa_2 = 2.67, pKa_3 = 6.16 and pKa_4 = 10.26. Thus, the main EDTA

species in 2.0 mM benzoate eluent, pH 6.9* are $[H_2EDTA]^{2-}$ and $[HEDTA]^{3-}$. However, polyvalent metal ions (M^{2+} and M^{3+}) form metal–EDTA anion complexes such as $[M(EDTA)]^{2-}$ and $[M(EDTA)]^{-}$, respectively. Attempts were initially made to complex calcium (Ca^{+2}) with EDTA, to produce $[Ca(EDTA)]^{2-}$ but no signal was observed. This result was not too surprising since a previous study showed that EDTA does not complex metal ions such as calcium(II) and magnesium (II) around neutral pH because of their low formation constants[77] . However, EDTA does complex many transition metal cations at neutral pH as shown by their large formation constants to form stable EDTA complexes [77-79]. Therefore, experiments were performed in which EDTA was added to the Cu(II) and Fe(III) metal ions. The results obtained showed that both $[Fe(EDTA)]^{-}$ and $[Cu(EDTA)]^{2-}$ form strong mono and divalent anion complexes in solution of neutral pH. $[Cu(EDTA)]^{2-}$ anion was eluted very late at about 35 minutes, and the peak was very broad. This was expected as $[Cu(EDTA)]^{2-}$ is a divalent anion and would have a high affinity for the anion exchange column. However, the monovalent anion $[Fe(EDTA)]^{-}$ was easily eluted from the column in a reasonable time. Since $Fe(III)(NO_3)_3$ was used for complexing EDTA, a negative peak of nitrate anion was observed

which partially overlapped with the positive peak of $[Fe(EDTA)]^-$.

Elution of the sorbate anion was promoted by using acetonitrile. Acetonitrile was effective in modifying the adsorption of sorbate onto the hydrophobic sites of the resin while the benzoate eluent was able to compete with analyte ions for the ion exchange sites. The structure of sorbate anion is show below.



SORBATE
(2,4-hexadienoic acid)

Borate is a weakly acidic anion and was not dissociated at the working pH of the eluent which was about 7.0. One of the problems in using a higher pH mobile phase for the separation of the borate ion in the saline solution would have been the precipitation of Fe(III). Usually Fe(III) precipitates to $Fe(OH)_3.nH_2O$ in basic solution. The control of eluent pH in the acidic condition prevented the precipitation of $Fe(OH)_3$, but on the other hand, it does not allow the determination of borate which can exist as an anion only in basic solution. An alternative procedure was employed to complex boric acid with fructose, since the boric acid-fructose complex is a stronger acid (pKa = 4) than the boric acid itself [2,74-76]. It seemed that complexation between fructose and boric acid was dependent

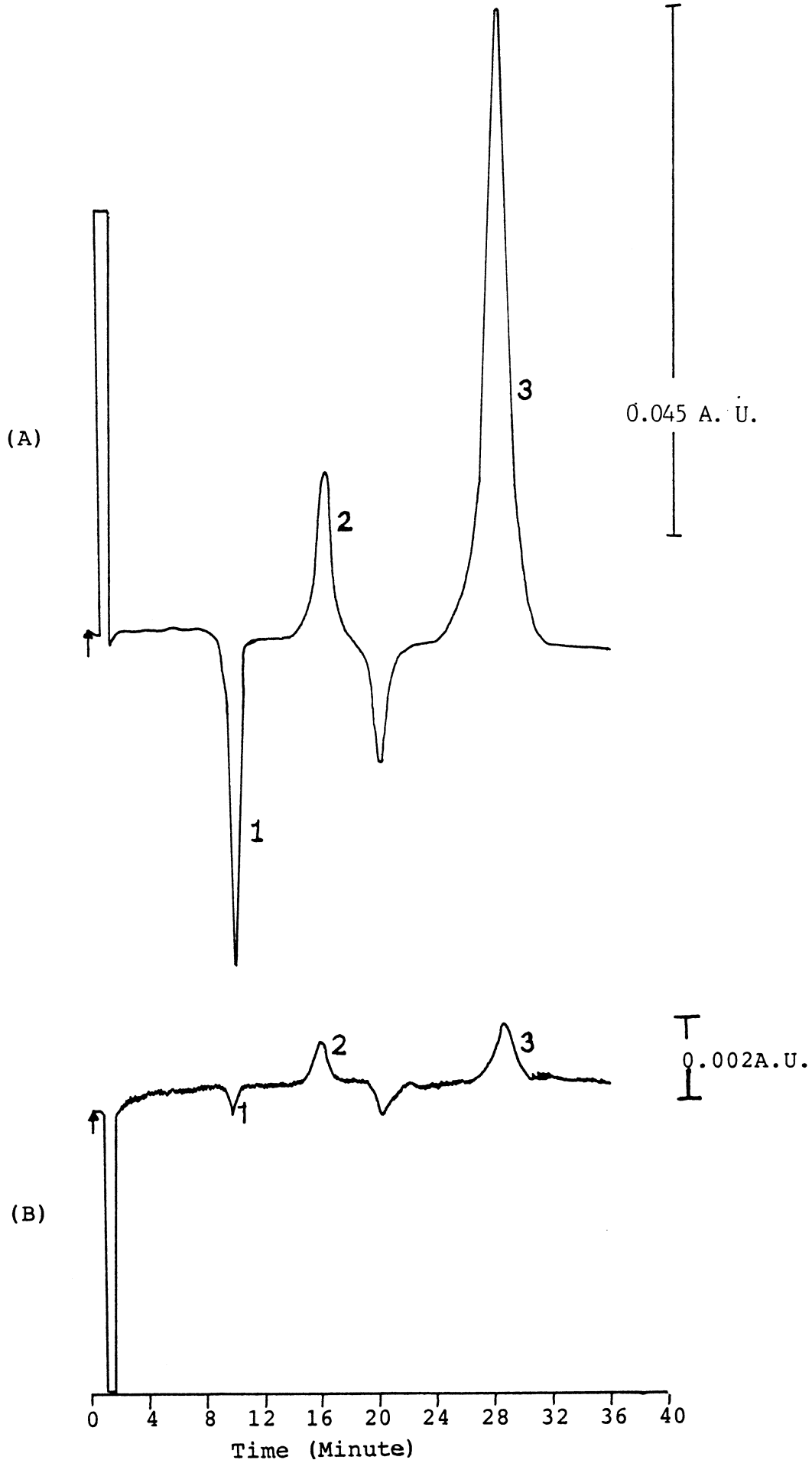
upon the relative proportion of the two reactants, pH etc. Because of time limitations no positive results were achieved for this approach, thus no further investigations was possible.

Ion exchange chromatography with UV detection is capable of good sensitivity. This fact is conveyed to some extent by Figure 24(A), which shows a separation of sorbate, chloride and EDTA as $[Fe(EDTA)]^-$ from the direct injection of 20 µL of saline solution. The analysis showed that the detector response of a sample depends on the relative absorbances of the sample and the eluent ions. In this case, $[Fe(EDTA)]^-$ and sorbate had higher molar absorptivities than the benzoate eluent at 260 nm so they appear as positive peaks, while the chloride ion gives a negative peak as it does not absorb any UV-light. Figure 24(B) shows a separation of a standard solution with the anions at a level close to their detection limits. The analysis time for the saline solution was about 28 minutes, but a faster separation could have been achieved at a higher flow-rate or by using a short column.

Quantitative measurement and detection limits

Because the $[Fe(EDTA)]^-$ peak partially overlapped with the nitrate peak, the quantitative measurement of the peak heights was used. Calibration was established after three injections by using the

Figure 24 Chromatogram of (A) Saline sample with (1) chloride (51.9 ppm), (2) $[Fe(EDTA)]^-$ containing (17.2 ppm) of *EDTA, (3) sorbate (19.1 ppm). (B) standard mixture of (1) chloride (1.25ppm), (2) $[Fe(EDTA)]^-$ containing (1.25 ppm) of *EDTA, (3) sorbate (0.5 ppm).at the level of detection limit.
Conditions : 2.0 mM sodium benzoate, 20% CH_3CN, pH* 6.9, 1.2 mL/min, 260 nm. 0.1 A.U.F.S for the saline sample, 0.04 A.U.F.S. for the standard mixture.


(A)
1
2
3
0.045 A. U.
(B)
1
2
3
0.002A.U.
0
4
8
12
16
20
24
28
32
36
40
Time (Minute)

combination anion solution indicated in Table III. Coefficients of variation (CV) ranged from 0.4 to 7.5% for standards and 0.5 to 3.4% for the saline sample. The average CV for the standard solution was 2.1%. From the slope and intercept value of the calibration plots, the concentration of the anions found in the saline solution were as follows: chloride, 51.9 ppm; *EDTA, 17.2 ppm; sorbate,19.1 ppm. Since 1 mL of commercial solution was diluted to 50 mL, the total amount of these anions in 1mL saline solution was : chloride, 2.59 mg; *EDTA, 0.86 mg; sorbate, 0.95 mg. The total amount of these anions found in the 355 mL commercial bottle was therefore: chloride, 919.5 mg; *EDTA, 305.3 mg; and sorbate, 337.3 mg. Plots of peak height vs. concentration of the anions are shown in Figure 25 (a-c). At concentrations tions higher than 25 ppm, the sorbate anion peak became unsymmetrical. There was a problem with peak height determination for EDTA, especially at concentrations higher than 100 ppm. The loss of peak height could be attributed to the increased overlap with the nitrate peak resulting in an undefined baseline. As a result, the calibration curve for *EDTA plateaus at concentrations higher than 100 ppm. For chloride, a linear response was obtained for the concentration range from 1.25 to 100 ppm.

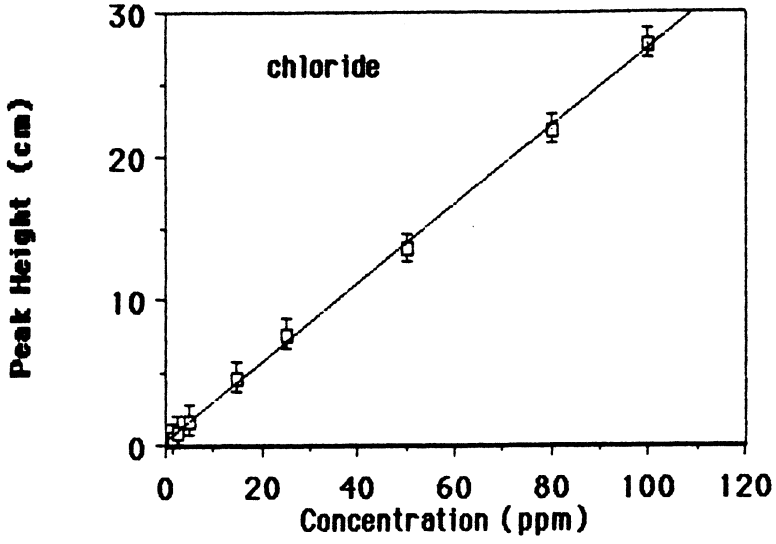
The regression parameters for peak height vs. concentration of standard anions injected are shown in Table IV. The correlation

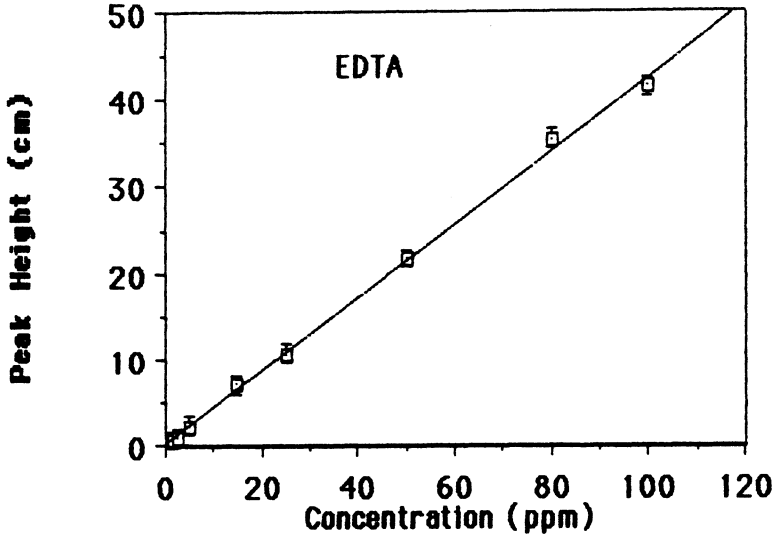
TABLE 3: LINEARITY RESPONSE OF ANIONS:***

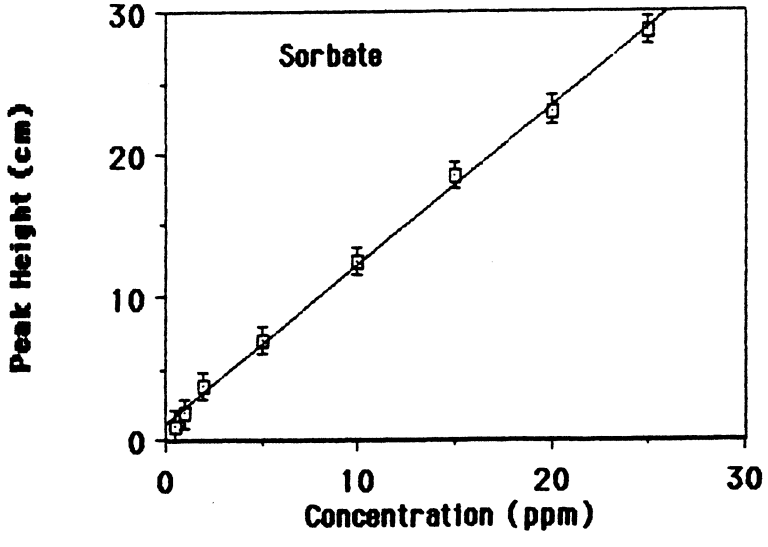
Anion Concentration	**Average peak Height (cm)	SD	CV (%)
Standards: A) Chloride			
100 ppm	27.85	±0.49	1.7
80 ppm	21.87	±0.09	0.4
50 ppm	13.62	±0.09	0.6
25 ppm	7.71	±0.11	1.5
15 ppm	4.75	±0.13	2.7
5 ppm	1.72	± 0.02	1.5
2.5 ppm	0.98	± 0.03	3.1
1.25 ppm	0.48	± 0.03	7.2
B)*EDTA			
100 ppm	41.50	± 3.13	7.5
80 ppm	35.50	± 0.39	1.1
50 ppm	21.82	± 0.25	4.6
25 ppm	10.66	± 0.31	3.0
15 ppm	7.05	± 0.07	1.0
5 ppm	2.32	± 0.02	1.1
2.5 ppm	0.95	± 0.03	3.8
1.25 ppm	0.60	± 0.03	5.8
C) Sorbate			
25 ppm	28.65	± 0.45	1.6
20 ppm	23.10	± 0.65	2.8
15 ppm	18.50	± 0.52	2.9
10 ppm	12.45	± 0.21	1.7
5 ppm	6.95	± 0.09	1.3
2 ppm	3.75	± 0.07	1.8
1 ppm	1.82	± 0.02	1.0
0.5 ppm	1.05	± 0.01	1.6
Saline solution			
A) Chloride 51.9 ppm	14.50	± 0.05	0.4
B) Edetate 17.2 ppm	7.57	± 0.16	1.9
C) Sorbate 19.1 ppm	22.34	± 0.75	3.4

n =3, * 2.0 mM Na benzoate 20% CH_3CN

Figure 25 : Calibration curves for chloride, *EDTA and sorbate. Conditions are the same as in Figure 24.


chloride
Peak Height (cm)
30
20
10
0
0
20
40
60
80
100
120
Concentration (ppm)


EDTA
Peak Height (cm)
50
40
30
20
10
0
0
20
40
60
80
100
120
Concentration (ppm)


Sorbate
Peak Height (cm)
30
20
10
0
0
10
20
30
Concentration (ppm)

coefficient varied from 0.9990 to 0.9995 for the standard anions studied.

The detection limits based on a 20 µL sample injection are shown in Table V. The detection limits (S/N ratio = 5) of anions studied were as follows: chloride, 25 ng; *EDTA, 25ng; and sorbate, 10ng. The detection limit of sorbate was relatively lower than *EDTA. This difference is due to the partial overlap of *EDTA with nitrate. However, the detection limits of these anions can be improved by using a larger sample injection loop. Table VI give results for the anions content of saline solution. The values obtained for chloride and EDTA contents were in good agreement with the certified values, however for sorbate content the value was found to be slightly lower than the value claimed in the label.

In summary, the IPC method described above is a very useful and selective method for the detection as well as the quantitation of chloride, sorbate and EDTA present in a commercial saline solution.

Table IV. Regression parameters for peak height versus concentration of the standard anion injected. n = 3. Condition as in Table III.

Anion	Slope	Intercept	Correlation coeffecient
Chloride	0.272 ± 0.003	0.384 ± 0.17	0.9995
*EDTA	0..424 ± 0.007	0.265 ± 0.38	0.9990
Sorbate	1.115 ± 0.02	1.09 ± 0.26	0.9990

Table V. Detection limits and calibrated range for Anions found in saline solution. Conditions as in Table III.

Anions	**Detection limits (ppm)	(ng)	(picomole)	Calibrated Range (ppm)
Chloride	1.25	25	700	1.25-100
*EDTA	1.25	25	400	1.25-100
Sorbate	0.5	10	90	0.5-25

**Signal-to- noise ratio = 5

TABLE VI : Sorbate, chloride and EDTA content of saline

Anion	Found % *	Expected Value %	% Error
Chloride	0.259 ± .006	0.255	+1.6
EDTA	0.086 ± .006	0.087	-1.2
Sorbate	0.096 ± .003	0.099	-3.0

*solute concentration calculated in 100 mL of solvent

CHAPTER IV

CONCLUSIONS

Indirect photometric chromatography (IPC) is a sensitive method for the determination of UV-transparent inorganic and organic anions. No special equipment is required for this method, and it should be applicable to a wide variety of inorganic and organic ionic compounds previously considered photometrically undetectable. Inorganic and organic anions were simultaneously determined on a polymer-based low capacity anion exchange column with sodium benzoate as an eluent. Although sodium benzoate has a high background absorbance (1.192) and does not have high molar absorptivity (ε = 596 L mol $^{-1}$ cm $^{-1}$ at 260 nm), it does have a strong eluting power at a concentration of 2.0 mM for the sensitive and reliable analysis of monovalent inorganic and organic anions

This approach of indirect photometric chromatography (IPC) can also be useful for the determination of weakly acidic anions and biological compounds in complex matrices at high pH because of the selectivity of ion exchange columns for UV transparent ionic compounds. The results have shown that the indirect UV absorption signal achieved at high pH (Figure 15) is clearly of limited value. For maximum sensitivity, the influence of hydroxide ion as an eluting species must be minimized. A carbonate system peak appears at high

pH. One obvious approach to eliminate the carbonate system peak is to work under a completely inert gas system for mobile phase preparation. The method proposed by Hawakawa [49] looks attractive, however it is not very cost effective. One can wonder about the availability of a simple and low cost alternative HPLC system for high pH work in IPC. There is no information on these points, and clearly this approach needs and deserves further investigation.

This separation and detection method was also successfully applied to several aliphatic carboxylic and sulfonic acids using the same eluent. The effect that an organic modifier (acetonitrile) had on the retention and selectivity of carboxylic and sulfonic acids was studied. The separations of mixtures of carboxylic and sulfonic acids as anions illustrate the effect of the combined adsorption/anion exchange mechanisms on selectivity and resolution. Only one kind of stationary phase was used in this investigation. Further investigations with other anion exchangers are necessary to determine the universality and magnitude of these kinds of reversed phase and ion-exchange interactions.

The hydrophobic and hydrophilic anions found in a commercial saline solution were determined by anion-exchange chromatography with a combined approach of indirect and direct detection. In this study, a mobile phase containing 2.0 mM sodium benzoate, 20%

acetonitrile at pH* 6.9, and UV detection at 260 nm was used to quantitate chloride and EDTA species in the saline solution in the range of 1.5 to 100 ppm, and sorbate in the range of 0.5 to 25 ppm. The detection limits for chloride and EDTA were 1.5 ppm, and sorbate was 0.5 ppm with a 20 μL injection loop. Quantitating these anions in the above mentioned range can be accomplished, but precision ultimately degrades to approximately 4.8 % CV at low concentrations of analytes. Although the chromatographic system was unhampered by co-elution problems with matrix components, the presence of high concentration of EDTA caused a decrease in the peak height of the $[Fe(EDTA)]^-$ signal. The detector was stable for periods of up to at least 24 hours of continous use during quantitation, and the column met the system suitability requirement (reproducibility of retention times) even after 100 injections.

Significant progress has been made in the understanding of IPC. Despite some of the theoretical shortcomings, the reality of practical IPC is now with us. The method permits the rapid, selective separation of charged or ionizable compounds and is amenable to both microanalytical and preparative studies. The availability of an improved array of chromatographic supports and the further development of mobile phase counter ions will extend the capabilites of this separation tool. The emphasis in the future, undoubtedly, will

be towards new ion-exchange and hydrophobic phases, thus permitting greater selectivity. As these new systems emerge, the inherent versatility of IPC will become increasingly important for the chromatographic separation of the constituents in biological samples.

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