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The use of liquid chromatography for the analysis of metal ions in aqueous solutions and for the determination of water in organic matrices

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The use of liquid chromatography for the analysis of metal ions in aqueous solutions and for the determination of water in organic matrices

by

Nancy Elaine Fortier

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For the Graduate College

Iowa State University
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1988

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DEDICATION

This work, the product of my sojourn in Iowa, is dedicated to my parents. Their guidance and supporting love are priceless.

GENERAL INTRODUCTION

History

High performance liquid chromatography (HPLC) is a separation technique that can be used to analyze a wide variety of samples. Liquid chromatography is a general term referring to a separation system comprised of a stationary phase packed in a column and a liquid mobile phase flowing through the column. Sample components are separated on the stationary phase based on the relative affinities of the solutes for the stationary and mobile phases and of the mobile phase for the stationary phase. When a sample is introduced at the head of the column, it is forced down the length of the column by the mobile phase. Solutes that have a weak affinity for the stationary phase will elute from the column first, followed by those sample components that are more strongly retained by the stationary phase. The greater the affinity of the mobile phase for the stationary phase, the faster will the sample components elute.

When liquid chromatography was first introduced, the mobile phase passed through the column by the force of gravity. As each analyte eluted, the fraction of the effluent containing it was collected and analyzed. The "high performance" in HPLC refers to the modern high pressure pumps used to accurately deliver the mobile phase at a controlled, relatively high, flow rate, as well as to the efficient stationary phases now used and the sensitive detectors that can be placed in-line with the column to automatically detect eluting species.

The range of samples that can be analyzed by HPLC is ever-

increasing. Many types of stationary phases are available, and detectors are becoming more and more sensitive. Depending on the stationary phase and eluent (mobile phase) chosen, polar and nonpolar molecular species, as well as cationic or anionic materials, can be separated and quantitatively determined.

When the analytes of interest and the active sites on the stationary phase are ionic species, the separation method is referred to as ion-exchange chromatography. Classical ion-exchange used stationary phases with a large number of ion-exchange sites available. Consequently, high concentrations of ionic eluents were needed to separate sample components. Fractions had to be collected and analyzed. However, ion-exchange chromatography has undergone substantial improvements recently. More efficient stationary phases (resins) with lower concentrations of active sites (capacities) have been developed. This permits the use of less concentrated mobile phases. It is no longer necessary to collect and analyze fractions, since detectors are now available which can sensitively detect eluting ions on-line. This "new and improved" type of ion-exchange chromatography is referred to as ion chromatography.

Ion chromatography can be used in two modes: single-column and dual-column ion chromatography. For the detection of ions, a conductivity detector is commonly used because it is a somewhat universal detector: solutions of ionic species conduct electricity, and therefore give signals in the conductivity detector. However, as with any HPLC detector, the <u>difference</u> in signal between the analyte and the eluent is what's important. To elute ions from an ion-exchange

resin, an ionic eluent is used. Consequently, a large background signal is seen, making it difficult to detect a small analyte signal. Single-column and dual-column ion chromatography solve this problem in different ways.

In dual-column anion chromatography, also called suppressed anion chromatography, the conductivity of the eluent is suppressed by a chemical reaction occurring in-line after the separation column (1). For example, when anions are separated with an eluent of $NaHCO_3/Na_2CO_3$, which is highly conducting, a second column in the H^+ form is placed after the separator column. The Na^+ ions of the eluent exchange for the H^+ ions in the column, with the result being the conversion of the $NaHCO_3/Na_2CO_3$ to carbonic acid, which is only slightly dissociated so has a low conductivity. A further benefit of this system is that the cations of the anionic sample components will also exchange for H^+, producing highly conducting acid anions. Thus not only is the background suppressed, but the analyte signals are enhanced. Since its introduction in 1975, suppressed ion chromatography has been modified so that now a hollow fiber suppressor is used instead of a packed suppressor column. The hollow fiber system has the advantage that it doesn't need to be regenerated as the suppressor column did, and also extra-column band broadening is reduced.

Single-column ion chromatography does not use a suppressor system. Rather, it uses very low capacity ion-exchange resins so that low concentrations of eluents can be employed. Additionally, eluents are judiciously chosen so that the eluent ion is only weakly conducting. These two factors taken together allow the background of the eluent to

be electronically zeroed out so that analyte ions may be detected (2). Unlike dual-column ion chromatography, single-column ion chromatography may be easily used for both anionic and cationic species (2, 3).

Ion-exclusion is another type of HPLC which employs an ion-exchange resin. However, it is used to separate molecular species. For example, carboxylic acids, which are only weakly ionized in neutral solutions and nearly fully protonated in acidic solutions, can be separated on a cation-exchange resin using an acid eluent (4). Ionic species in the sample do not partition into the resin because of Donnan exclusion at the charged resin surface. Consequently, ionic species are not retained. However, neutral molecular species, such as the carboxylic acids, can partition, so these are retained and separated on the column. In this case, negative conductance peaks are seen when the carboxylic acids elute since their conductivity is lower than that of the acid eluent.

Overview

This dissertation is divided into four sections, each of which is a distinct project. Sections I, II, and III all deal with single-column ion chromatography. The separation mechanism in Section IV is believed to be ion-exclusion.

The first section is an ion chromatographic method for the determination of trivalent aluminum. Cation chromatography was used in conjunction with a conductivity detector. Not only does this section show how aluminum can be accurately determined in the presence of an excess of divalent metals, but the general utility of a p-

phenylenediammonium eluent for cation chromatography is shown. This section is an expanded version of a publication that resulted from this work (5).

The second section is a discussion of postcolumn reaction in liquid chromatography. A general overview of the utility of this detection mode is followed by an examination of the chelating agent disodium 1-(2-thiazolylazo)-2-naphthol-3,6-disulfonate (TAN-3,6-S), and how it can be used as a postcolumn reagent for the detection of metal ions in ion chromatography.

Section III is an examination of how and why elevated temperature affects ion chromatographic retention times. Approximate enthalpies of reaction were calculated for several ion-exchange reactions. Additionally, it was demonstrated that the shifts in retention times which occur as the temperature of a chromatographic system is raised can be used to improve chromatographic resolution. This section also was previously published (6).

The last section covers two methods to determine trace amounts of water in organic matrices. The uniqueness of these chromatographic determinations of water lies not in the separation but the detection schemes. In the first method, solvatochromism is used to detect an eluting water peak with an absorbance detector. In the second method, an equilibrium is established between cinnamaldehyde and methanol in the eluent. This equilibrium is shifted by the addition of water if an acid catalyst is present. The acid catalyst is in the form of a second column placed in-line after the separation column and filled with a cation-exchange resin in the H^+-form. The magnitude of the equilibrium

shift is proportional to the amount of water in an injected sample, and can be readily measured with a UV-visible absorbance detector. A patent for the second method has been filed.

Although each of the four sections is an individual entity, related to the others only by the fact that they all involve HPLC, the sections are not meant to stand alone. Occasionally, in some of the later sections, the reader is referred to material presented in earlier sections.

SECTION I. SEPARATION AND DETERMINATION OF ALUMINUM USING SINGLE-COLUMN ION CHROMATOGRAPHY

INTRODUCTION

The determination of aluminum in a variety of matrices has become an increasingly important problem recently. Several spectroscopic techniques are widely used for aluminum determination, both with and without sample preconcentration. Although many of the techniques used are sensitive, they are often plagued by interferences.

Acid rain and snow have caused a dramatic increase in the "geochemical mobility" of aluminum (1). The relatively high levels of aluminum present in watersheds in Eastern North America and Scandinavia pose a potential threat to fish and other aquatic life forms.

Patients undergoing hemodialysis are at risk from elevated aluminum levels in dialysis fluids. Blood, water, and the concentrate which is added to water to yield the dialysis fluid must be monitored. It has been shown (2) that aluminum is responsible for the occurrence of a progressive fatal neurological condition in patients receiving hemodialysis treatments. The level of aluminum in the dialysis fluids is a key factor, and reducing this level below 10 µg/L can prevent the occurrence of this syndrome (3).

The semiconductor industry also has a need for the determination of low levels of aluminum. A compound such as trimethylgallium can contain aluminum, iron, copper, and magnesium. The properties of the semiconductor material formed from the trimethylgallium can be drastically altered if these contaminants are not kept well below the microgram-per-gram range (4).

Graphite furnace atomic absorbance spectroscopy (GFAAS) is a

commonly used spectroscopic technique for the determination of aluminum. It is very sensitive, but prone to interference by matrix constituents (5). The addition of micromolar concentrations of phosphoric acid has been shown to improve the determination of aluminum using this technique (6). When analyzing dialysis concentrate, which has high levels of minerals, erratic results obtained with GFAAS were improved when approximately 40% nitric acid was added as a matrix modifier (3). Weisel et al. showed that the limit of detection for aluminum determination could be decreased by using the coprecipitation of aluminum with ferric oxide as a preconcentration step prior to analysis by GFAAS (7).

Inductively coupled plasma atomic emision spectrometry (ICP-AES) is frequently used for aluminum determination, but the signal can be affected slightly by the sample matrix. The addition of cesium was found to reduce signal variation due to different matrix compositions as well as increase the emission signal (8). Uehiro et al. showed that by using a vacuum ultraviolet emission line (167.1 nm) of aluminum in ICP-AES, very sensitive determinations with few spectral interferences could be achieved (9). A chelating resin has been used to preconcentrate aluminum prior to determination with ICP-AES, improving detection limits and reducing interferences (10).

Spectrophotometry can be used for aluminum determination by adding a complexing agent to solutions containing aluminum. Pyrocatechol violet (PCV) reacts with aluminum after ten minutes to form a complex that can be detected at a wavelength of 580 nm (11). A flow injection analysis spectrophotometric method for aluminum determination using

eriochrome cyanine R and cetyltrimethylammonium bromide (ECR/CTA) at pH 7.5 has been presented (12). The author of that paper points out that other spectrophotometric methods require a pH of 5-6 for complexation, where anionic interferences can be troublesome. Specifically, in this pH range fluoride and phosphate complex readily with aluminum and compete with the complexation reaction between aluminum and the spectrophotometric reagent. ECR/CTA exhibited high sensitivity for aluminum in a pH range of 5.5 to 8. At a pH of 7.5, fluoride and phosphate interferences were greatly reduced, due to the decrease in stability of the aluminum complexes with these anions at pHs above 7. However, other metals, such as iron, beryllium, lanthanum, and cerium, which also complex with ECR/CTA, interfere.

Methods for the determination of aluminum using dc argon plasma emission spectrometry often employ a preconcentration step. A method developed by Sarzanini et al. (13) uses pyrocatechol violet to form anionic complexes of Al(III). These complexes are taken up by an anion-exchange resin. The metal is recovered by acidic elution, then determined by dc argon plasma emission spectrometry. A similar method employs anionic complexation of the Al(III) with Tiron, 1,2-dihydroxy-3,5-benzenedisulfonic acid, followed by sorption, elution and determination (14).

Saarl and Seltz (15) used a fiber optic with morin (3,5,7,2′,4′-pentahydroxyflavone) immobilized on the end to determine aluminum. Morin forms highly fluorescent complexes with Al(III). However, several metals interfere with the method. Beryllium causes an increase in signal, while Fe(III) and Cu(II) quench the fluorescence.

The problem of matrix effects in aluminum determination was alleviated by using ^{27}Al nuclear magnetic resonance (NMR) spectrometry, a rapid, nondestructive technique for quantifying aluminum (16). However, the equipment needed for such a determination is not readily accessible to many laboratories.

Since so many methods for aluminum determination are plagued by interference problems, a separation step prior to determination seems appropriate. Chromatography should lend itself to solving the problem. However, although a vast quantity of research has been done utilizing chromatography to separate and quantify divalent and rare earth metal ions, relatively little work has been done on developing a chromatographic method for the determination of aluminum.

Several classical chromatographic methods were developed for Al(III) determination, but these generally used gravity eluent flow and required collection of effluent fractions from the column. These fractions were then analyzed in a variety of ways, described below.

Brajter and Olbrych-Śleszyńska used an anion-exchange resin to separate anionic xylenol orange complexes of metals, including aluminum (17). Fractions were analyzed by atomic absorption spectrometry (AAS). Phillips and Fritz (18) used an N-methylhydroxamic acid chelating resin to concentrate Al(III), then eluted it by rinsing the column with 0.1 M oxalic acid. Atomic absorption spectrometry was used to quantify the aluminum in the eluted solution. A third group, Varshney et al. (19), also used AAS for Al(III) determination. Their method employed the inorganic cation-exchangers zirconium(IV) arsenophosphate (ZAP) and zirconium(IV) arsenosilicate (ZAS). They separated aluminum and iron

from other metals in alloys and silicate rocks by passing solutions through the cation-exchange resins. Aluminum and iron were sorbed while other metals were eluted with an eluent of 0.01 M nitric acid. A solution of 1 M nitric acid eluted the iron and aluminum, which were then quantified by AAS.

Ethylenediaminetetraacetic acid (EDTA) titration has also been shown to be a useful way to quantify aluminum in chromatographic fractions. Inorganic cation-exchangers were used to separate metals by gravity elution; the metals in collected fractions were analyzed by titrating with EDTA (20-22).

Sen Sarma and Majumdar used a strong base anion-exchanger (Dowex 1-X8) in the chloride form to separate aluminum and iron chlorides (23). The aluminum fraction was analyzed colorimetrically by extraction with 8-hydroxyquinoline in $CHCl_3$. Strelow et al. (24) also used an anion-exchange resin to separate elements with an oxalic-hydrochloric acid mixture. The amount of Al(III) in the appropriate fraction was determined using a complexometric procedure involving excess DCyTA (1,2-diaminocyclohexane-tetraacetic acid), back-titration with a standardized zinc solution, and an indicator of xylenol orange at pH 5.5.

Maslowska and Pietek used a low pressure system to separate p-hydroxybenzoate complexes of aluminum(III), iron(III), and cerium(III) on an ion-exchange column in the $p\text{-}C_6H_4(OH)COO^-$ form (25). A spectrophotometric detector was used, but even with an eluent flow rate of 2 mL/min, retention times were between 32 and 68 minutes.

Modern ion chromatography has been shown to be useful for

separating monovalent (26), divalent (26, 27), and the rare earth metal cations (27). However, with the exception of the rare earths, trivalent metal ions, such as Al(III), have posed a problem in ion chromatography. One difficulty with ion chromatography is that frequently the eluents can be detrimental to the pumps and detectors used. Specifically, quite often high concentrations of strong acids and/or salts are employed. A case in point is a method Dionex Corporation has developed for aluminum(III) determination (28). Using an eluent of 0.01 M H_2SO_4/0.2 M $(NH_4)_2SO_4$ and a cation-exchange column, they were able to elute Al(III) in about 3.5 minutes. The Al(III) was detected by reacting it with the postcolumn reagent Tiron (4,5-dihydroxy-m-benzene disulfonic acid disodium salt, monohydrate) and using a UV/visible detector set at 310 nm. The Dionex method has been shown to be useful for separating Al(III) and Fe(III).

Low concentrations of protonated amines are less caustic than acid eluents. In the present research, the elution of metal cations from a cation-exchange column of low capacity, using protonated amine eluents, is studied. The eluent contains the diprotonated cation of either N-phenylethylenediamine or p-phenylenediamine, and conductivity detection is employed. The original objective was to study how the retention times of various cations change with the type of eluent used. However, aluminum(III) was found to elute later than most divalent metal ions and well before other trivalent metal ions. This forms the basis of a selective and convenient ion-exchange method for determining aluminum(III).

EXPERIMENTAL SECTION

Apparatus

The instrument used consists of a Model AA-94 Eldex dual channel pump, a Model 1116 Eldex column heater equipped with a 50-microliter sample loop, a model #269-004 Wescan catex column (capacity 0.03 meq/g, 12-16 micron particles, 5% crosslinked gel, sulfonated polystyrene divinylbenzene, 25 cm in length, 2 mm i.d.), a Model 213A Wescan conductivity detector, and a Curken strip chart recorder.

Eluents

Reagent grade p-phenylenediamine (Fischer) and reagent grade perchloric acid were used without further purification. N-phenylethylenediamine (Aldrich) was used both as received and after distillation under vacuum. The amine eluent solutions were prepared by dissolving the amine in perchloric acid and diluting with deionized water, obtained from a Barnstead NANOpure II water purification system. The eluents were filtered through a 0.45-micron membrane and degassed. The pH of the eluents was adjusted with perchloric acid to a value of 3. The purpose of the pH adjustment was to ensure sufficient protonation of the diamines used, as well as to maintain a constant pH so the relative strengths of the diamines as eluents could be compared. Once prepared, the eluents were immediately stored under a helium atmosphere and protected from light to prevent decomposition.

Sample Solutions

The Al(III) standard stock solution was prepared by dissolving 0.4707 g of Alcoa Research Laboratories standard aluminum wire SCI-A in about 25 mL of concentrated hydrochloric acid with heating, then diluting to 1.00 L with deionized water. The Al(III) solutions used for the calibration curve were prepared by diluting aliquots of this stock solution.

The stock solution of NBS Standard Sample 94a, a zinc-base alloy (see Table I), was prepared by dissolving the bulk of a 0.5305 g sample in perchloric acid and deionized water with heating. When effervescence ceased a black residue remained. This went into solution upon addition of a small amount of concentrated nitric acid. The solution was diluted to 1.00 L with deionized water to yield a solution that was 0.767 mM in Al(III).

Other metal ion solutions were prepared using reagent grade salts, concentrated perchloric acid, and deionized water. The pH of all metal ion solutions was adjusted with perchloric acid to approximately 3 merely to prevent hydrolysis.

Table I. National Bureau of Standards Standard Sample 94a, zinc-base alloy (die-casting)

Al	3.90%
Cu	1.08%
Mg	0.042%
Mn	0.015%
Fe	0.015%
Pb	0.006%
Ni	0.005%
Sn	0.005%
Cd	0.002%

RESULTS AND DISCUSSION

Since protonated ethylenediamine was shown to be an adequate eluent for cation chromatography (26, 27), it was surmised that other amines might also be useful. The first one tested was N-phenylethylenediammonium perchlorate. Retention times for several divalent metal ions are presented in Table II. While at first this appeared to be a satisfactory eluent, it was soon noted that the retention times obtained with this eluent were not reproducible. The retention times were much shorter when an attempt was made to reproduce the table. Further experimentation showed that something in the eluent, either an impurity or a decomposition product, was gradually occupying exchange sites on the column. As time passed, the capacity of the column was reduced and retention times were drastically shortened. Figure 1 shows how the retention times for an Al(III) solution changed as more and more eluent was pumped through the column. Attempts to prevent this phenomenon by purifying the amine through distillation under vacuum and slowing decomposition by keeping the eluent under helium and protected from light were unsuccessful; retention times continued to decrease with elapsed time. Although an injection of nitric acid seemed to effectively remove the species that was occupying the column sites and restored retention times to their initial values, the trend of decreasing retention times with increasing elapsed time merely began again as soon as the nitric acid was eluted. Therefore this eluent was abandoned.

The second amine tested was p-phenylenediamine. An eluent

Table II. Approximate relative (see text) retention times for some cations using an eluent of 1.99 mM N-phenylethylenediamine, pH 3.1. Flow rate was 1.01 mL/min, temperature was 25°C

Cation	t′ (mins)
Mg(II)	0.52
Zn(II)	0.62
Mn(II)	0.64
Co(II)	0.67
Ca(II)	0.89
Cu(II)	0.89
Sr(II)	1.08
UO_2^{2+}	1.52
Pb(II)	3.31
Al(III)	7.69
Lu(III)	29.75

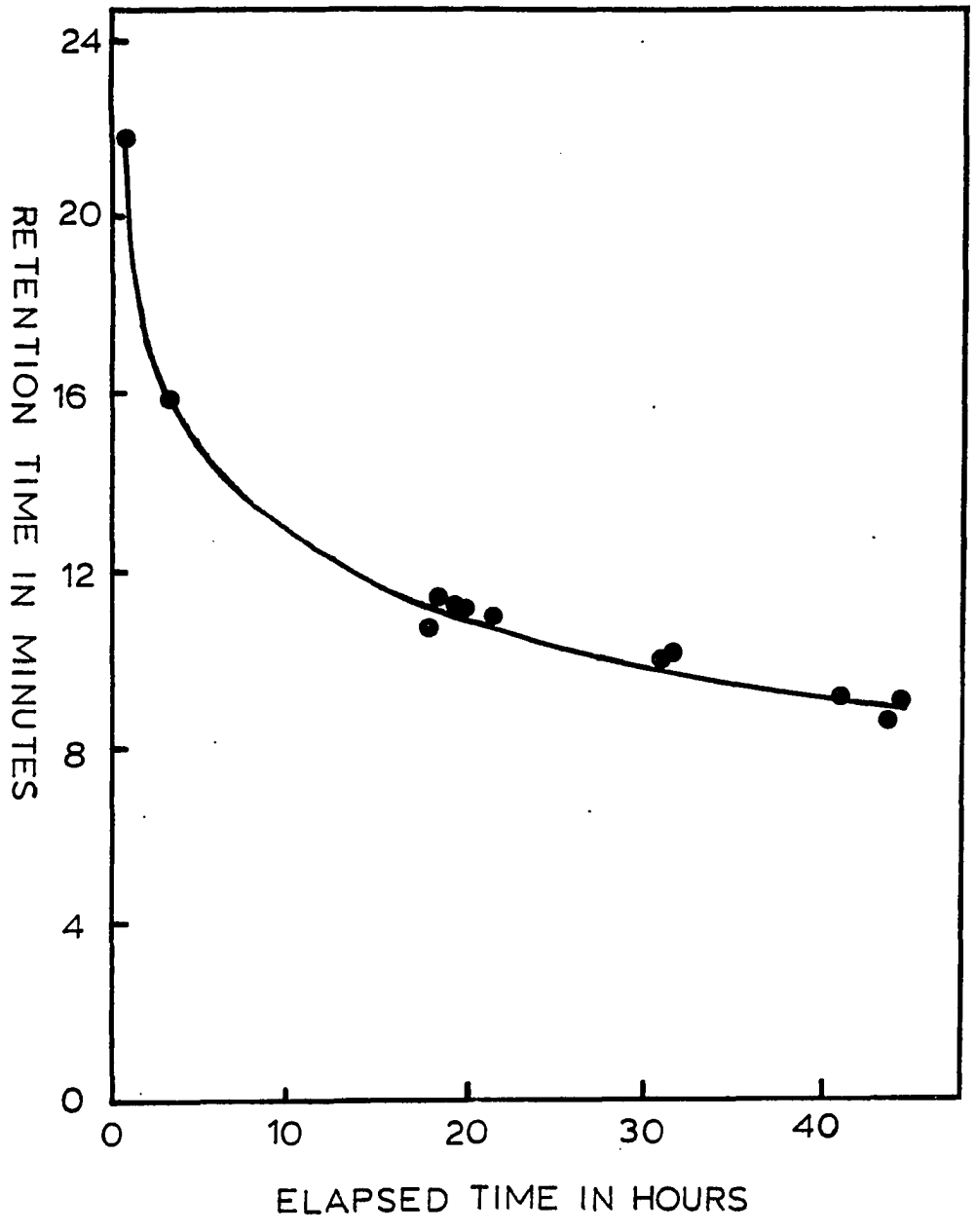

Figure 1. Plot of the retention time of Al(III) peak vs. time elapsed since column equilibration achieved. Conditions: eluent, 0.994 mM N-phenylethylenediamine, pH 3.1; temperature, 26°C; flow rate, 1.02 mL/min

prepared by doubly protonating p-phenylenediamine with the addition of perchloric acid worked well. Since for p-phenylenediamine $pK_{a,1} \simeq 6.2$ and $pK_{a,2} \simeq 2.9$ (29), at pH 3 the p-phenylenediamine is about 44% singly protonated and 56% doubly protonated. It is desirable to have the amine as fully protonated as possible, but a pH of 0.9 would be needed in order for the amine to be 99% double protonated. At pH 0.9 the concentration of H^+ is so high that it will compete with the p-phenylenediammonium ion as eluent cation. Also, the background conductivity of an eluent of pH 0.9 cannot be electronically zeroed out. At a pH of 3, the p-phenylenediamine eluent had sufficient eluting power, yet its absolute conductivity was easily electronically zeroed. At a flow rate of 1 mL/min, the column back pressure was about 1200 psi. The retention times given in Table III were found to be very reproducible. Occasionally nitric acid was injected as a precautionary measure to prevent any long-term buildup on the column, although this treatment may have been superfluous. Retention times of ions were unaffected by this treatment.

Theoretical Considerations

It is well known that the retention time of a given ion is dependent upon the concentration of eluent. The cation exchange equilibrium for a divalent eluent ion can be represented by

$$yE^{2+}R_2 + 2M^{y+} \rightleftharpoons yE^{2+} + 2M^{y+}R_y \qquad (1)$$

where E^{2+} is the eluent ion, M^{y+} the metal ion, and R the resin matrix;

Table III. Retention times for some divalent and trivalent metal ions. The temperature was 27°C, the flow rate was 1 mL/min. For the divalents, the concentration of p-phenylenediamine was 1.99 mM; for the trivalents, the concentration of p-phenylenediamine was 6.04 mM. The retention time of the unretained peak, t_o, was 0.44 minutes

Cation	t (mins)[a]	σ_{rsd}
Mg(II)	1.63	0.0%
Mn(II)	2.02	1.8%
Zn(II)	2.06	0.0%
Co(II)	2.17	1.7%
Ca(II)	2.83	1.3%
Sr(II)	3.92	2.4%
Al(III)	8.6	1.9%
Lu(III)	22.3	1.3%
Tm(III)	23.0	2.2%

[a]Each is an average of at least three runs.

the subscript on R is the number of exchange sites occupied by an ion (27). The equilibrium constant for this reaction, K_E^M, also called the selectivity coefficient, is given by the equation

$$K_E^M = \frac{[E^{2+}]^y[M^{y+}R_y]^2}{[E^{2+}R_2]^y[M^{y+}]^2} \qquad (2)$$

If the amount of analyte metal ion injected is small relative to the column capacity (the number of ion-exchange sites, $--SO_3^-$, per gram of resin), then essentially all of the ion-exchange sites will be taken up by the eluent cations. Consequently, $[E^{2+}R_2]$ is approximated by one half the column capacity, cap. In addition, the ratio $[M^{y+}R_y]/[M^{y+}]$ is defined to be the chromatographic mass distribution coefficient, also called the capacity factor, k. Therefore, the expression for K_E^M can be rewritten

$$K_E^M = \frac{[E^{2+}]^y k^2}{(cap/2)^y} \qquad (3)$$

Taking the log of both sides and rearranging yields

$$\log k = -(y/2)\log [E^{2+}] + (y/2)\log (cap/2) + 1/2 \log K_E^M \qquad (4)$$

The capacity factor, k, can be described in terms of the adjusted retention time for an eluted peak (t′) and the time it takes for an unretained species to elute (t_o):

$$k = t'/t_o \qquad (5)$$

where t' is the difference between the measured retention time and t_o. Therefore, equation 4 can be rewritten

$$\log t' = -(y/2)\log[E^{2+}] + \log t_o + (y/2)\log (cap/2) + 1/2 \log K_E^M \qquad (6)$$

where the last three terms on the right side are constant for a given eluent, column, and analyte ion M. This last equation shows that plots of log t' vs. log $[E^{2+}]$ should be linear with a slope of $-y/2$, y being the charge on the analyte ion. Divalent metal ions should have a slope of -1, while trivalent metal ions should have a slope of -1.5. However, this derivation is based on the assumption that the only sorption mechanism occurring is one of ion-exchange (30). If ion-pairing or some other type of partitioning occurred instead of or in addition to the ion-exchange, the experimental slopes would be different from those calculated according to equation 6. Conversely, if the experimental slopes were different from the calculated slopes, it would be an indication that a mechanism other than ion-exchange is occurring.

Note that the experimental slopes of log t' vs. log [p-phenylenediamine] are very close to the calculated slopes for the divalent and trivalent metals studied (see Figures 2 and 3 and Table IV). However, the slopes for the trivalent metals are consistently higher than the calculated value. This means that another sorption

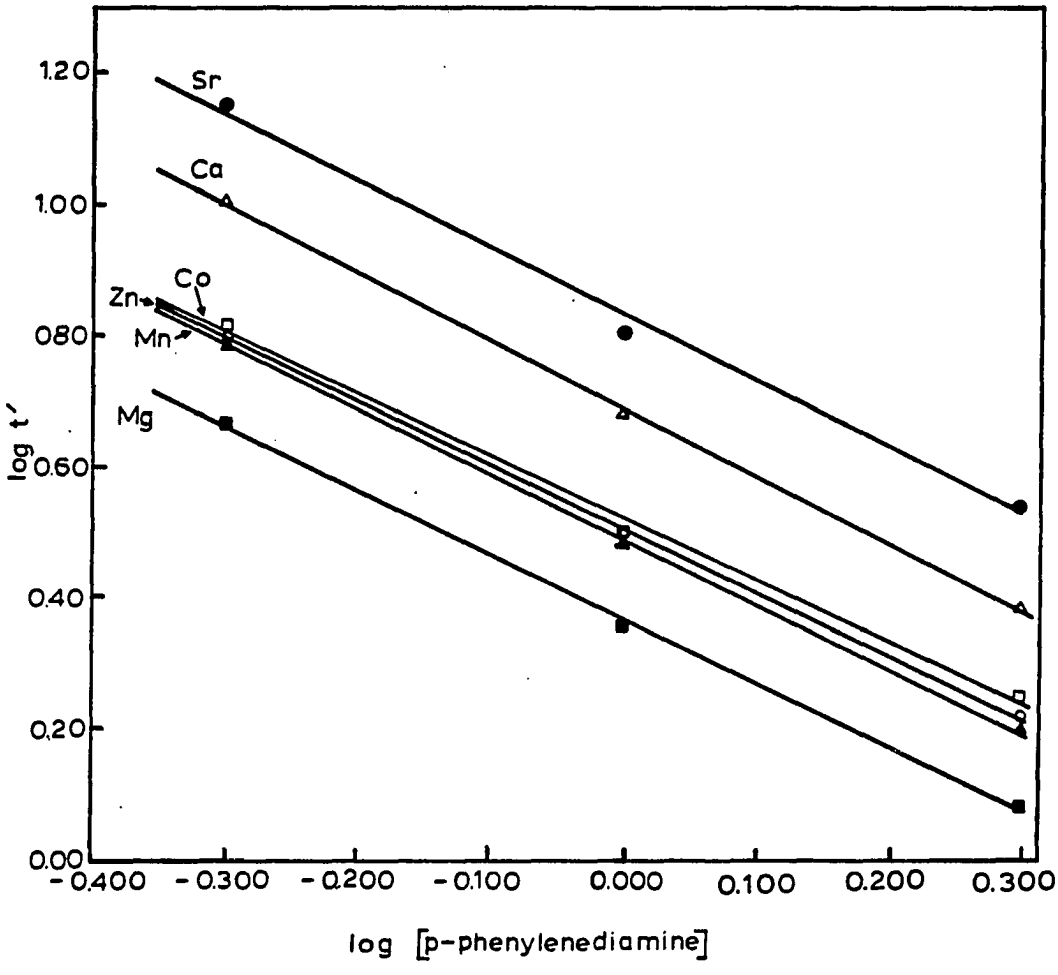

Figure 2. Plot of log adjusted retention time of divalent metal ions vs. log concentration of p-phenylenediamine. Conditions: concentrations of p-phenylenediamine used were 0.50, 1.00, and 1.99 mM, pH 3.0; temperature, 27°C; retention times normalized to correspond to a flow rate of 1.00 mL/min (actual flow rates were between 1.00 and 1.03 mL/min)

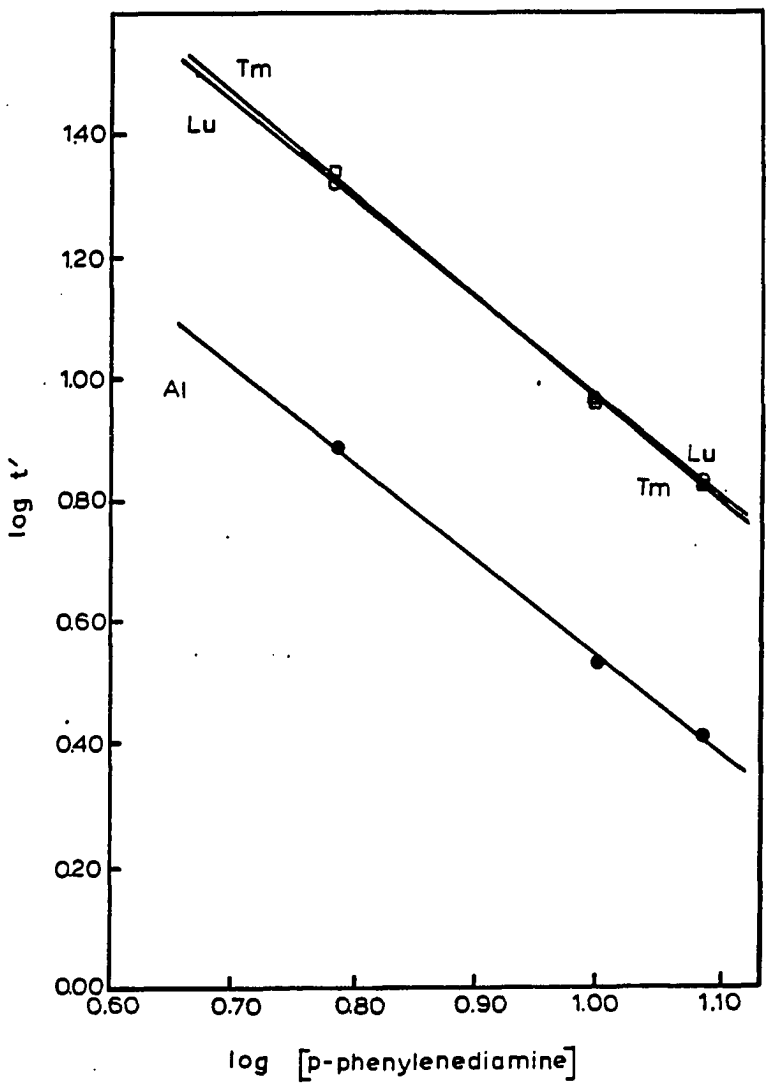

Figure 3. Plot of log adjusted retention time of trivalent metal ions vs. log concentration of p-phenylenediamine. Conditions: concentrations of p-phenylenediamine used were 6.04, 9.91, and 12.07 mM, pH 3.0; temperature, 27°C; retention times normalized to correspond to a flow rate of 1.0 mL/min (actual flow rates were between 0.96 and 1.0 mL/min)

Table IV. Correlation coefficients and slopes of plots of log t' vs. log concentration p-phenylenediamine. Concentrations of p-phenylenediamine used were 0.50, 1.00, and 1.99 mM for the divalent metal ions and 6.04, 9.91, and 12.07 mM for the trivalent metal ions, temperature was 27°C, and since flow rates varied slightly, all retention times were normalized to correspond to a flow rate of 1.0 mL/min

Cation	Corr. Coef.[a]	Slope
Mg(II)	-1.00	-0.99
Mn(II)	-1.00	-1.00
Zn(II)	-1.00	-0.98
Co(II)	-0.998	-0.95
Ca(II)	-1.00	-1.04
Sr(II)	-0.997	-1.02
Al(III)	-0.999	-1.61
Lu(III)	-1.00	-1.63
Tm(III)	-1.00	-1.69

[a]All are for an average of at least three runs at each concentration.

mechanism occurs, probably in addition to ion-exchange (as opposed to instead of ion-exchange, since the differences between calculated and experimental slopes are slight).

Aluminum Determination

In order to obtain a sharp, early peak for Al(III), it was necessary to increase the concentration of p-phenylenediamine; 8.23 mM was used. At this concentration, the divalent metal ions eluted quickly as a group and did not interfere with the Al(III) peak. Therefore, a study was undertaken to see if the quantitative determination of Al(III) was possible using protonated p-phenylenediamine as eluent. All solutions were injected under the following conditions: eluent -- 8.23 mM p-phenylenediamine, pH 3.00; temperature -- 27°C; flow rate -- 0.97 mL/minute. Since an electronic integrator was not available, a calibration curve was prepared by plotting peak weight vs. concentration of the Al(III) standard solutions. Six different concentrations from 0.1 to 0.7 mM gave a linear plot (see Figure 4).

To see if the amount of aluminum in a solution could be accurately determined when other metal ions were present, injections were made of the solution of NBS Standard Sample 94a. In order to keep the peak for the solution on scale, the stock solution was diluted by a factor of two. However, it was noted that a representative Al(III) peak could not be obtained due to the presence of a large negative peak caused by the excess acid needed to dissolve the alloy. The pH of the undiluted stock solution was found to be only 0.95, whereas the pH of the Al(III)

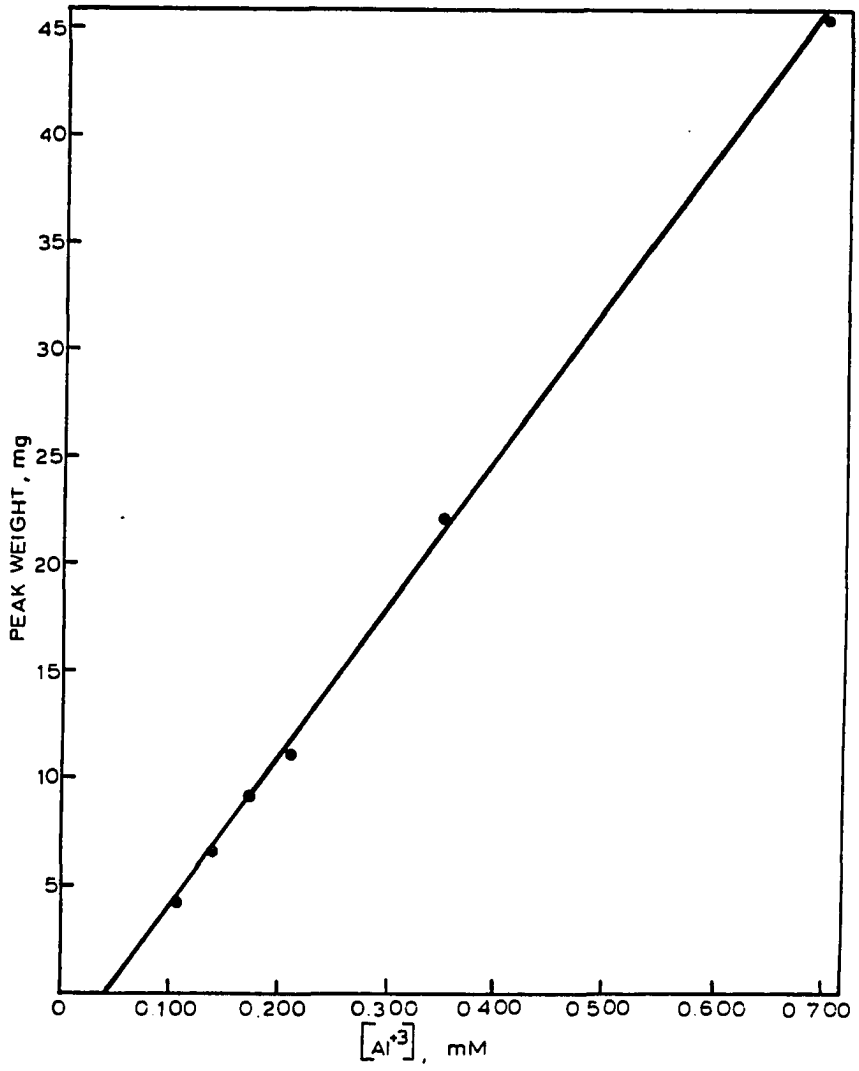

Figure 4. Calibration plot for aluminum standard solutions -- peak weight in mg vs. concentration of Al(III) in mM. Conditions: eluent, 8.23 mM p-phenylenediamine, pH 3.0; temperature, 27°C; flow rate, 0.97 mL/min. Correlation coefficient is 0.9996

standard solutions ranged from 2.45 to 3.29. Therefore the pH of a 50.0 mL aliquot of the stock solution was adjusted to 2.57 with concentrated ammonium hydroxide; the solution was then diluted to 100.0 mL (final pH was 2.94). Injections of this solution gave peaks whose areas could be easily measured (see Figure 5).

Injections were also made of two other solutions containing Al(III): Al(III) in a 114-fold molar excess of uranyl ion, chosen because uranyl is a late-eluting divalent ion (Figure 6), and Al(III) in a solution containing six other metal ions in the following molar excesses: 12-fold Ca(II); 13-fold each of Mg(II), Zn(II), Sr(II); 14-fold each of Co(II) and Mn(II) (Figure 7).

The results of the aluminum determinations are tabulated in Table V. These show that the chromatographic method described is capable of giving quantitative results for aluminum(III), even in samples containing numerous other metal ions.

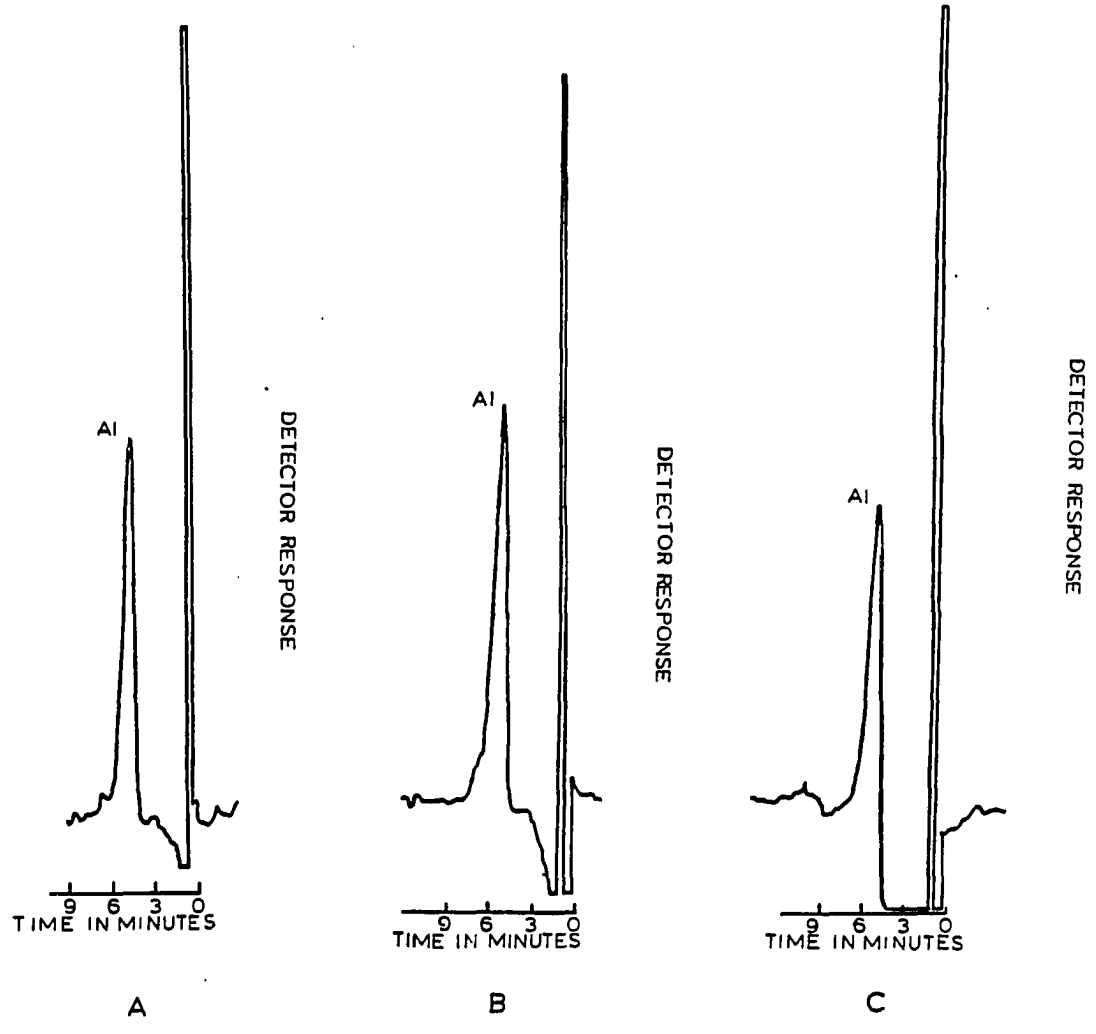

Figure 5. Conditions as in Figure 4. A.) Standard 0.349 mM Al(III) solution, B.) solution of NBS Standard Sample 94a, 0.383 mM in Al(III), pH 2.94, C.) solution of NBS Standard Sample 94a, 0.383 mM in Al(III), pH 0.95

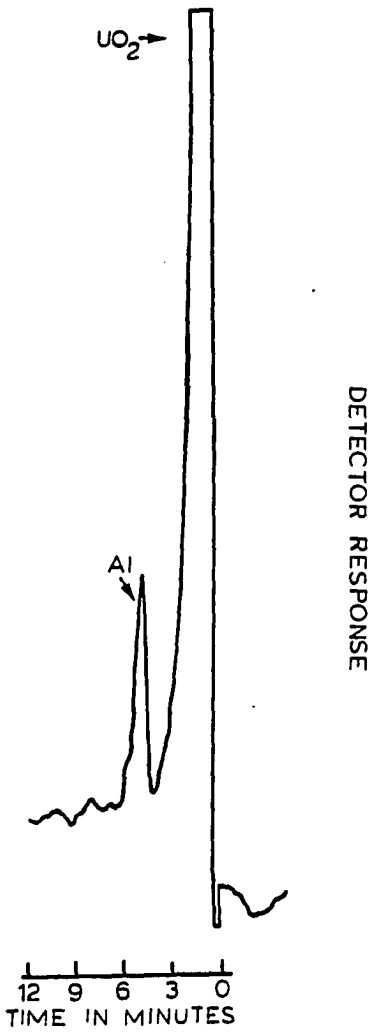

Figure 6. Separation of Al(III) from UO_2^{2+}. Concentration of Al(III) is 0.173 mM, concentration of uranyl ion is 19.80 mM. Conditions as in Figure 4

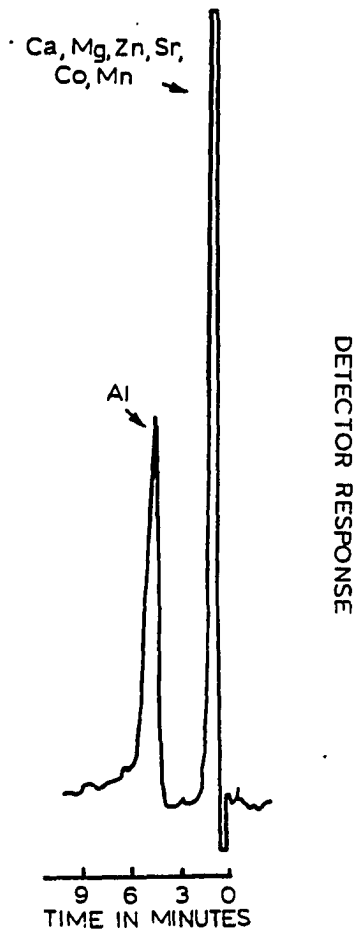

Figure 7. Separation of Al(III), 0.286 mM, from six other metal ions: 3.44 mM Ca(II); 3.77 mM each of Mg(II), Zn(II), Sr(II); 4.10 mM each of Co(II), Mn(II). Conditions as in Figure 4

Table V. Aluminum determination results

Solution	Concentration of Al(III) present	Concentration of Al(III) found[a]	σ_{rsd}	Error
0.2653 g/L of NBS Standard Sample 94a, a zinc-base alloy	0.383 mM	0.389 mM	6.1%	1.6%
Al(III) in a solution of 19.80 mm UO_2^{+2}	0.173 mM	0.173 mM	6.4%	0.0%
Al(III) in a solution of Ca(II) (3.44 mM), Mg(II) (3.77 mM), Zn(II) (3.77 mM), Sr(II) (3.77 mM), Co(II) (4.10 mM), Mn(II) (4.10 mM)	0.286 mM	0.261 mM	3.6%	-8.7%

[a]Calculated from an average of at least three peak areas.

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SECTION II. THE USE OF DISODIUM 1-(2-THIAZOLYLAZO)-2-NAPHTHOL-3,6-DISULFONATE (TAN-3,6-S) AS A POSTCOLUMN REAGENT FOR THE DETECTION OF METAL IONS IN ION CHROMATOGRAPHY

INTRODUCTION

In high performance liquid chromatography (HPLC), three of the most powerful detectors used today are UV-visible absorbance, fluorescence, and electrochemical detectors (1). However, in order for these detectors to be used for the determination of sample components, the sample components must be species which cause a change in the output of the detector. For example, direct detection with a UV-vis absorbance detector requires that the analyte molecules have a chromophore. Consequently, the range of samples that can be analyzed with HPLC detectors can be increased if methods are available to convert species with poor detection properties into species which can be sensitively detected.

Fortunately, such methods are available. One such method is postcolumn reaction (PCR). A postcolumn reaction system is, as the name implies, a chromatographic system in which a reagent is added to the effluent stream immediately after the separation column. The reagent mixes with the sample analytes in a mixing chamber, a reaction occurs, and the reaction products then move downstream to the detector, where they are sensitively detected.

A typical PCR system is shown in Figure 1. "P_1" is the eluent pump, "I" is the injection valve, "C" is the separation column, "T" is the mixing or reaction chamber, "P_2" is a second pump for delivering the reagent, and "D" is the detector.

As can be seen from Figure 1, a PCR system requires several additional pieces of hardware over a conventional HPLC system. So why

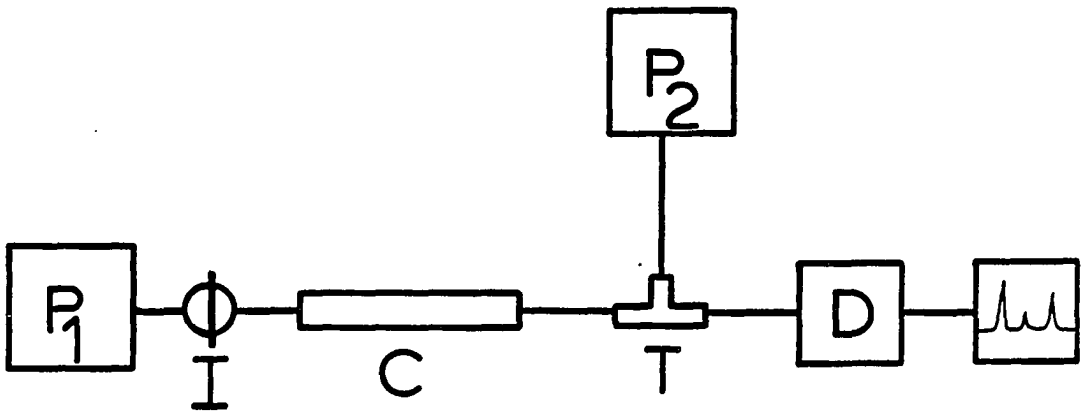


Figure 1. A typical postcolumn reaction chromatographic system. P_1 = eluent pump, I = injection valve, C = separation column, T = mixing or reaction chamber, P_2 = reagent pump, D = detector

not just derivatize the samples before injecting them? There are several advantages of using postcolumn derivatization instead of precolumn derivatization (1). For one thing, since the reaction occurs after the separation has taken place in PCR, the separation can be optimized independently of the detection scheme. Published separation methods can be used, leaving the analyst free to concentrate on the detection aspect. If precolumn derivatization is used, sample cleanup is needed to remove side products and artifacts of the reaction. If not removed, these can interfere with the separation, or possibly foul the column. It is also important that the reaction goes to completion, so excess reagent will not cause problems with the separation and the determination of analytes will be quantitative. Stability of reaction products is another important consideration when using precolumn derivatization. The reaction products must be stable long enough to traverse the length of the column. Degradation on the column can be a problem.

With PCR, artifact formation, completeness of reaction, and stablility of products do not cause problems, since the reaction occurs after the separation. All that is needed is for the reaction products to be stable long enough to get from the reaction chamber to the detector cell. The only other requirement is that the reaction be reproducible (1).

There are a few disadvantages of a postcolumn reaction system. Each of the extra pieces of hardware causes problems of its own. The reaction chamber must be designed so that intimate mixing of the effluent and reagent streams occurs; poor mixing results in an

inhomogeneous stream, which leads to baseline noise. However, it is important that the dead volume be kept to a minimum so that the efficiency of the separation is not destroyed. This design can be a challenge if the reaction is not an instantaneous one, since some sort of delay chamber must be incorporated to give the reaction time to occur. Examples of reactor and mixing chamber designs will be given later.

The reagent pump can be a source of baseline noise. Since, in the case of a UV-vis absorbance detector, two streams of different optical properties are being mixed, any small changes in the flow rate of one of the streams contributes to the baseline noise. If a piston pump is used to deliver the reagent, the pulsing caused by the piston going back and forth will be the dominant source of noise (2). This noise is proportional to the concentration of reagent in the stream, so excess reagent can interfere with the sensitivity of the method.

It was mentioned earlier that the detection scheme can be optimized independently of the separation. However, sometimes modification of the separation method must be done if the optimum eluent for separation is not the optimum medium for the reaction.

Mixing Chamber and Reactor Designs

There are two types of situations when postcolumn reaction is employed: those where the reaction is fast and little holdup volume is needed, and those where the reaction kinetics are slower, so a reaction coil or some other type of delay must be used. Usually, for fast reactions (up to 30 seconds), an open tubular reactor (OTR) is used.

For slower reactions (0.5-4.0 minutes), a packed bed reactor (PBR) is needed (1). These reactors can either be preceded by a mixing chamber, or they can be incorporated into a mixing chamber.

When reactions are instantaneous, as is often the case with inorganic systems, no reactor is needed; the mixing chamber itself serves as reactor. The most important features of a mixing chamber are its small dead volume and its ability to provide intimate mixing. Both of these features are present in the designs shown in Figure 2. The design in Figure 2A was conceived by Elchuk and Cassidy (3), while that in Figure 2B was conceived by Cassidy et al. (2). Both are made of a bored-out Swagelok stainless steel chromatography tee. The tubing is 1/16 inch outer diameter (o.d.).

The first design consists of two tubes cut at a 45° angle and butted against each other, so that the reagent and effluent streams impinge directly upon each other. The outlet tube, leading to the detector, is set flush with these tubes. The diagram shows that the only way for the mixed stream to enter the outlet tubing is by seeping through the natural spaces between the tubes. Consequently, this design has essentially a dead volume of zero, and yields very efficient mixing.

The second design also has a zero dead volume and gives slightly better mixing than the first. Again, the only way for the reagent stream to reach the outlet tubing is by seeping through the "cracks." The reagent enters the effluent stream and mixes with it via a 120-mesh stainless steel screen placed in the line. The main advantage this design has over the first is the ease with which it may be built.

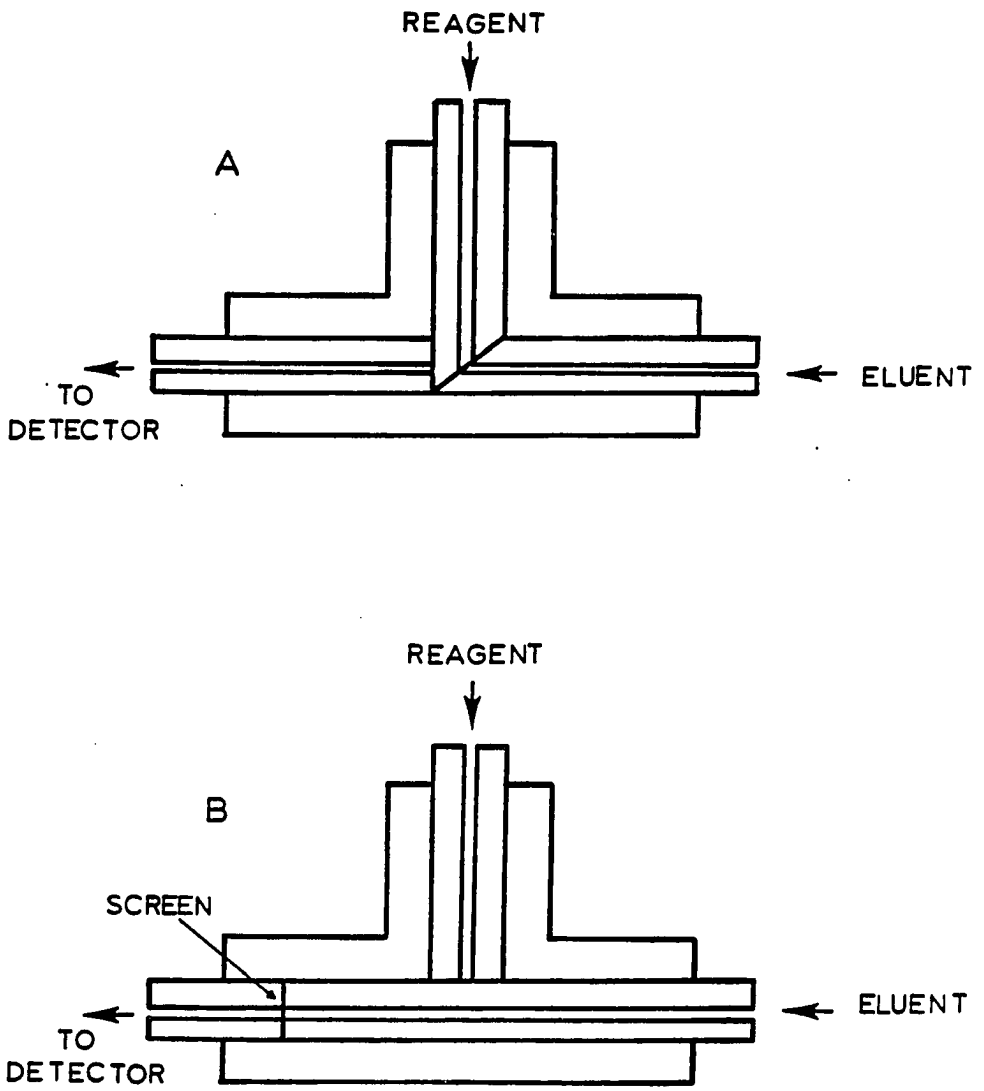


Figure 2. Mixing tees used for combining effluent and reagent streams in a postcolumn reaction chromatographic system. A.) Design by Elchuk and Cassidy (3), B.) design by Cassidy et al. (2)

A mixing chamber of a slightly more complex design is the "whirlpool, divided tangential entry chamber" devised by Sickafoose (4). This 16 microliter mixing chamber has two side inlets positioned opposite each other, one for the effluent and one for the reagent stream. Each inlet branches out in a "V" inside the device. The four segments of the two "V"'s meet in the center chamber where a whirlpool-like mixing occurs; the homogeneous stream then exits through the outlet at the top.

A commercially available mixer is marketed by the Lee Company Technical Center. This design is known as the Lee Visco-Jet Mixer. It consists of a series of "spin chambers," which yields a progression of tiny swirling mixers. As the reagent stream passes from one chamber to the next, it must reverse its direction of rotation, which results in a vigorous mixing process (5). The mixer has an internal volume of 10 microliters.

If a reaction is fast but not instantaneous, a short reaction coil may be placed in-line after the mixing chamber. This is called an open tubular reactor. A very simple design is that patented by Halasz et al. and licensed by Kratos for use in their commercially available postcolumn reactor. By using as a reactor a tube with a noncircular cross section wound in a tight helix, band broadening is minimized (6).

Another way to miminize band broadening is to use a "knitted" piece of tubing as a reaction chamber. By putting loose knots in the tubing to form a knitted open tubular (KOT) reactor (7), mixing in the longitudinal direction is decreased (1). This type of reactor is used if slightly longer reaction times are needed.

Hollow fibers lend themselves readily to a postcolumn reaction system. They serve as both mixing chamber and reactor. Reagent is placed outside the fiber and allowed to diffuse into the effluent stream, which is pumped through the interior of the fiber. This can be accomplished using a stagnant system, where the hollow fibers are placed in a large reservoir of reagent (8). The hollow fibers may also be placed into a larger concentric tube, so that the reagent stream is pumped from one end of the exterior tube to the other; this design allows fresh reagent to be constantly in contact with the fibers (9). The hollow fiber reactor can be coiled, filled with tiny inert beads (10), or have a nylon filament placed inside it (2) to increase mixing efficiency and decrease band broadening.

For kinetically slow reactions, a packed bed reactor is needed. This is a large column packed with small, inert glass beads. Since the dead volume is relatively large in a PBR, this type of reactor should only be used if it is absolutely necessary, i.e., when a reaction time of 0.5-4.0 minutes is needed (1).

An extension of the inert PBR is a column filled with a material that will somehow participate in the reaction (1). Examples of this are a support containing a reagent or a catalytic substance. One disadvantage of the former is that the reactor column must be regenerated as the reagent gets used up. Some catalysts that have been used in solid-phase reactors are ion-exchange resins, metal-loaded surfaces, and immobilized enzymes.

The solid-phase reactor is an example of a pumpless reaction unit (1). These types of reaction units require no reagent pump or mixing

device. In the case of a column filled with a reagent, no second pump is needed because the reagent is a solid. In the case of a catalytic column, all the reagents can be present initially in the mobile phase, so again no second pump is needed. No reaction occurs during the separation in the absence of the catalyst. When the catalytic column is reached, after the separation has occurred, the reaction takes place and the reaction products can be detected.

A thermally sensitized reaction is another situation where postcolumn reaction occurs without the use of a reagent pump (1). This method can be used when the desired reaction is kinetically slow at room temperature. Like the case where a catalytic column is used as a reactor, reagents for the postcolumn reaction are added to the mobile phase. After the separator column, the effluent stream is fed to a reactor where a temperature jump is applied. At the elevated temperature the reaction readily proceeds.

Applications

Postcolumn reaction can be used in conjunction with conductivity, electrochemical, fluorescence, and UV-vis absorbance detectors. It has been used for detection in reversed-phase, anion, and cation chromatography.

An example of PCR in ion chromatography using a conductivity detector is a method developed by Stevens et al. (9). They used an eluent of $NaHCO_3/Na_2CO_3$ to separate anions on a proprietary Dow ion-exchange resin. However, the eluent has a high conductance, so in order to reduce the conductivity of the eluent and increase the

conductivity of the analyte anions, the authors employed a postcolumn reaction system. They used a bundle of eight low-density sulfonated polyethylene hollow fibers six feet in length as the reactor. Each fiber had a 300 micrometer inner diameter (i.d.). Effluent from the separation column was pumped through the fibers. The bundle of hollow fibers was sealed in a 2 mm i.d. stainless steel tube. Sulfuric acid was pumped through the outer tube. Sodium ions in the effluent stream are allowed to permeate out through the hollow fiber wall and enter the sulfuric acid stream, while hydrogen ions permeate in. The result is that the effluent is converted to weakly conducting carbonic acid. Additionally, analyte anions are converted from their sodium (or other alkaki metal) salts to the acid form, resulting in enhanced conductivity. Donnan exclusion prevents the sample anions from permeating the sulfonated hollow fiber wall. This is a case of suppressed ion chromatography, where the second, suppressor column has been replaced by the hollow fiber suppressor.

Chromatographic resolution was improved by using a packed hollow fiber in the system described above (10). The empty hollow fibers caused some band broadening, which decreased resolution. By packing 800 micron i.d. Nafion 811-X hollow fibers with 500 micron styrene-divinylbenzene copolymer beads, and coiling the hollow fibers around a 1.25 inch diameter plexiglass rod, resolution was improved due to the reduction in band spreading.

PCR was successfully used in conjunction with another type of electrochemical detector, a coulometric detector. Takata and Muto (11) separated carboxylic acids by ion exclusion on a strongly acidic

cation-exchange resin with an eluent of methylcellosolve/water. However, carboxylic acids are not electroactive. Therefore p-benzoquinone was added with a peristaltic pump to the effluent stream to serve as an electrolyte for the "auxiliary electrode reaction." The carboxylic acids were detected with the secondary electrode reaction of benzoquinone and a proton:

$$\underset{\text{p-benzoquinone}}{Q} + 2H^+ + 2e^- \longrightarrow \underset{\text{hydroquinone}}{H_2Q} \qquad (1)$$

This paper showed that even electroinactive substances can be detected by introducing secondary electrochemical reactions.

A fluorescence detector may be used for the determination of anions if detection is preceded by PCR. Lee and Field (12) used anion-exchange chromatography to separate nitrite, thiosulfate, and iodide. A solution of Ce(IV) sulfate was added to the column effluent, and the combined stream was passed through a packed bed reactor consisting of a 6 mm i.d. x 20 cm Teflon tube packed with 100/120 mesh glass beads. The analyte anions are oxidized by the Ce(IV), producing Ce(III). The Ce(III) is subsequently measured by fluorescence detection. The PBR was needed due to the slow reaction time of the Ce(IV) with the analyte anions.

Nitrate may also be determined by modifying the method slightly. If a copperized cadmium (Cu-Cd) reactor is placed in-line after the separator column but before the mixing tee, nitrate is reduced to nitrite. The nitrite is then oxidized to nitrate by the Ce(IV),

producing Ce(III) which is measured as before. This is an example of a system which employs a packed bed reactor filled with a reagent instead of an inert material.

Fluorescence detection may also be used for the determination of metals. A fluorogenic complexing agent which undergoes a transition from nonflourescent to fluorescent upon complexation with a great many metals lends itself readily to a postcolumn reaction system. 8-Hydroxyquinoline-5-sulfonic acid (HQS) is such a complexing agent, preferred over oxine (8-hydroxyquinoline) because of the latter's limited water solubility. Soroka et al. (13) used this reagent to determine 25 pmole of Zn, 6 pmole of Cd and 500 pmole of Mg. Their method used a reactor consisting of a microporous hollow fiber with a monofilament nylon fishing line inside. They noted that a stainless steel screen-tee reactor could not be used because iron contamination led to fluorescence quenching.

Absorbance detection seems to be the most common method for detecting species formed in a postcolumn reaction. Both UV and visible light are used.

Haginaka et al. (14) determined β-lactamase inhibitors in serum and urine with a hollow fiber postcolumn reactor and detection in the UV. The hollow fiber reactor was suspended in sodium hydroxide solution. Upon diffusion of the sodium hydroxide into the methanolic effluent stream, the β-lactamase inhibitors rapidly degraded. The degradation products are readily detected with a UV spectrophotometric detector.

A unique way to detect sulfate involves a postcolumn solid-phase

reaction followed by detection in the visible region (15). One advantage of a solid-phase reaction system is that no second pump is needed for reagent delivery. This method is based on the indirect colorimetric determination of sulfate by barium chloranilate. A postcolumn reaction column was filled with a 1:1 mixture of silica gel and solid barium chloranilate. Sulfate was separated from other anionic components via anion-exchange with a slightly acidic eluent. In the reactor, sulfate precipitated as barium sulfate, thus releasing an equivalent amount of acid chloranilate ions:

$$SO_4^{2-} + BaC_6Cl_2O_{4(s)} + H^+ \longrightarrow BaSO_{4(s)} + HC_6Cl_2O_4^- \quad (2)$$

The acid chloranilate is detected at 530 nm. Although the reactor must be regenerated daily, the author of this paper noted that it could be used for 13 hours without any depletion in signal.

It is also important to note that the complete precipitation of barium sulfate takes ten minutes, which at first seems to be an unacceptable length of time for an on-line reaction. However, chemical engineering principles state that in a continuous-flow packed bed reactor, due to the intimate mixing of dissolved and solid reactants and the removal of reaction products by convective and diffusion mechanisms, the reaction rate of a heterogeneous reaction can be greatly increased. Also, it is not necessary for a postcolumn reaction to go to completion.

Another method which doesn't need a reagent delivery pump is one

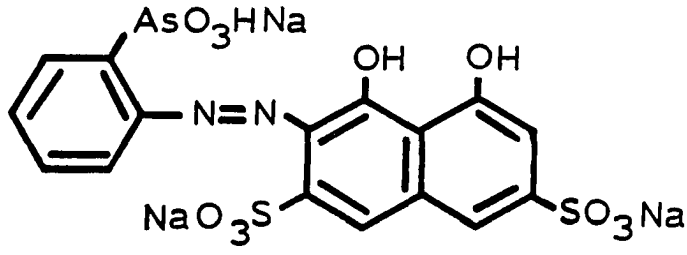
for the determination of amines (16). Amines are commonly detected by reaction with ninhydrin. This method utilizes the fact that the reaction between ninhydrin and amines is very slow. Consequently, the ninhydrin can be added to the mobile phase, and no reaction occurs during the chromatographic separation of the amines. After separation has occurred, the effluent is passed through a reaction coil thermostatted at 140°C. At this temperature the ninhydrin reacts rapidly with the amines, and the resulting complexes are detected at 570 nm.

Several researchers have extensively studied the use of postcolumn reaction for the detection of metal ions with colored chelating agents (2, 3, 17, 18). Since few detectors are available for the direct detection of low concentrations of metals, postcolumn derivatization followed by detection at a visible wavelength seems to be an obvious solution to the problem.

Early work by Elchuk and Cassidy (3) used the mixing tee shown in Figure 2A. (The design in Figure 2B is an improvement on that in Figure 2A, conceived later in time.) The postcolumn reactions they studied all were instantaneous, so no reaction coil was needed. They detected the lanthanide metal ions after separation on bonded phase and ion-exchange resins.

Three colored complexing reagents were examined: Alizarine Red S (alizarine sodium monosulfate), Arsenazo I (2-(1,8-dihydroxy-3,6-disulfo-2-naphthylazo)benzenearsonic acid, trisodium salt), and PAR (4-(2-pyridylazo)-resorcinol monosodium salt). The structures of Arsenazo I and PAR, as well as Arsenazo III, are shown in Figure 3.

A



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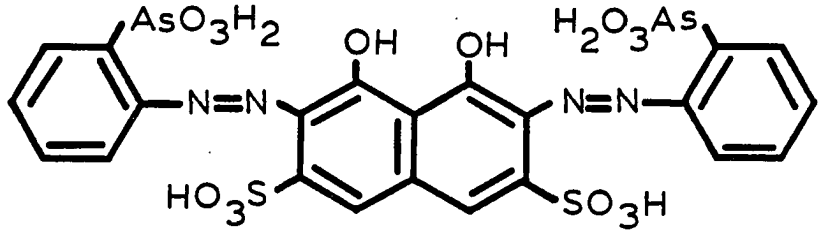

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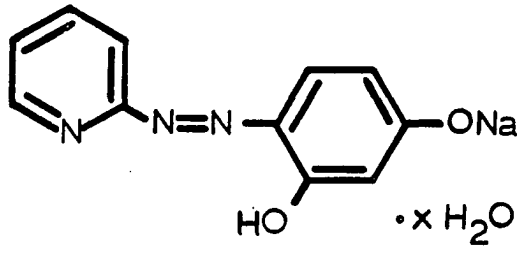

Figure 3. Structure of A.) Arsenazo I, B.) Arsenazo III, and C.) PAR

Spectrophotometric work showed that for all three reagents, complexation was complete upon mixing, and the complexes were stable for more than 30 minutes. The molar absorptivities of the lanthanide complexes in $L \cdot mol^{-1} \cdot cm^{-1}$ are ~ 9×10^3 for Alizarine Red S at 535 nm, ~ 3×10^4 for Arsenazo I at 585 nm, and ~ 6×10^4 for PAR at 520 nm.

The lanthanides were eluted with hydroxyisobutyric acid (HIBA), which is a complexing reagent and consequently can interfere with the postcolumn complexation. However, when tested, the HIBA was found to interfere only with the Alizarine Red S.

For postcolumn reaction, either a 1.3×10^{-4} M solution of Arsenazo I in 3 M ammonia was used, or a 2×10^{-4} M solution of PAR in 2 M ammonia/1 M ammonium acetate. The high pH of the postcolumn reagent solutions was necessary since metal complexation with these reagents is optimized under very alkaline conditions. Using a syringe pump to deliver the reagent solutions, the authors obtained detection limits of approximately 0.1 ng with PAR and approximately 1 ng with Arsenazo I for most of the lanthanides examined.

Fritz and Story (17) used the whirlpool, divided tangential entry chamber designed by Sickafoose (4) as a mixing device in their chromatographic system. They separated several metal ions on low capacity, macroreticular resins, using either PAR, Arsenazo I, or Arsenazo III as a postcolumn reagent, depending on the analyte metals. In another study by these same authors (18), the lanthanides were similarly detected, using PAR for in-stream colorimetric analysis.

TAN-3,6-S

A complexing reagent similar in structure to PAR and Arsenazo I has been shown to be useful for spectrophotometric determination of metal ions (19-22). This reagent, disodium 1-(2-thiazolylazo)-2-naphthol-3,6-disulfonate, abbreviated TAN-3,6-S, is shown in Figure 4. The sulfonated form of TAN (1-(2-thiazolylazo)-2-naphthol) is commonly used because the unsulfonated reagent is only slightly soluble in water (23).

TAN-3,6-S can be used to spectrophotometrically determine Pd(II) (19). Absorbance of a solution of the Pd/TAN-3,6-S complex, which is green in color at acidic pH, was measured at 654 nm. (The reagent is orange at acidic pH.) The reaction between TAN-3,6-S and Pd(II) takes ten minutes to reach completion. Palladium (II) reacts in a 1:1 molar ratio with the TAN-3,6-S, and the extinction coefficient at 645 nm is 1.2×10^4. Beer's law was followed over a palladium concentration range of 1 to 10 micrograms/mL.

Several derivatives of TAN were used to determine Zn spectrophotometrically (20). Most of the chelates formed were found to have a metal to reagent ratio of 1:2; this was true for Ni^{2+} and Co^{2+} as well as the Zn^{2+}. However, Cu^{2+} forms two types of chelates: one in a metal to reagent ratio of 1:1, the other 1:2. The structure of the metal chelates which form with unsulfonated TAN is shown in Figure 5.

Although most of the TAN derivatives give 1:2 complexes, TAN-3,6-S gives a 1:1 chelate with Zn^{2+} at a pH of 6.5. Absorption maxima for

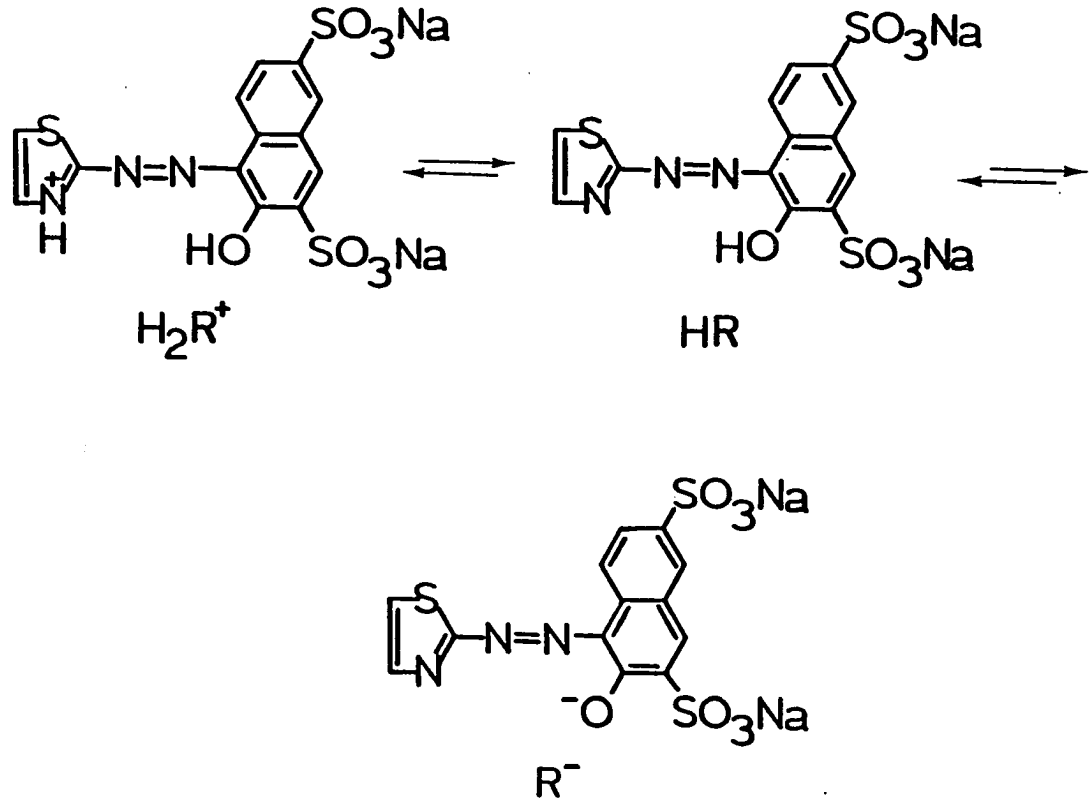

Figure 4. Structure of TAN-3,6-S, showing the acidic (H_2R^+), neutral (HR), and basic (R^-) forms

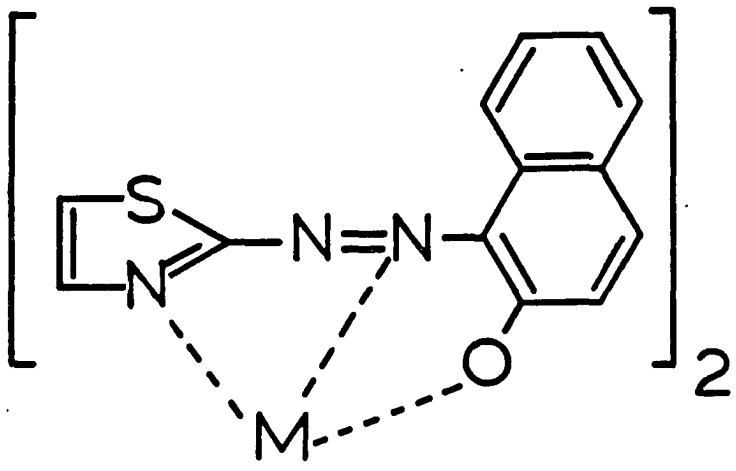

Figure 5. Structure of metal chelates which form with unsulfonated TAN (20). M = Cu(II), Ni(II), Co(II), Zn(II)

the red-violet Zn/TAN-3,6-S complex are 550, 525, and 394 nm. The optimum wavelength for determination was either 570 or 590 nm, and the optimum pH was 6.5. The molar extinction coefficient was found to be 1.15×10^4 at a wavelength of 590 nm. The author pointed out a great need for masking agents when Zn^{2+} is determined by this method in the presence of other metals, since so many metals are complexed by TAN derivatives.

When unsulfonated TAN was used for Zn^{2+} determination, the complexation reaction was complete within five minutes. Beer's law was followed over a range of 0.1 to 8.0 micrograms.

Gallium (III), indium (III), and thallium (III) can be spectrophotometrically determined with TAN-3,6-S (21). Extinction coefficients of the chelates at a wavelength of 580 nm were found to be 1.93×10^4 (Ga), 2.40×10^4 (In), and 2.98×10^4 (Tl). All three metal complexes exhibit two absorbance maxima: 530 and 570 nm for Ga, 540 and 570 nm for In, and 550 and 580 nm for Tl. Since the reagent itself absorbs minimally at the higher wavelength, the 570-590 nm region was used for metal determination.

For gallium and indium determination, the optimal pH with an excess of reagent was 3.0-4.0, while for thallium it was 2.3-2.8. At pH greater than 3.5-4.0 precipitation occurs due to hydrolysis. A fourfold excess of the reagent was found to be necessary for complete complexation. All three metal complexes reached their maximum absorbance immediately after metal and reagent solutions were mixed.

Gallium and indium were found to form 1:1 molar ratio complexes with TAN-3,6-S, while thallium (III) forms a 1:2 complex. Beer's law

was followed over the concentration ranges 0.2-2.5 micrograms Ga/mL, 0.5-5.0 micrograms In/mL, and 0.5-7.0 micrograms Tl/mL.

The authors noted that indium can be easily determined in the presence of gallium because a ten-fold excess of tartrate breaks down the gallium/TAN-3,6-S complex, while the indium complex is stable even in the presence of a 250-fold excess of tartrate. All three metals were determined without interference from nitrate, chloride, sulfate, or aluminum.

TAN-3,6-S has also been used as a complexometric indicator for the EDTA titration of Ga(III), In(III), and Tl(III) (21). Color changes were from red to orange-yellow (Ga) and violet or blue-violet to yellow (In and Tl). The color change was easily detected for In and Tl, but it was noted a more distinct end point was obtained when a hot solution of Ga was titrated.

The complexation of copper by TAN-3,6-S has been studied extensively by Langova et al. (22). Job plots, or the method of continuous variations, showed that for a pH range of 1.05 to 9.45, a 1:1 complex formed between Cu^{2+} and TAN-3,6-S, even in solutions with excess reagent. It is, however, desirable to avoid a large excess of reagent because at high reagent concentrations, an unexplained decrease in the absorbance of the complex, relative to solutions with equimolar amounts of metal and reagent or excess metal, occurred.

Copper (II) should be spectrophotometrically determined with TAN-3,6-S in a pH range of 2.5 to 4.5. The absorbances of the complex and the reagent are constant over this pH range. Additionally, interferences from other metals are minimized at this low pH, since

most other metals complex more readily with the reagent at a higher pH. The molar absorptivity at a wavelength of 575 nm, pH 2.6, is 2.1×10^4.

The examples above show that TAN-3,6-S complexes many metals, and the molar absorptivities of the chelates are fairly high. The reagent and the complexes are water soluble, and the complexation reaction occurs quickly. There is a fairly large difference in color between the reagent and the metal complexes, the reagent being orange at acidic pH and most metal chelates being violet. These characteristics make TAN-3,6-S an excellent choice for a postcolumn reagent for the detection of metal ions.

EXPERIMENTAL SECTION

Apparatus

The basic chromatographic instrument consisted of a model 302 Gilson single piston pump for eluent delivery, a model 7125 Rheodyne injector equipped with a 100-microliter sample loop, a model LP-2 Scientific Systems, Inc. Lo-Pulse pulse dampener in-line between the eluent pump and injector, a TSKgel IC-Cation-SW sulfonated cation-exchange column (silica based, 0.45 meq/g capacity, 5 μ particles, 50 mm x 4.6 mm i.d.), a Spectroflow 783 Kratos UV-visible spectrophotometric detector, and a Curken strip chart recorder. The following accessories were added so that postcolumn reaction could be used: a second model 302 Gilson single piston pump for postcolumn reagent delivery, a second pulse dampener made of a 4.6 mm x 10 cm Teflon column packed with Bio-Glas-200 100-200 mesh porous glass beads and placed in-line between the reagent pump and the mixing tee, and either of the mixing tees shown in Figure 2. The chromatograms in Figure 7 were obtained using the mixing tee design shown in Figure 2A, while the quantitative data reported in Table IV were obtained with the mixing tee shown in Figure 2B.

Visible spectra were obtained with a model DMS-100 Varian UV-visible spectrophotometer.

Eluents

Reagent grade ethylenediamine and tartaric acid were used without further purification. Sodium hydroxide was used to adjust eluent pH to

the desired value. Deionized water was obtained from a NANOpure II water purification system (Barnstead). Eluents were filtered through a 0.45-micron membrane and degassed by suction, then used under a nitrogen atmosphere to prevent decomposition.

Sample Solutions

Metal ion solutions were prepared by dissolving reagent grade salts in deionized water. All metal solutions were made acidic by addition of a small amount of nitric acid merely to prevent hydrolysis.

Postcolumn Reagent Solutions

Reagent grade triethanolamine (TEA) and nitric acid were used without purification. TAN-3,6-S was synthesized as described below (24). The postcolumn reagent solutions were buffered at the desired pH by adding concentrated nitric acid to a 0.5 M solution of TEA in deionized water, then dissolving the TAN-3,6-S and diluting to volume with the TEA/$TEAH^+$ buffer.

TAN-3,6-S was synthesized as follows. All materials used in the synthesis were reagent grade and used as received. Concentrated HCl (30 mL) was used to dissolve 1.1 g of 2-aminothiazole; 50 g of ice was added, followed by a solution of 0.7 g $NaNO_2$ in 10 mL water. The mixture was then kept at -5°C for 15 minutes by standing it in an ice/methanol bath. Urea (3 g) was added followed by a slurry of 3.5 g 2-naphthol-3,6-disulfonic acid disodium salt (Pfaltz & Bauer) and 50 g of sodium acetate in 50 mL water. This produced a brown mixture the consistency of pudding, which was suction filtered to yield a brown

paste. When the product was dissolved in water, a dark brown solution resulted. This was boiled to reduce the volume then cooled to below the boiling point. Activated charcoal was added to remove brown impurities. The mixture was hot filtered to remove insoluble impurities and the charcoal. The volume of the filtrate was reduced, and a crystalline solid precipitated upon placement of the solution into an ice bath. The red-orange crystals were isolated by suction filtration, then washed only once with a small volume of cold water. As the red-orange crystals dried, they changed in color to brown.

Chromatographic Conditions

Chromatography was done with an eluent of 3 mM ethylenediamine/4 mM tartaric acid, pH 4.5. The eluent flow rate was 0.7-0.8 mL/min, and the postcolumn reagent stream flow rate also was 0.7-0.8 mL/min. A detection wavelength of 570-600 nm was used. For the detection limit and working range study, the concentration of TAN-3,6-S was optimized to 4.4×10^{-4} M.

RESULTS AND DISCUSSION

Separation Conditions

The purpose of this work was to study the use of TAN-3,6-S as a postcolumn reagent for the determinaton of metal ions in single-column ion chromatography. Therefore, little work was done on developing separation schemes. Rather, separations already developed by others were used. Sevenich and Fritz (25) showed that ethylenediamine/tartrate is an excellent eluent for elution of divalent metals from a lightly sulfonated polystyrene-divinylbenzene cation-exchange column. This separation scheme was therefore relied upon.

Choice of Mixing Device

Elchuk and Cassidy (3) and Cassidy, Elchuk and Dasgupta (2) extensively studied mixing chamber designs for PCR. They designed several devices that gave excellent mixing yet had a low dead volume. Two of these designs are given in Figure 2. The third design consisted of a monofilament nylon fishing line placed inside a 400 micrometer i.d. Celgand X-20 microporous hollow fiber. The hollow fiber was coiled on a 3 mm outer diameter glass rod and immersed in boiling water. Upon cooling the hollow fiber remained in a helical configuration. This was then sealed in a 100 mm x 6.4 mm o.d. tube equipped with a reagent inlet and outlet. Effluent flowed through the interior of the fibers while the postcolumn reagent flowed around the outside of the hollow fibers. The reagent entered the effluent stream through the hollow fiber's pores.

The authors of these studies (2, 3) compared these designs to other mixing devices, both those described in the literature and some that are commercially available. They found that these three designs gave the best performance in terms of efficient mixing and minimal band spreading. Of the three, the membrane reactor and the tee reactor shown in Figure 2B gave the best performance, with the latter being the easiest to construct.

In the work presented here, the mixing tee originally used was that shown in Figure 2A. However, towards the end of this study, the superiority of the design in Figure 2B became known. Consequently that mixing tee was used for the remaining portion of the work, which comprised the quantitative portion of this study.

Spectrophotometric Work

Before the TAN-3,6-S was used as a postcolumn reagent for the detection of metal ions in ion chromatography, spectrophotometric data were obtained to see what metals form chelates with the TAN-3,6-S. The results are shown in the last column of Table I. Data in Table I for Arsenazo I, Arsenazo III, and PAR were taken from an article by Fritz and Story (17) and are included for comparison purposes. In all cases except Pd(II) the chelates formed with TAN-3,6-S were violet in color; Pd(II) forms a green chelate. Although no kinetic studies were done, it appeared the complexation reactions with TAN-3,6-S were instantaneous. The wavelengths for maximum absorbance (λ_{max}) of the chelates are given in Tables II and III, along with the molar absorptivities (ε) at those wavelengths. For Tables II and III, a 20-

Table I. Reactions of postcolumn reagents with metal ions. X indicates a positive reaction

Metal Ion	Reagent			
	Arsenazo I[a]	Arsenazo III[a]	PAR[a]	TAN-3,6-S[b]
Ag(I)				X
Mg(II)	X			
Ca(II)	X	X		
Ba(II)		X		
Sr(II)		X		
Mn(II)			X	X
Fe(II)			X	X
Co(II)			X	X
Ni(II)			X	X
Cu(II)		X	X	X
Zn(II)		X	X	X
Pd(II)				X
Cd(II)			X	X
Hg(II)			X	X
Pb(II)		X	X	X
Al(III)		X		
Cr(III)		X		
Fe(III)			X	X
Ga(III)				X
Y(III)				X
In(III)				X
Ln(III)[c]		X	X	X
Tl(III)				X
Bi(III)			X	
V(IV)			X	X
Zr(IV)		X	X	
Hf(IV)		X	X	
Th(IV)		X	X	X
U(VI)				X

[a]Taken from reference 17.

[b]Original data.

[c]Lanthanides.

Table II. Absorbance data for complexes of divalent metals with TAN-3,6-S

metal ion	pH	λ_{max} [a,b]	ε [c] x 10^{-4}	approximate optimum pH for detection at 600 nm
Mn(II)	4.0	487	1.32	6.6
	5.7	494	1.29	
		581(sh)	0.22	
	6.6	535	1.43	
		576(sh)	0.90	
Fe(II)	3.1	500	1.27	6.8
		520(sh)	1.17	
		559(sh)	0.60	
	4.8	502(sh)	1.25	
		526	1.61	
		557(sh)	1.39	
	6.8	500(sh)	1.59	
		526	1.98	
		557(sh)	1.81	
Co(II)	3.3	509	1.03	5.0-6.6
		589(sh)	0.49	
	5.0	542	1.24	
		589(sh)	0.90	
	5.9, 6.6	542	1.27	
		587(sh)	0.96	
Ni(II)	3.0	544	1.67	3.0-6.2
		563(sh)	1.58	
		585(sh)	1.45	
	4.8, 6.2	544	1.77	
		563(sh)	1.71	
		585(sh)	1.59	
Cu(II)	3.0, 4.9	574	1.81	3.0-4.9
	6.7	574	1.66	

[a]Units of nm.

[b]Shoulder is abbreviated sh.

[c]Units of $L \cdot cm^{-1} \cdot mole^{-1}$.

Table II (Continued)

metal ion	pH	λ_{max} [a,b]	ε[c] x 10^{-4}	approximate optimum pH for detection at 600 nm
Zn(II)	3.3	494	1.17	5.0-6.3
		576(sh)	0.30	
	5.0	536	1.83	
		549(sh)	1.81	
		568(sh)	1.69	
	6.3	545	1.93	
		568(sh)	1.81	
Pd(II)	1.9, 2.3, 3.2	603(sh)	0.56	1.9-3.2[d]
		650	1.01	
		693(sh)	0.87	
Cd(II)	3.5	491	1.21	5.4-6.4
		579(sh)	0.13	
	5.4	554	1.58	
	6.4	554	1.83	
Hg(II)	3.2	551	1.52	3.2
		582	1.54	
	5.3	546	1.35	
		573(sh)	1.30	
	6.7	516(sh)	1.08	
		542	1.26	
		579(sh)	1.04	
Pb(II)	3.4	498	1.09	5.0-6.4
		582(sh)	0.50	
	5.0	559	1.50	
	6.4	559	1.56	

[d]Use detection wavelength greater than 600 nm.

Table III. Absorbance data for complexes of metals of valency other than +2 with TAN-3,6-S

metal ion	pH	λ_{max} [a,b]	ε [c] x 10^{-4}	approximate optimum pH for detection at 600 nm
Ag(I)	4.0	490	1.38	7.0
	5.9	494	1.32	
		582(sh)	0.28	
	7.0	506	1.23	
		572(sh)	0.72	
Fe(III)	3.0	574	0.92	3.0
	4.0, 4.7	549(sh)	0.83	
Ga(III)	2.3	501	1.11	2.7-3.4
		536(sh)	0.93	
		573(sh)	0.61	
	2.7	501(sh)	1.03	
		530	1.30	
		567(sh)	1.16	
	3.4	542	1.76	
		559	1.69	
In(III)	1.9	544	1.42	2.5-3.2
		567(sh)	1.36	
	2.5	544	1.80	
		567	1.80	
	3.2	544	1.87	
		567	1.87	
Tl(III)	1.8	558(sh)	1.64	1.8
		590	1.80	
	2.7	515(sh)	0.81	
		547	1.02	
		581(sh)	0.88	
	3.3	524	0.74	
		590(sh)	0.40	
Y(III)	3.3	489	1.37	4.8-7.0
	4.8	533	1.70	
	7.0	541	1.98	

[a]Units of nm.

[b]Shoulder is abbreviated sh.

[c]Units of $L \cdot cm^{-1} \cdot mole^{-1}$.

Table III (Continued)

metal ion	pH	λ_{max} [a,b]	$\varepsilon^{c} \times 10^{-4}$	approximate optimum pH for detection at 600 nm
La(III)	3.5	486	1.21	5.3-7.0
	5.3	534	1.31	
	7.0	541	1.54	
Ce(III)	3.4	491	1.18	5.0-7.0
	5.0	538	1.51	
	7.0	538	1.46	
Pr(III)	3.4	493	1.24	5.1-6.9
		577(sh)	0.19	
	5.1	543	1.71	
	6.9	545	1.90	
Nd(III)	3.4	496	1.3	5.3-6.8
		574(sh)	0.39	
	5.3, 6.8	537	1.94	
Sm(III)	3.4	530	1.21	4.7-6.9
		569(sh)	0.92	
	4.7	545	1.73	
	6.9	545	1.78	
Eu(III)	3.4	499	1.13	5.1-6.9
		572(sh)	0.45	
	5.1, 6.9	546	1.74	
Gd(III)	3.3	496	1.27	5.0-6.7
		572(sh)	0.51	
	5.0	545	1.98	
	6.7	545	2.01	
Tb(III)	3.5	507	1.14	5.3-7.0
		572(sh)	0.62	
	5.3	543	1.84	
	7.0	543	1.95	
Dy(III)	3.5	516	1.19	5.4-6.7
		568(sh)	0.75	
	5.4, 6.7	543	1.87	

Table III (Continued)

metal ion	pH	λ_{max} [a,b]	$\varepsilon^{c} \times 10^{-4}$	approximate optimum pH for detection at 600 nm
Ho(III)	3.2	491	1.32	4.8-6.7
		576(sh)	0.31	
	4.8	540	1.98	
	6.7	540	2.05	
Er(III)	3.5	497	1.22	5.0-6.6
		575(sh)	0.40	
	5.0	541	1.76	
	6.6	544	1.88	
Tm(III)	3.5	511	1.24	5.5-6.7
		571(sh)	0.74	
	5.5, 6.7	543	2.01	
Yb(III)	3.3	506	1.13	5.7-6.8
		568(sh)	0.59	
	5.7, 6.8	542	1.79	
Lu(III)	3.3	509	1.13	5.1-6.7
		572(sh)	0.63	
	5.1, 6.7	545	1.81	
V(IV)	3.1	522(sh)	0.84	4.0
		550	0.89	
		582(sh)	0.83	
	4.0	509(sh)	0.80	
		556	1.08	
		577(sh)	1.05	
	5.6	491	0.91	
		582(sh)	0.58	
Th(IV)	2.9	536	1.62	2.9-3.5[d]
	3.5	538	1.57	
	4.6	519	1.62	
	5.5	519	0.96	

[d]For detection wavelength less than 600 nm.

Table III (Continued)

metal ion	pH	λ_{max} [a,b]	ε [c] x 10^{-4}	approximate optimum pH for detection at 600 nm
U(VI)	3.4	536	1.24	5.1-6.7
		572(sh)	1.09	
	5.1	551(sh)	1.62	
		569	1.65	
	6.7	551(sh)	1.65	
		569	1.68	

fold excess of metal ion over reagent was used. Extinction coefficients were calculated using the assumption that with excess metal, all metals form a 1:1 complex with TAN-3,6-S. Although in some cases a λ_{max} of ~ 490 nm is reported for the complex, this absorbance is probably due to uncomplexed reagent present when the solution pH is not favorable for complex formation.

It is important to note that the reagent itself can exist in more than one colored form. Figure 4 shows the TAN-3,6-S is involved in acid-base equilibria. At acidic pH the reagent is orange, changing to a rose color at a pH slightly greater than 7, and finally turning purple at basic pH. Since postcolumn reaction relies on detecting chelates in the presence of excess reagent, it is important that the reagent have minimal absorbance at the wavelength used to detect the chelates. Consequently, in a postcolumn reaction system using TAN-3,6-S, the reagent must be kept in its acidic form for maximum sensitivity.

Fortunately, the metals studied all complex readily with TAN-3,6-S at slightly acidic pH. This is one of the advantages TAN-3,6-S has over PAR, Arsenazo I, and Arsenazo III. The latter three reagents all require a strongly alkaline pH for metal complexation to occur; large amounts of ammonia must be added to the reagent solution (17). One of the reasons many people use a peristaltic pump for delivery of a postcolumn reagent is because of the caustic nature of the reagent solutions; peristaltic pumps use disposable plastic tubing, so there is no danger of harming a pump head.

Figure 6 shows spectra of TAN-3,6-S and its complex with Ni^{2+} at a

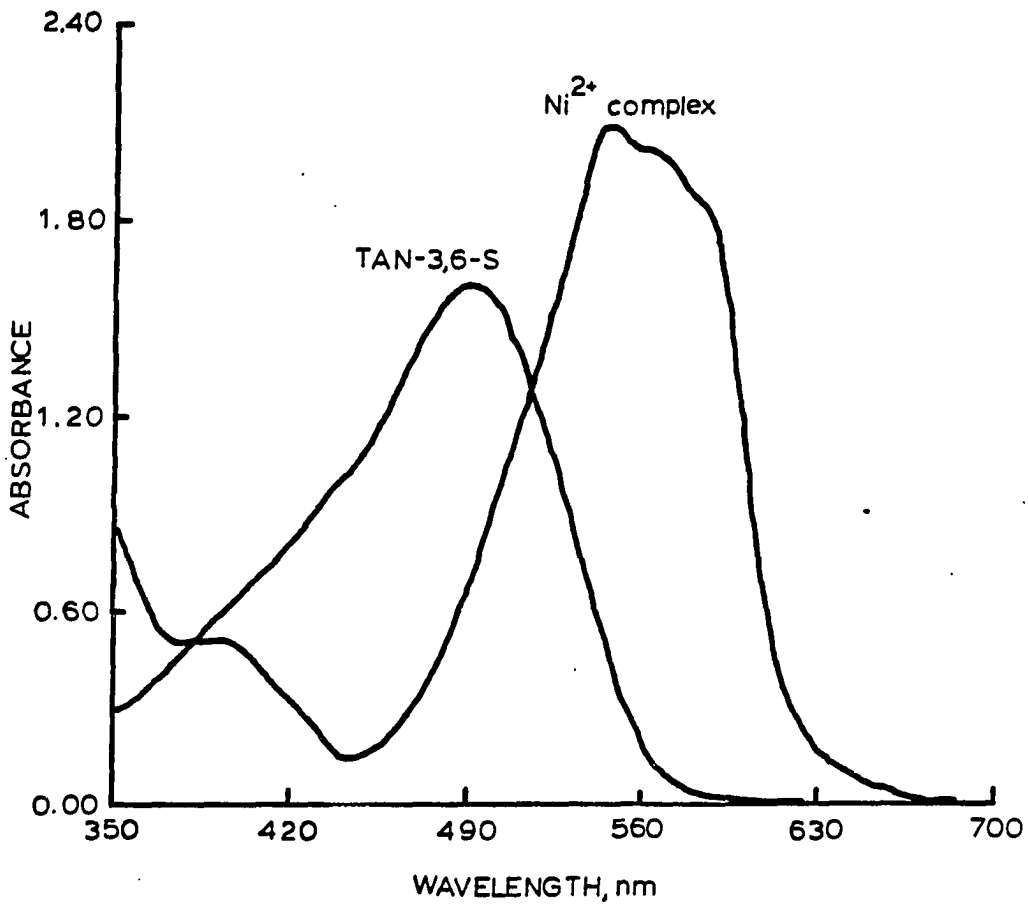

Figure 6. Visible spectra of aqueous 0.12 mM TAN-3,6-S, pH 6.2, and 0.12 mM TAN-3,6-S mixed with aqueous 0.20 mM Ni(II), pH 5.3

pH of 5-6. Most of the transition metals have similar spectra; Ni^{2+} was randomly chosen to illustrate the difference in the absorbance maxima.

Postcolumn Reaction Parameters

Choosing the optimal detection wavelength is critical in a postcolumn reaction system (3). Although it is relatively easy to get an adequate signal with PCR, the important task to accomplish is maximizing the signal to noise ratio. For example, the wavelength of maximum absorbance for PAR/metal chelates is 490 nm, but 540 nm is used for detection since here the noise due to the excess PAR is minimized. With the TAN-3,6-S, a detection wavelength of 600 nm was used, since the signal to noise ratio was optimized at that wavelength.

The buffer system chosen for this work was 0.5 M TEA/$TEAH^+$. TEA has a pK_a of 6.2 (26), so the buffer capacity is fairly high at a pH of 5-6. This pH was chosen since it gives the best signal to noise ratio for a large number of metals. When choosing a buffer the complexing ability of the buffering agent must be considered. In this case the TEA did not compete with the TAN-3,6-S for the metal ions. This was checked by complexing the Ni^{2+} in two solutions at a pH of 6; one was complexed by adding TAN-3,6-S at a pH of 6, while the other was complexed by adding TAN-3,6-S after the addition of a TEA/$TEAH^+$ buffer at pH 6. The absorbance spectra of the two solutions were identical.

The complexing ability of the eluent must also be considered. It has been shown (3) that Alizarin Red S cannot be used as a postcolumn reagent with an eluent of HIBA, because the HIBA interferes with the

complexation of the metals by the Alizarin Red S. With TAN-3,6-S as a postcolumn reagent, eluent tartrate concentrations of 4 mM were used successfully to determine transition metals. Spectrophotometrically, even a 600-fold excess of tartrate over TAN-3,6-S did not interfere with the complexation of Ni^{2+}. In preliminary experiments, 22 mM HIBA was also used without any apparent interference with the complexation by TAN-3,6-S.

Another important point to consider in a postcolumn reaction system is the concentration of the reagent. A high enough concentration of reagent must be used to permit the determination of a wide concentration range of metals. However, baseline noise is dependent on the concentration of reagent. When two streams of different optical properties are mixed, small fluctuations in the flow rate, such as those caused by the stroking of the piston, or in the composition of the stream due to imperfect mixing, will lead to baseline noise (2, 3). Since this noise is dependent on the difference in absorbance between the effluent and reagent streams, it is advantageous to keep the concentration of the reagent as low as possible. Therefore, the concentration of reagent must be experimentally optimized. Elchuk and Cassidy (3) found the optimal concentration of PAR was 2.0×10^{-4} M, while 1.3×10^{-4} M was used for Arsenazo I. These concentrations gave a linear working range of 10 ng to 600 ng for most of the metals they examined.

The optimal concentration of TAN-3,6-S was found to be 4.4×10^{-4} M. The detection limits and linear working ranges for the metals studied are given in Table IV. Unless otherwise noted, the

Table IV. Limit of detection (LOD) and linear dynamic range for the detection of some transition metals with TAN-3,6-S. A detection wavelength of 600 nm was used; other conditions given in text

metal	pH	LOD[a]	linear dynamic range[b]	correlation coefficient
Mn^{2+}	5.8	1100	0.102-2.03[c,d]	0.9996
Fe^{2+}	5.8	6	0.00109-0.434	0.9998
Co^{2+}	5.8	1[e,f]	0.000163-0.204	0.9995
Ni^{2+}	5.8	13[f,g]	0.0108-0.216[d]	0.9993
Cu^{2+}	4.4	64[h,i]	0.010-0.40[c,d]	0.9996
Zn^{2+}	5.8	1	0.000103-2.06[c]	0.9999
Cd^{2+}	5.8	99[i,j]	0.0088-0.220[c]	0.9997

[a]Units of ng.

[b]Units of mM.

[c]Minimum working range.

[d]Log-log plot.

[e]S:N ≃ 16.

[f]Limited by system peak.

[g]S:N ≃ 26.

[h]S:N ≃ 10.

[i]Lowest concentration injected.

[j]S:N ≃ 17.

limit of detection is where the signal to noise ratio (S:N) is equal to three. In some cases, the sensitivity for a particular metal was further limited by a system peak which eluted near the metal peak, obscuring the metal peak at low levels. The working ranges reported are those for which a plot of peak height vs. concentration was linear. In some cases, pointed out in Table IV, a plot of log peak height vs. log concentration yielded a larger linear working range. For several of the metals, a "minimum working range" is reported. This notation means the calibration plot was linear over the entire concentration range injected, and the true linear dynamic range is actually larger than that reported.

The flow rate of the postcolumn reagent stream can also be optimized. Usually mixing efficiency improves as total (reagent plus eluent) flow rate increases (3). One limitation, however, is that the introduction of the reagent stream dilutes the sample components; at equal reagent and eluent flow rates, the samples are diluted by a factor of two. For this work, a reagent flow rate of 0.7-0.8 mL/min, coupled with an eluent flow rate of 0.7-0.8 mL/min, was used.

Selectivity

Another advantage TAN-3,6-S has over PAR and Arsenazo I and III is that in addition to being a near-universal reagent, as Table I shows, TAN-3,6-S can also be a selective reagent. Although most metals form chelates with TAN-3,6-S at a pH of 6-7, some selectivity can be added to the detection method by choosing a pH where complexation of analytes of interest is optimized and complexation of interferences is

minimized. Note the last column in Tables II and III.

An example of this is shown in Figure 7. The chromatograms were obtained under identical conditions except for the pH of the postcolumn reagent solution. If manganese were of particular interest, these chromatograms show a high pH should be used. If manganese were an interference, by choosing a low pH for analysis the peak for Mn^{2+} can be eliminated.

The Cu^{2+} peak is an even more striking example. Copper is different from the other transition metals in that it complexes more readily at lower pH's; for most of the transition metals, complexation increases as pH increases. Therefore, for sensitive determination of Cu^{2+}, a lower pH should be used. Conversely, if Cu^{2+} were an interference, a high pH should be used for analysis.

Even at a pH where most metals complex with TAN-3,6-S, this detection method can still be considered a selective method. With conductivity detection, most cations will give a signal. However, only those metals which form chelates with the TAN-3,6-S should be detected with this PCR system. Consequently, potential interferences such as the alkali or alkaline earth metals, which do not complex with TAN-3,6-S, should not pose a problem, even when present in large excesses.

Sensitivity

Cassidy et al. (2) showed that good mixing efficiency is crucial for adequate sensitivity in PCR. However, they also showed that when high-performance reciprocating pumps are used for eluent delivery, 90-100% of the baseline noise (BLN) is due to pump pulsations. The

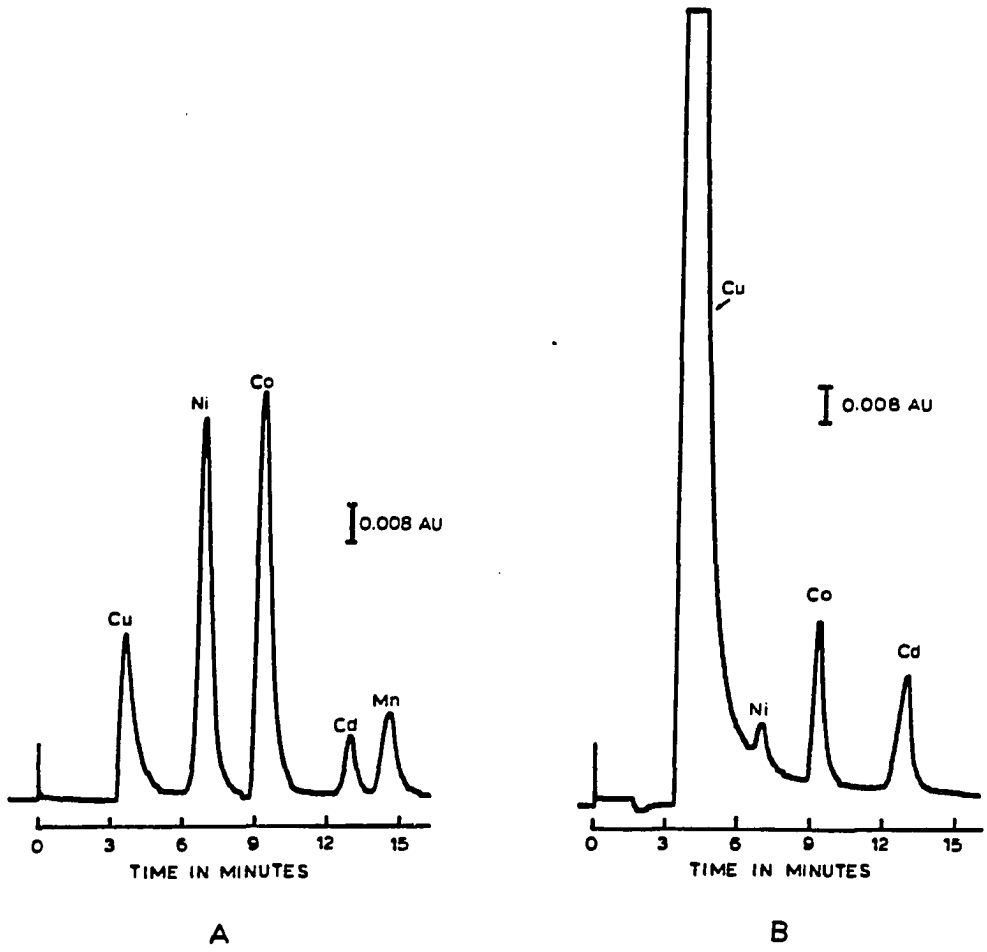

Figure 7. Separation of some transition metals on a TSKgel IC-Cation-SW silica cation-exchange column. Eluent is 3.2 mM ethylenediamine/4.0 mM tartaric acid, pH 4.5, flow rate of 0.7 mL/min. Postcolumn reagent stream is 0.09 mM TAN-3,6-S buffered with TEA, flow rate of 0.65 mL/min. Detection wavelength is 580 nm. Sample: mixture of 3.1 mM Cu(II), 0.31 mM Ni(II), 0.32 mM Co(II), 3.4 mM Cd(II), and 3.2 mM Mn(II). A.) Effluent pH = 6.9, B.) effluent pH = 4.9

factors that contribute to BLN are (2): flow pulsations (FN), mixing noise (MN), cell noise (CN), and detector noise (DN), related as shown below.

$$(BLN)^2 = (DN)^2 + (CN)^2 + (MN)^2 + (FN)^2 \qquad (3)$$

DN is noise observed when no liquid or a nonabsorbing liquid is in the cell. CN, usually a result of thermal effects, is a function of the background absorbance (BA) of a homogeneous liquid flowing through the detector. MN is noise due to mixing inefficiency. "In most PCR systems the BLN is dominated by the inhomogeneity of the liquid entering the detector, irrespective of whether the inhomogeneity is caused by flow pulsations (FN) or mixing inefficiency (MN) of the reactor" (2).

In order to measure mixing efficiency for several mixing chamber designs, the authors of the above-cited work used a pulseless delivery system for both eluent and reagent delivery. Either a pneumatic delivery system or a syringe pump was used. They found that BLN drastically increased when reciprocating pumps were substituted for the pulseless delivery systems. Consequently, for maximum sensitivity, pulseless delivery systems must be used.

The detection limits shown in Table IV were obtained with single piston reciprocating pumps used for both eluent and reagent delivery. Limits of detection could be decreased by perhaps as much as a factor of 50 (2) if pulseless delivery systems were used. A packed bed reactor (PBR) also could be installed to reduce pump pulsations;

however, this would increase band broadening due to the extra dead volume present in a PBR. Cassidy et al. (2) found that by introducing a 3 mm x 15 cm PBR filled with 20-25 mesh beads, BLN for a single piston pump was reduced from 11 x 10^{-4} to 1.7 x 10^{-4} AU, but with a 25% loss in efficiency.

FUTURE WORK

TAN-3,6-S has been shown to be an excellent postcolumn reagent for the chromatographic determination of transition metals. However, as Tables II and III show, the reagent complexes many other metals as well.

It should be possible to sensitively determine lanthanides using TAN-3,6-S. However, because the λ_{max} for these metal complexes is closer to the λ_{max} of the reagent itself, limits of detection will probably not be as low as those seen for the transition metals.

The detection selectivity due to a judicious choice of pH can be put to good use for the determination of In(III). At a pH of about 2, complexation of In(III) with TAN-3,6-S is optimized; above pH 3.5 the In(III) precipitates. However, at such a low pH, complexation of most divalent metals is minimal. Therefore, PCR with TAN-3,6-S followed by detection at 600 nm should be an excellent way to determine In(III) in the presence of divalent interferences. Similar selective methods for the determination of Ga(III), Tl(III), Th(IV), and Pd(II) can also be developed.

Since Pd(II) forms a green chelate with TAN-3,6-S while other metals form purple chelates, further selectivity for Pd(II) determination can be added by selecting an appropriate detection wavelength. The long wavelength for the maximum absorbance of the Pd(II) chelate should allow a limit of detection lower than those seen for the transition metals.

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SECTION III. THE EFFECT OF TEMPERATURE ON SINGLE-COLUMN ION CHROMATOGRAPHY OF METAL IONS

INTRODUCTION

The importance of temperature in gas chromatography is well known and exploited. However, the use of elevated temperature to improve liquid chromatographic separations, especially in ion-exchange chromatography, is much less common. While the temperature effects seen in ion-exchange chromatography are not as vast as those in gas chromatography, it is becoming apparent that the influence of temperature is not as slight as previously imagined.

Several research groups have investigated the effect of temperature on ion-exchange chromatography (1-23). Selectivity coefficients were found to either increase or decrease as a function of temperature, depending on the ion-exchange reaction (1-8). Changes in separation factors with temperature have also been noted (2, 4, 7, 9, 10). Systems studied include the cation-exchange of alkali (1-3, 8-10), alkaline earth (2, 3), transition (3), and rare earth metal ions (3) on sulfonated cation-exchangers, cation-exchange of alkali metals on phenolsulfonic ion-exchangers (1, 9-11), and the anion-exchange of rare earth complexes (4-7, 10, 11). Both isocratic (12) and gradient (13) anion-exchange of polyphosphates have been investigated at high temperatures, as have the analyses of alcohols, aldehydes, ketones, organic acids and carbohydrates using a strong cation-exchange column (14). In addition, elevated temperature has been shown to speed up the equilibrium between complexing eluents and metal ions, thus yielding peaks of better shape (15, 16).

The thermodynamic functions ΔH^*, ΔG^*, and ΔS^* have been calculated

for ion-exchange reactions as a function of temperature (1, 3-7). (The superscript asterisk notation will be explained later.) These were found to differ substantially even among chemically similar ions. One explanation for this is based on the differences in hydration of different metal ions (1, 5, 6). The free energy of reaction, ΔG^* term, is due to several factors. One factor is the difference in energy of interaction between the analyte (exchanged) ions and the resin functional groups. Another is the relative energy and entropy contributions from the dehydration of ions entering the resin and the hydration of ions initially present in the resin. Metal ions are hydrated to different extents at room temperature and the changes in the hydration spheres as temperature is raised vary from one ion to another.

Gregor and Bregman (8) and Gregor (24) stressed solvation effects in ion-exchange. For the exchange reaction

$$RY + X \longrightarrow RX + Y \qquad (1)$$

where R represents the resin and X and Y are ionic solutes, the following equation holds under conditions where activity coefficient effects are negligible in comparison to the swelling energy:

$$\ln K_Y^X = \frac{\pi}{RT} (Z_X V_Y - Z_Y V_X) \qquad (2)$$

where K_Y^X is the selectivity coefficient, π is the swelling pressure, R is the gas constant, T is the absolute temperature, Z is the absolute value of the ion charge, and V is the solvated molar volume (24).

Since π is directly proportional to temperature, the equation can be rewritten as:

$$\ln K_Y^X = A (Z_X V_Y - Z_Y V_X) \quad (3)$$

where A is a constant (8). This demonstrates that for ions of like charge, ions with the smaller solvated volume will be preferred by the resin. Therefore, since temperature affects the solvation of ions, it should affect the selectivity of the ion-exchange resin towards those ions. This was demonstrated by Gregor and Bregman (8) when they determined K_Y^X for ions of similar and dissimilar hydration. When the exchange reactions for ions hydrated to similar extents were studied, K_Y^X was found to be approximately independent of temperature. However, K_Y^X decreased with an increase in temperature when the two cations were hydrated to different extents.

Separation factors, α, as well as selectivity coefficients can vary with temperature (4, 9). An example of this is found in the work of Dybczyński (4). He found that the ion-exchange resolution of rare earth/EDTA (ethylenediaminetetraacetic acid) complexes at higher temperatures improves because the separation factors relative to europium are temperature dependent. These results are significant when one considers the well-known similarities of the rare earths as well as the fact that they all form complexes with EDTA of identical stoichiometric composition. Since the separation factor of a rare earth with respect to europium is a function of the capacity factor, k, of the rare earth, which in turn is a function of ΔH^* of the ion-exchange reaction (to be shown later), the separation factors vary

with temperature because the values of ΔH^* vary widely among the rare earths.

It has been shown that not only can selectivity coefficients and separation factors increase or decrease as a function of temperature, but column efficiency measured as plate height H can also change in either a favorable or unfavorable direction as temperature increases (2, 4, 7, 9, 10). An additional term to account for longitudinal diffusion in the resin phase needs to be added to equations used to calculate H, such as the Glueckauf equation. The theoretical expression for plate height (2) then appears as

$$H = 1.64r + \frac{(\lambda')(0.142r^2\mu)}{(\lambda' + i)^2 D_S} + \frac{(\lambda')^2(0.266r\mu)}{(\lambda' + i)^2(1 - i)D_L(1 + 70r\mu)} + \frac{(D_L)(i\sqrt{2})}{\mu} + \frac{2\gamma_S D_S \lambda'}{\mu i} \qquad (4)$$

r = mean radius of resin particles

D_S and D_L = diffusion coefficients in the resin and in solution, respectively

μ = linear flow rate of the eluent

i = fractional free volume of the resin bed

$\lambda' = \lambda d_z$

d_z = density of the resin bed

λ = weight distribution coefficient (amount of ion per gram of dry resin/amount of ion per mL of solution)

γ_S = mean activity coefficient of the stationary phase

Usually the last two terms of equation 4 are neglected when theoretical plate height is being discussed, since at room temperature they are negligible compared to the other terms. With the exception of the last two terms, the diffusion coefficients in the equation are in the denominator. Since an increase in temperature increases the diffusion coefficients in both the stationary phase and in solution, a consideration of only the first three terms of equation 4 would lead to an expectation that a rise in temperature leads to a decrease in H. While this is observed in many cases, there have been instances where an increase in temperature led to an increase in H (2, 7, 9, 10). It seems probable that at elevated temperatures longitudinal diffusion in the mobile and stationary phases can become important, so that the fourth and fifth terms in equation 4, where diffusion coefficients are in the numerator, make significant contributions to the plate height.

Ion-exchange chromatography has been used extensively for the separation of amino acids. Elevated temperature has been shown to improve resolution (17). Although ion-exchange separations of amino acids are most often carried out at above-ambient temperatures, typically between 50°C and 70°C (25), usually no explanation for the improvements in resolution is offered. Partridge and Brimley noted a change in the order of displacement of amino acids from sulfonated polystyrene divinylbenzene at elevated temperatures (18). They postulated that the order of displacement should be that of pK_1 values of acid dissociation of the amino acids. However, at room temperature this is not the case due to hydration and van der Waals forces. At high temperatures, these forces diminish and the order approaches that

expected due to pK_1 values. This and other explanations (17, 25) are based on the premise that separation in ion-exchange chromatography is due not only to the ionic interaction of solutes with the charged support but also to hydrophobic interactions with the polymer portion of the resin. A more rigorous mathematical approach by Hamilton, Bogue and Anderson (19) notes the decrease in peak variance and increase in solid phase diffusion coefficients of amino acids when temperature is raised. Several factors affect the variation of the diffusion coefficients with temperature, including the extent of hydration of the resin, the equilibrium of the adsorbed ions, and the diffusion process itself.

Elevated temperature has also been used in the ion-exchange separations of lanthanides and actinides (15, 16, 20-23). In separating citrate complexes of the rare earths, Ketelle and Boyd found that resolution increased with increasing temperature so therefore carried out their separations at 100°C (20). Since high temperature increased diffusion and the rate of exchange, tailing was reduced. In addition, the stability constants of the citrate complexes varied with temperature, so at elevated temperature retention of the cations was increased, leading to better separations. Increased retention at high temperature was seen also with the separation of lanthanides and actinides on anion-exchange resins with α-hydroxyisobutyrate (21) and LiCl eluents (16); in the latter case a decrease in peak half-widths was also noted. Furthermore, Schädel et al. (22) demonstrated that with the system they employed for separating the lanthanides, plate height decreased as temperature increased.

Studies of ion-exchange reactions at various temperatures, besides being useful to improve separations, are also valuable for shedding light on the structure of complex ions in solution. From experimentally obtained thermodynamic data, Dybczyński (5) and Dybczyński and Wódkiewicz (6) were able to deduce whether ligands such as EDTA or DCTA (trans-1,2-diaminocyclohexane-N,N′-tetraacetic acid) act as penta- or hexadentate ligands toward the various rare earth cations. They studied the anion-exchange of the rare earth complexes on strongly basic anion-exchange resins. From ΔH^*, ΔS^*, and ΔG^* for the ion-exchange reactions, the extent of hydration of the rare earth cations, and consequently the number of coordination sites taken up by the ligand, could be determined.

Retention mechanisms occurring in more complex stationary phases can also be elucidated through a thermodynamic study (1, 9). An example of this is a study done of the thermodynamics of cation-exchange of alkali metals on sulfonic and phenolsulfonic acid resins (1). Temperature effects on the enthalpy and entropy of exchange were found to correlate well with the amount of dehydration occurring as the metal entered the resin phase. The phenolsulfonic acid resin was more highly crosslinked than the sulfonic acid resin. At low temperatures the exchange of Na^+ for H^+ was more exothermic with the sulfonic acid resin than with the phenolsulfonic acid resin because fewer water molecules are stripped off the Na^+ ion as it enters the stationary phase; the higher the crosslinking, the more stripping occurs. At higher temperatures the Na^+ ions are already partially dehydrated. Consequently, further dehydration has little effect and the exchange is

more exothermic with the phenolsulfonic acid resin based on the energy of interaction of the Na^+ with the stationary phase.

With the Cs^+ ion, which is hydrated to a small extent, at low temperatures the exothermicity of the exchange reaction is nearly twice as great with the phenolsulfonic acid resin as with the sulfonic acid resin. This behavior is the reverse of that seen with the highly hydrated Na^+. In fact, with the phenolsulfonic acid resin, ΔH^* for the Cs^+-H^+ exchange is much more exothermic than is common for ion-exchange reactions on organic ion-exchangers. This suggests that the phenol groups of the resin provide additional stability by coordinating the Cs^+ ion with their oxygen atoms (1), shown in Figure 1. The small amount of hydration and large ionic radius of the Cs^+ ion make such complex formation feasible from both an energy and spatial consideration point of view. This retention mechanism is further supported by the relative changes in entropy observed for the sulfonic acid and the phenolsulfonic acid resin. At low temperature the entropy decrease for the Cs^+-H^+ exchange is much greater with the phenolsulfonic acid resin. This is due to the loss of configurational entropy of the phenolic groups which occurs when the complex forms.

Dybczyński (11) pointed out another use for selectivity coefficient vs. temperature data: they can be used to identify unknown analytes. Standard plots of log K vs. 1/T over a wide range of temperatures can be prepared. Then, instead of having to rely on one retention time measurement for identification, the retention time at a few different temperatures can be measured and the curve matched to the appropriate standard curve. While similar ionic species may have

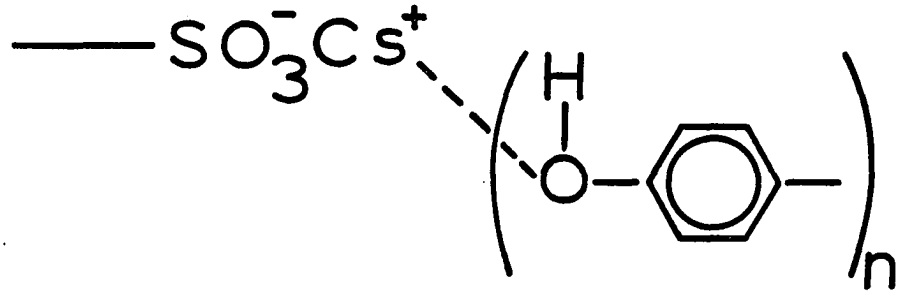

Figure 1. Schematic diagram showing how oxygen atoms of phenol groups on a phenolsulfonic acid resin can stabilize a Cs^+ ion (1)

identical retention times at room temperature, it is unlikely that plots of log K vs. 1/T for the ions will completely coincide.

The goal of this work was to show that by operating a cation chromatographic system at temperatures above ambient, the retention times of metal ions can be changed substantially. Retention times may increase or decrease with an increase in temperature, depending both on the nature of the eluting cation and on the individual analyte metal ion. Improved separations can result when an increase in temperature causes an increase in the retention of metal ions.

EXPERIMENTAL SECTION

Apparatus

The instrument used consisted of a model 302 Gilson single piston pump, a model 1116 Eldex column-heater, a model 7125 Rheodyne injector equipped with a 20-microliter sample loop, a Li-Chroma-Damp II coil type pulse-dampener, a model No. 269-004 Wescan catex column (capacity 0.03 meq/g, 12-16 micron particle size, 5% cross-linked, sulfonated polystyrene-divinylbenzene gel, 25 cm in length, 2 mm i.d.), and a Curken strip-chart recorder. Two modes of detection were used, either conductivity or postcolumn reaction and spectrophotometry. A model 213A Wescan conductivity detector was used for conductivity detection, and a Spectroflow 783 Kratos UV-visible spectrophotometric detector was used with the postcolumn system. The postcolumn mixer consisted of a bored-out stainless steel tee as described by Elchuk and Cassidy (26). The postcolumn color-forming reagent was delivered with a Minipuls 2 Gilson peristaltic pump. A model PD-60-LF Fluid Metering Inc. pulse-dampener was placed in line between the peristaltic pump and mixing tee. The eluent was preheated and thermostatted with a Haake A80 water bath. All connecting tubing was insulated by wrapping with sheets of styrofoam packing material. The conductivity cell was also insulated with styrofoam.

Eluents and Color-forming Reagents

Reagent grade p-phenylenediamine, p-phenylenediamine dihydrochloride, ethylenediamine, tartaric acid, perchloric acid,

4-(2-pyridylazo)-resorcinol (PAR), Arsenazo I, ammonia, and ammonium acetate were used without further purification. The p-phenylenediamine eluent was prepared by dissolving the solid in perchloric acid, diluting with deionized water (NANOpure II system, Barnstead), and adjusting the pH to 3 to ensure sufficient protonation of the amine. The ethylenediamine/tartaric acid eluent was prepared by dissolving the appropriate amounts of both ethylenediamine and tartaric acid in deionized water, diluting to volume, and adjusting the pH with aqueous sodium hydroxide. The p-phenylenediamine dihydrochloride eluent was prepared by dissolving and diluting the solid with deionized water; the perchloric acid eluent was prepared by diluting 70% perchloric acid. The amine eluents were freshly prepared each day and stored under a helium atmosphere and protected from light to prevent decomposition. All eluents were filtered through a 0.45 micron membrane and degassed.

The Arsenazo I solution was prepared by dissolving 3×10^{-3} moles Arsenazo I and 3 moles of ammonia in 1.0 liter of deionized water. The PAR solution was prepared by dissolving 5.6×10^{-4} moles PAR and 9×10^{-3} moles ammonia in about 400 mL deionized water, then adding a solution of 1 mole ammonium acetate in about 350 mL deionized water, and diluting to 1.0 L. All postcolumn reagent solutions were filtered through a 0.45 micron membrane prior to use.

Sample Solutions

All metal ion solutions were prepared by using reagent-grade salts, concentrated perchloric acid, and deionized water. The pH of all metal ion solutions was adjusted with perchloric acid to

approximately 3 merely to prevent hydrolysis.

Chromatographic Conditions

All chromatography was done at a flow rate of 0.6 mL/min. Additional eluent degassing was carried out on-line by continually sparging the eluent in the reservoir with helium. With each change of temperature or eluent, the system was allowed to equilibrate overnight (at least five hours).

With amine eluents, conductivity detection was employed. When perchloric acid was the eluent, postcolumn reaction and spectrophotometric detection were used. Mg^{2+} and Ca^{2+} were detected at 585 nm with Arsenazo I as the postcolumn reagent, while other metal ions were detected at 520 nm with PAR.

RESULTS AND DISCUSSION

For an eluent ion, E, of charge x and a sample metal ion, M, of charge y, the following exchange reaction can be written,

$$yE^{x}R_{x} + xM^{y} \rightleftharpoons xM^{y}R_{y} + yE^{x} \qquad (5)$$

where R_x and R_y represent the number of resin exchange sites occupied by E and M ions, respectively. This exchange reaction can be either exothermic or endothermic, depending on the ions involved. Consequently, the exchange equilibrium may be shifted in either direction as the temperature is increased, resulting in a change in the capacity factor, k, of the metal ion.

If for exchange reaction 5 the metal ion is present in trace amounts and the composition of the stationary phase remains essentially unchanged, then in the resin the mole fraction of metal is essentially zero and the mole fraction of eluent is essentially one: $X_M = 0$, $X_E = 1$. The thermodynamic values for the ion-exchange reaction are given the symbols ΔG^* (free energy change), ΔH^* (enthalpy change), and ΔS^* (entropy change), and can be used for comparison purposes instead of the standard values of these functions (5, 6).

The relationship among the thermodynamic parameters is given by the well-known Gibbs equation:

$$\Delta G^* = \Delta H^* - T\Delta S^* \qquad (6)$$

Capacity factor, k, which is used as a measure of chromatographic retention, is related to the thermodynamic equilibrium constant by the equation below (27):

$$k = \phi K \qquad (7)$$

where ϕ is the phase ratio, a characteristic constant for a given column. The free energy change, ΔG^*, for the ion-exchange reaction 5 is given by (28):

$$\Delta G^* = -RT\ln K = -RT\ln(k/\phi) \qquad (8)$$

The relationship between capacity factor, k, and system temperature in degrees Kelvin, T, is obtained by substituting equation 8 into equation 6 (27, 28):

$$\ln k = \frac{-\Delta H^*}{RT} + \frac{\Delta S^*}{R} + \ln\phi \qquad (9)$$

A plot of ln k versus reciprocal temperature, commonly called a Van't Hoff plot, should be linear with a slope equal to $-\Delta H^*/R$. Linearity is dependent on the exchange mechanism and the enthalpy of the reaction both being constant with temperature. If such a plot has a positive slope, reaction 5 is exothermic; if the plot has a negative slope, the exchange reaction is endothermic. Figures 2 and 3 show that with a stationary phase of sulfonated polystyrene divinylbenzene and divalent

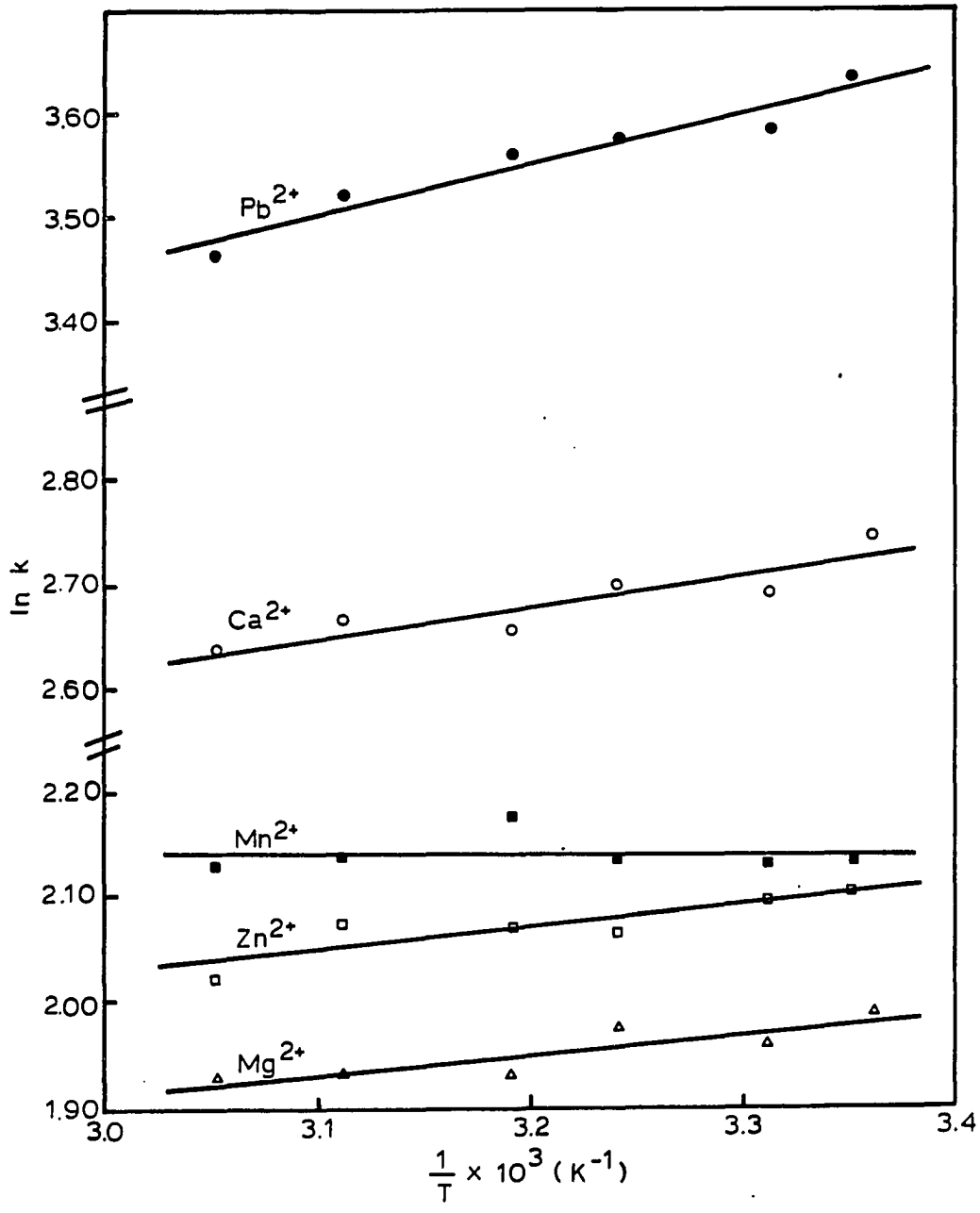

Figure 2. Plot of natural log of capacity factors of divalent metal ions as a function of reciprocal system temperature. Eluent: 0.12 M $HClO_4$

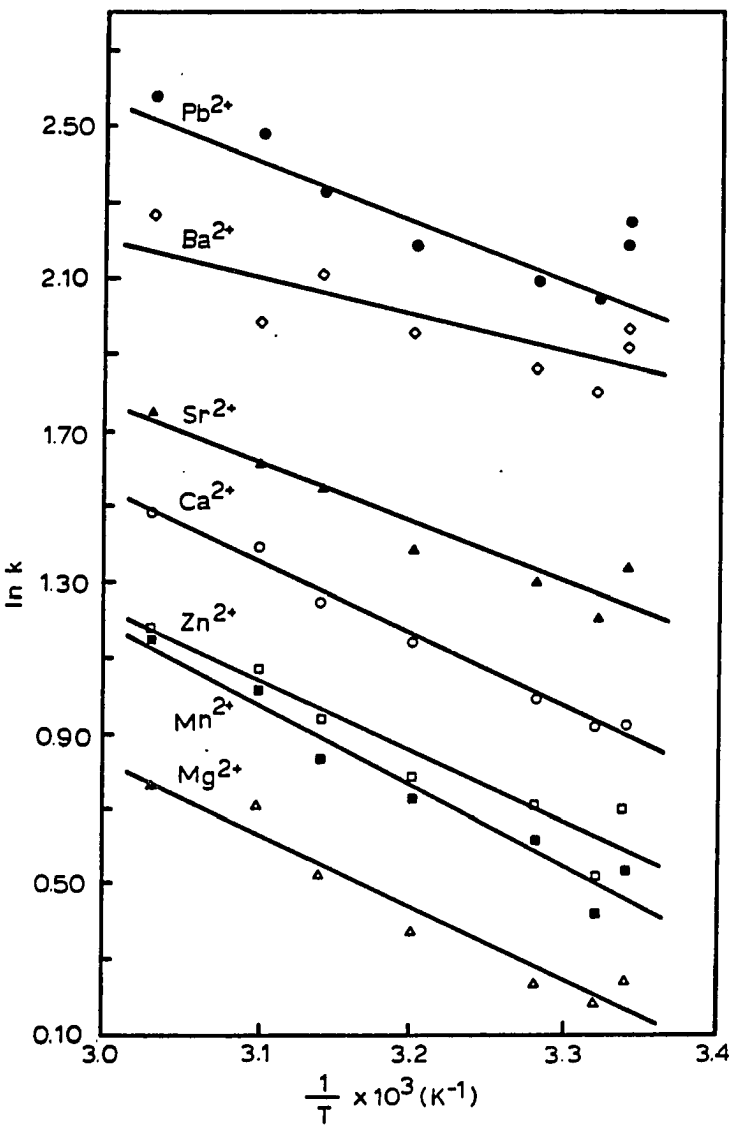

Figure 3. Plot of natural log of capacity factors of divalent metal ions as a function of reciprocal system temperature. Eluent: 3.0 mM p-phenylenediamine dihydrochloride

analyte metal ions, either situation can occur, depending on the eluent cation. With H^+ as eluent, equation 5 is exothermic; however, with doubly protonated p-phenylenediamine as eluent, the same reaction is endothermic.

Figures 2-5 show that the exo- or endothermicity of equation 5 is dependent not only on the eluent cation but also on the metal ion. While with perchloric acid as an eluent the reaction is exothermic for divalent metal ions, it is endothermic for rare earth metal ions. With p-phenylenediamine dihydrochloride as eluent the reaction is endothermic for both the di- and trivalent metal ions examined. The results of the runs with perchloric acid eluent are summarized in Table I and those with p-phenylenediammonium eluent are give in Table II. These values of ΔH^* agree with those found in the literature for cation-exchange on a strongly acidic cation-exchange resin of the $--SO_3H$ type: ΔH^* should be 0-3 kcal/equiv (5).

According to Melander et al. (28), curvature of a Van't Hoff plot indicates one of two things: the retention mechanism is not constant over the temperature range studied, or ΔH^* is not constant with temperature. Dybczyński (10) states that for ion-exchange ΔH^* can vary with temperature if the heat capacity for a reaction ΔC_p^* is not negligible:

$$\Delta H^* = \Delta H_o^* + \Delta C_p^* T \qquad (10)$$

Additionally H. F. Walton, in a review article on ion-exchange, mentions that "large singly-charged cations, such as the

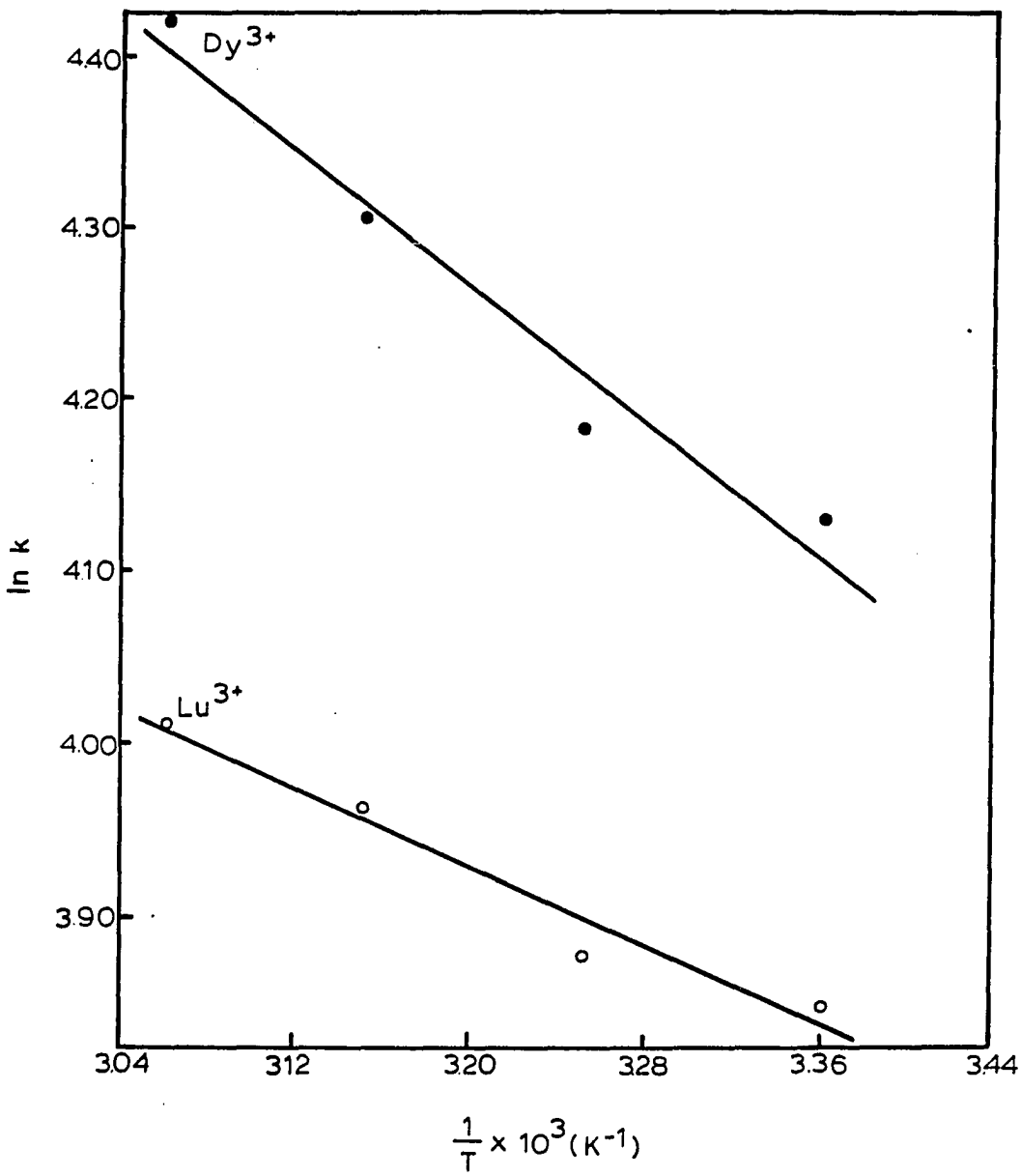

Figure 4. Plot of natural log of capacity factors of rare earth metal ions as a function of reciprocal system temperature. Eluent: 0.29 M $HClO_4$

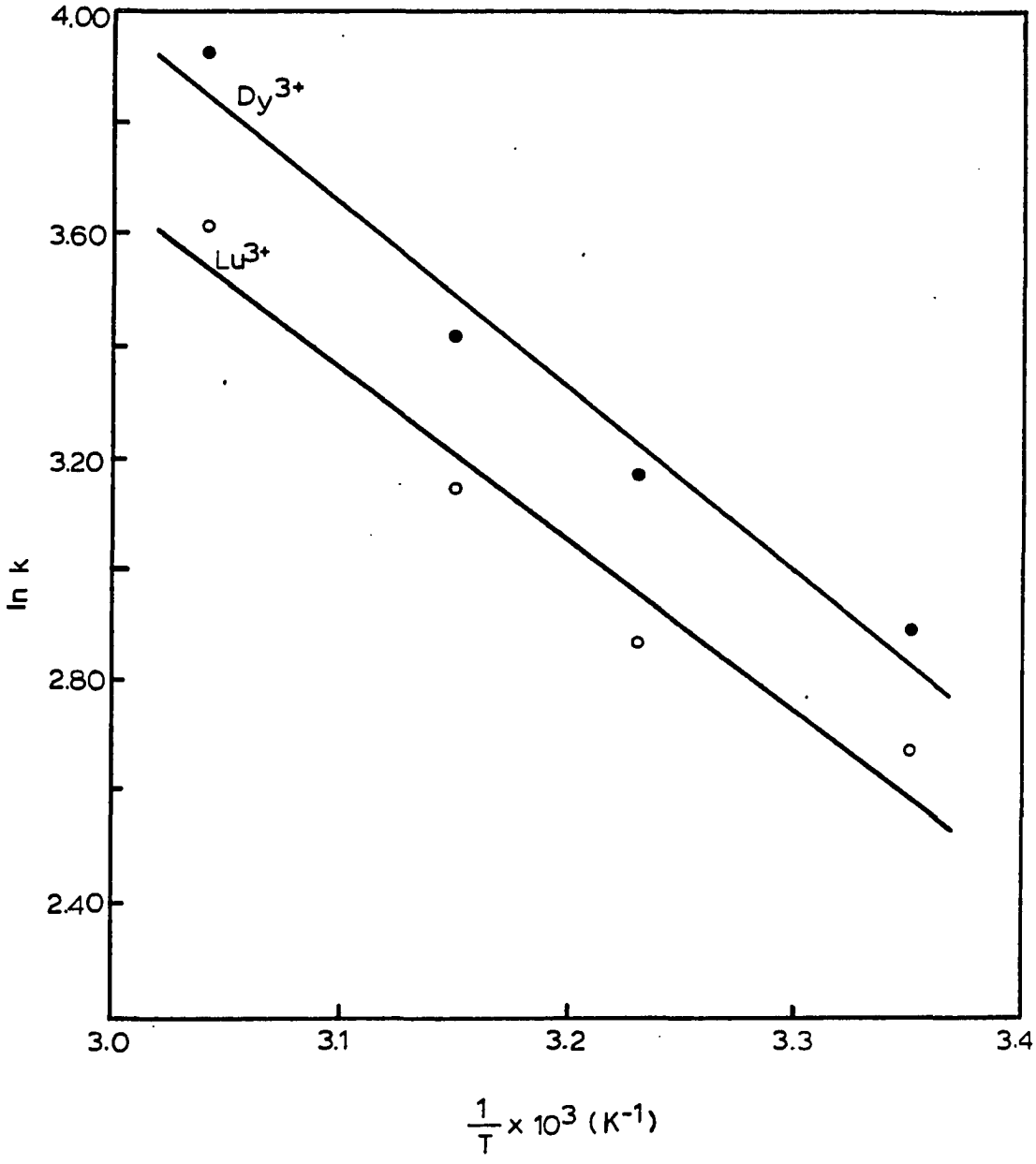

Figure 5. Plot of natural log of capacity factors of rare earth metal ions as a function of reciprocal system temperature. Eluent: 7.8 mM p-phenylenediamine dihydrochloride

Table I. Linear regression data for plot of ln k vs. reciprocal temperature for an eluent of perchloric acid. Concentration of eluent was 0.12 M for the divalent metal ions and 0.29 M for the rare earth cations

cation	slope = $\frac{-\Delta H^*}{R}$	correlation coefficient	ΔH^* (kcal/mole)
Mg^{2+}	185	0.851	-0.37
Zn^{2+}	216	0.870	-0.43
Mn^{2+}	-18	-0.115	0.04
Ca^{2+}	295	0.901	-0.59
Pb^{2+}	489	0.970	-0.97
Lu^{3+}	-572	-0.980	1.14
Dy^{3+}	-994	-0.978	1.98

Table II. Linear regression data for plot of ln k vs. reciprocal temperature for an eluent of p-phenylenediamine dihydrochloride. Concentration of eluent was 3.0 mM for the divalent metal ions and 7.8 mM for the rare earth cations

cation	slope = $\frac{-\Delta H^*}{R}$	correlation coefficient	ΔH^* (kcal/mole)
Mg^{2+}	-1945	-0.971	3.87
Mn^{2+}	-2161	-0.976	4.29
Zn^{2+}	-1870	-0.952	3.72
Ca^{2+}	-1924	-0.993	3.82
Sr^{2+}	-1575	-0.954	3.13
Ba^{2+}	-965	-0.794	1.92
Pb^{2+}	-1318	-0.855	2.62
Lu^{3+}	-3068	-0.975	6.10
Dy^{3+}	-3300	-0.985	6.56

tetrabutylammonium ion, produce structure in water and increase the heat capacity when they move outside of the resin" (29). Thus perhaps the slight curvature seen in the data points of some of the plots in Figures 2-5 is due to ΔH^* for the ion-exchange reaction being not quite constant over the temperature range examined. However, in order to calculate approximate ΔH^* values so that comparisons could be made, this curvature was ignored.

Initially, it was suspected that the decomposition of the amine eluent may have been the reason for increased retention times at elevated temperatures. The decomposition of the eluent would yield a lower effective eluent strength and consequently longer retention times. In fact, although p-phenylenediamine, when doubly protonated by the addition of perchloric acid, is a stable and satisfactory eluent at room temperature (30), it was found to decompose too quickly at higher temperatures to be useful. However, an eluent prepared from p-phenylenediamine dihydrochloride was found to be entirely stable and satisfactory at the higher temperatures. Its stability was evidenced by the facts that: UV spectra of the fresh eluent and the same eluent after being kept at 55°C all day were exactly superimposable, the eluent was not discolored at the end of the day (as was the p-phenylenediamine mixed with perchloric acid), and retention times obtained at room temperature with an eluent that had been used at 55°C and then cooled to room temperature were the same as those obtained at room temperature with a freshly made eluent.

The retention times of metal ions eluted with a p-phenylenediammonium eluent are considerably longer at higher

temperatures, as shown by Table III. In some cases the difference in retention times of two metal ions is larger at a higher temperature. This can be useful in obtaining better chromatographic separations.

Figures 6 and 7 show the advantage of operating an ion chromatographic system at elevated temperatures when reaction 5 is endothermic. With both an ethylenediamine/tartaric acid eluent (Figure 6) and a p-phenylenediamine dihydrochloride eluent (Figure 7), increased temperature led to longer retention times and improved separations. Figure 6 shows improved resolution of calcium(II) and manganese(II) peaks at 54°C as compared to 25°C. Figure 7 shows that the separation of calcium(II) and zinc(II) is much better at the higher temperature.

It has been pointed out that operation of liquid chromatography systems at high temperatures can lead to problems caused by radial and axial temperature gradients in the column (31-33). No such problems were encountered with the system used. This was attributed to the use of a narrow-bore column (2 mm i.d.) as well as to the preheating of the eluent in the constant temperature bath. These precautions agree with those suggested in the literature (31).

Note also that it is possible to use conductivity detection at elevated temperatures. Although the background conductivity of the eluent increases at the rate of about 2% per °C (34), this is easily electronically zeroed; in addition, the conductivity of the analyte ions increases at the same rate.

One final point worth mentioning is an additional advantage of running a liquid chromatographic system at high temperature. As

Table III. Adjusted retention times in minutes for some divalent and trivalent metal ions at two system temperatures. Flow rate was 0.6 mL/min. For the divalents the concentration of p-phenylenediamine dihydrochloride was 3.0 mM; for the trivalents 7.8 mM was used

cation	27.9°C	56.9°C	25.4°C	55.8°C
Mg^{2+}	0.97	1.73		
Mn^{2+}	1.23	2.55		
Zn^{2+}	1.36	2.63		
Ca^{2+}	2.03	3.59		
Sr^{2+}	2.71	4.66		
Ba^{2+}	4.92	7.82		
Pb^{2+}	6.26	10.63		
Lu^{3+}			11.72	30.28
Dy^{3+}			14.61	40.94

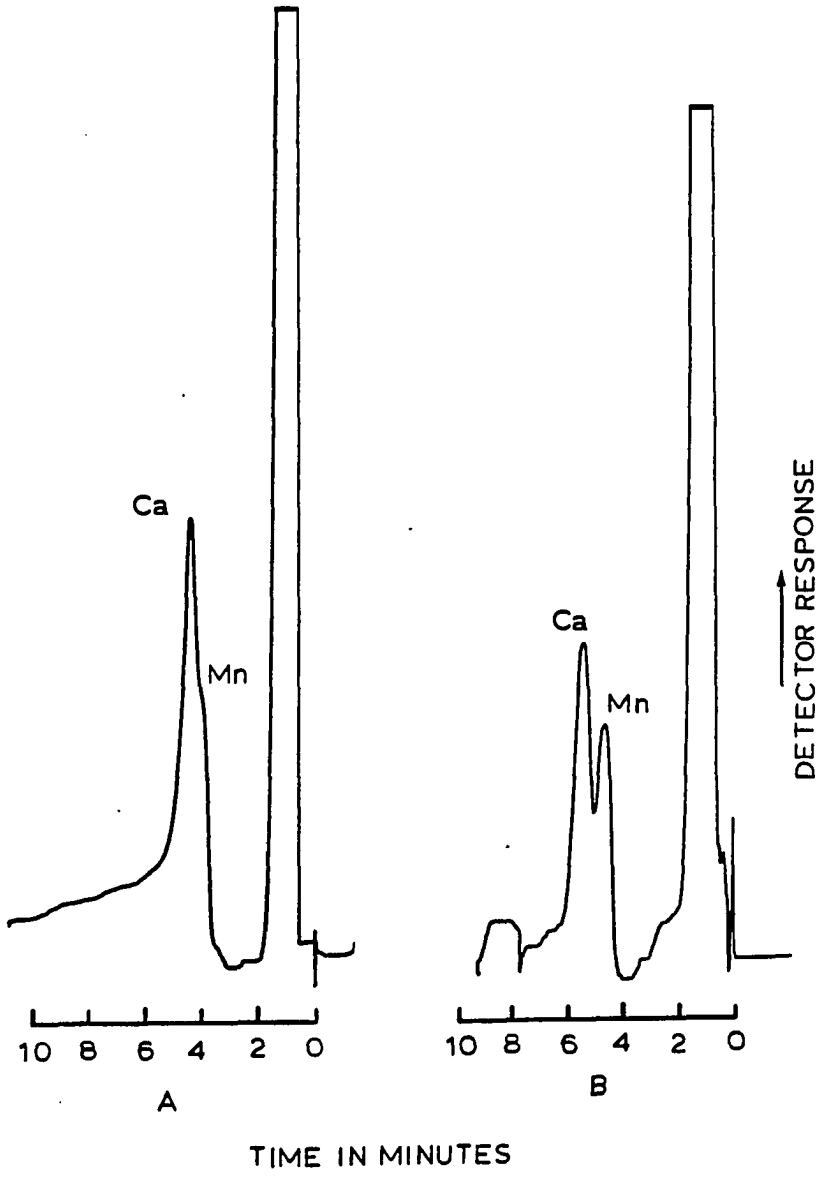

Figure 6. Separation of Mn^{2+} and Ca^{2+}. Eluent: 1.0 mM ethylenediamine, 2.0 mM tartaric acid, pH 4.5. A.) Temperature: 25°C, B.) Temperature: 54°C

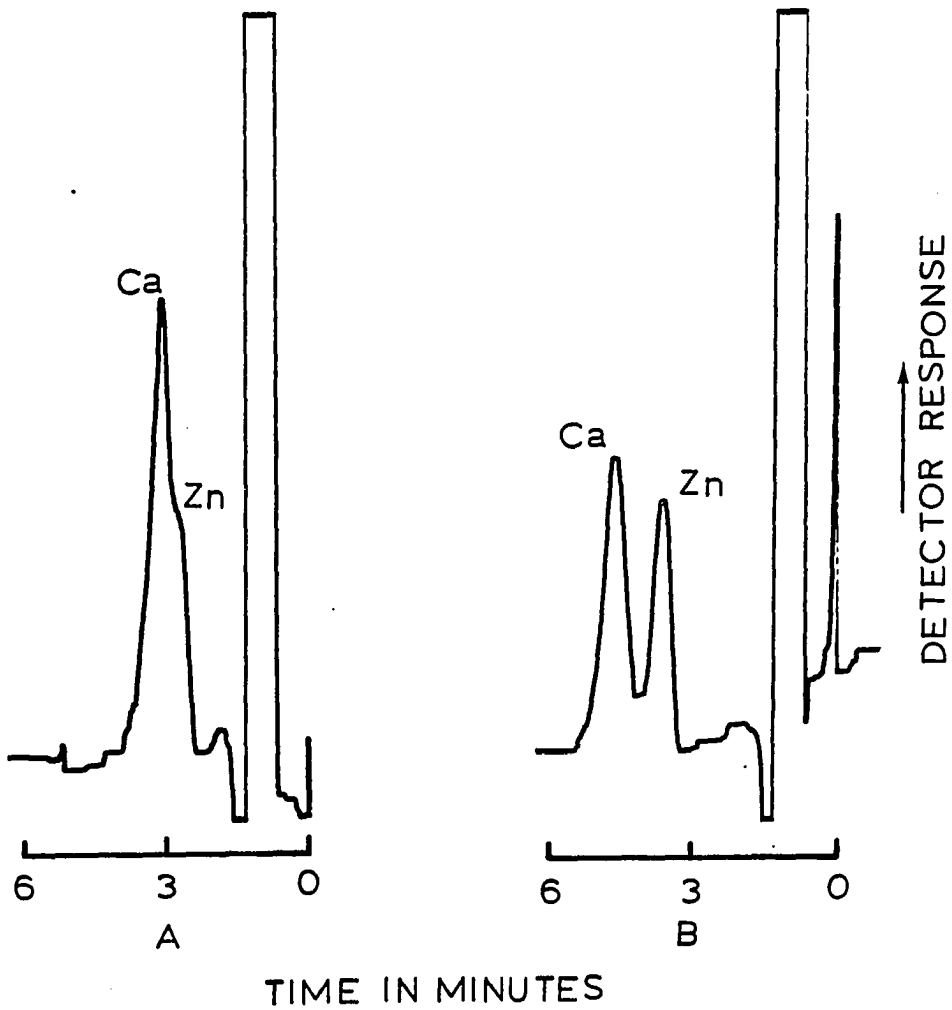

Figure 7. Separation of Zn^{2+} and Ca^{2+}. Eluent: 3.0 mM p-phenylenediamine dihydrochloride. A.) Temperature: 27°C, B.) Temperature: 57°C

temperature is increased, the viscosity of the mobile phase decreases (4), leading to a drop in column back pressure. For the system studied here, a drop from 1070 psi at 28°C to 730 psi at 55°C was observed. Consequently, it is possible to use higher flow rates at elevated temperatures without exceeding the pressure limitations of the column or fittings. An upper limit on the useful temperature range is the temperature at which degassing of the mobile phase at the column outlet becomes a problem.

FUTURE WORK

It was mentioned that both a nonzero heat capacity ΔC_p^* and a change in retention mechanism over the temperature range studied can lead to nonlinear Van't Hoff plots. The second reason would be expected to come into play if a silica column were used for separating ions, since free silanol groups, in addition to the ion-exchange sites, are present on the resin surface (35). It would be valuable to repeat this study using a silica-based cation-exchange column, noting the temperature trends and also the degree of curvature of the Van't Hoff plots. Since it has been shown that both the metal ion and the eluent ion affect the temperature dependence of ion-exchange, the next logical step is to see what influence the stationary phase has on plots of ln k vs. reciprocal temperature.

The effect of elevated temperature on complexing eluents in cation chromatography should be examined. For instance, an eluent of ethylenediamine/tartaric acid could be compared to an eluent of doubly protonated ethylenediamine. What effect does increased temperature have on the chromatography when complexation is important? Would the retention times as well as the width of the peaks be affected?

One problem with amine eluents is that they often require long equilibration times. Could this requirement be shortened by equilibrating at a higher temperature?

The particular metal involved in the ion-exchange reaction has been shown to determine whether the reaction is exothermic or endothermic. Does the charge on the metal ion have an effect as well?

This could be briefly examined by studying metals with more than one common valence state, such as Fe(II) vs. Fe(III).

One broad area to be examined is temperature programming in ion chromatography. This would not be possible with conductivity detection because of the change in background conductance which occurs as temperature is changed. But what about using postcolumn reaction detection? Temperature programming has already been shown to be viable with microbore chromatography. In fact, due to the need for low dead volumes, especially in the mixing chamber, when solvent gradients are used in microbore chromatography, temperature programming was actually shown to be simpler and cheaper than solvent programming (36). Additionally, thanks to the drop in column back pressure which occurs at elevated temperature, it should be possible to combine temperature programming with flow programming.

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SECTION IV. LIQUID CHROMATOGRAPHIC DETERMINATION OF WATER USING A SPECTROPHOTOMETRIC DETECTOR

INTRODUCTION

Karl Fischer Reagent

The quantitative determination of water in organic and inorganic materials is one of the most important and frequently encountered of all analytical problems. Analytical methods include chemical, gravimetric, thermal, separation, electrical, spectroscopic, spectrophotometric, and physical methods (1). By far the most widely used method for water determination is titration with the Karl Fischer Reagent (KFR).

The Karl Fischer Reagent method is a titration in which water is consumed. It is based on the fact that in the presence of sulfur dioxide, iodine reacts quantitatively with water:

$$I_2 + SO_2 + 2\ H_2O \longrightarrow 2\ HI + H_2SO_4 \qquad (1)$$

However, the reduction of iodine by sulfur dioxide reverses once the concentration of acidic products increases:

$$H_2SO_4 + 2\ HI \longrightarrow I_2 + H_2SO_3 + H_2O \qquad (2)$$

Therefore, to prevent this reversal, it is necessary to add a substance which will combine with the reaction products of equation 1; pyridine is particularly well suited, and also combines with the sulfur dioxide, lowering the latter's vapor pressure (2). Methanol is the solvent of choice since it readily dissolves the amine salts produced in the

consumption of water.

In the usual solvent of methanol, the reaction with water occurs in two steps:

$$I_2 \cdot pyr + SO_2 \cdot pyr + pyr + H_2O \longrightarrow 2pyrH^+I^- + SO_3 \cdot pyr \quad (3)$$

$$SO_3 \cdot pyr + CH_3OH \longrightarrow pyrH^+CH_3SO_4^- \quad (4)$$

The simplest use of the KFR for the determination of water is to titrate to the first appearance of iodine. With this visual endpoint, samples containing 125-200 mg of water can be titrated with an accuracy and precision of 0.1-0.2 percent (2). With a small amount of practice the endpoint is easily discernible, and the titration can be completed rapidly.

Electrometric methods have also been used. These include potentiometric, biamperometric, and coulometric titration methods. They were found to be more sensitive than the visual endpoint method, and also can be used with dark-colored solutions. The biamperometric (or "dead-stop") titration is easiest to use. The potentiometric method gives the highest precision, while coulometry provides the best sensitivity. Coulometric generation of iodine is advantageous because the complete KFR (with iodine in it) has a very limited shelf life; it deteriorates rapidly due to side reactions of iodine with other components in the mixture.

One problem with KFR titrations is that they are prone to interferences. Since the method is based on a simple oxidation-

reduction reaction, many species can quantitatively consume the reactants. Table I lists some of the substances which react stoichiometrically with the Karl Fischer reagent.

If an independent method for the quantification of the interference is available, then a correction can be applied to the titration for water determination. Also, for some interferences, the Karl Fischer titration procedure can be modified so that interferences are eliminated. An example of this is the determination of water in mercaptans. Since mercaptans are oxidized by the iodine of the KFR, a direct titration of the water in the presence of the mercaptan is not feasible. However, if an initial addition reaction of the mercaptan to an active unsaturated compound is carried out, the addition compound is inert towards the KFR and a titration may then be undertaken. However, this addition reaction itself is also prone to interferences.

Gas Chromatographic Methods

Gas chromatography (GC) has been used extensively for the determination of water in both organic and inorganic samples. It can be used either as a direct determination method or in conjunction with an initial chemical reaction, with one of the reaction products being sensitively detected.

One of the first ways to determine water with GC following a chemical reaction involved using a precolumn filled with calcium carbide. Water reacts with the carbide, forming acetylene. The acetylene is separated from other sample components using a column of dionyl phthalate on diatomaceous earth, then detected with thermal

Table I. Substances that react stoichiometrically with the Karl Fischer Reagent (2)

l-ascorbic acid	cupric salts
hydrazine salts	ferric salts
mercaptans	metal hydroxides
alkali bicarbonates	metal oxides
alkali carbonates	sodium arsenate
alkali sulfites	sodium arsenite
alkali pyrosulfties	sodium tetraborate
boric acid and oxides	sodium thiosulfate
	stannous chloride

conductivity. Detection limits of 10 ppm water can be obtained. A modification of this method used a column of 25% squalane on 80-100 mesh silanized Chromosorb W, on which the acetylene can be eluted prior to all hydrocarbons. A flame ionization detector was used, allowing detection of 1 ppm water (1). One problem with this method, however, is that alcohols interfere, since they too form acetylene (3).

Another GC method utilizes the acid catalyzed reaction between 2,2-dimethoxypropane (DMP) and water to form acetone and methanol. The amount of acetone formed is directly proportional to the amount of water in the sample. Excess DMP is added to a sample, along with methanesulfonic acid as a catalyst. Using a gas chromatograph with a column of 30% THEED (tetrahydroxyethylenediamine) on Chromosorb-W, excess DMP, acetone, and methanol are separated, with the acetone peak used to quantify the water in the sample. A thermal conductivity detector is used. The method was originally used to determine water in ethanol and p-dioxane (4).

Several applications of the DMP/GC method for water determination have been published. One of these uses a 30% THEED on Varaport 30 column and a flame ionization detector (FID) to determine water in nitroglycerin-nitrocellulose pastes (5). Another paper compares direct and indirect (DMP) GC methods for the determination of water in natural products (3). The author points out that when water is detected directly by GC, a thermal conductivity detector, which is fairly insensitive, must be used because a flame ionization detector is unsuitable for water detection. However, with the DMP/GC method, an FID can be easily used, making the method more sensitive than a direct

GC determination of water.

The acetic acid formed when water in a sample reacts with lead tetraacetate can also be detected with GC. The vaporized sample is carried in a hydrogen stream through a reactor packed with lead acetate held at a temperature of 70 to 80°C. The acetic acid produced by the reaction of the lead acetate and water is then separated in the GC and detected by flame ionization or thermal conductivity (1).

Initial attempts to determine water directly with gas chromatography had marginal success due to the poor column stationary phases available. Peaks were broad and unsymmetrical, and sensitivity, accuracy and precision were poor. However, direct separation of water using GC is now very reliable due to improved liquid and solid stationary phases. Some column packing materials commonly used include di-isodecyl phthalate, Carbowax 1500, Apiezon L grease, and polypropylene glycol, all on 60-80 mesh Chromosorb, as well as aromatic polymer porous beads, carbon molecular sieves, diatomite, fluorocarbon resins, and modified dextran. These have been used to determine water in both inorganic and organic substances (1).

Many of the determinations of water in inorganic samples deal with gaseous matrices: water in air, hydrogen, oxygen, chlorine. However, water has also been quantified in ammonium nitrate, lithium chlorate, and tributyl phosphate (1).

Inorganic hydrates have been analyzed by GC. Following removal of water of crystallization by extraction with 2-ethoxy or 2-methoxy-ethanol, analysis by GC gave the total water of hydration for sodium tartrate dihydrate, barium chloride dihydrate, and

$Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$. However, the method yielded only four moles of water for $CuSO_4 \cdot 5H_2O$ (1).

A vast number of gas chromatographic methods for the determination of water in organic matrices has been developed. Porous polymer column packings have a low affinity for compounds of high polarity, and are therefore good for water analysis (6). The columns have been used for analysis of water in matrices which include hydrocarbons, halides, alcohols, glycols, ketones, amines, nitriles, nitroparaffins, and acids. Water peak sharpness is considerably improved with the porous polymer beads. Hydrocarbon systems examined include heptane, benzene, naphtha, kerosene, C_{10}-C_{14} olefins, and C_{10-12} alicyclics (1). With a Porapak Q column (50-80 mesh) and a thermal conductivity detector, water was determined in hydroxylated hydrocarbons, paraffins, and halogenated hydrocarbons in the range of about 10 ppm to about 1% (6).

Water may be determined in polymers using GC. Volatile components from the molten polymer are passed through a column of 20% Carbowax 1500 on Haloport F and detected with a thermal conductivity detector. This method can also be used for the determination of water in carbon black, titanium dioxide, and aspirin (1).

A method has been developed for the direct determination of water on cotton (7). The fabric samples were extracted with acetone, which was injected into a GC equipped with a Poropak T column and a thermal conductivity detector. Although, as is common with the porous polymer packings, the water peak eluted early (six minutes), the large acetone peak was not fully eluted for thirteen minutes. This example shows that GC methods for water determination can be limited by other sample

components.

In addition to the examples given above, matrices in which water may be determined by GC, using a wide variety of column packings, include: aldehydes, dimethylsulfoxide, esters, natural gas, ethers, and toluene (1).

Liquid Chromatographic Methods

As in gas chromatography, both direct and indirect separation and detection of water have been accomplished using liquid chromatographic techniques. However, examples of these are extremely scarce compared to the abundance of GC methods available.

Fehrmann and Schnabel (8) used a styragel column, commonly used in gel permeation chromatography, to determine water in hydrocarbons. With an eluent of toluene, water was strongly retained. They used a differential refractometer to determine water down to 1.1×10^{-4} M. For a qualitative determination, the limit of detection was 10^{-5} M water.

Stevens et al. (9) used a cation-exchange column in the hydrogen-form, a methanol eluent containing a low concentration of sulfuric acid, and a conductivity detector to determine water in a wide variety of samples. The water lowered the conductivity of the eluent, so water peaks of decreased conductivity (negative peaks) were seen. It was possible to detect 30 ppm water in CCl_4, and no interference was seen for samples containing mercaptans. However, dimethylsulfoxide interfered, as did ketones, aldehydes, and hydroxide ion.

Determination of water after a chemical reaction to convert water

to an easily detectable species was shown to be possible by Björkqvist and Toivonen (10). The reaction of phenyl isocyanate and water produces N,N'-diphenylurea (DPU), which is easily separated on a reversed-phase column with an eluent of water/acetonitrile. Since the molar absorptivity of DPU is 3.6×10^4 at 256 nm, the DPU can be detected with a UV detector down to the nanogram level, which corresponds to water amounts below one nanogram. A major drawback of this method is the sample preparation time; total reaction time prior to the chromatography is 45 minutes, even though the chromatography itself takes only about six minutes.

In this section, two liquid chromatographic methods for the determination of water are presented. Both employ a high-capacity cation-exchange column, which has been shown to give efficient separations of water using a methanol eluent (9). The separation is probably based on an ion-exclusion mechanism. Ions cannot penetrate the resin beads and therefore pass rapidly through the column. Most molecular organic material has a strong affinity for the methanol eluent and also passes through the column rather quickly. However, water can enter the resin beads and therefore is delayed in its passage through the column. Ion-exclusion chromatography has been shown to be a fast and efficient way to separate and determine molecular compounds such as carboxylic acids, carbon dioxide (as carbonic acid) (11), and neutral substances such as alcohols and sugars (12). The determination of water by ion-exclusion should therefore be possible provided a suitable detection method is available.

The two methods for water determination presented here differ in

the detection schemes utilized. The first method makes use of solvatochromism, which is discussed in detail below. The second method employs a postcolumn reaction to convert water in the sample to a compound that is easily detected with a UV-absorbance detector.

DETECTION OF WATER IN ORGANIC MATRICES USING SOLVATOCHROMISM

Introduction

It is well known that absorption spectra are solvent-dependent. The polarity of the solvent can affect the intensity, shape and position of UV/visible absorbance bands. These spectral changes are caused by intermolecular solute-solvent interactions, such as ion-dipole, dipole-dipole, dipole-induced dipole, and hydrogen bonding. Interactions such as these alter the energy difference between the ground and excited states of the chromophore (the unsaturated group responsible for electronic absorption). Theories dealing with solvent effects on absorbance spectra assume that there is no difference in the chemical states of the solvated and isolated chromophores; the effects are treated as physical perturbations of the chromophore's molecular energy levels. Consequently, information about solute-solvent interactions can be obtained by examining solvent effects on absorbance spectra (13).

Solvatochromism is defined to be a change in position, intensity, or peak shape of a spectroscopic band due to a change in the polarity of the medium (14). Negative solvatochromism refers to a hypsochromic, or blue, shift (shift to a shorter wavelength) which occurs as solvent polarity is increased. Positive solvatochromism is a shift to a longer wavelength (bathochromic or red shift).

Both the type of chromophore and the particular electronic transition involved ($\sigma \Rightarrow \sigma^*$, $n \Rightarrow \sigma^*$, $\pi \Rightarrow \pi^*$, $n \Rightarrow \pi^*$, and charge-transfer) determine how large an effect the solvent will have on a

spectrum. Charge-transfer, $\pi \rightarrow \pi^*$, and $n \rightarrow \pi^*$ electronic transitions exhibit the largest solvatochromic effect (13). Figure 1 shows a representation of these energy levels (15).

For isolated chromophores, such as carbonyl or nitro groups, $n \rightarrow \pi^*$ transitions (R-bands) are forbidden and therefore have low molar absorptivities (usually less than 100). They exhibit a hypsochromic shift when solvent polarity is increased. Spectra of molecules with conjugated π-systems show $\pi \rightarrow \pi^*$ transitions (K-bands), which usually have high molar absorptivities (greater than 10^4). These absorptions appear in spectra of molecules such as butadiene or mesityl oxide, as well as in aromatic molecules with chromophoric substituents (styrene, benzaldehyde, acetophenone) (15).

The solvatochromism exhibited by $\pi \rightarrow \pi^*$ transitions is dependent on the chromophoric system. In diene and polyene systems, since the hydrocarbon double bonds are nonpolar, the K-bands are unaffected by solvent polarity. However, in enone systems (carbonyl in conjugation with an ethylenic group), the K-bands exhibit a bathochromic shift, often with an increase in intensity, as solvent polarity increases. This is probably due to a decrease in the π^* energy level caused by dipole-dipole and hydrogen bonding interactions (15).

As mentioned above, large solvatochromic effects are only observed in molecules with π-electrons for which the charge distributions (and therefore the dipole moments) of the ground and excited electronic states differ to a large extent. The intramolecularly ionic meropolymethine dyes exhibit vast solvatochromic effects as solvent polarity is changed. These dyes have an electron-donating group linked

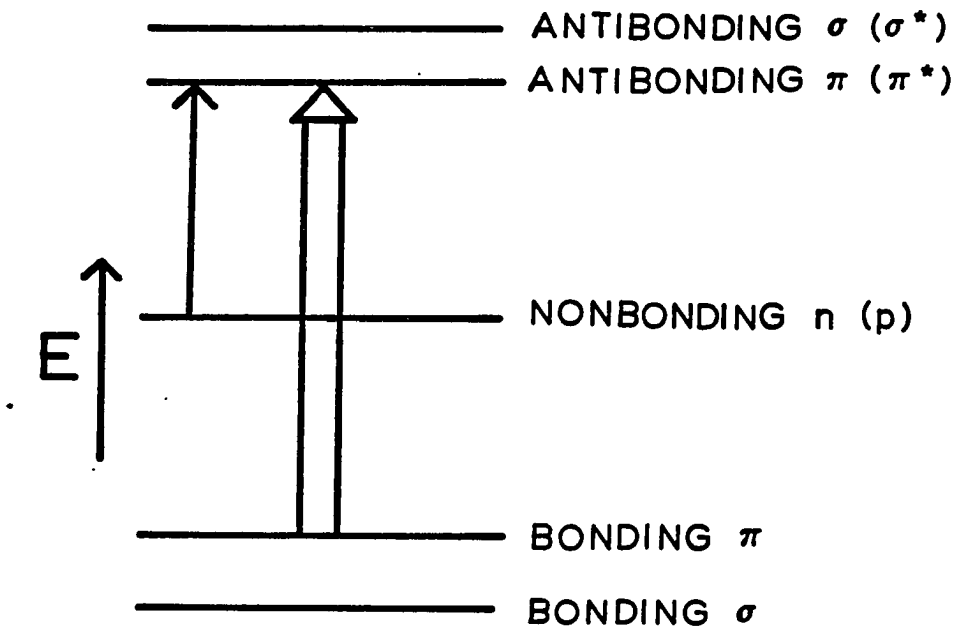


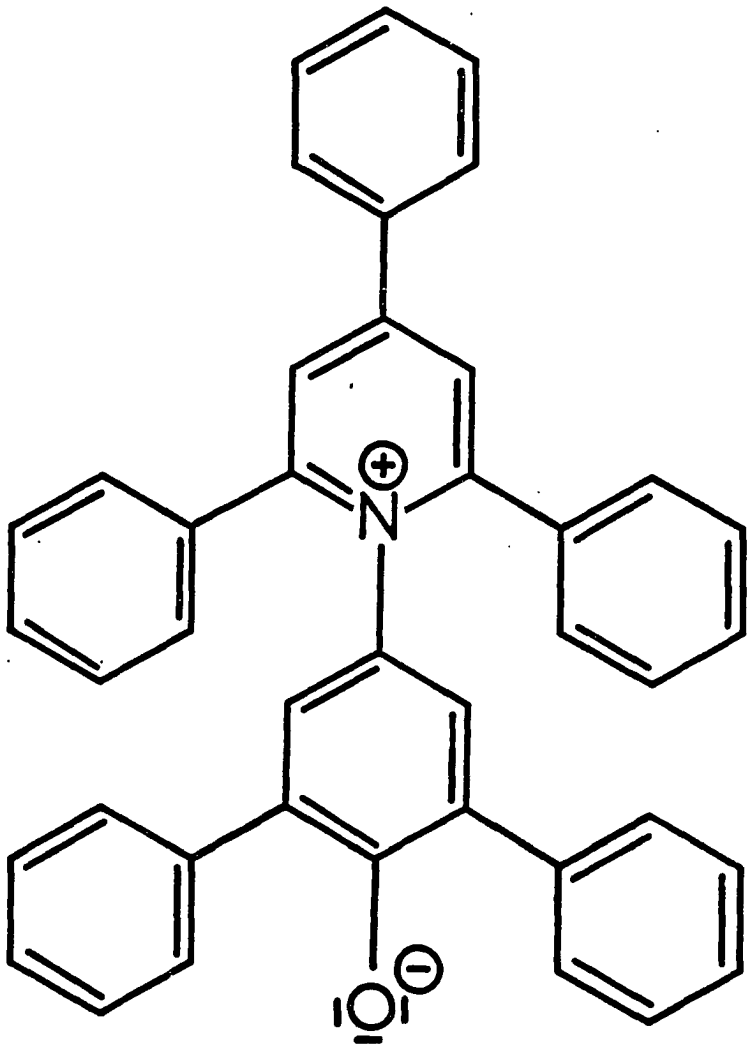
Figure 1. Energy transitions commonly exhibiting solvatochromism. Fine arrow is the n → π* transition, heavy arrow is π → π* transition (15)

by a conjugated system to an electron-accepting group. An intramolecular charge-transfer between these two groups produces an excited state with a dipole moment quite different from that of the ground state; the electronic transition between these states consequently is highly affected by the polarity of the solvent (13).

It is the relative magnitude of the dipole moments of the ground and excited states of molecules which determines the direction of the wavelength shift. The phenomenon is explained by the Franck-Condon principle. On the time scale of optical absorption, solvent molecules do not have enough time to rearrange themselves around a solute. This leads to preferential solvation of either the ground or the excited state, and consequently a shift in the energy of the electronic transition (16).

Several types of dyes exhibit substantial solvatochromism, either positive or negative. If the excited state is more dipolar than the ground state, positive solvatochromism is observed. If the ground state is more dipolar than the excited state, negative solvatochromism occurs.

An example of a dye exhibiting large spectral changes as solvent polarity is varied is pyridinium-N-phenolate betaine, also called 2,6-diphenyl-4-(2,4,6-triphenyl-N-pyridinio)phenolate and abbreviated ET-30; it is sold under the commercial name "Reichardt's dye" (Aldrich Chemical) (14). Its structure is shown in Figure 2. In going from the solvent diphenylether to water, the long wavelength band shifts 357 nm. The dye solution is red in methanol, violet in ethanol, blue in isoamylalcohol, green in acetone, and yellow in phenetole (13).



ET-30

Figure 2. Structure of pyridinium-N-phenolate betaine, also called Reichardt's dye or ET-30

Because of the large wavelength shifts observed, this molecule can be used as a sensitive measure of solvent polarity. $E_T(30)$ values are defined to be:

$$E_T(30) \text{ (kcal mole}^{-1}) = 28592 / \lambda_{max} \qquad (5)$$

where λ_{max} is the position of the charge transfer peak in nm (17). The constant in the above equation is the product of Avagadro's number, the speed of light, and Planck's constant. This $E_T(30)$ scale has been used to assign relative polarity values to over 200 solvents (17). Note that $E_T(30)$ refers to the polarity scale while ET-30 refers to the dye itself.

This dye can also be used to determine the composition of binary solvent mixtures, although the variation in the $E_T(30)$ polarity as a function of either mole fraction or volume percent is usually not linear (17). ET-30 is extremely sensitive to small amounts of water in aprotic solvents; λ_{max} shifted 46 nm when 2 % v/v was added to the acetonitrile solvent. The effect is not as large with protic solvents. However, the authors of this paper pointed out that with proper calibration curves, the measurement of λ_{max} for ET-30 dissolved in a given solvent could be used an an alternative to the Karl Fischer titration for the determination of water.

Kumoi et al. (18) used several betaine dyes as indicators for the determination of water in organic solvents. Their method employed measurement of absorbance at a fixed wavelength. They examined ET-30, 2,4,6-triphenyl-N-(hydroxyphenyl)-pyridinium betaine (P-1), 1-

hydroxyquinolizinium betaine (1-QZ), 2-methyl-5-hydroxyisoquinolinium betaine (2,5-IQ), 1-methyl-6-hydroxyquinolinium betaine (1,6-Q), and 1-methyl-8-hydroxyquinolinium betaine (1,8-Q) as potential dyes for the spectrophotometric determination of water.

Their criteria for selecting suitable dyes were: solubility in a wide variety of organic solvents, low degree of photosensitivity, and high sensitivity to low levels of water. The dye P-1 was not sufficiently soluble, while the dye 1-QZ decomposed upon exposure to light. Therefore, only ET-30, 1,8-Q, 1,6-Q, and 2,5-IQ were studied. The structures of the latter three dyes are given in Figure 3.

These four dyes were fairly sensitive for determining water in several solvents. Calibration plots of absorbance at a designated wavelength vs. water content (mg/mL) were not linear since the method is not based on Beer's law. ET-30 was found to be very sensitive; 0.01 mg of water per mL of dioxan, and 0.2 mg/mL in pyridine could be determined. The dye 1,6-Q also gave good results: 0.1 mg water per mL of pyridine, 0.06 mg/mL of acetonitrile. The authors noted that the dyes were much more sensitive to a change in water content in aprotic solvents than in protic solvents.

Because the method is based on solvatochromism, and is therefore not specific for water, potential interferences are any other polar components of the sample. The method is consequently limited to solvents of high purity or of known composition. However, this method could perhaps be applied to the determination of low levels of polar impurities (other than water) in organic matrices.

The work presented here describes the use of solvatochromism to

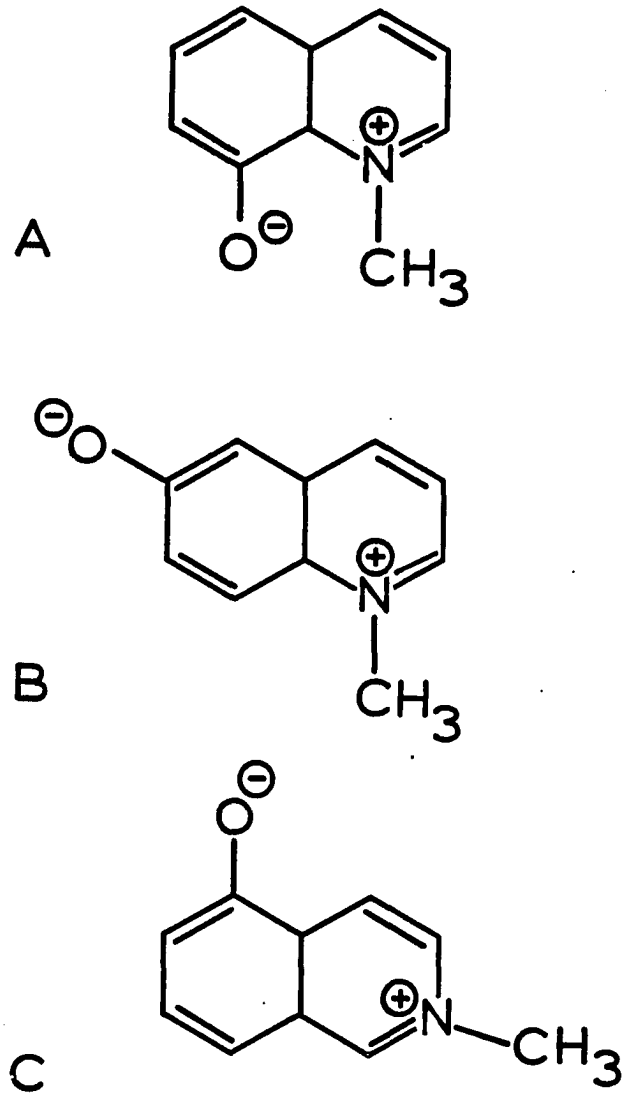


Figure 3. Structure of betaine dyes used for the spectrophotometric determination of water. A.) 1-methyl-8-hydroxyquinolinium betaine (1,8-Q), B.) 1-methyl-6-hydroxyquinolinium betaine (1,6-Q), C.) 2-methyl-5-hydroxyisoquinolinium betaine (2,5-IQ)

spectrophotometrically detect water in organic solvents after a liquid chromatographic separation. In one portion of the work solutions of chromophoric materials were used as eluents; when water eluted the spectrum of the chromophore was shifted, resulting in a change in absorbance at the detector wavelength. In the second portion, betaine dyes were added to the effluent in a postcolumn reaction mode. Again, the elution of water altered the spectrum of the betaine dye, producing either a positive or negative peak.

Experimental

Apparatus

The instrument used consisted of a model 302 Gilson single piston pump, a model 7125 Rheodyne injector equipped with a 20-microliter sample loop, a model LP-2 Scientific Systems, Inc. Lo-Pulse pulse dampener, one of several columns, described below, a Spectroflow 783 Kratos absorbance detector, and a Curken strip-chart recorder. When postcolumn reaction was used, a second model 302 Gilson single piston pump was used to deliver the reagent. The mixing chamber design was a stainless steel screen-tee described by Cassidy et al. (19). A Li-Chroma-Damp II coil type stainless steel pulse dampener was placed in line after the reagent pump but before the mixing tee.

The columns used were a 10 cm x 4.6 mm Teflon or stainless steel column packed with TSK-SCX gel cation-exchange resin (5 μ particle size, capacity 4.2 meq/g) in the H^+-form, a 10 cm x 8 mm glass column packed with TSK-SCX in the H^+-form, and a 25 cm x 2 mm stainless steel column packed with Bio-Rad Aminex Q-150S cation-exchange resin

(20-34 μ particle size, capacity 1.7 meq/g, 8% crosslinked) in the H^+-form. These were packed using upward slurry packing. However, a balanced density method was not used. Due to the large degree of shrinking and swelling that occurs in polystyrene divinylbenzene resins when a change in solvent occurs, it was necessary to pack the column in the solvent used in the mobile phase.

UV-visible spectra were obtained with a model DMS-100 Varian UV-Visible Spectrophotometer set at a bandwidth of 1.0 nm.

Eluent and sample solutions

Reagent grade benzyl alcohol, benzophenone, acetophenone, 2-sulfobenzoic acid hydrate, benzoic acid, trans-cinnamaldehyde, sodium benzoate, benzaldehyde, 1,3-diphenyl-1,3-propanedione, acetone, methanol, tetrahydrofuran, and HPLC grade acetonitrile were used without purification. The deionized water used to make up samples was obtained from a Barnstead NANOpure II System. No special measures were taken to dry solvents prior to use.

Postcolumn reagent solutions

All postcolumn reagent solutions were made in the solvent used as the mobile phase. The ET-30 (2,6-diphenyl-4-(2,4,6-triphenyl-N-pyridinio)phenolate) was purchased from Aldrich Chemical Co. under the name "Reichardt's Dye." The 1,8-Q (1-methyl-8-hydroxyquinoline betaine) was synthesized as described below. This synthesis is a combination and modification of several published syntheses (20-22), since the published syntheses either did not produce the desired

product or were unsafe (i.e., used benzene as solvent).

The quantity 0.5 g of 8-hydroxyquinoline was dissolved in 50 mL of acetone, then 15 mL methyl iodide was added. This was refluxed for one hour, then left standing at room temperature. After cooling in an ice bath for two hours, a tiny amount of precipitate formed. The flask was left in a refrigerator for 15 days, allowing bright yellow crystals to precipitate. These were isolated and washed with cold ether. Aqueous 2 M potassium carbonate was added, at which point a bright red solution formed. This solution was extracted with chloroform; the chloroform layer was purple. Sodium carbonate was added to the chloroform solution to dry it; it was allowed to stand for 30 minutes. The solution was then concentrated on a rotary evaporator. Upon addition of petroleum ether "C" (boiling point 86-100, n-heptane), orange crystals precipitated. These were isolated and dried in a vacuum desiccator, then in a vacuum oven at ~ 50°C. The resulting product was in the form of red-brown crystals. According to Saxena et al. (21), the orange crystals obtained after precipitation with ligroin should be converted to violet-red needles upon drying under vacuum.

Chromatographic conditions

Chromatography was done at a flow rate of between 1 and 2.3 mL/min. When postcolumn reaction was used, the reagent flow rate was the same as the eluent flow rate. In all cases the detector wavelength and concentration of solvatochromic species were optimized to give the best signal to noise ratio.

Results and Discussion

Solvatochromic eluents

The goal of this work was to use an ion-exclusion separation method in conjunction with a UV-visible absorbance detection scheme for the determination of water in organic matrices. The separation method is based on one recently developed by Stevens et al. (9), which uses a cation-exchange column and a methanol eluent for water determination. The idea of using solvatochromism to detect water occurred serendipitously.

Initially, an eluent of benzyl alcohol/methanol was used to elute water. This eluent was chosen because alcohols were shown to be useful for eluting water from a cation-exchange column (9), and benzyl alcohol absorbs in the UV; the methanol was added since water is not miscible with benzyl alcohol. It was hoped that when a water peak entered the detector set at a wavelength where benzyl alcohol absorbs, the water, which had exchanged with the benzyl alcohol in the eluent, would produce a peak of decreased absorbance. However, a peak of increased absorbance was seen. This makes sense when the spectra in Figure 4 are considered. With no water in the solution of benzyl alcohol/methanol, spectrum A is obtained. When 20% water is added, the spectrum shifts to longer wavelengths (B), causing an increase in absorbance at the detector wavelength of 300 nm. Therefore it is reasonable to observe peaks of increased absorbance when water elutes in an eluent of benzyl alcohol/methanol.

This method was briefly shown to be feasible for water determination. An eluent of 50/50 benzyl alcohol/methanol, a 10 cm x 8

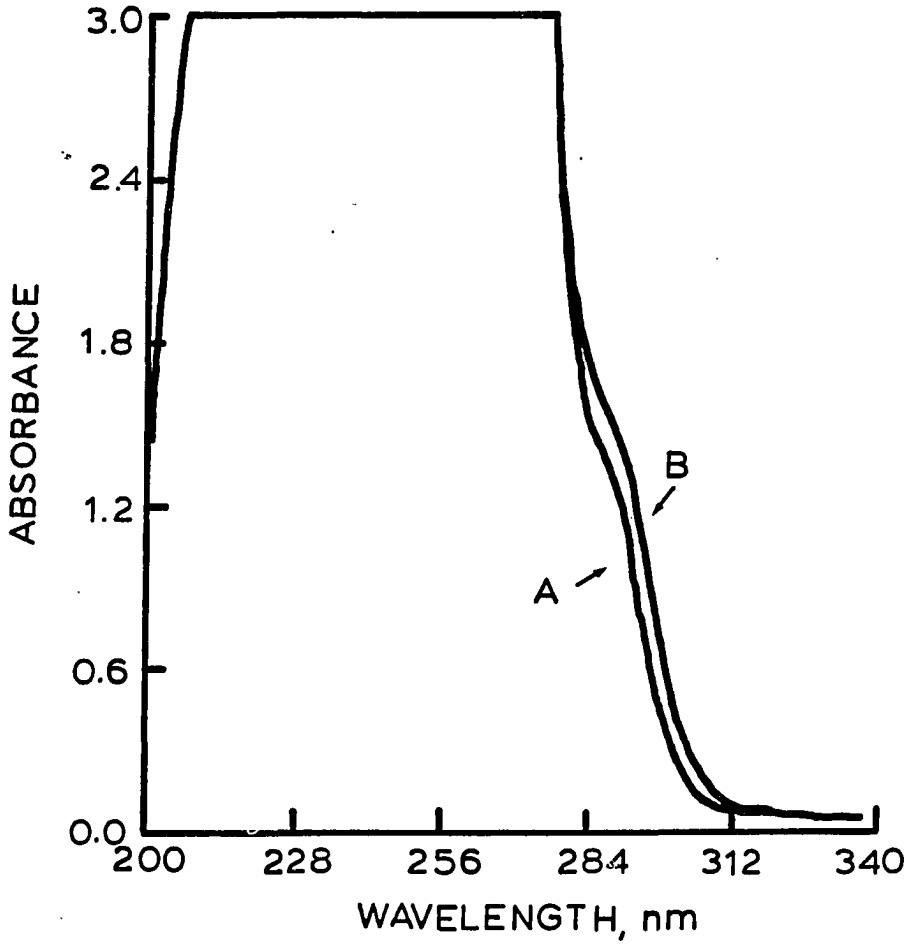


Figure 4. Spectra of benzyl alcohol. A.) A solution of 1:4 benzyl alcohol:methanol (v:v), B.) a solution of 1:3:1 benzyl alcohol:methanol:water (v:v:v)

mm TSK-SCX cation-exchange column in the H^+-form, and a detection wavelength of 300 nm were used. A calibration plot for standards of water in eluent was linear from 0.10 to 1.0% water (correlation coefficient for linear regression = 0.99988). However, no interference study was done, and the method is relatively insensitive (limit of detection ~ 0.10% H_2O).

Since enones have been shown to exhibit solvatochromism, a few available compounds with enone functional groups were examined to see if they were suitable for the determination of water. Compounds studied were benzoic acid, sodium benzoate, 2-sulfobenzoic acid hydrate, benzophenone, acetophenone, benzaldehyde, trans-cinnamaldehyde, and 1,3-diphenyl-1,3-propanedione. Their spectra in methanol and in 80% methanol/20% water show that all except 2-sulfobenzoic acid hydrate exhibit positive solvatochromism.

However, when these compounds were added to a methanol eluent for the chromatographic determination of water, results were only fair, with the notable exception of trans-cinnamaldehyde. An eluent of 1,3-diphenyl-1,3-propanedione was insensitive. Acetophenone (0.85 mM) was adequately sensitive at a wavelength of 267 nm (see Figure 5); the limit of detection was less than 0.02% water. However, the water peak appeared as a shoulder on the pseudo peak. Benzophenone (0.26 mM) gave a limit of detection of ~ 0.02% water at a wavelength of 272 nm (see Figure 6). Benzaldehyde, although examined spectrophotometrically, was not tested in a liquid chromatographic system. With an eluent of 0.54 mM benzoic acid and a detection wavelength of 240 nm (see Figure 7), a calibration plot from 0.20% to 2.0% water in eluent was linear. These

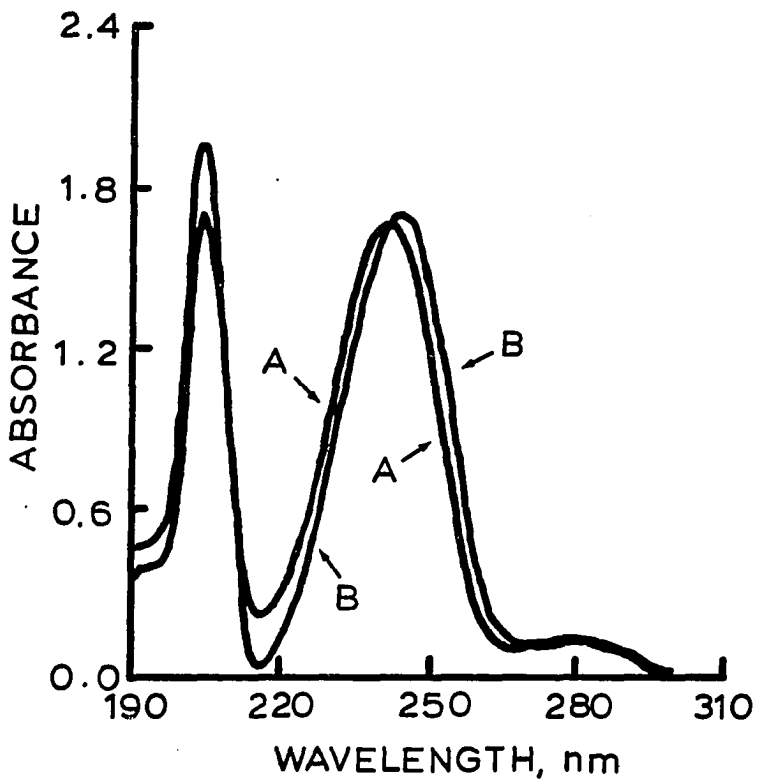


Figure 5. Spectra of acetophenone. A.) Acetophenone in methanol, B.) the same concentration of acetophenone in 80% methanol, 20% water

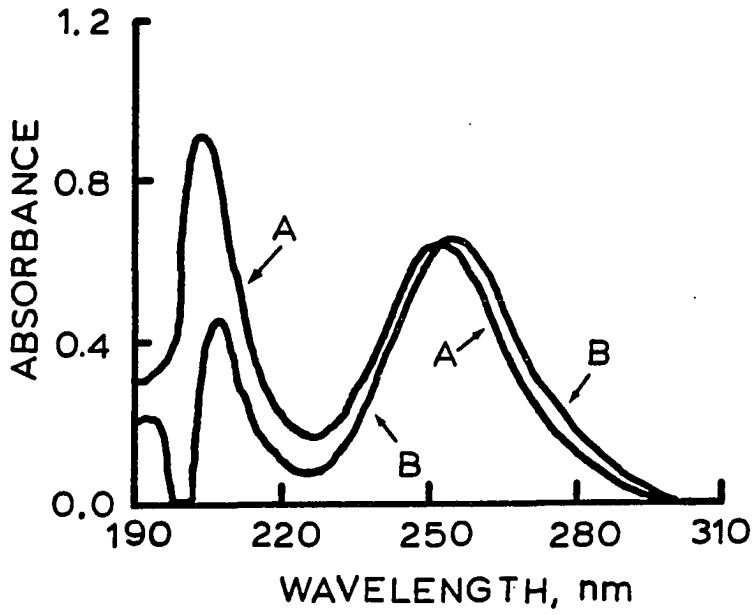


Figure 6. Spectra of benzophenone. A.) Benzophenone in methanol, B.) the same concentration of benzophenone in 80% methanol, 20% water

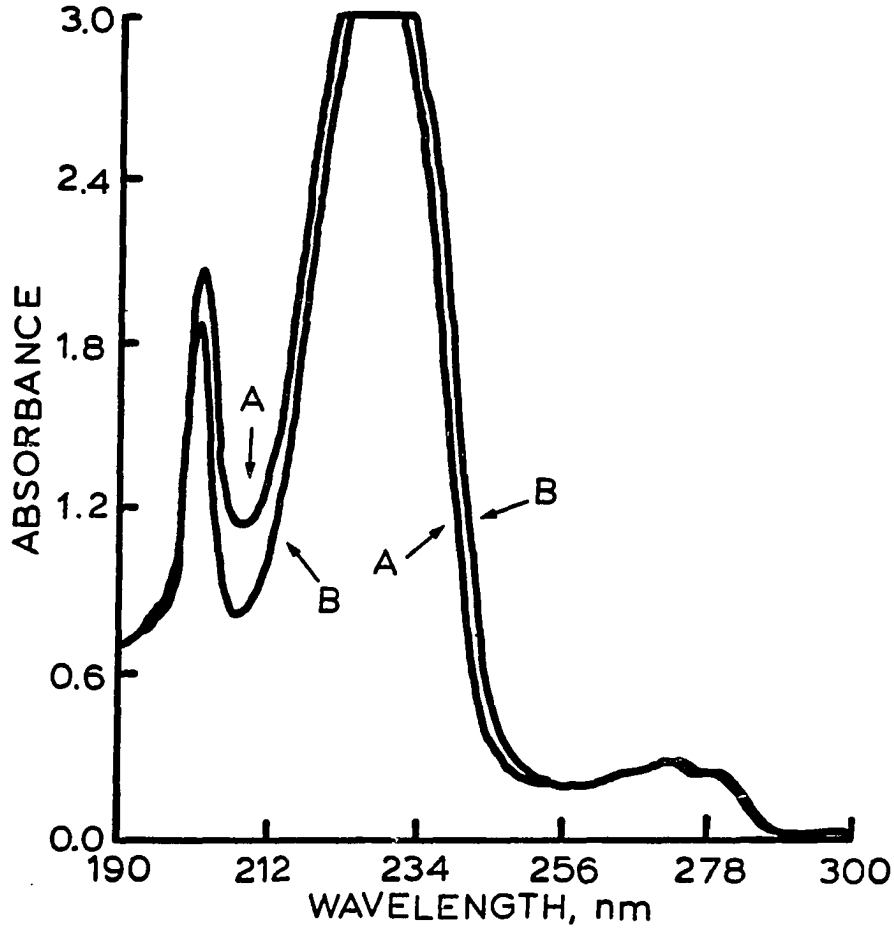


Figure 7. Spectra of benzoic acid. A.) Benzoic acid in methanol, B.) the same concentration of benzoic acid in 80% methanol, 20% water

chromatographic results were obtained on a 2 mm x 25 cm Aminex Q-150S column in the H^+-form. The small size (low i.d.) of this column contributed to the poor resolution of the water peak in some cases.

It was noted that the direction of the water peaks corresponded to the solvatochromism exhibited in the spectra. For example, with benzoic acid, at 207 nm the peaks were negative (decreased absorbance), while at 245 nm they were positive (increased absorbance). With the benzophenone, at a wavelength of 272 nm, water peaks were positive.

For some reason, the trans-cinnamaldehyde yielded much more sensitive determinations of water than the other compounds tried, even though its spectra with and without water are similar to those of the other compounds tested (see Figure 8). The reason for this is the subject of the last portion of this dissertation, "The Use of a Postcolumn Reaction for the Spectrophotometric Detection of Water in Organic Matrices."

Solvatochromic dyes

Since meropolymethine dyes are known to exhibit such large solvatochromic shifts, two of these were studied as possible postcolumn reagents for water determination: ET-30 and 1,8-Q (1-methyl-8-hydroxyquinolinium betaine). Their structures are shown in Figures 2 and 3, respectively. They were studied as postcolumn reagents instead of eluent additives because they irreversibly sorb to polystyrene based chromatographic columns.

These dyes are most sensitive as indicators for water in aprotic solvents (18). Therefore, tetrahydrofuran (THF) and acetonitrile were

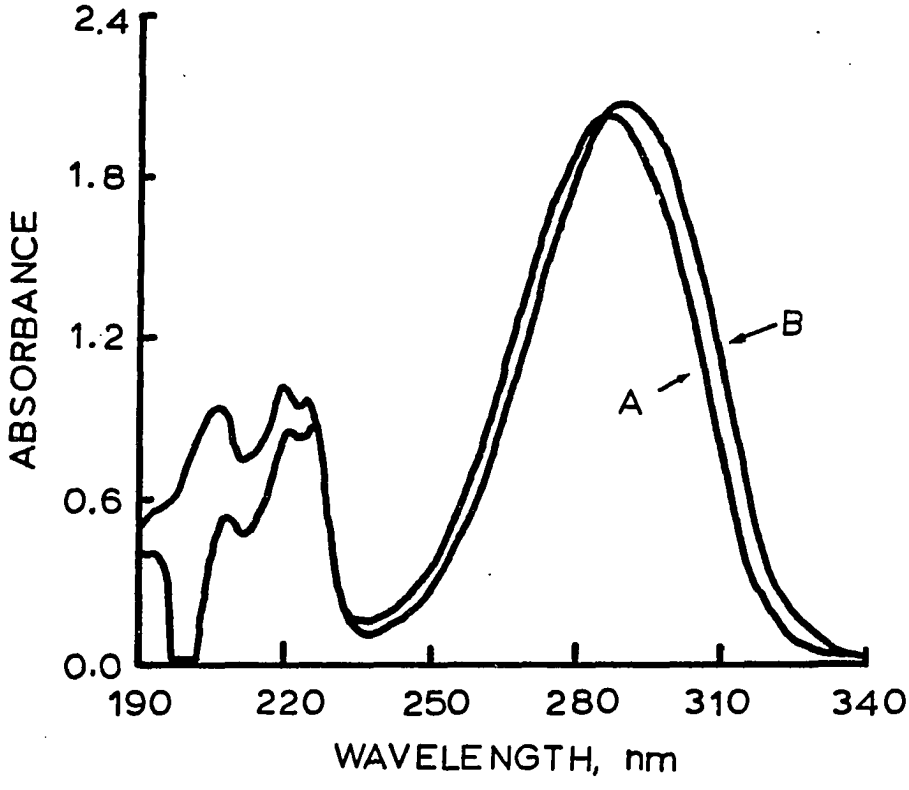


Figure 8. Spectra of trans-cinnamaldehyde. A.) Trans-cinnamaldehyde in methanol, B.) the same concentration of trans-cinnamaldehyde in 80% methanol, 20% water

initially chosen as eluents. Figures 9 and 10 show that with ET-30 in either THF or acetonitrile, although negative solvatochromism occurs, a wavelength can be chosen where the elution of water should yield a peak of increased absorbance. This is important since due to the problem of baseline noise in PCR, which is directly proportional to background absorbance of the reagent, it is not possible to sensitively measure decreased absorbance peaks with a PCR system. These figures show the large shifts in the spectra of ET-30 that occur with the addition of very small amounts of water.

With an 8mm x 10 cm TSK-SCX column in the H^+-form, an eluent of THF, and a postcolumn reagent of ET-30 in THF, a water peak was seen in the positive direction at a wavelength of 550 nm, but 2% water was the limit of detection (LOD). The retention time for water with a flow rate of 1.1 mL/minute was 5.1 minutes.

When acetonitrile was tried as eluent and solvent for the PCR ET-30, the retention time for water increased to about 30 minutes with a flow rate of 1.2 mL/min. Due to the large amount of baseline noise, the LOD could not be decreased below 2% water (see Figure 11). However, because of the long retention time for water, it was possible to chromatographically resolve water from low molecular weight alcohols such as methanol, ethanol, isopropanol, and n-butanol. Unfortunately, the LOD for the latter three samples was ~ 16%, while for methanol the LOD was ~ 8%.

Figure 12 shows the spectra of 1,8-Q in acetonitrile with and without water added. Using a 4.6 mm x 10 cm TSK-SCX column (smaller diameter), and a flow rate of ~ 2 mL/minute, the retention time of

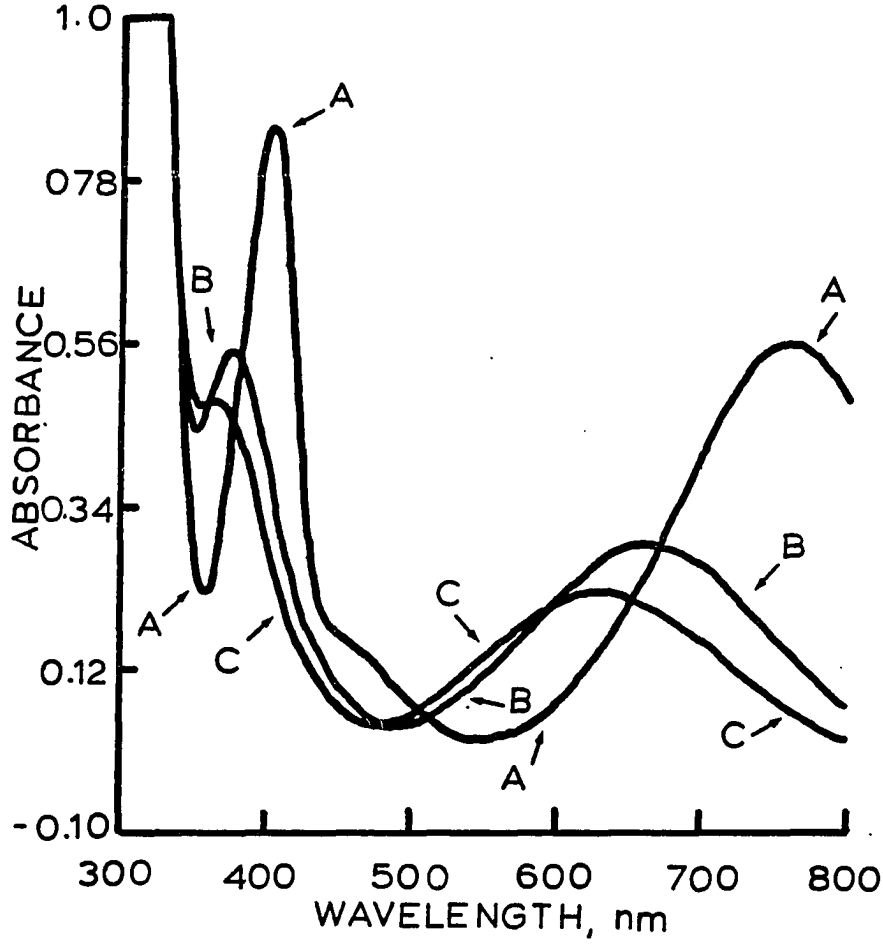


Figure 9. Spectra of ET-30. A.) ET-30 in THF, B.) ET-30 in 97% THF, 3% water, C.) ET-30 in ~ 94% THF, ~ 6% water

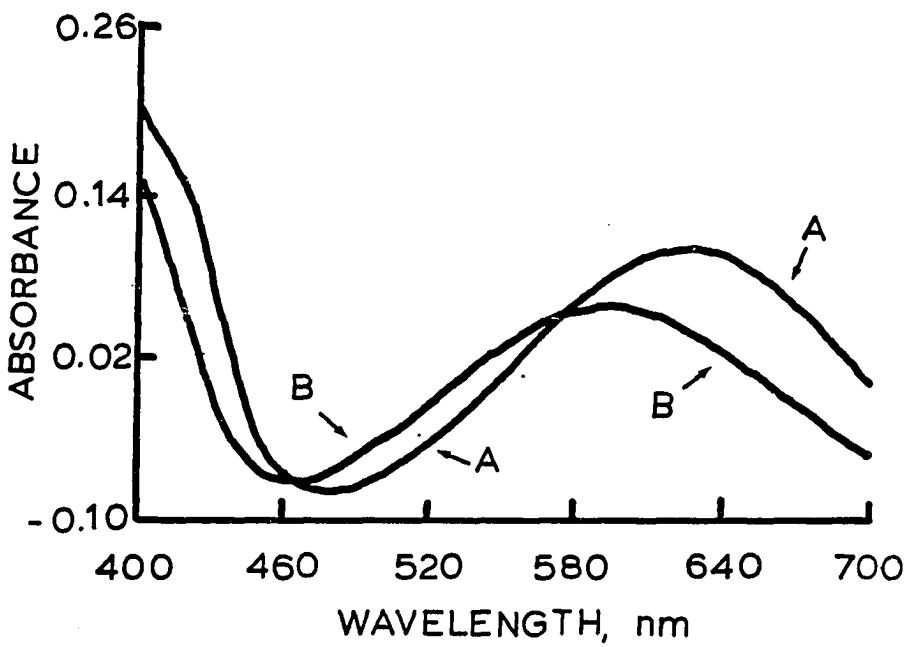


Figure 10. Spectra of ET-30. A.) ET-30 in acetonitrile, B.) ET-30 in 98.9% acetonitrile, 1.1% water

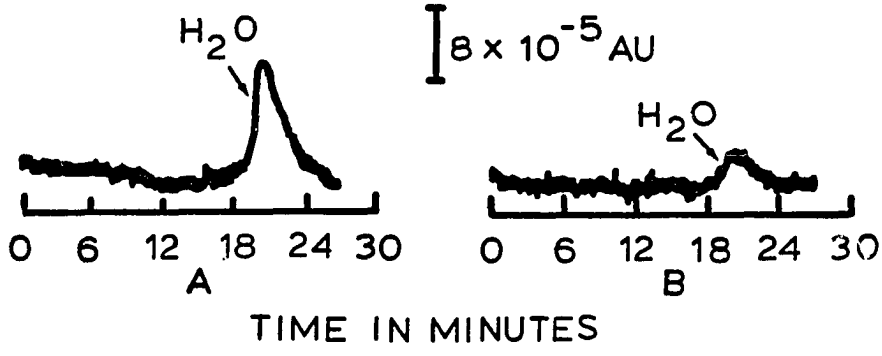


Figure 11. Chromatograms of samples containing A.) 12% water and B.) 2% water. Column was 8 mm x 10 cm TSK-SCX, eluent was acetonitrile, PCR was ET-30 in acetonitrile (0.013 g/L), eluent and PCR stream flow rates were 2.25 mL/minute, detection wavelength was 490 nm

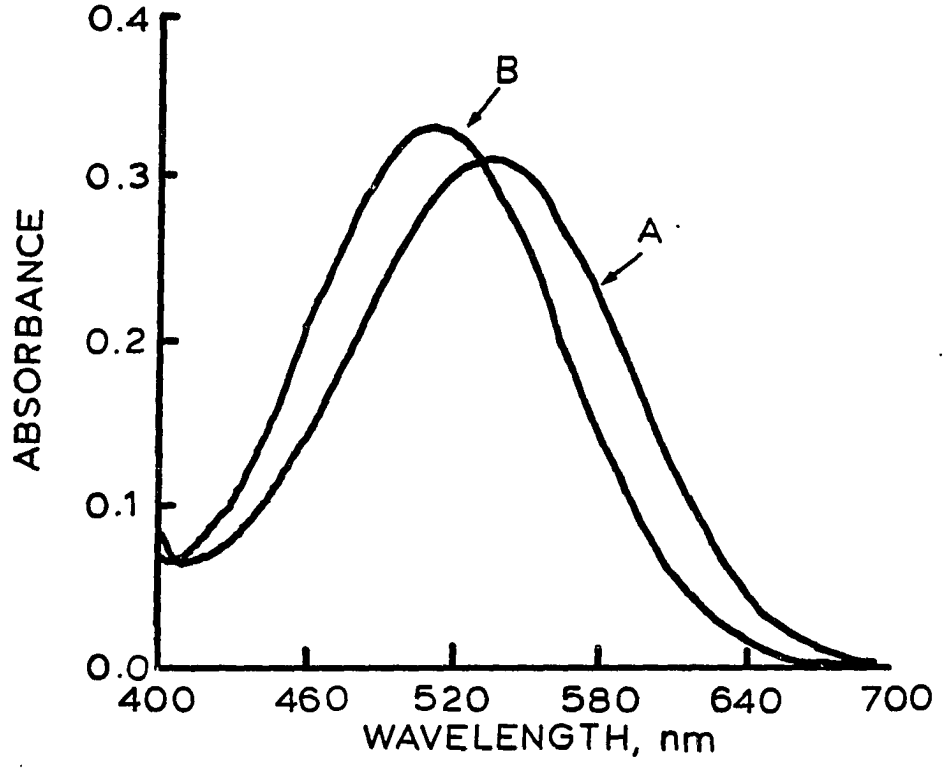


Figure 12. Spectra of 1,8-Q. A.) 1,8-Q in acetonitrile, B.) 1,8-Q in 98.6% acetonitrile, 1.4% water

water was decreased to about six minutes. The direction of the water peak corresponds to the spectral shift shown in Figure 12. A positive peak was seen at 470-505 nm, while a negative peak was seen at 580 and 595 nm. The LOD with this system also was 2% water.

Conclusions

Although the methods presented here are not very sensitive, it has been shown that solvatochromism can be used for the detection of water in a liquid chromatographic determination. These results show that it should be possible to similarly determine other polar species, such as alcohols.

THE USE OF A POSTCOLUMN REACTION FOR THE SPECTROPHOTOMETRIC DETECTION OF WATER IN ORGANIC MATRICES

Introduction

Water has been determined by gas and liquid chromatography after a reaction to convert the water to an easily detected species. However, these GC and LC methods require the addition of reagents to the water samples prior to injection. As mentioned in the section of this dissertation dealing with postcolumn reaction systems, precolumn derivatization is generally less desirable than on-line postcolumn reaction. Especially when dealing with samples containing low amounts of water, due to the relative ease with which most samples absorb moisture from the environment, it is highly desirable to keep sample handling to a minimum.

In U.S. Patent 3,607,782, Rosen (23) presents a method to determine water based on the shift in an equilibrium caused by water. A cobalt-substituted sulfonated strong cation-exchanger was used as an indication of moisture content in refrigeration systems using halogenated refrigerants. The indicator is deep blue violet when the ambient humidity is low (0-1%), changing to various shades of violet/pink as humidity increases, and finally to salmon pink when humidity reaches 5%. This is an example of a system in which the shift in an equilibrium is proportional to the amount of water present, and that shift is readily detected (in this case by a visible color change).

The liquid chromatographic detection method presented here also involves the shift of an equilibrium caused by water. The separation is again carried out on a high-capacity cation-exchange resin, where the mechanism is believed to be ion-exclusion. The eluent is a mixture of methanol and acetonitrile containing a low concentration of cinnamaldehyde. After passing through the separation column, a short catalytic column causes the cinnamaldehyde and methanol to react, apparently producing water plus an acetal that has a much lower absorbance at an appropriate UV wavelength than the free cinnamaldehyde. The water peak appears to partially reverse this reaction, thus giving an increase in absorbance for detection of the water peak. The new method is fast and sensitive; it has few interferences and a large linear dynamic range.

A second method for water determination is also presented. This uses one column as both the separation and catalytic column, and does not require the addition of acetonitrile to the eluent. However, it is more prone to interferences than the two-column method.

Experimental

Apparatus

The instrument used consisted of a model 302 Gilson single piston pump, a model 7125 Rheodyne injector equipped with either a 20-microliter or a 100-microliter sample loop, a model LP-21 Scientific Systems, Inc. Lo-Pulse pulse-dampener, either a glass 8 mm x 10 cm or a stainless steel 4.6 mm x 5 cm column packed with Bio-Rad Aminex Q-150S in the Li^+-form (separation column), a 2 mm x 10 cm stainless steel

column packed with Bio-Rad Aminex Q-150S in the H^+-form (catalyst column), a Spectroflow 783 Kratos absorbance detector, and a Curken strip-chart recorder. The one-column method used either a stainless steel 4.6 mm x 10 cm or a glass 8 mm x 10 cm column packed with Aminex Q-150S in the H^+-form. The columns were packed using upward slurry packing. However, a balanced density method was not used. Due to the large degree of shrinking and swelling that occurs in polystyrene-divinylbenzene resins when a change in solvent occurs, it was necessary to pack the column in the solvent used in the mobile phase.

Eluent and sample solutions

Trans-cinnamaldehyde, 99% (Aldrich Chemical Co.), was used without purification. Reagent grade methanol (Mallinckrodt) and HPLC grade acetonitrile (Fischer Scientific) were dried by storing over activated 3 angstrom molecular sieves (Aldrich) for at least a week. The 3-mercaptopropionic acid (Aldrich) was 99+% pure. All other samples were reagent grade and used without purification.

For maximum sensitivity and reproducibility, the eluent and all samples were prepared in a nitrogen-filled glove bag. Once prepared, the eluent was protected from atmospheric moisture by bubbling nitrogen through the solution and out through a drying tube filled with anhydrous calcium sulfate (Drierite). All sample solutions were placed in vials equipped with Mininert valves (Supelco) prior to removal from the glove bag. The valve and septum of the Mininert caps allowed removal of an aliquot without exposing the remainder of the sample to atmospheric moisture.

Titrations

Karl Fischer Reagent (titer 2.8 mg/mL) was purchased from Aldrich and standardized. The burette was flushed with nitrogen prior to being filled and then blanketed with nitrogen during the titration. A large (50 mL) burette was used so that a standard and three samples could be titrated without refilling the burette. Samples were titrated in volumetric flasks blanketed with nitrogen to minimize exposure to atmospheric moisture. A visual endpoint was used.

Chromatographic conditions

For the two-column method, chromatography was done at a flow rate of either 1 mL/min (the long separation column) or 0.8 mL/min (the short separation column). A detector wavelength of 310 nm was used. The eluent was 0.79 mM trans-cinnamaldehyde in 60/40 acetonitrile/methanol. This concentration of trans-cinnamaldehyde gave the best signal to noise ratio for water determination.

When only one column in the H^+-form was used, the eluent was 0.32 mM trans-cinnamaldehyde in methanol. With the 4.6 mm diameter column a flow rate of 1 mL/min was used, while with the 8 mm diameter column a flow rate of 1.2 mL/min was used. Detection was again at 310 nm.

Results and Discussion

Since a method for separating water from organic sample constituents, using a cation-exchange column and a methanol eluent, has already been devised (9), efforts were concentrated on developing a

useful detection scheme. However, it was found that when a cation-exchange column in the Li^+-form was used as a separator column, better resolution of the water peak from early-eluting material was obtained using a 60:40 mixture of acetonitrile and methanol as the eluent. Aminex Q-150S ion-exchange resin was chosen because it has a crosslinking (8%) high enough to prevent gradual dissolution by acetonitrile in the eluent.

Detection system

A stable and sensitive detection system is critical to the success of any chromatographic method for the separation and determination of water. The method presented here is based on the shift in a chemical equilibrium caused by low concentrations of water. This shift in equilibrium causes an increase in absorbance, measured spectrophotometrically at an appropriate wavelength, that is proportional to the amount of water.

A good detection system is set up by adding a low concentration (0.8 mM) of cinnamaldehyde to the eluent. Cinnamaldehyde has the potential to react with methanol in the eluent to form an acetal plus water. However, spectral evidence indicates that reaction only occurs when a catalyst is present, such as when the eluent passes through a cation-exchange column in the H^+-form:

$$C_6H_5CH{=}CHCHO + 2\ CH_3OH \xrightleftharpoons{H^+} C_6H_5CH{=}CHCH(OCH_3)_2 + H_2O \qquad (6)$$

The cinnamaldehyde absorbs strongly at the detector wavelength of 310 nm, while the acetal of cinnamaldehyde has little absorbance at this wavelength (see Figure 13). When a solution of cinnamaldehyde in methanol (with no catalyst present) was allowed to stand for two days, the absorbance at 310 nm remained unchanged. Although the spectrophotometric data presented in Figure 13 appear to indicate a wavelength lower than 310 nm should be used for maximum sensitivity, the optimal signal to noise ratio was obtained at 310 nm, where the background absorbance is quite low.

Water in a sample injected into the chromatographic column forces the equilibrium in the reverse direction when an acid catalyst is present:

$$H_2O + C_6H_5CH{=}CHCH(OCH_3)_2 \xrightleftharpoons{H^+} C_6H_5CH{=}CHCHO + 2\ CH_3OH \qquad (7)$$

This was spectrophotometrically confirmed (see Figure 14). Usually the amount of water from the sample will be substantially greater than the background water. The extent of the shift in equilibrium is measured by the increased absorbance at 310 nm, which is proportional to the amount of water in the sample.

Separation - detection systems

Initially, only one column was used, filled with Aminex Q-150S in the H^+-form. As mentioned in the portion of this dissertation titled "Detection of Water in Organic Matrices Using Solvatochromism," the

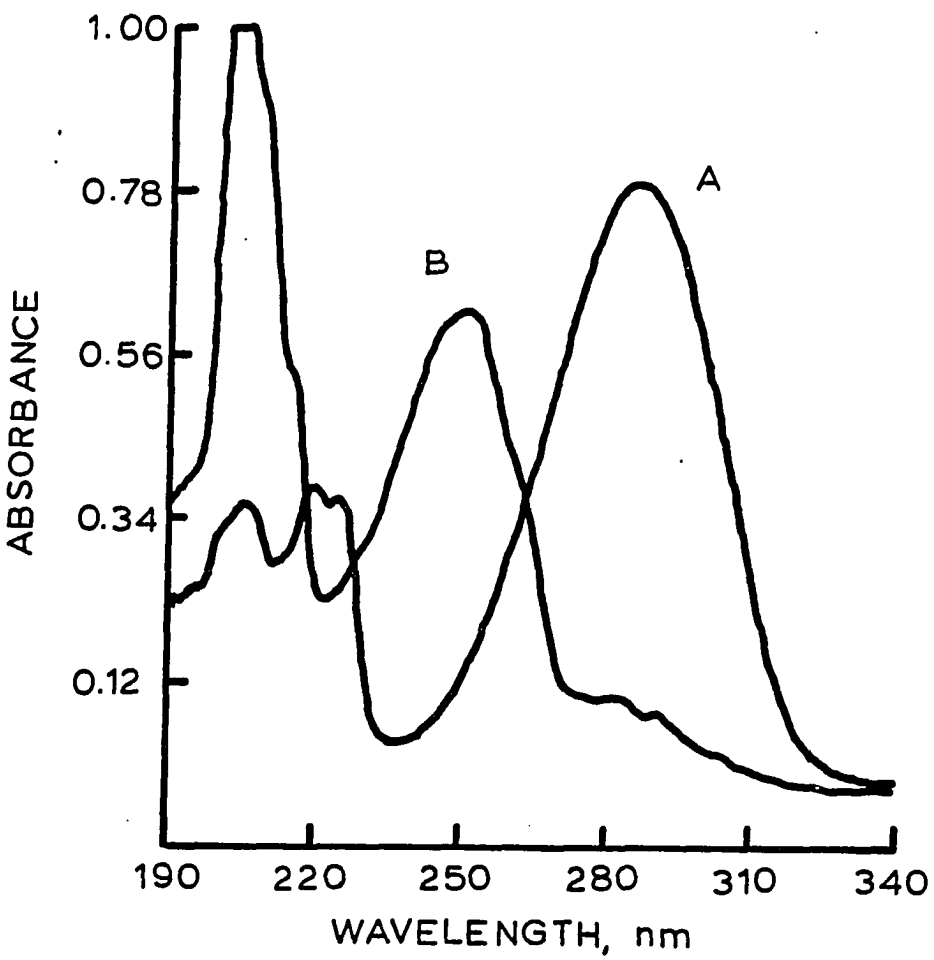


Figure 13. Spectra of 0.0318 mM trans-cinnamaldehyde in methanol. A.) Immediately after solution prepared, B.) after solution was shaken with Aminex Q-150S in the H^+-form

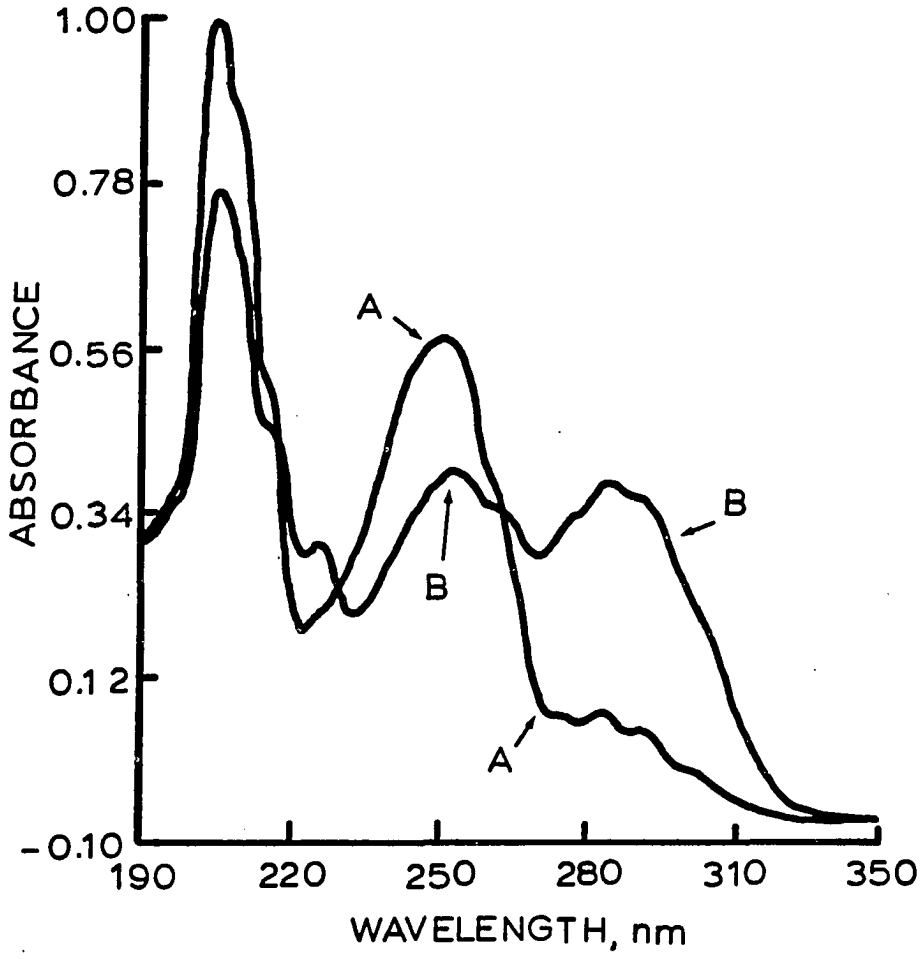


Figure 14. Spectra of 0.0318 mM trans-cinnamaldehyde in methanol. A.) After solution was shaken with Aminex Q-150S in the H^+-form, B.) after a small amount of water was added to the above solution followed by additional shaking with Aminex Q-150S in the H^+-form

detection mechanism was believed to be a solvatochromic shift of the spectrum of trans-cinnamaldehyde, caused by the eluting water. However, the method was far more sensitive than other methods using similar enones as eluent additives. The realization that the sensitivity was due to a chemical reaction occurring on the separation column led to the development of the method presented here.

When water entered the separation column in the H^+-form, it was converted to cinnamaldehyde in the first part of the column by shifting the equilibrium in equation 7 to the right. The species actually separated on the column was the cinnamaldehyde produced. This was confirmed by using an eluent of only methanol and injecting cinnamaldehyde. Two peaks were obtained, one large peak eluting very early in the chromatogram, and one small peak eluting at the retention time seen for water when cinnamaldehyde was in the eluent. The detector wavelength was varied and peak heights vs. wavelength were plotted. Since the plots for the early and late peaks matched the UV spectra of the acetal and cinnamaldehyde respectively, it was concluded the early peak was the acetal and the peak with the same retention time as water was cinnamaldehyde.

With the one-column method, aldehydes and ketones were substantially retained; all those tested had approximately the same retention time as cinnamaldehyde, and therefore interfered with the determination of water. However, most organic solvents were not retained by the column, and consequently posed no problem. Table II gives retention times for some organic compounds obtained with the 8 mm x 10 cm column in the H^+-form. An eluent of methanol only (no

Table II. Retention times for organic compounds on a column (8 mm x 10 cm) packed with Aminex Q-150S in the H^+-form

compound	detector wavelength[a]	retention time[b]
cinnamaldehyde	310	12.1
acetone	310	12.8
acetophenone	310	13.4
benzaldehyde	310	13.0
toluene	250	2.6
benzyl alcohol	250	3.0
o-nitrophenol	250	3.3
naphthalene	250	3.5
benzoic acid	250	2.9

[a]Units of nm.

[b]Units of minutes.

cinnamaldehyde) was used, with the detector set at a wavelength where the compounds have substantial absorbance.

Sensitivity with the one-column method was very good; an injection of 10 ppm water in methanol using a 20-microliter sample loop yielded an easily measured peak. A calibration plot of standard solutions of water in methanol was linear from 0.00512 to 2.40% water (correlation coefficient for linear regression of 0.9995). However, in addition to the interference from aldehydes and ketones due to their retention, there is also the possibility that aldehydes and ketones in the sample can partially react with the methanol in the eluent to form their corresponding acetals or ketals plus water. This would result in erroneously high values for the determination of water. A large interference was also seen when metal hydroxides were present in samples. This is because the metal cation exchanged with the H^+, which reacted with the OH^- to form water, again yielding high results for water determination.

These difficulties should be avoided by using a cation-exchange column in the Li^+-form for the chromatographic separation of water, followed by a short cation-exchange column in the H^+-form to catalyze the reaction needed for detection. No reaction occurs in the first column (Li^+-form); the water itself is separated from organic and inorganic material in the sample. When the eluent enters the second, catalytic column, the reaction in equation 6 takes place and gives a low background absorbance. But when the water peak enters the catalytic column, the equilibrium is shifted to the formation of more aldehyde in proportion to the amount of water injected.

The system described here is an example of a postcolumn reaction system which uses a solid-phase reactor. The set-up is simple and works very well. The reactants are already present in the mobile phase. The reaction simply doesn't occur until the catalyst column is reached. No additional reagents are mixed with the effluent stream. There is no need for the additional hardware (second pump, mixing tee, or reaction chamber) commonly used in a postcolumn reaction system. Consequently the problems inherent in a typical postcolumn reaction system are avoided. These include mixing problems and excess dead volume in the tee and reaction coil, and baseline noise due to the reagent pump.

This method is so powerful for the determination of water because it combines a selective detection method with the selectivity of chromatography. Because the reaction occurs <u>after</u> the separation, water can be determined in the presence of substances which would interfere if the separation column were not employed, i.e., aldehydes and ketones, which react with methanol in the presence of an acid catalyst to form water. This was tested by determining water in acetone, a ketone (Figure 15). Acetone would be expected to react with methanol to form water (plus a ketal) when it entered a catalytic column. However, it is already separated chromatographically from the water in the sample before it reaches the catalytic second column and no interference is encountered.

The same reasoning holds for sample components that absorb at the detection wavelength. They do not interfere with the quantitative determination of water as long as they are separated

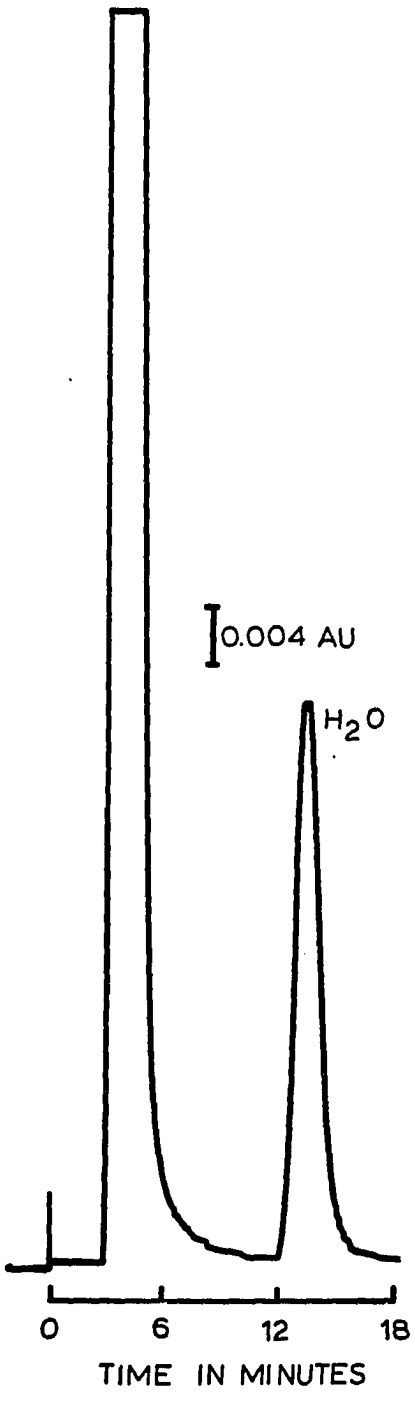


Figure 15. 0.321% water in acetone. Sample loop: 20 microliters. Separation column: 8 mm x 10 cm. Flow rate: 1 mL/min. Other conditions given in text

chromatographically before entering the catalytic detection system. Cinnamaldehyde works better than many aldehydes in the detection system because the wavelength of 310 nm is above the UV-cutoff for many organic solvents.

Although fairly good results were obtained with the one-column method for water determination, and the eluent is simpler and less toxic due to the lack of acetonitrile, since fewer interferences were encountered with the two-column method, the remaining discussion focuses on the latter method for water determination.

Column length

Many of the separations were done on a fairly long column (10 cm x 8 mm with a 10 cm x 2 mm catalyst column) in order to obtain good resolution of the water peak in some difficult samples. The separation of water from acetone shown in Figure 15 and the separation of water from a sample of 3-mercaptopropionic acid in Figure 16 are examples.

In many cases a shorter column can be used and the chromatographic separation of water greatly speeded up. Using a short column (5 cm x 4.6 mm with a 10 cm x 2 mm catalyst column) good separations were obtained for 367 ppm water in isopropyl alcohol (Figure 17) and for 184 ppm water in toluene (Figure 18).

Calibration curves

Calibration plots were obtained with both the long and short columns using methanol containing varying amounts of water as standards. For the long column a plot of points ranging from 0.00128%

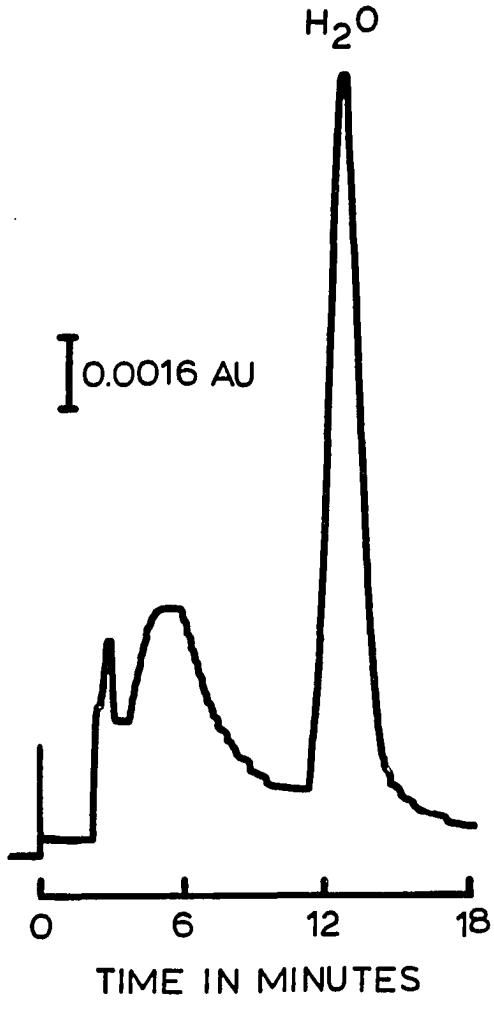


Figure 16. 0.138% water in a methanolic solution of 1.15 M 3-mercaptopropionic acid. Sample loop: 20 microliters. Separation column: 8 mm x 10 cm. Flow rate: 1 mL/min. Other conditions given in text

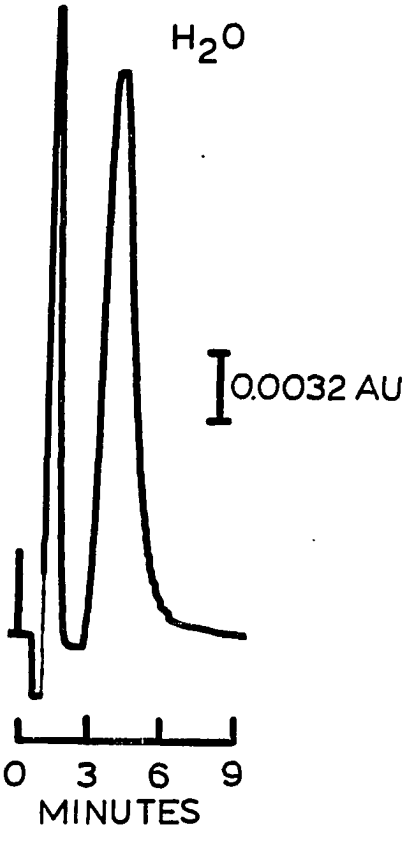


Figure 17. 367 ppm water in isopropanol. Sample loop: 100 microliters. Separation column: 4.6 mm x 5 cm. Flow rate: 0.8 mL/min. Other conditions given in text

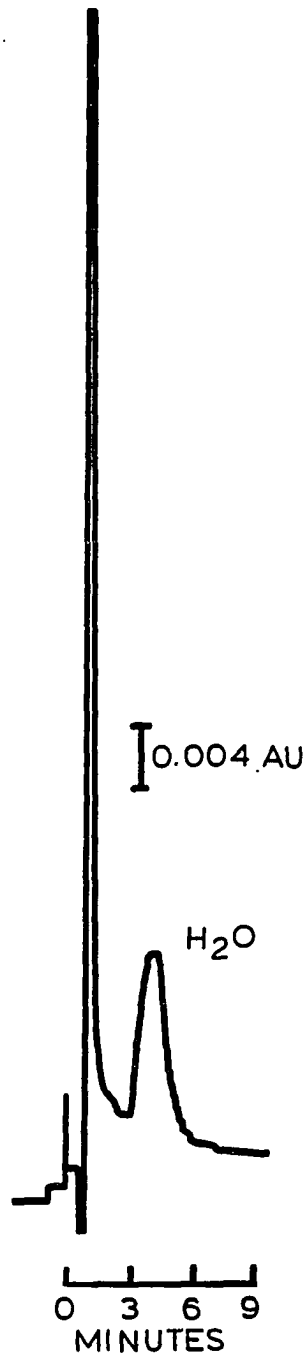


Figure 18. 184 ppm water in toluene. Sample loop: 100 microliters. Separation column: 4.6 mm x 5 cm. Flow rate: 0.8 mL/min. Other conditions given in text

water to 3.40% water had a correlation coefficient for linear regression of 0.9998. Graphical plotting showed a slight curvature at the higher concentration end of the plot (above 3.0%). The lower end of the plot appeared to be strictly linear, a correlation coefficient of 0.999996 being obtained from 0.00128% to 0.0800% water. Similar results were obtained for calibration plots with the short column. In this case the plot ranged from 0.0064% to 0.50% water.

The response factor of the chromatographic detection system for water was measured in the following units:

$$RF = \frac{\text{signal in AU at 310 nm}}{\text{0.1\% water in sample}} \qquad (8)$$

Response factors of 0.012 and 0.071 were obtained for the long column with a 20-microliter sample loop and the short column with a 100-microliter sample loop, respectively. These are quite good, considering that the baseline noise with this system was approximately 2×10^{-5} AU.

The limit of detection for water will depend on the response factor, the size of sample loop used and on the amount of water in the eluent. The water content of the eluent can be estimated by extrapolating a linear plot of peak height vs. ppm water in standards to zero peak height. Such an extrapolation of data from the short column gave approximately 30 ppm as the water content of the eluent. Injections of samples containing less water than the eluent give negative peaks at the retention time for water. This effect was

previously noted with a different chromatographic system for water (9). In principle, these negative peaks could be used to determine lower sample concentrations of water than in the eluent, but a reasonable calibration curve for the negative peaks was not obtained.

The most critical factor in obtaining extremely low detection limits for water seems to be to prepare and use an eluent of exceptionally low water content. Because of the possibility of obtaining negative peaks when the sample contains less water than the eluent, it is not legitimate to take a positive water peak of substantial height and estimate the limits of detection by dividing the peak height (and concentration) by 2.5 times the noise (9). By injecting 20 microliter samples of decreasing water concentration onto the long column, a detection limit of approximately 13 ppm water (260 ng absolute) at S/N = 3 was obtained (see Figure 19).

Scope and quantitative results

The utility of this method was demonstrated by separating and determining water on the long column in each of the following samples: toluene, ethyl acetate, acetone, 3-mercaptopropionic acid, ascorbic acid (dissolved in methanol), and copper(II) chloride dihydrate (dissolved in methanol). Samples analyzed for water on the short column included isopropyl alcohol, toluene, ethylacetate, and "absolute" ethanol. Analysis of the mercaptan, ascorbic acid and copper(II) chloride samples by the Karl Fischer method would not be possible. Likewise, aldehydes and ketones interfere in a liquid chromatographic method that uses a different detection system (9).

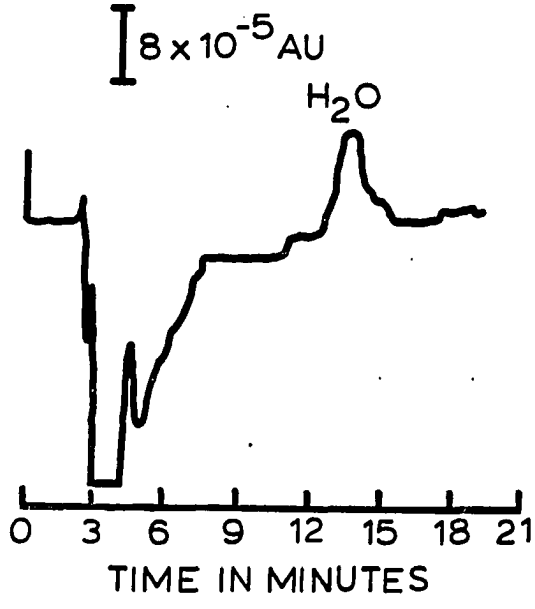


Figure 19. Limit of detection as obtained with the long separation column (8 mm x 10 cm): 260 ng water. Injected: 12.8 ppm water in methanol. Sample loop: 20 microliters. Flow rate: 1 mL/min. Other conditions given in text

Results are shown in Tables III and IV. The results in Table III, for toluene, ethyl acetate and acetone, were checked by comparison with the Karl Fischer method. The ethyl acetate sample was further checked by spiking it with 0.100% water. Recovery was excellent. Table IV shows results for those samples for which a Karl Fischer titration would have yielded erroneous results (2). These samples were checked by analyzing a blank, then spiking the sample and reanalyzing. In the case of the 3-mercaptopropionic acid, due to the small amount of mercaptan available, a 10.00% solution of the mercaptan in methanol was spiked. Recoveries were again very good.

One final application of this method is that it may be used for the determination of water of hydration in inorganic salts. A sample of $CuCl_2 \cdot 2H_2O$ was analyzed and found to contain 1.99 moles H_2O per mole of $CuCl_2$. This determination could not be done with a Karl Fischer titration since the Cu(II) would be reduced to Cu(I) by the reagent (2).

Interferences

Of the organic samples tested, only dimethyl sulfoxide (DMSO) was found to interfere. DMSO produced a very large peak that obscured the water peak. However, it has been pointed out that certain organic acids can react with methanol to form esters, producing water as a by-product (24). This would lead to high values for water determination.

Of the inorganic samples tested, metal hydroxides interfered. Since the columns involved are ion-exchange columns, any cation injected will cause the release of a corresponding amount of H^+. In

Table III. Liquid chromatographic determination of water in organic solvents

sample	% water added	% water found (this method)	% water found (Karl Fischer)
neat toluene	0	0.0253	0.0251
neat acetone	0	0.321	0.325
neat ethyl acetate	0	0.0950	---
ethyl acetate spiked with 0.100% water	0.100	0.196	0.198

Table IV. Liquid chromatographic determination of water in the presence of common interferences

sample	% water added	% water calculated (spike plus blank)	% water found
neat 3-mercaptopropionic acid	0	N.A.	0.494
methanolic solution of 10.00% 3-mercaptopropionic acid, spiked with 0.200% water	0.200	0.249	0.246
methanolic solution of 0.057M ascorbic acid	0	N.A.	0.0080
methanolic solution of 0.057M ascorbic acid spiked with 0.100% water	0.100	0.108	0.104

the case of metal hydroxides, this H^+ will react with the OH^- to form water. However, with the two-column system, the OH^- should be well-resolved from the water in the sample by the time the catalyst column is reached. Consequently the reason for the observed interference of metal hydroxides is unknown.

Samples containing large amounts of ionic materials can pose a problem by displacing more and more Li^+ from the separation column. The Li^+ removed from the separator column will exchange with the H^+ of the catalyst column, decreasing its ability to catalyze the reaction. Likewise, a large number of acidic samples will put much of the separation column in the H^+-form and thereby cause a change in the retention time of the water peak. Periodic regeneration or possibly the use of a replaceable Li^+-form precolumn should alleviate these difficulties.

Future Work

A chromatographic separation followed by postcolumn reaction between water and an acetal, forming an aldehyde which can be detected with a spectrophotometric detector, has been shown to be a sensitive way to determine water. Cinnamaldehyde works well since a detector wavelength of 310 nm can be used, which is above the UV cut-off of many organic solvents.

In theory, this method should work for any aldehyde or ketone, since both aldehydes and ketones absorb UV light and react with an alcohol in the presence of an acid catalyst to form an acetal or a ketal, respectively. Two desirable criteria for selecting an aldehyde

or ketone to be used in this method are absorption at a long wavelength and a large difference in the spectra of the aldehyde (ketone) and acetal (ketal) at the detection wavelength. This first criterion will minimize interference from organic matrices, while the second is needed for high sensitivity. A large molar absorptivity at the detection wavelength is also desirable for sensitive water determination.

Several sets of rules have been devised which allow one to calculate how the λ_{max} of a compound will shift as organic functional groups are added to the original molecule (15). Therefore it should be a fairly simple matter, requiring no experimentation, to determine which aldehydes and ketones will be suitable for water determination from the point of view of absorbing light of a long wavelength.

The second desirable quality, a large difference in the spectra of the aldehyde (ketone) and acetal (ketal) at the detection wavelength, can be investigated very quickly and easily using a UV-vis spectrophotometer. The spectrum of a solution of the aldehyde or ketone in methanol is obtained before and after shaking with a small amount of cation-exchange resin in the H^+-form, as shown in Figure 13 for cinnamaldehyde.

By "designing," through the addition of various functional groups, an aldehyde or ketone that absorbs at a very long wavelength, it may be possible to use this detection method in a flow injection analysis (FIA) system. If the detector wavelength is long enough that no other sample components absorb there, the need for a chromatographic separation is eliminated. However, interference would be expected if other aldehydes or ketones were present in the sample, since these

would react in the catalyst column to form additional water.

A dual cell spectrophotometric detector can be used in an FIA system. If the stream were split in half, with one half going through one detector cell and the other half passing through the catalyst column first and then the other cell, it should be possible to cancel out interferences that absorb at the detection wavelength. Again, this would not work for interferences that react to form water in the catalyst column.

The catalyst column used for this work was 2 mm x 10 cm. This size column was not optimized but was used because it was readily available. It should be possible to use an even smaller catalyst column, thereby decreasing band broadening due to the second column. However, the importance of increasing efficiency of the separations must be weighed against the frequency with which a small catalyst column requires regeneration.

Although a catalyst of cation-exchange resin in the H^+-form works well, other types of acid catalysts can be investigated.

Are there any reactions involving water that are base catalyzed? Perhaps a method for water determination can be devised which uses a catalyst column of anion-exchange resin in the OH^--form.

There should be many other instances where an acid catalyzed spectral change occurs, permitting the determination of water or other species. For example, the acid catalyzed reaction of dimethoxypropane with water and subsequent measurement of the resulting acetone has been used to determine water by GC. Could this same reaction be used to determine water with LC? Addition of dimethoxypropane to the eluent

and the use of an H^+ catalyst column, coupled with a detection wavelength where acetone has a high extinction coefficient, should work. This on-line reaction eliminates the extensive sample preparation needed when this reaction is used in conjunction with GC.

The general idea of using an acid catalyzed spectral change does not have to be limited to only reactions involving water. Perhaps similar methods can be devised which will allow the determination of alcohols, amines, etc.

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OVERALL CONCLUSIONS

Doubly protonated p-phenylenediamine was shown to be an effective eluent for the elution of divalent and trivalent metal ions from a low capacity cation exchange resin. It is especially useful for separating aluminum (III) from divalent metals. The Al(III) separation and quantification method presented here is rapid, precise, and accurate. No interferences were found among the metals tested.

TAN-3,6-S can be used as a postcolumn reagent for the sensitive detection of metal ions. Since the reagent complexes a wide variety of metals, it can be used as a near-universal reagent for metal detection. However, selective methods for determining metals of interest while minimizing interferences from other metals can be devised by judiciously choosing the reaction's pH.

Temperature was presented as another parameter to be optimized in ion chromatography.. In cases where the exchange reaction is endothermic, an increase in system temperature can lead to improved separations. Especially with the use of an electric column heater, increasing the temperature can be done very quickly, more quickly than preparing and reequilibrating an eluent of a different concentration. Temperature should be viewed not as a substitute for other chromatographic parameters such as the concentration or nature of eluent, but as an additional parameter to take into consideration when optimizing chromatographic conditions.

Although numerous methods for determining water by GC are

available, a method that works well for one sample may not work at all for a different sample. The actual method and stationary phase chosen are dependent on the sample matrix. Gas chromatographic methods are slow for samples containing late-eluting compounds. Decomposition of samples leading to fouling of the column can also be a problem. GC methods often cannot be used at all for samples containing non-volatile constituents, unless the chromatography is preceeded by a quantitative extraction of volatile analytes. Some GC methods for water determination require a derivatization of the sample prior to injection.

The method presented here for the determination of water using ion-exclusion chromatography and UV detection after a postcolumn reaction can be used for a wide variety of samples. No sample pretreatment is necessary for homogeneous solutions. The method is fast and accurate, has a low detection limit, and a large linear dynamic range. No interference was seen for some of the materials known to interfere with the Karl Fischer method: mercaptans, ascorbic acid, and easily reduced metals. Additionally, no interference from sample ketones was seen; aldehydes and ketones do interfere in a recently published liquid chromatographic method for water determination (9).

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I can honestly say I would not have made it through graduate school without the love and encouragement given to me by my family. My parents, Robert and Shirley, and my brothers and sisters, Robbie, Sally, Gary, and Janet, have given me immeasurable support. Even though they've been far away these last five years, I've never been closer to them. I've lost count of the number of times I wanted to quit, but thanks to their pep talks and willingness to listen, their understanding of my fears and acceptance of my faults, I somehow

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