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Michael David Arguello
*Iowa State University*

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# Forced-flow chromatographic determination of calcium and magnesium with continuous spectrophotometric detection

by

Michael David Arguello

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Chemistry
Major: Analytical Chemistry

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In Charge of Major Work

For the Major Department

For the Graduate College

Iowa State University
Ames, Iowa

1977

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# I. INTRODUCTION

## A. Purpose

The fact that forced-flow chromatographic methods employing continuous detection can be used for the rapid and accurate determination of trace amounts of metal ions is now well established. The advantages associated with such methods include:

1) The ability to handle samples in the tens of microliters range containing microgram amounts of sample ions.
2) Extremely rapid analysis times compared to gravity-flow chromatographic methods employing collection of fractions followed by separate analysis of each fraction.
3) Minimal sample handling and manipulation.
4) Relatively simple and inexpensive equipment requirements compared to most other instrumental methods of analysis.
5) The possibility of doing routine analysis of particular kinds of samples by using a dedicated instrument designed expressly for that purpose.

Because of the importance of rapidly and accurately determining calcium and magnesium in such diverse materials as hard water and blood serum, it was felt desirable to try to develop forced-flow chromatographic methods for calcium and magnesium that would offer some of the advantages outlined above. As in the development of any chromatographic method, it was then necessary to investigate such things as the proper choice of resin, the proper choice of eluent, and the proper choice of operating conditions. And since it was desired to employ continuous spectrophotometric detection, it was also necessary to investigate the proper choice of color-forming reagent and buffer.

As a result of these investigations, analytical methods for calcium and magnesium were developed which offer all of the advantages associated with any forced-flow chromatographic method employing continuous detection. This was done by taking advantage of the rapid separations which are obtainable using low capacity cation exchangers based on partially sulfonated XAD-2. Elution was with ammonium chloride or ethylenediammonium chloride and Arsenazo I or PAR-ZnEDTA color-forming reagents were used for the continuous spectrophotometric detection of calcium and magnesium. In order to demonstrate their usefulness, the methods developed were used for the analysis of synthetic samples and also for the analysis of hard water and blood serum.

## B. Literature Survey

## (Separation of Calcium and Magnesium)

The separation of calcium and magnesium prior to their individual determination has been a problem of continuing analytical interest because of the many different kinds of materials in which significant amounts of these two elements occur together. Examples of some materials containing significant amounts of both calcium and magnesium include hard waters, limestones, cements, plant tissue, bone ash, milk and milk ash, blood serum, and soil. As a result, many different approaches to the separation of calcium and magnesium have been described in the literature. A number of different procedures using cation exchangers, anion exchangers, and chelating resins have been reviewed by Samuelson (1).

The majority of procedures described for the separation of calcium and magnesium have employed cation exchangers and a representative selection of these is given in Table I. As can be seen from the table, various kinds of eluents have been used to elute calcium and magnesium from cation exchangers. In some cases, simple mineral acids have been used as eluents whereas in other cases complexing agents or mixtures of mineral acids and organic solvents have been used in order to achieve increased separation factors. In most cases, however, the separations described are rather

Table I. Cation exchange separations of Ca and Mg

| Cation Exchanger | Ca eluted with: | Mg eluted with: | Elution order | Application | Reference |
|---|---|---|---|---|---|
| Dowex 50 | (retained on column) | 1.05 *M* HCl | Mg,Ca | Analysis of limestones and dolomites | Campbell and Kenner (2) |
| Bio-Rad AG 50W-X8 | 1.25 *M* HNO$_3$ | 1.25 *M* HCl | Mg,Ca | Ion exchange determination of major and minor elements in silicate rocks | Strelow *et al.* (3) |
| Microcrystalline cellulose | 70:30 methanol-HCl | 70:30 methanol-HCl | Mg,Ca | Separation of alkaline earths | Fritz and Peters (4) |
| Bio-Rad AG 50W-X8 | 3 *M* HCl | 3 *M* HCl-60% ethanol | Mg,Ca | Separation of alkaline earths | Strelow and Van Zyl (5) |
| Zeo-Karb 225 | 1 *M* NH$_4$Cl | 0.5 *M* NH$_4$Cl | Mg,Ca | Ion exchange determination of Ca and Mg in silicate rocks | Abdullah and Riley (6) |
| Amberlite CG 120 | 1 *M* ammonium acetyl-acetonate | 0.35 *M* NH$_4$Cl | Mg,Ca | Ion exchange determination of major cations in sea water | Greenhalgh *et al.* (7) |
| Dowex 50W-X8 | 1 *M* ammonium acetate | 1 *M* ammonium acetate | Mg,Ca | Analysis of dolomite and limestone | De and Sen (8) |

Table I. (Continued)

| Cation Exchanger | Ca eluted with: | Mg eluted with: | Elution order | Application | Reference |
|---|---|---|---|---|---|
| Amberlite IRC 50, Dowex 50W-X8 | EGTA pH 6.5-7 | 2-3 M HCl | Ca,Mg | Analysis of artificial sea water | Marhol and Cheng (9) |
| Dowex 50 | 1.5 M ammonium lactate | 1.5 M ammonium lactate | Mg,Ca | Separation of alkaline earths | Milton and Grummitt (10) |
| Dowex 50W-X8 | 1 M α-hydroxy-isobutyric acid pH 5 | 0.8 M α-hydroxy-isobutyric acid pH 4.15 | Mg,Ca | Separation of alkaline earths | Pollard *et al.* (11) |

slow and oftentimes the presence of complexing agents or organic solvents complicates the determination of the individual ions.

Work of a more general nature related to the cation exchange separation of calcium and magnesium is that of Strelow and Weinert (12) who compiled an extensive tabulation of distribution coefficients of the alkaline earths on AG 50W-X8 with various complexing agents and mineral acids. Strelow (13) has also reviewed the cation exchange behavior of the alkaline earths and discussed the merits of various eluents.

## C. Literature Survey
## (Forced-flow Chromatography)

Salmon (14) in 1971 first suggested that ion-exchange chromatography needed to be coupled to a continuous detection process in order to achieve a flexibility of operation comparable to that of gas chromatography. He proposed the use of ion-selective electrodes as continuous detectors for ion-exchange chromatography.

Since then many procedures have been described in the literature employing forced-flow ion-exchange chromatography of metal ions with continuous detection. A representative selection of such procedures is given in Table II.

Table II. Forced-flow chromatographic separations of metal ions employing continuous detection

| Ion Exchanger | Eluent | Pumping System | Ions Separated | Detection System | Reference |
|---|---|---|---|---|---|
| A-26 anion exchanger | 2 *M* HCl | Gas pressure | Fe(III) from other ions | UV absorption of iron chloride complex | Seymour *et al.* (15) |
| A-26 anion exchanger | 0.4 *M* NaClO$_4$ pH 3.75 | Gas pressure | Cr(VI) from other ions | UV absorption of Cr(VI) | Fritz and Sickafoose (16) |
| A-26 anion exchanger | HCl-HClO$_4$ mixtures | Gas pressure | As(III), Bi(III), Sb(III); Ni, Pd(II), Pt(IV); Pb(II), Cu(II), Fe(III); Hg(II), Sn(IV) | UV absorption of chloride complexes | Seymour and Fritz (17) |
| Dowex 1-X8 anion exchanger | 8 *M* HCl | Gas pressure | Pb(II) from other ions | UV absorption of lead chloride complex | Seymour and Fritz (18) |
| A-15 cation exchanger | 0.5 *M* HBr | Gas pressure | Hg(II), Bi(III), Cd(II), Sb(III) | UV absorption of bromide complexes | Willis and Fritz (19) |
| A-200 or A-15 cation exchanger | HCl-organic solvents | Chromatronix metering pump | Cd(II), Zn(II), Fe(III), Pb(II), Cu(II), Co(II), Mn(II) | PAR or PAN color-forming reagents | Kawazu and Fritz (20) |

Table II. (Continued)

| Ion Exchanger | Eluent | Pumping System | Ions Separated | Detection System | Reference |
|---|---|---|---|---|---|
| Low capacity cation exchangers | Varied | Chromatronix metering pump | Zn, Pb(II), Cu(II), Mn(II), Ni; Ca, Mg; Zr, Th; La, Th | PAR, Arsenazo I or Arsenazo III color-forming reagents | Fritz and Story (21) |
| Dowex 50W-X8 cation exchanger | 2-Hydroxy-isobutyric acid pH gradient | Chromatronix metering pump | 13 rare earths | PAR color-forming reagent | Story and Fritz (22) |
| Surface sulfonated cation exchangers, surface agglomerated anion exchangers | HCl, $AgNO_3$<br>$Cu(NO_3)_2$<br>Aniline•HCl<br>$AgNO_3$-$HNO_3$,<br>NaOH<br>Na(phenate) | Milton Roy metering pump | alkali metals, amines, and quaternary ammonium ions;<br>Ca, Mg;<br>inorganic and organic anions | Conductimetric | Small *et al.* (23) |
| Zipax SCX cation exchanger | 0.01 *M* $HNO_3$<br>0.2 *M* citric acid | Waters Model 6000 Solvent Delivery System | Ca, Sr, Ba;<br>rare earths | Flame emission | Freed (24) |
| Amberlite CG-120 cation exchanger | 1 *M* $NH_4Cl$ | Fuji midget pump, Mitsumi peristatic pump | Zn from other ions | Zincon color-forming reagent | Matsui (25)<br>(25) |

Table II. (Continued)

| Ion Exchanger | Eluent | Pumping System | Ions Separated | Detection System | Reference |
|---|---|---|---|---|---|
| Zirconium phosphate cation exchanger | 1 M $NH_4Cl$ | Hydrostatic pressure | Alkali metals | Flame ionization (Intermittent rather than continuous) | Araki *et al.* (26) |
| IRA-200 cation exchanger | 0.05 M $HClO_4$ | Gas pressure | Se(VI) from other ions | Anodic stripping voltammetry | Andrews and Johnson (27) |

Forced-flow chromatographic separations of inorganic ions employing supports other than simple cation or anion exchangers have also been described in the literature.

Fritz and Goodkin (28) used adsorption chromatography on XAD-11, a macroporous polyacrylate resin with amide functional groups, to separate tin (IV) from 23 other metal ions. They eluted tin (IV) from the resin with 0.1 M HCl using gas pressurization and detected it by means of the UV absorbance of its chloride complex.

Moyers and Fritz (29) separated silver (I), mercury (II), bismuth (III), and gold (IV) on a chelating resin containing hexylthioglycolate functional groups. Using gas pressurization, they eluted the first three metals with hydrochloric acid and detected them by the UV absorption of their chloride complexes. Gold (III) was then eluted with thiourea and detected by the UV absorption of its thiourea complex.

Using liquid-liquid chromatography on Zipax controlled surface porosity supports coated with tricaprylmethyl ammonium chloride, Horwitz and Bloomquist (30) were able to separate a number of different metal ions. Various mixtures of different metal ions were separated including a mixture that contained lead (II), bismuth (III), rhenium (III), cadmium (II), mercury (II), and polonium (IV). Stepwise elution with mineral acids and thiocyanate solutions was done using gas pressurization and the eluted ions were detected radiochemically.

## II. EXPERIMENTAL

### A. Apparatus

I. The forced-flow chromatograph has been described previously (31). The chromatographic system used in this work is shown schematically in Figure 1 and the same system in somewhat greater detail is shown in Figure 2. Modifications to the basic chromatograph that were made in the course of this work included the following:

1) Milton Roy minipumps (Instrument Model and Model 396) and a Chromatrix CMP-2 "Cheminert" metering pump as well as gas pressurization were used for solvent delivery. The use of metering pumps permitted both reproducible and accurate control of flow rates regardless of changes in system back pressure and provided the capability of changing from one flow rate to another rapidly and with a minimum of manipulation.

2) The single tank used for containing color-forming reagent was replaced with a system of pressurized bottles similar to that described previously (32). This permitted rapid changes from one color-forming reagent to another without the necessity of having to empty and refill the single tank. Additionally, the system of bottles used to contain color-forming reagent could be pressurized

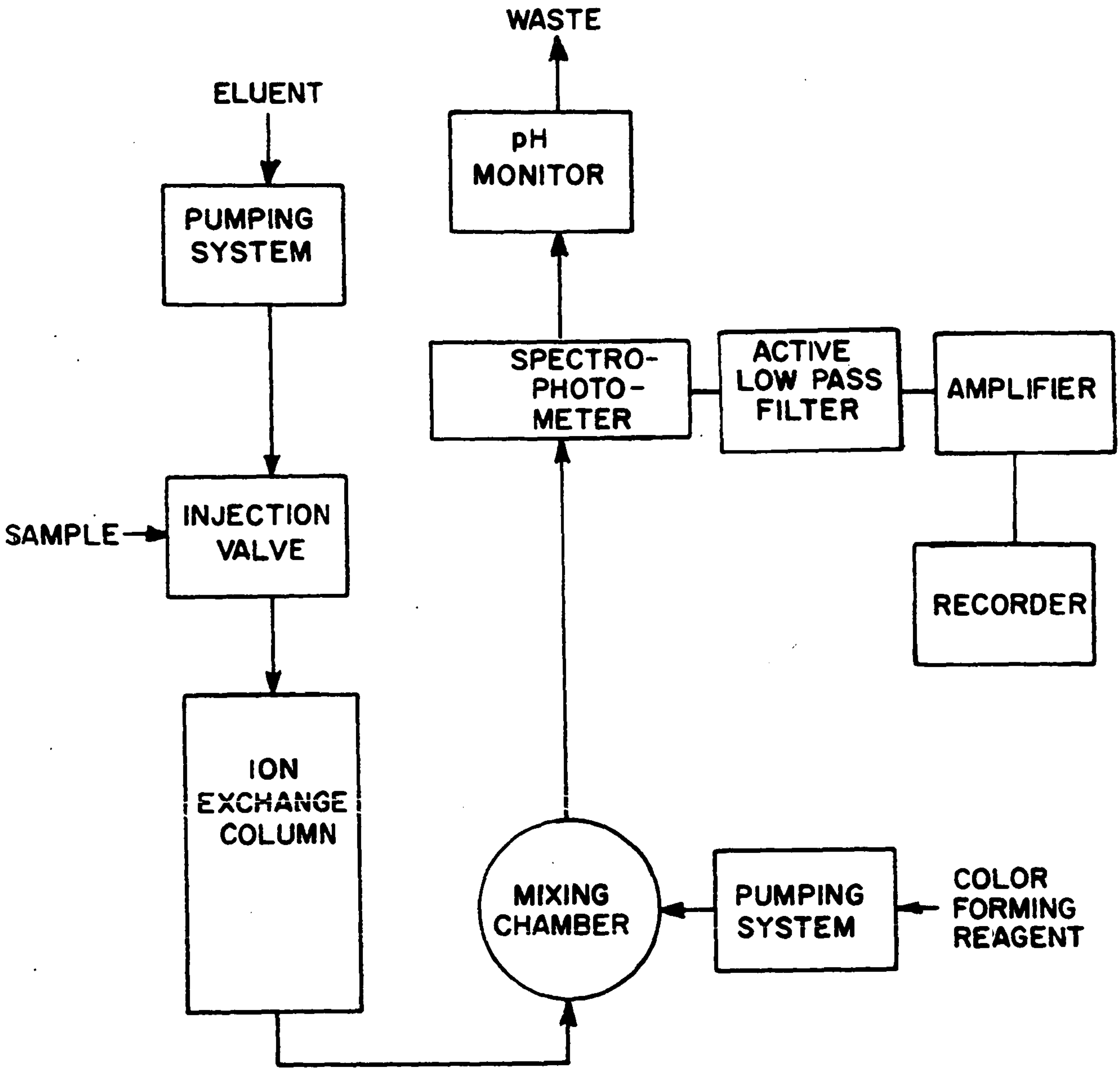


Figure 1. Block diagram of forced-flow chromatograph

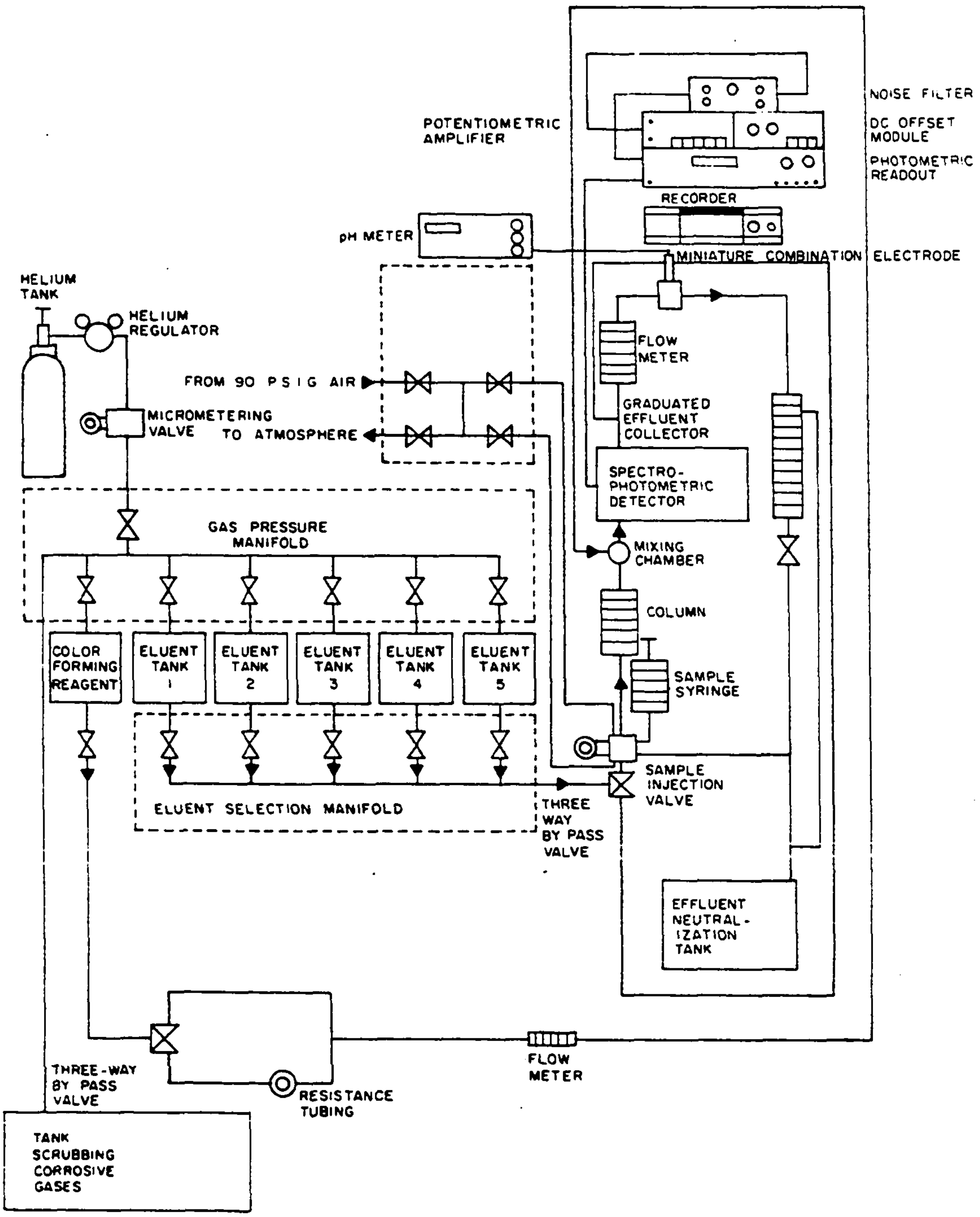


Figure 2. Schematic diagram of forced-flow chromatograph

separately from the eluent tanks thereby allowing the flow rate of color-forming reagent to be regulated independently of the flow rate of eluent.

3) A system to continuously monitor the pH of the final effluent was incorporated within the chromatograph. This system is shown schematically in Figure 3.

4) An active low-pass filter was incorporated within the chromatograph between the photometric readout unit and the amplifier. The circuit diagram of the filter is shown in Figure 4. Use of the filter eliminated high frequency noise due to lamp instability, photomultiplier shot noise, and pump pulsations and permitted a considerably increased amplification of the chromatographic signal. Chromatograms illustrating filter performance are shown in Figures 5 and 6.

5) The heavy iron tanks used to contain the pressurized polyethylene eluent bottles were replaced with lightweight, corrosion resistant plastic tanks constructed from heavy-walled 6 inch i. d. Plexiglass pipe epoxied into a grooved polycarbonate base plate. Similarly, the metal collars used to retain the bottles within the tanks were replaced with ones constructed of polycarbonate and the steel bolts

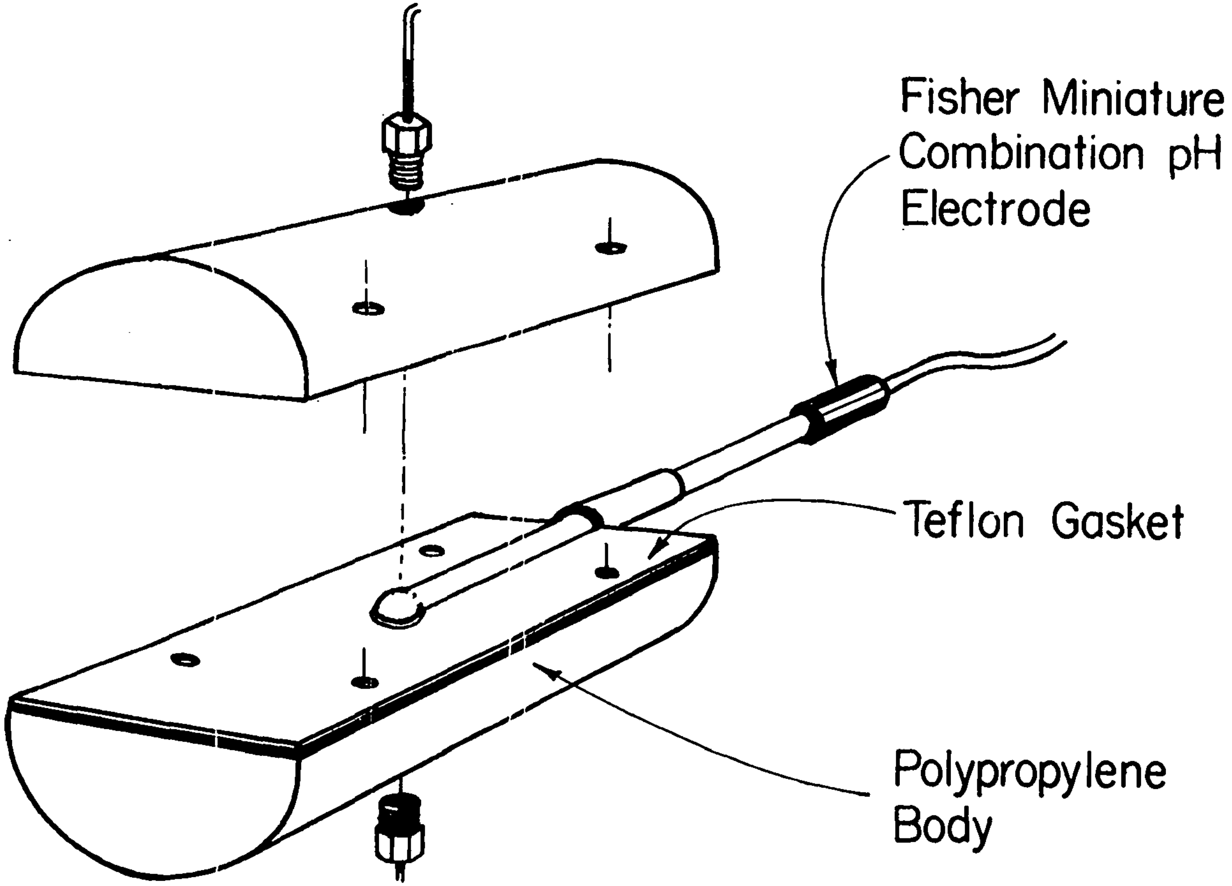


Figure 3. Schematic diagram of pH monitor

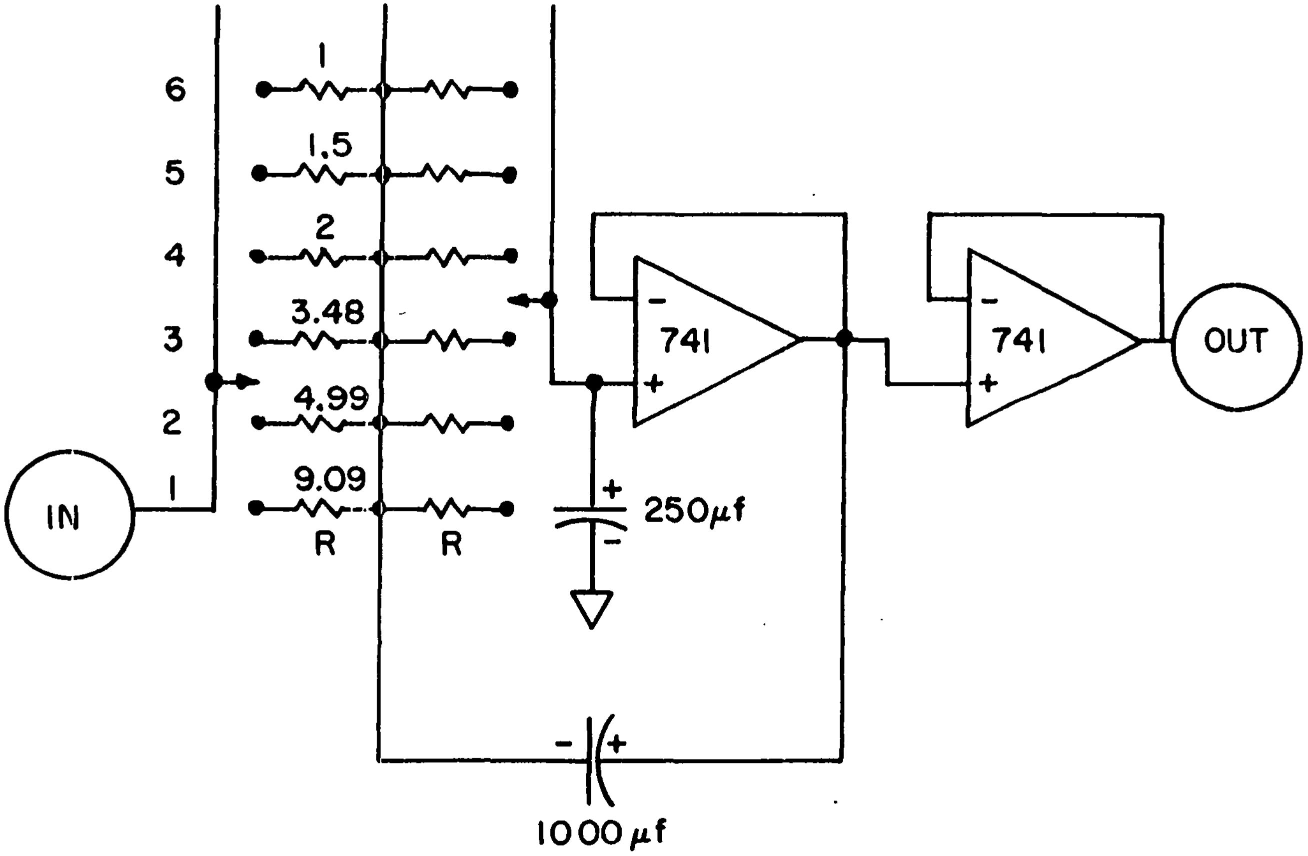


Figure 4. Circuit diagram of active low-pass filter. R values shown are in units of Kilo Ohms

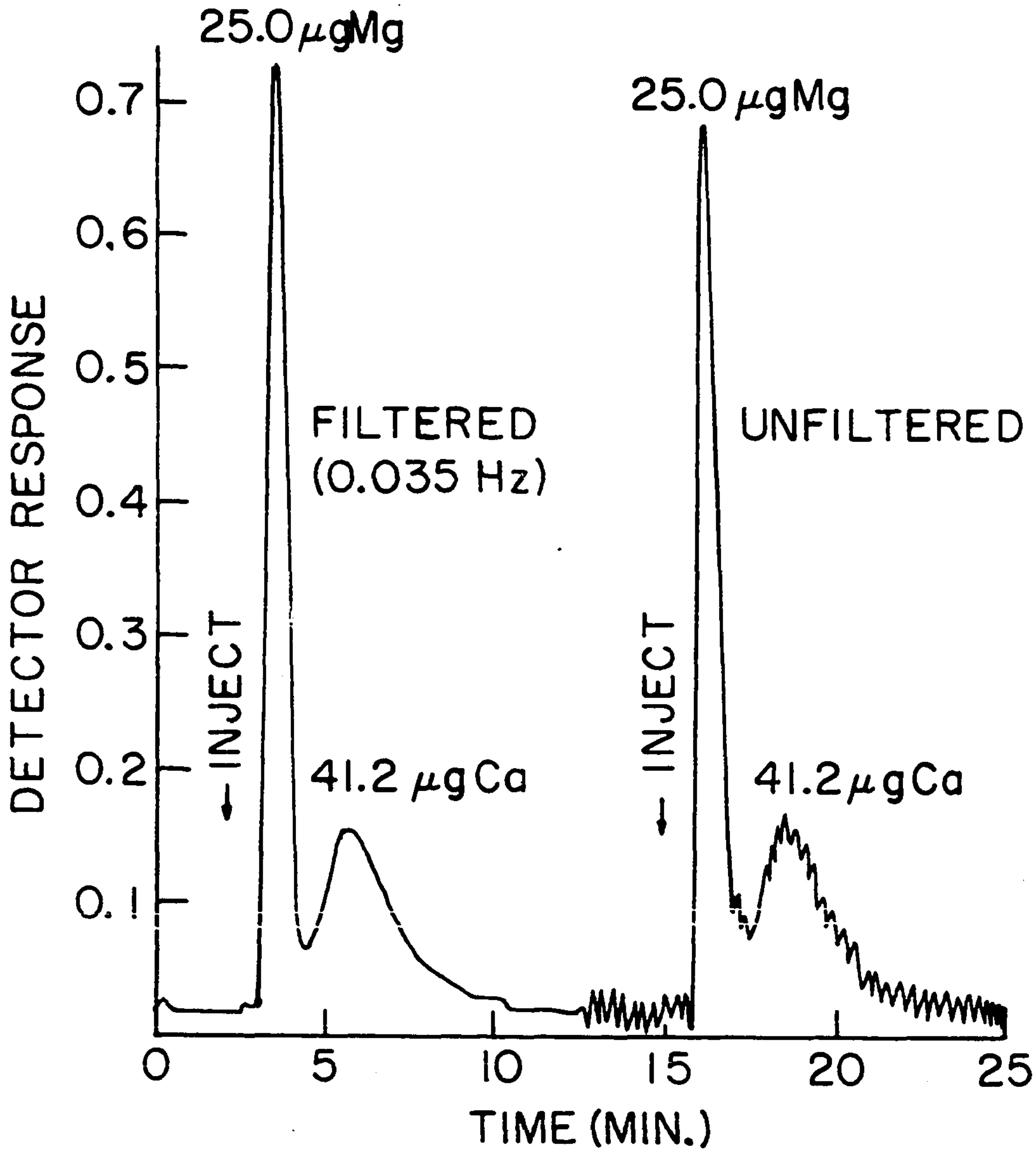


Figure 5. Chromatogram illustrating performance of active low-pass filter

Column: 1.9 meq/g low capacity catex; 7 cm x 0.4 cm

Eluent: 1 M $NH_4Cl$; 2 ml/min

Detection: Arsenazo I color-forming reagent; 0.9 ml/min

Sample: 51.4 μl

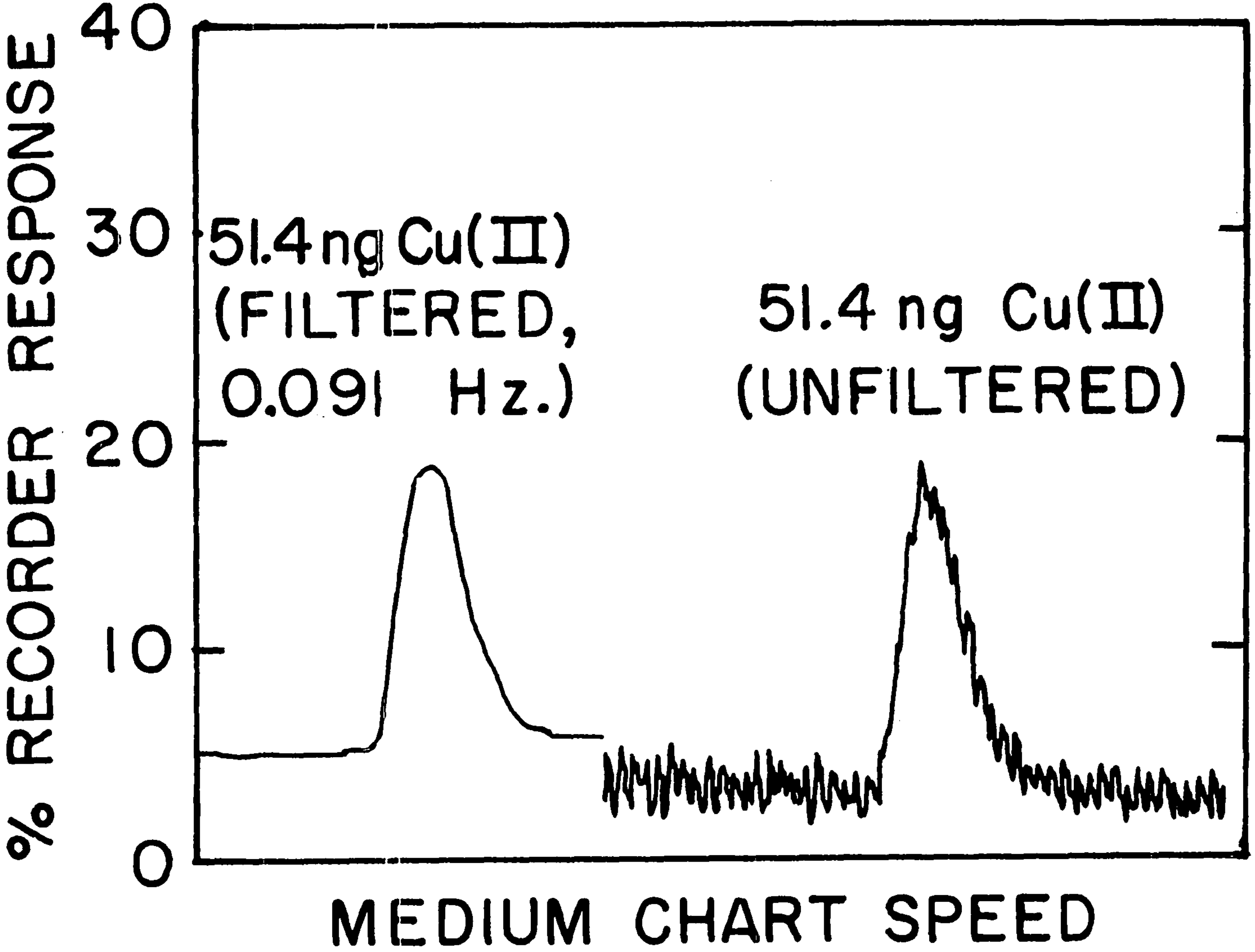
Figure 6. Chromatogram illustrating performance of active low-pass filter

Column: None; direct injection into flowing stream

Eluent: 0.1 M HCl; 2 ml/min

Detection: Beckman Model B equipped with flow cell (20)
PAR color-forming reagent; 0.5 ml/min

Sample: 51.4 μl


% RECORDER RESPONSE
0
10
20
30
40
51.4 ng Cu(II)
(FILTERED,
0.091 Hz.)
51.4 ng Cu(II)
(UNFILTERED)
MEDIUM CHART SPEED

were replaced with nylon pins. The plastic tanks gave trouble-free operation and could be used continuously at pressures as high as 50 psi.

II. Columns were constructed from lengths of 4 mm i.d. Pyrex tubing onto which Altex 200-28 glass connectors had been fused. The use of Viton O rings between the polypropylene bushings and caps of the glass connectors resulted in columns that were both leak-tight and chemically inert. Additionally, the columns were easy and inexpensive to construct. Broken or discarded columns were easily salvaged by cutting off the glass connectors which could then be reused for the construction of new columns.

Columns were packed by inserting the smallest possible plug of glass wool into one end of the column so as to retain the resin and then filling the column from the other end with a slurry of the resin in acetone using an Altex 200-19 male luer adapter and a syringe.

III. All plumbing components (tubing, tube-end fittings, couplings, plugs, tees, valves, sample loops, etc.) used were either purchased from Laboratory Data Control or Altex or were constructed in the Ames Laboratory shops.

## B. Reagents

1. 0.01 M stock solutions of metal ions were prepared from the best available grade of metal salts. In most cases reagent grade chloride salts were used but in a few cases it was necessary to use metal oxides or other salts. Sufficient hydrochloric acid was added to each stock solution to prevent hydrolysis.
2. Magnesium standard solution was prepared by dissolving 1.000 g of magnesium metal in a minimum amount of hydrochloric acid and diluting to 1.000 ℓ with distilled, deionized water.
3. Calcium standard solution was prepared by dissolving 2.500 g of primary standard calcium carbonate in a minimum amount of hydrochloric acid and diluting to 1.000 ℓ with distilled, deionized water.
4. Ethylenediammonium chloride (en•2HCl) was prepared by dissolving 100 g of ethylenediamine (J. T. Baker) in 1 ℓ of distilled water. The solution was cooled in an ice bath and 330 ml of concentrated hydrochloric acid (Dupont reagent grade) was added slowly with stirring. The en•2HCl was crystallized by the addition of 2-propanol, filtered with suction, washed with acetone, and air dried.

5. All other chemicals (acids, bases, indicators, solvents, etc.) used in the preparation of various solutions were the best grade available and were used as received.

# III. EXPERIMENTAL PARAMETERS

## A. Choice of Resin

The general usefulness of low capacity cation exchangers based on partially sulfonated XAD-2 was first demonstrated by Fritz and Story (21) who used such resins for the forced-flow chromatographic separation of many different metal ions. Among the many separations demonstrated were rapid, qualitative separations of calcium and magnesium.

Low capacity cation exchangers based on partially sulfonated XAD-2 offer a number of advantages for use in forced-flow chromatography over both conventional gel-type cation exchangers and cation exchangers of the superficially porous or pellicular type. As against conventional gel-type cation exchangers, these advantages include both better mechanical stability and increased efficiency of separation. Compared to superficially porous or pellicular resins, the advantages include greater economy and ease of preparation as well as the ability to accommodate larger samples due to the larger capacities.

Other workers have pointed out additional advantages. Hansen and Gilbert (33) in a theoretical study of support design for high-speed ion-exchange chromatography considered the effects of particle size, intra-particle diffusion, and film diffusion on resolution. They concluded that optimum resolution should occur with pellicular supports having capacities greater than those then currently available ($\leq$0.100 meq/g). Hansen and Gilbert (34) also did an experimental study of resin design in which they prepared a series of cation exchangers of varying capacity (0.123 - 0.642 meq/g) by partially sulfonating styrene-divinyl benzene copolymer. They used the 200-300 mesh fraction for the separation of uracil, cytosine, and adenine and found that resolution increased with increasing capacity due to greater retention of the late eluting peaks.

### B. Preparation of Partially Sulfonated XAD-2

Partially sulfonated XAD-2 was prepared by a procedure similar to that described by Fritz and Story (21). XAD-2 resin, 20-50 mesh, obtained from Rohm and Haas was Soxhlet extracted with methanol and air dried. The cleaned resin was ground in a Wiley mill and sieved dry. A 5 to 10-g portion of the 250-325 mesh fraction was suspended in concentrated sulfuric acid (Dupont Reagent grade) and stirred for 30 minutes at 102-103°C. Close control of temperature was maintained by means of a well regulated, electrically heated oil bath. At the end of the reaction period, the reaction mixture was poured over ice and the partially sulfonated resin filtered off. The resin was washed repeatedly with distilled water and finally with acetone. It was then air dried overnight.

The hydrogen capacity of each batch of resin was measured by equilibrating a weighed amount of resin with a known excess of standard base. After shaking for several hours on a wrist action shaker, the resin was filtered off and the remaining base titrated with standard acid. Capacities of resins prepared in the manner described above ranged from 1.8 to 2.0 meq hydrogen per gram of air-dried resin.

### C. Choice of Color-forming Reagents

#### 1. General requirements

In order for any given color-forming reagent to be useful for the continuous spectrophotometric analysis of forced-flow chromatographic effluents, it is necessary that the reagent exhibit certain characteristics. Among the most important of these are the following:

1) The product of the color-forming reaction should have a large effective molar absorptivity in order that it may be used for the accurate analysis of trace amounts of metal ions.
2) Color development should be fast enough that the use of a delay loop can be avoided since such loops always result in a decrease in resolution due to band spreading (35).
3) The color-forming reagent should be insensitive to the presence of background electrolyte from the eluent and buffer used.

4) The products of the color-forming reaction should be soluble because reactions that result in the formation of lakes and precipitates can cause coating of cell windows.
5) The reagent should be stable in solution and easy and economical to prepare.
6) The selectivity of the reagent should be suited to the requirements of the particular analysis being performed. In cases in which two or more ions are completely resolved and it is desired to analyze each of them in turn, it is necessary to use a nonspecific reagent. In cases in which two or more ions are incompletely resolved and it is desired to analyze only one of them, a reagent of greater specificity must be used.

### 2. Arsenazo I reagent

With these criteria in mind, a number of reagents that have been used or suggested for the colorimetric determination of calcium and/or magnesium were first briefly examined by means of the following experiment.

Stock solutions of the various reagents including Arsenazo I, Arsenazo III, Sulfonazo III, Methyl Thymol Blue, Murexide, Titan Yellow, Sodium Rhodizonate, Eriochrome Black

T, Chlorophosphonazo III, and TAR (4-(2-Thiazolyl-azo)-resorcinol) were prepared in distilled water. Measured aliquots of each stock solution were mixed with an equal volume of buffer (pH 4.2, 4.9, 6.1, 7.0, 8.0, 9.0, 10.0, 11.0, or 12.0) and the final solutions made approximately 0.001 M in calcium or magnesium. The extent of reaction, if any, compared to that of blanks containing only color-forming reagent and buffer, was judged on the basis of color contrast, color intensity, and time of development. Based on these considerations, it was concluded that of the reagents surveyed, Arsenazo I appeared best suited for use in the continuous spectrophotometric detection of calcium and/or magnesium in column effluents.

These results are in accord with the previous use of the reagent for that purpose (21) and with its use as an indicator in water hardness titrations (36). It has also been used for the determination of magnesium in alloys (37) and for the determination of calcium and magnesium in blood serum (38). Other uses of the reagent have been reviewed by Savvin (39).

In the preparation of Arsenazo I reagent, 121.1 g of THAM (Tris(hydroxymethyl)aminomethane) was dissolved in approximately 750 ml of distilled water. Sufficient concentrated hydrochloric acid was added to adjust the pH of the solution to 9.0 and the volume was made up to 1 liter using distilled water. 0.100 g of Arsenazo I was then added with stirring. The resulting solution could be used immediately and was stable at room temperature for a month or longer.

THAM was chosen as a buffer since it is pure, nonhydroscopic, and only weakly complexing (40). Additionally, it is nonvolatile and this is an advantage since the use of ammonia-based buffers may result in clogging of the helium supply lines with deposits of ammonium chloride and in corrosion of brass fittings.

### 3. PAR reagent

PAR (4-(2-Pyridylazo)-resorcinol) is one of the most versatile color-forming reagents available. It has been used or proposed for the colorimetric determination of many different metal ions including vanadium (V) (41), manganese (II) (42), iron (III) (43), cobalt (II) (44), nickel (II) (45), copper (II) (46), zinc (II) (42), lead (II) (47), gallium (III) (48), indium (III) (48), thallium (III) (48), osmium (IV) (49), palladium (II) (50), tantalum (V) (51), niobium (V) (52), scandium (III) (53), the rare earths (54), uranium (VI) (55), neptunium (V) (56), and thorium (IV) (57).

The first use of PAR as a color-forming reagent for the continuous spectrophotometric analysis of forced-flow chromatographic column effluents was reported by Sickafoose (46) who used it for the determination of copper (II) and zinc (II). PAR has since been employed in a similar fashion by Fritz and Story (21) for the continuous determination of lead (II), nickel (II), the rare earths, and zinc (II). Additionally, Kawazu and Fritz (20) and Kawazu, Shibata, and Kakiyama (58) have used it for the determination of cadmium (II), cobalt (II), copper (II), iron (III), lead (II), manganese (II), and zinc (II). PAR is probably the best single reagent available for the nonspecific detection of metal ions in forced-flow chromatographic column effluents due to its extreme sensitivity, immediacy of reaction, indifference to background electrolyte, and stability in solution.

In the preparation of PAR reagent 0.100 g of PAR (Eastman #7714) was dissolved with stirring in 1 ℓ of THAM-HCl buffer (pH 9). The buffer solution was prepared in an identical manner to that of the THAM-HCl buffer used in the preparation of Arsenazo I reagent. The solution of PAR reagent was $4.65 \times 10^{-4}$ $\underline{M}$ in PAR. It could be used immediately and was stable indefinitely.

### 4. PAR-ZnEDTA reagent

In order that small samples containing quite low levels of calcium and/or magnesium might be analyzed, it was deemed necessary to employ a color-forming reagent with greater sensitivity than that offered by Arsenazo I. Additionally, a reagent was sought that offered good sensitivity for the detection of strontium and barium. Various possibilities were considered and it was concluded that the use of a displacement reaction offered the greatest chance of success. Many such reactions, in which one ion displaces another from a complex thereby freeing the second ion to participate in a reaction, have been discussed by Perrin (59).

For the detection of the alkaline earths, it was decided to use a displacement reaction based on complexation by EDTA because EDTA forms strong complexes with the alkaline earths at higher pH values. It is also relatively inexpensive and readily available. Zinc was chosen as the ion to be displaced because it forms EDTA complexes intermediate in stability between those of the alkaline earths and most other metal ions (60). Additionally, detection of zinc with PAR is extremely sensitive (42).

Other examples of displacement reactions that have been utilized for the spectrophotometric determination of one or more of the alkaline earths include the use of Mg-EDTA and Calmagite to determine calcium (61), the use of Zn-EGTA and Zincon to determine calcium in the presence of magnesium (62), and the use of SR-EGTA and Eriochrome Black T to determine magnesium in the presence of calcium (63).

Use of a color-forming reagent based on the reaction of a metal ion with Zn-EDTA and PAR to form the EDTA complex of the metal ion and Zn-PAR resulted in a sensitive and accurate determination of the alkaline earths. Additionally, all of the metal ions that can be detected using PAR alone could be detected as well or better using the mixed reagent. The PAR-ZnEDTA reagent was kinetically fast enough to be used without a delay loop and was not affected by the presence of background electrolyte. It was stable in solution and easily prepared. The extent of the color-forming reaction is affected by pH and in practice it was found necessary to ensure that the pH of the final mixture of effluent and color-forming reagent was $\geq 9$ for sensitive, accurate detection of the alkaline earths. The PAR-ZnEDTA reagent was developed in the latter part of this work. It was used both for the analysis of serum samples and for the determination of column distribution coefficients of the alkaline earths.

A stock solution of Zn(EDTA) was prepared by reacting stoichiometric amounts of zinc and EDTA stock solutions. The correct ratio was determined by prior titration of the zinc stock solution with the EDTA stock solution using xylenol orange as indicator at pH 5. Several NaOH pellets were added to each liter of freshly prepared solution to raise the pH sufficiently to prevent the precipitation of the free acid form of EDTA. The Zn(EDTA) concentration of the final solution was $7.65 \times 10^{-3}$ $\underline{M}$.

50 ml of this solution was added to each liter of PAR reagent and the final solution was made 0.200 $\underline{M}$ in sodium hydroxide. The resulting solution of PAR-Zn(EDTA) reagent

was $4.43 \times 10^{-4}$ $\underline{M}$ in PAR and $3.64 \times 10^{-4}$ $\underline{M}$ in Zn(EDTA) and the pH was approximately 12.

### 5. Chromazural S reagent

Chromazurol S (3"-Sulfo-2",6"-dichloro-3,3'-dimethyl-4-hydroxyfuchsone-5,5'-dicarboxylic acid) has been used for the determination of aluminum in iron ores, sinters, and open hearth slags (64) and in steel, zinc, and galvinized coating (65). Other applications of the reagent have been listed by Bishop (66).

The reagent was used for the continuous spectrophotometric analysis of aluminum ions because of the excellent color contrast between the free (yellow-orange) and bound (violet) forms of the reagent. Extended use of the reagent resulted in coating of the lines and flow cell surfaces with the violet products of the color-forming reaction and it was necessary to periodically flush the lines with hydrochloric acid in order to remove this coating. The use of stabilizers and dispersing agents including Triton X-100, Tween 80, and methanol was tried but not extensively investigated in an effort to minimize formation of this coating.

### 6. Effective molar absorptivities of color-forming reagents

Experimental conditions employed in determining the effective molar absorptivities of the different color-forming reagents are given in Table III. In all cases, pH 9 THAM-HCl buffer was used to adjust the pH and all absorbance measurements were made using a Cary 16 double-beam spectrophotometer and 1- or 10-cm cells. Similarly, all measurements were run *versus* reagent blanks containing concentrations of color-forming reagent, buffer, and background electrolyte identical to those of the solution being measured.

Table III. Experimental conditions employed in determining effective molar absorptivities of different color-forming reagents

| $\lambda$ | Concentration of color-forming reagent | Concentration of metal ion | Concentration of background electrolyte |
|---|---|---|---|
| 490 nm | $4.2 \times 10^{-5}$ M PAR<br>$4.6 \times 10^{-5}$ M ZnEDTA | $2.00 \times 10^{-5}$ M<br>Mg, Ca, Sr, Ba | --- |
| 590 nm | $7.0 \times 10^{-5}$ M<br>Arsenazo I | $2.50 \times 10^{-5}$ M<br>Mg, Ca | --- |
| 590 nm | $1.24 \times 10^{-4}$ M<br>Arsenazo I | $2.00\text{-}4.00 \times 10^{-5}$ M<br>Mg, Ca | 0.8 M $NH_4Cl$ |
| 590 nm | $1.24 \times 10^{-4}$ M<br>Arsenazo I | $2.00\text{-}4.00 \times 10^{-5}$ M<br>Mg, Ca | 0.03 M en•2HCl |

Table IV. Effective molar absorptivities, $M^{-1}cm^{-1}$

| Metal Ions | Color-Forming Reagents | | | |
|---|---|---|---|---|
| | Arsenazo I (0.8 $\underline{M}$ $NH_4Cl$)[a] | Arsenazo I (0.03 $\underline{M}$ en. 2HCl)[a] | Arsenazo I | PAR-Zn(EDTA) |
| Mg | 1,900 | 4,600 | 8.300 | 17,000 |
| Ca | 870 | 1,700 | 6,800 | 22,000 |
| Sr | --- | --- | 2,500 | 19,000 |
| Ba | --- | --- | 1,400 | 18,000 |

[a] Values in parentheses refer to background electrolyte.

### 7. Wavelength of detection

Optimum wavelength settings for use with the different color-forming reagents were determined by injecting identical aliquots of metal ions under fixed conditions and measuring the resulting peak heights as a function of wavelength. Results of some of these studies are shown in Figures 7 and 8.

As can be seen from Figures 7-8, the range of wavelengths giving maximum sensitivity is somewhat smaller when using Arsenazo I as a color-forming reagent than when using PAR-ZnEDTA. This presumably is related to the greater shift in absorbance between the free and bound forms of PAR (yellow to red) compared to Arsenazo I (red-orange to violet). Based on these studies, a wavelength setting of 590 nm was chosen for use in conjunction with Arsenazo I and a setting of 495 nm for use with PAR-ZnEDTA.

## D. Choice of Eluents

In forced-flow chromatography with continuous spectrophotometric detection it is often advantageous to employ salt solutions as eluents rather than acidic solutions since it is then much easier to buffer the final pH of the mixture of effluent and color-forming reagent to any desired value. This makes for more convenient, more precise spectrophotometric detection, particularly in cases where close pH control of the color-forming reaction is required for maximum

Figure 7. Peak height of calcium and magnesium as a function of wavelength of detection using Arsenazo I color-forming reagent

Column: 1.9 meq/g low capacity catex;
7 cm x 0.4 cm

Eluent: 1 M $NH_4Cl$; 1.15 ml/min

Detection: Arsenazo I color-forming reagent;
0.5 ml/min

Sample: 51.4 µl; 179 µg Ca and 53.8 µg Mg

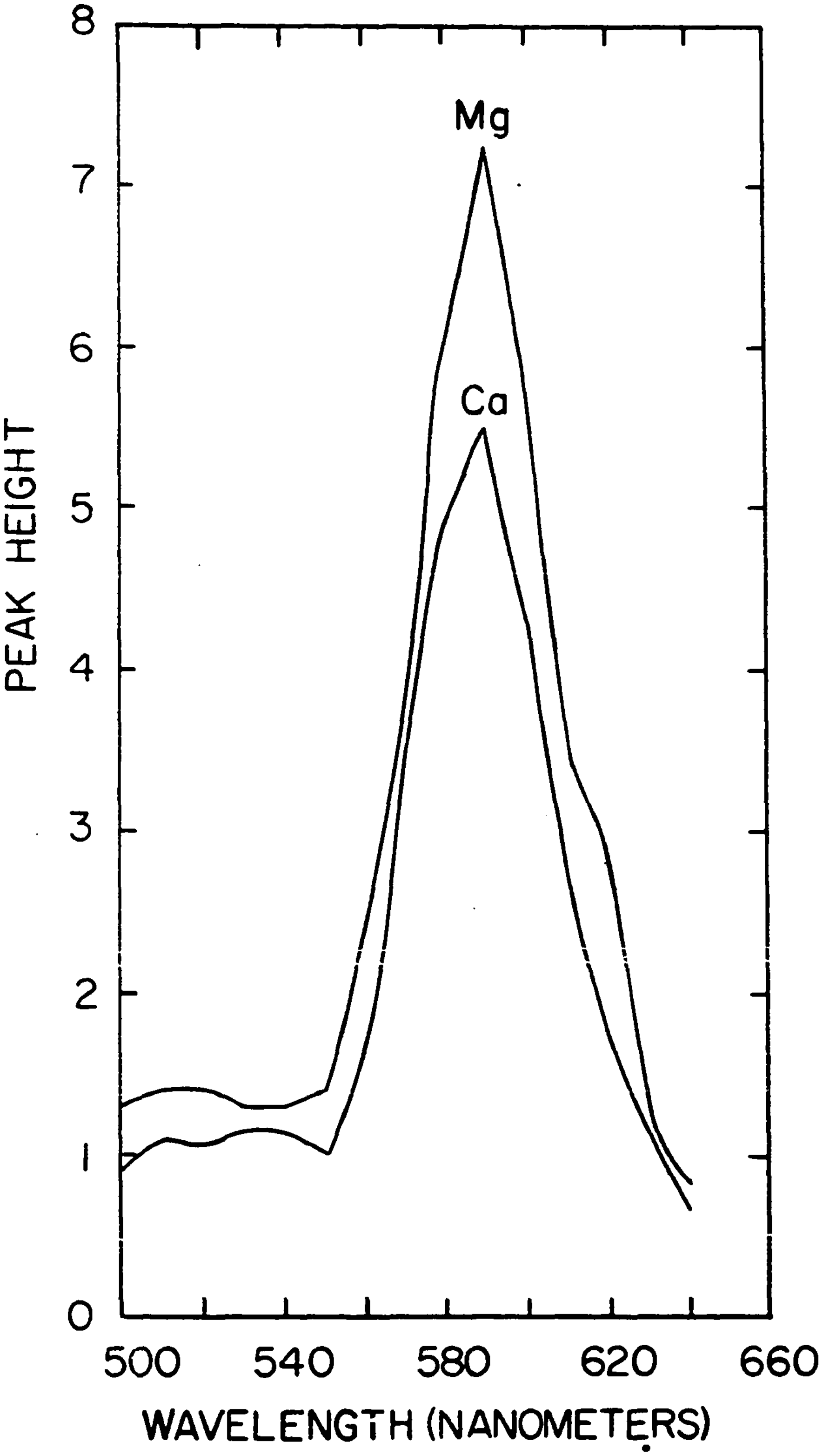

Mg
Ca
PEAK HEIGHT
0
1
2
3
4
5
6
7
8
500
540
580
620
660
WAVELENGTH (NANOMETERS)

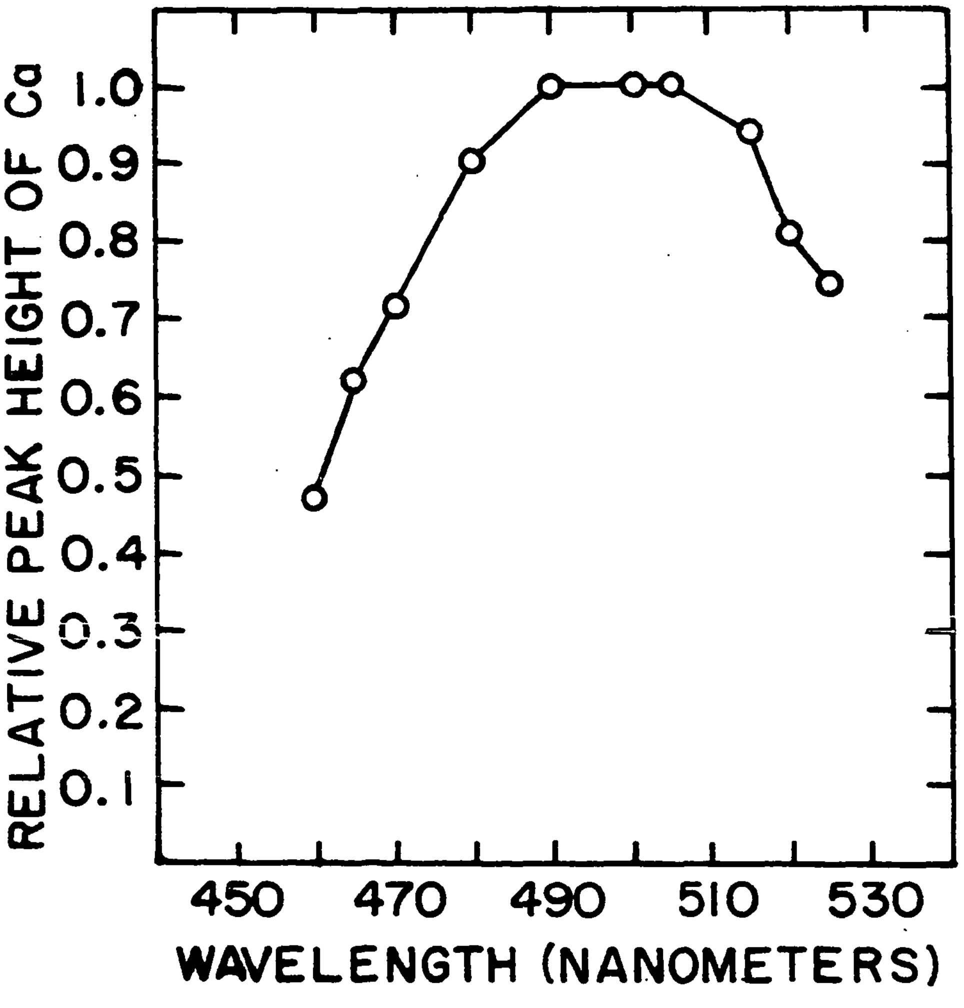
Figure 8. Relative peak height of calcium as a function of wavelength of detection using PAR-ZnEDTA color-forming reagent

Column: 2.0 meq/g low capacity catex; 7 cm x 0.4 cm

Eluent: 0.035 M en•2HCl; 2 ml/min

Detection: PAR-ZnEDTA color-forming reagent; 0.25 ml/min

Sample: 42.2 μl; 2.1 μg Ca


RELATIVE PEAK HEIGHT OF Ca
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
450
470
490
510
530
WAVELENGTH (NANOMETERS)

sensitivity. Additionally, it is possible to control the flow rates of eluent and color-forming reagent independently of each other over a relatively broad range while still maintaining a desired final pH. Accordingly, it was decided to employ salt solutions as eluents wherever possible in this work.

### 1. Ammonium chloride

An experiment was performed in which solutions of various salts were evaluated for possible use of eluents in the separation of calcium and magnesium on low-capacity cation exchangers. This was done by using the same concentration of various salts to elute identical aliquots of a mixture of calcium and magnesium under fixed conditions. Results of this study are shown in Table V together with results obtained with a solution of hydrochloric acid. Based on these results, it was concluded that of the different salts studied, ammonium chloride appeared to be the best choice from the standpoints of detection, $D_{Mg}$, $D_{Ca}$, and $\alpha_{Mg}^{Ca}$. Accordingly, solutions of ammonium chloride were chosen for use as eluents in the initial phase of this work.

### 2. Ethylenediammonium chloride

Fritz and Karraker (67) were the first to use ethylenediammonium chloride solutions as eluents in ion-exchange

Table V. Comparison of various eluents for the separation of Ca and Mg

| Eluent | $D_{Mg}$ | $D_{Ca}$ | $\alpha^{Ca}_{Mg}$ | Comments |
|---|---|---|---|---|
| 0.6 M HCl | 7.6 | 36 | 4.7 | --- |
| 0.6 M LiCl | 30 | >75 | --- | Poor detection of Mg, Ca not eluted |
| 0.6 M NaCl | 13 | 64 | 4.9 | Considerable tailing of both Ca and Mg |
| 0.6 M $NaNO_3$ | 13 | 49 | 3.8 | Considerable tailing of both Ca and Mg |
| 0.6 M $NH_4Cl$ | 8.0 | 32 | 4.0 | --- |
| 0.6 M $NH_4NO_3$ | 8.6 | 27 | 3.1 | --- |

chromatography. They used 0.1 M ethylenediammonium chloride to separate zinc from lanthanum on Dowex 50 X 8 and then titrated the eluted zinc with EDTA using a visual indicator. They also pointed out some of the advantages of ethylenediammonium chloride as an eluent. These included being able to use a much lower concentration of ethylenediammonium chloride to elute zinc than is possible with simple mineral acids and also the noninterference of the ethylenediammonium ion with subsequent analytical operations.

Fritz and Karraker (68) also used ethylenediammonium perchlorate to separate divalent ions from trivalent ions. They eluted most divalent ions with 0.1 M ethylenediammonium perchlorate and then stripped the trivalent ions with 0.5 M ethylenediammonium perchlorate. The separated ions were subsequently analyzed by EDTA titrations.

Ethylenediammonium chloride solutions offer several advantages for use as eluents in forced-flow cation exchange chromatography of doubly charged metal ions.

The first of these advantages derives from the similar affinity for the cation-exchange resin of the +2 ethylenediammonium ion compared to the affinities of the +2 metal ions. Because of these similar affinities, it is possible to rapidly elute +2 metal ions from the resin with small volumes of eluent containing low concentrations of ethylenediammonium chloride. Much more concentrated solutions are required using eluents containing +1 ions such as hydrogen or ammonium since +1 ions have much smaller affinities for the resin than do +2 metal ions. Thus, it is possible to use 0.035 M ethylenediammonium chloride in place of 1 M ammonium chloride for the rapid elution of calcium and magnesium from low capacity cation exchangers.

A second advantage relates to the noninterference of the ethylenediammonium ion with detection of the eluted metal ions by means of their reaction with color-forming reagents. Here the ability to use quite dilute solutions as eluents is also important because background caused by metal ion impurities in the eluent is minimized. Additionally, such dilute solutions are easily buffered to any desired final pH required for successful color formation. Finally, the ethylenediammonium chloride can be easily and economically prepared in pure form.

# IV. RESULTS AND DISCUSSION

## A. Distribution Coefficients

Both batch and column distribution coefficients were measured using established procedures (69). For the determination of column distribution coefficients, the amount of metal ion injected was chosen so that no more than 3% and in most cases less than 1% of the total available column capacity was used. The column dead volume, $V_m$, was determined by injecting concentrated hydrochloric acid and measuring the volume in which it eluted. The unretained hydrochloric acid was detected by the pronounced change in absorbance which resulted from its passage through the detector. Different metal ions were detected by means of appropriate color-forming reagents. The alkaline earths were detected using Arsenazo I and PAR-Zn EDTA, aluminum was detected using Chromazural S, and the remaining metal ions were detected using PAR and PAR-Zn EDTA. Column distribution coefficients were computed in the usual way by means of the expression:

$$D = \frac{V_r - V_m}{W}$$

where D is the distribution coefficient, $V_r$ is the peak retention volume, $V_m$ is the dead volume, and W is the weight of dry resin.

For the determination of batch distribution coefficients, the amount of metal ion partitioned was chosen to be no more than 10% of the total available resin capacity. Equilibration was carried out by shaking a weighed amount of resin with a measured volume of solution containing a known concentration of metal ion. After equilibration, the resin was filtered off and the concentration of metal ion remaining in solution was determined by atomic absorption spectroscopy. Batch distribution coefficients were then calculated by means of the expression:

$$D = \frac{\text{amount of ion/g of resin}}{\text{amount of ion/ml of solution}}$$

Column distribution coefficients for calcium, magnesium, and a representative selection of other metal ions on 1.9 meq/g low capacity cation exchanger as a function of ammonium chloride concentration are shown in Figures 9-10 and Figures 12-16. Calcium/magnesium separation factors on the same resin as a function of ammonium chloride concentration are shown in Figure 11. Distribution coefficients and separation factors on AG 50W-X8 were taken from Strelow and Weinert (12) and are shown for the purpose of comparison. Column distribution coefficients for calcium, magnesium, and several other divalent metal ions on 2.0 meq/g low capacity cation exchanger as a function of ethylenediammonium chloride concentration are shown in Figure 17.

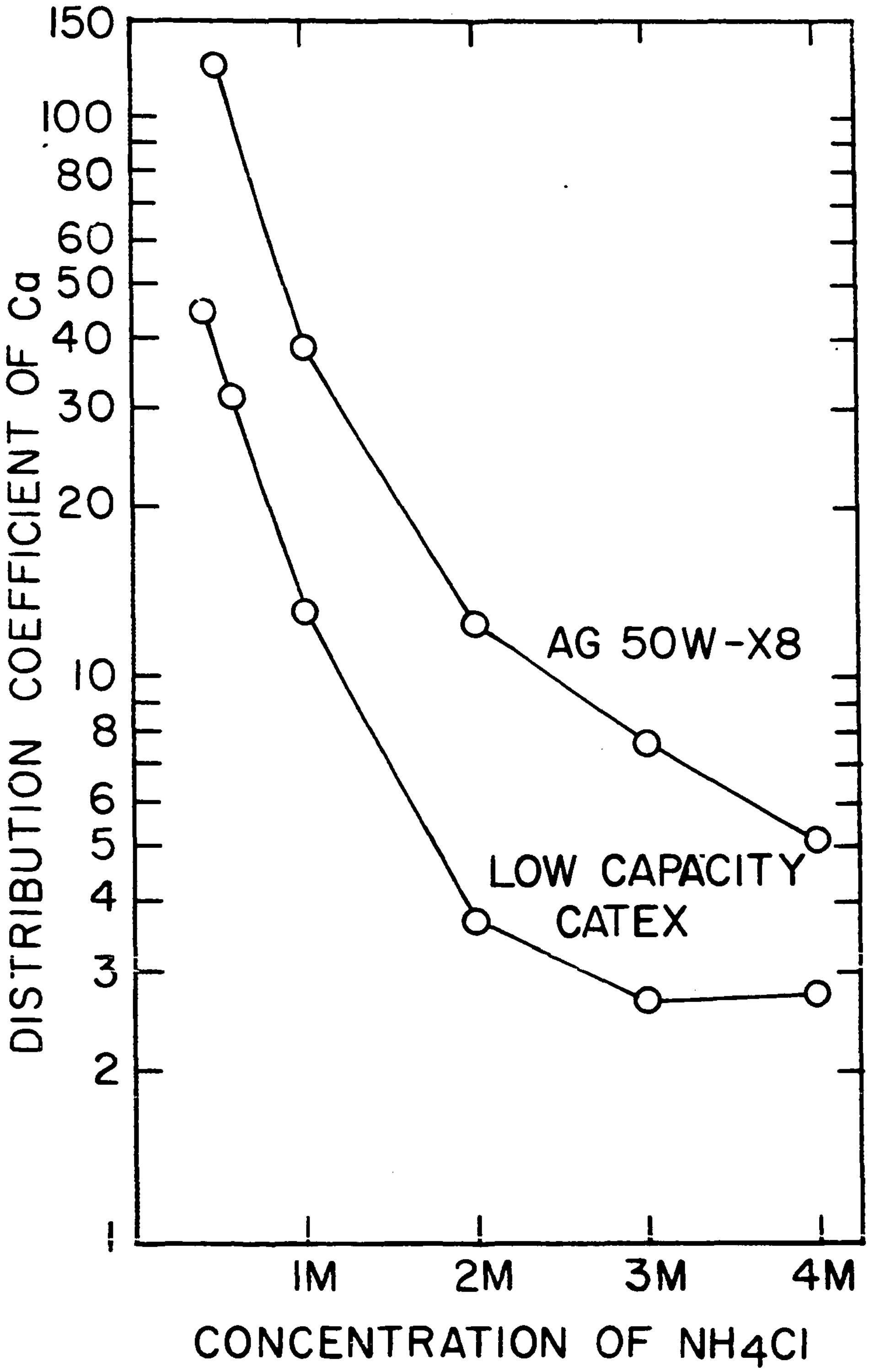


Figure 9. Distribution coefficient of calcium on AG 50W-X8 and 1.9 meq/g low capacity catex as a function of ammonium chloride concentration

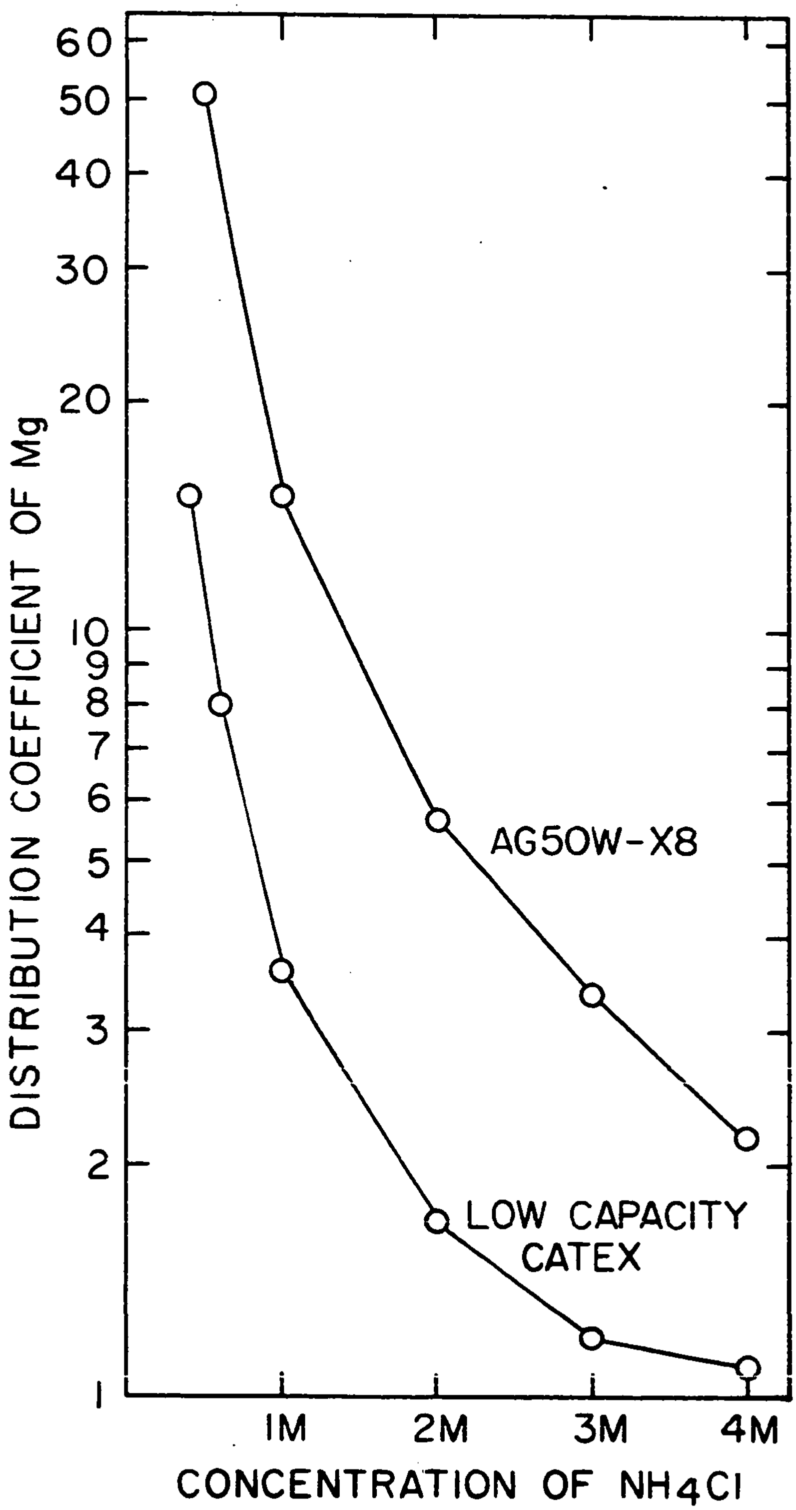


Figure 10. Distribution coefficients of magnesium on AG 50W-X8 and 1.9 meq/g low capacity catex as a function of ammonium chloride concentration

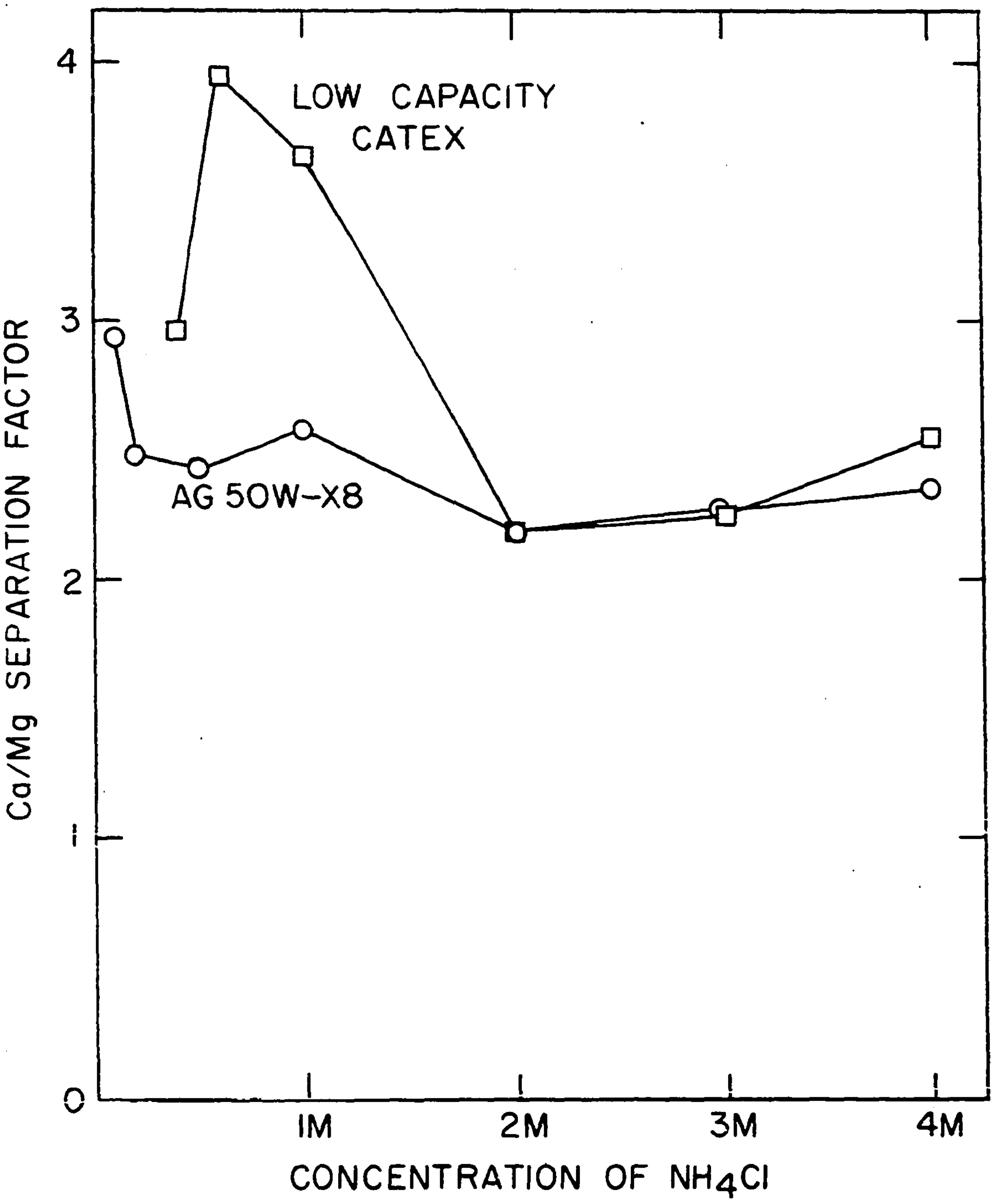


Figure 11. Calcium/magnesium separation factors on AG 50W-X8 and 1.9 meq/g low capacity catex as a function of ammonium chloride concentration

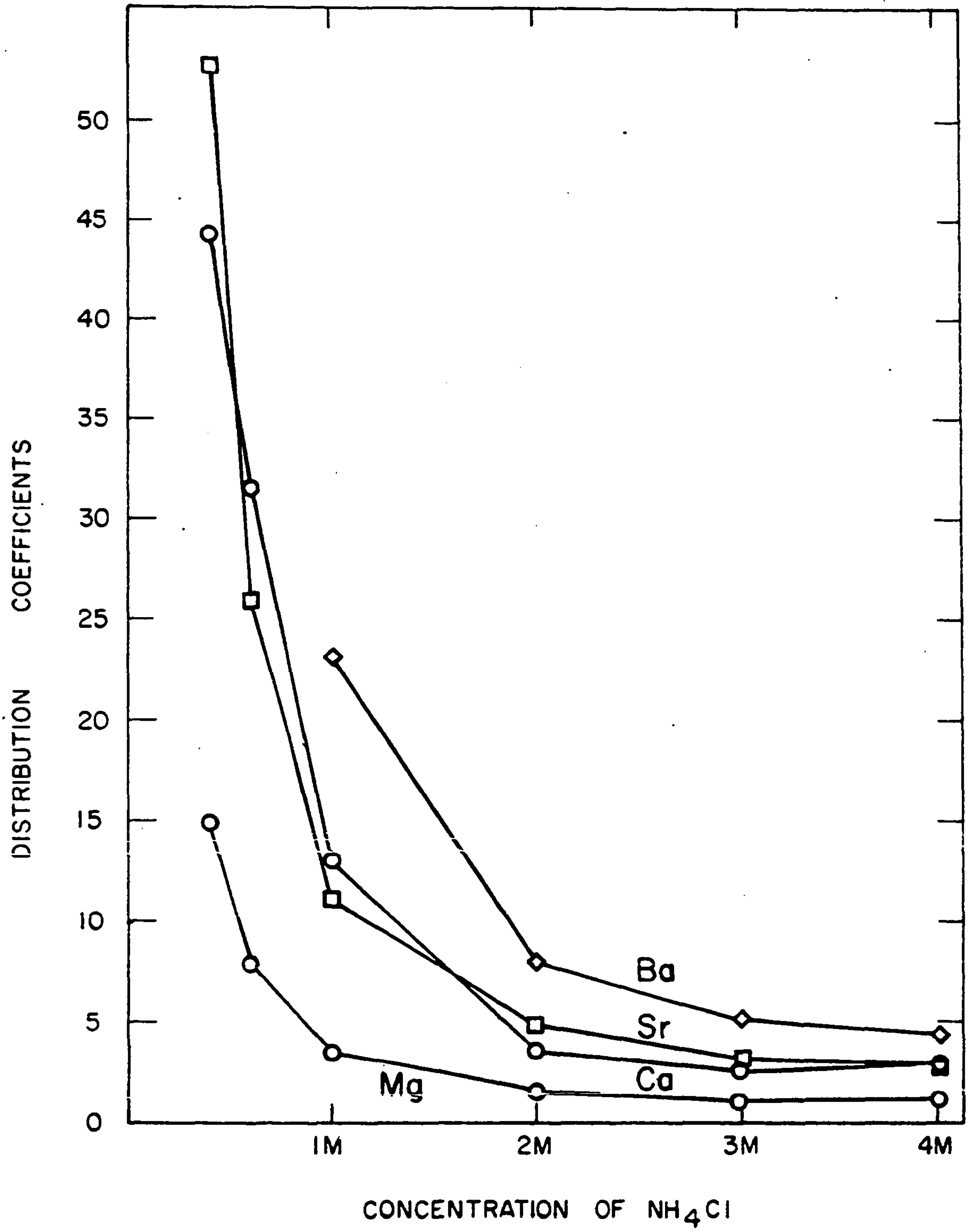


Figure 12. Distribution coefficients of calcium, magnesium, strontium, and barium on 1.9 meq/g low capacity catex as a function of ammonium chloride concentration

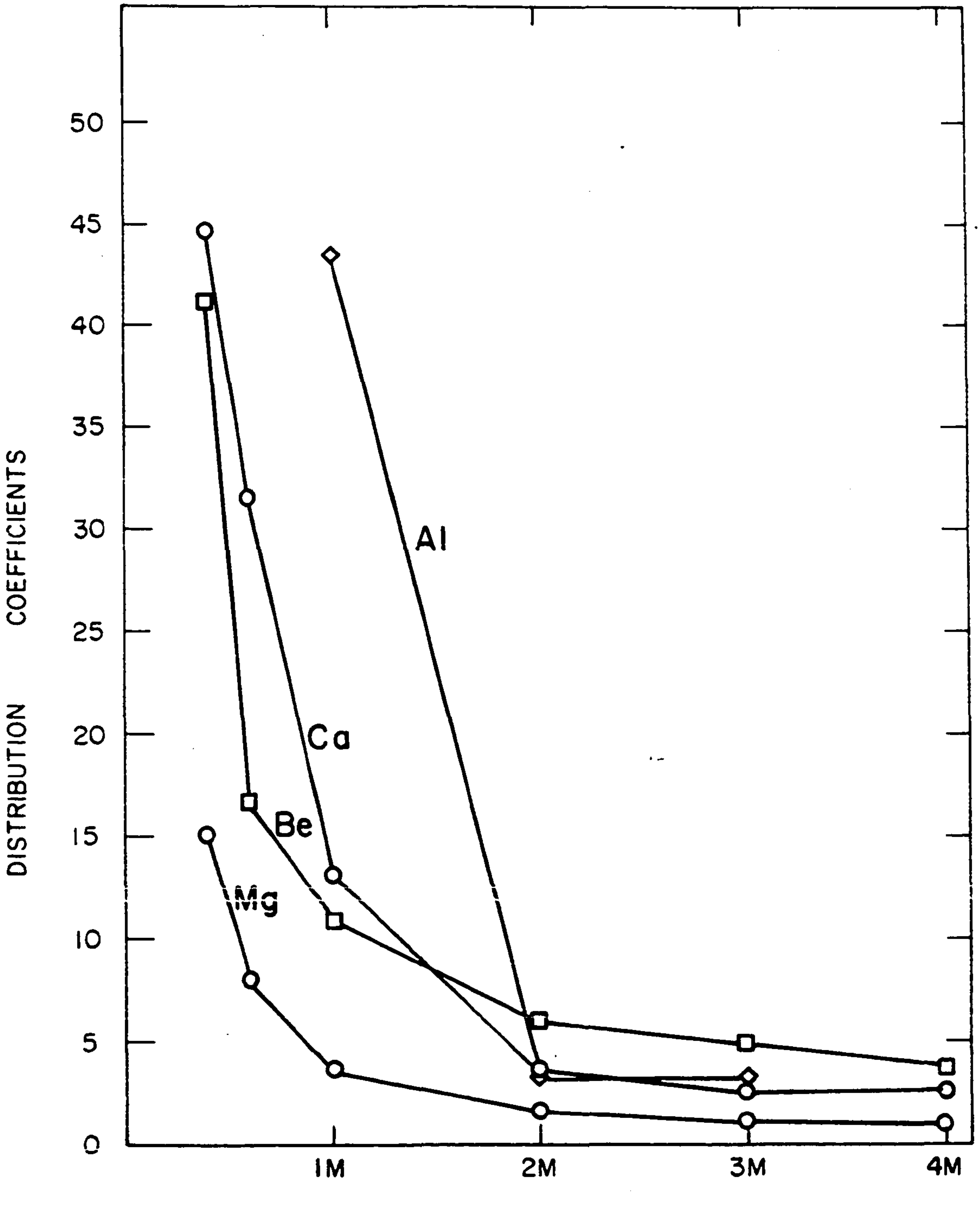


Figure 13. Distribution coefficients of calcium, magnesium, beryllium, and aluminum on 1.9 meq/g low capacity catex as a function of ammonium chloride concentration

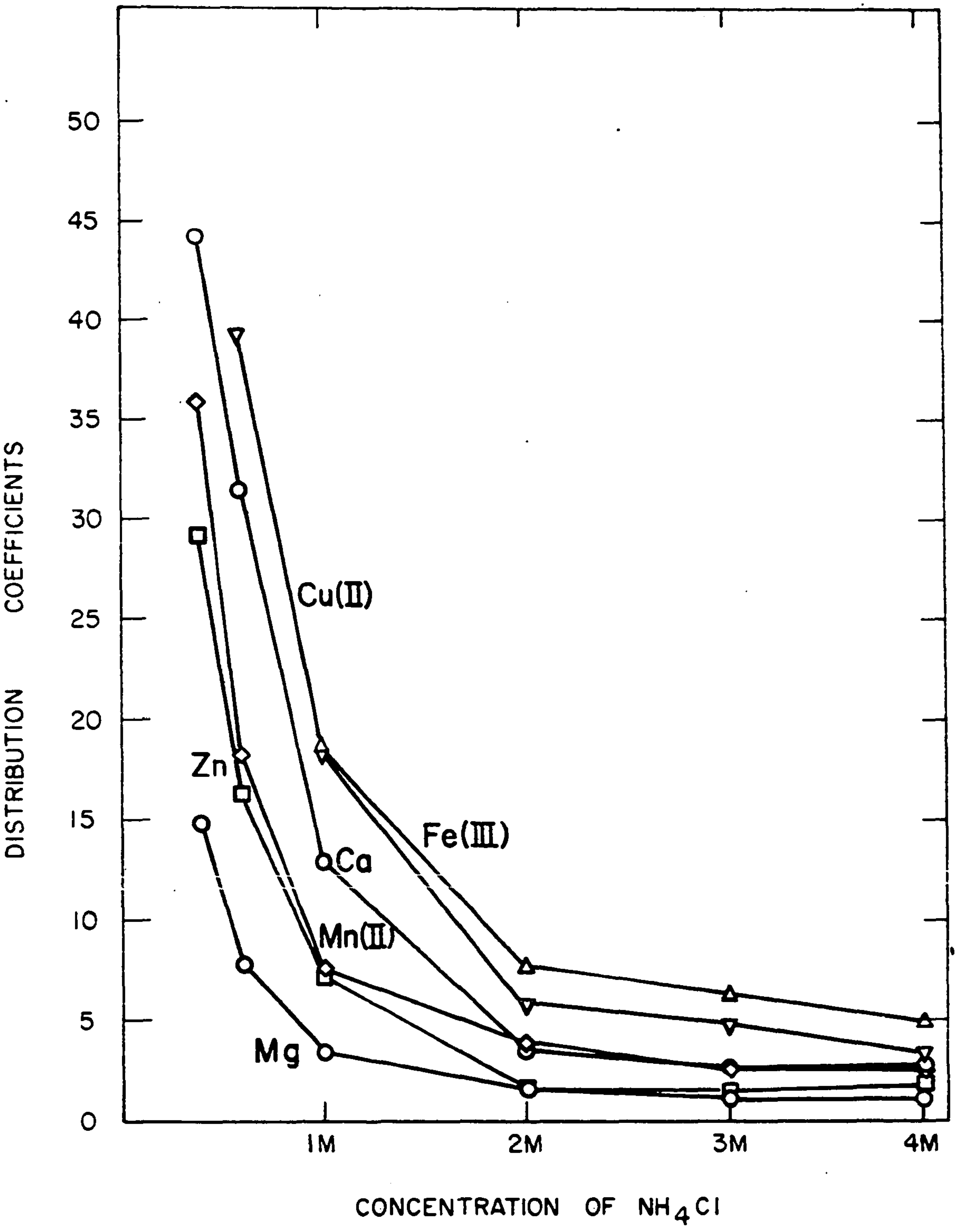


Figure 14. Distribution coefficients of calcium, magnesium, manganese (II), iron (III), copper (II), and zinc of 1.9 meq/g low capacity catex as a function of ammonium chloride concentration

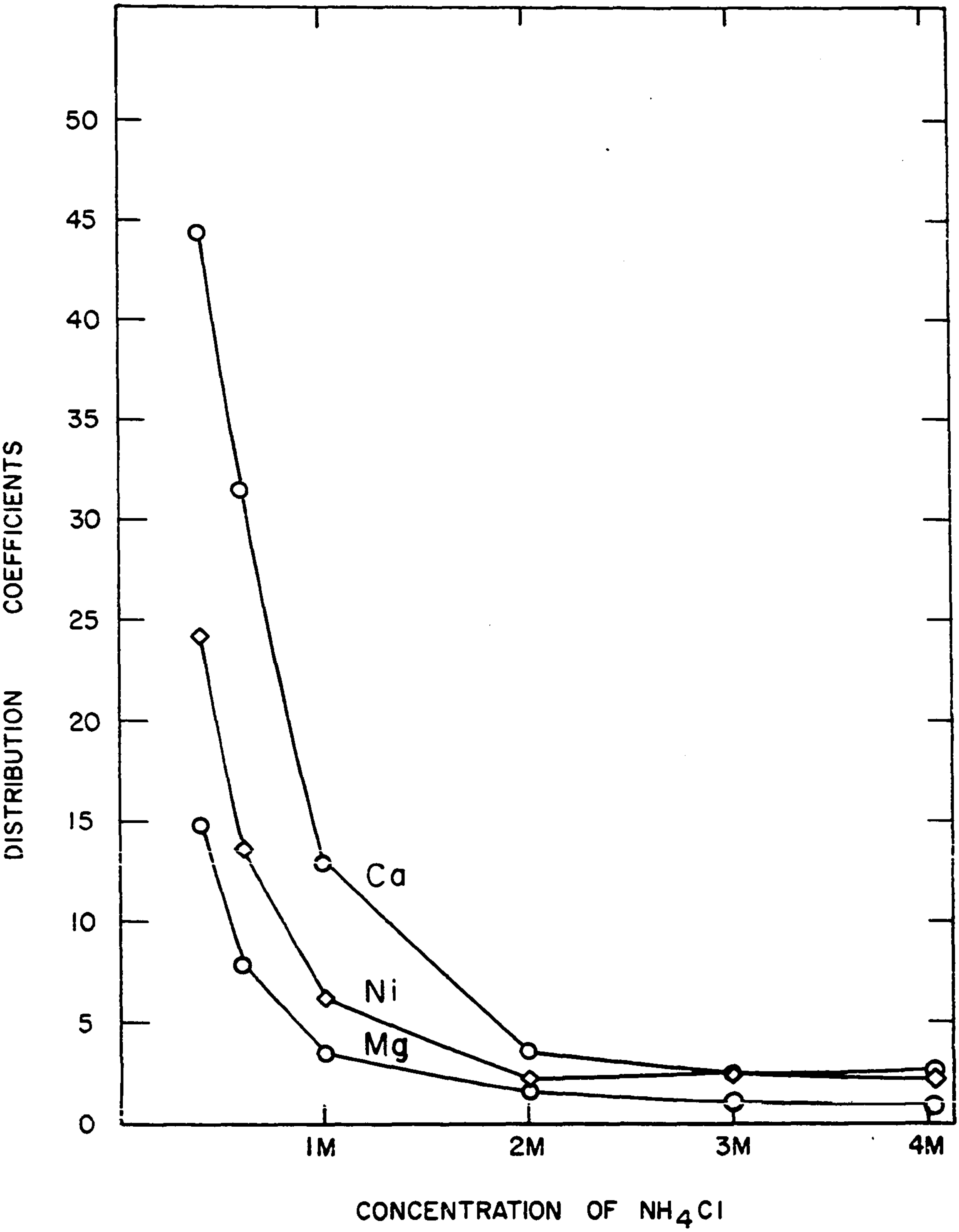


Figure 15. Distribution coefficients of calcium, magnesium, and nickel on 1.9 meq/g low capacity catex as a function of ammonium chloride concentration

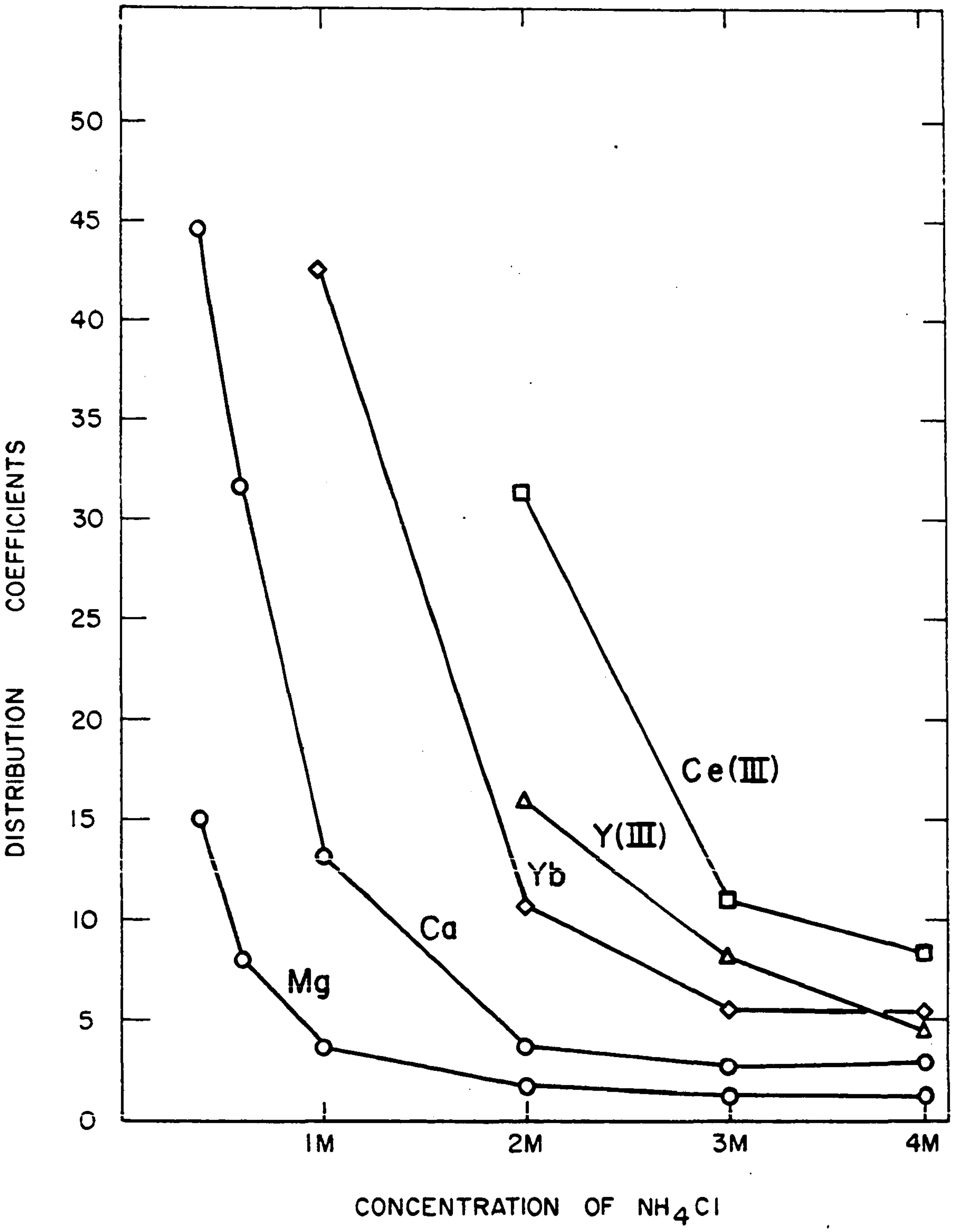


Figure 16. Distribution coefficients of calcium, magnesium, ytterbium, yttrium, and cerium (III) on 1.9 meq/g low capacity catex as a function of ammonium chloride concentration

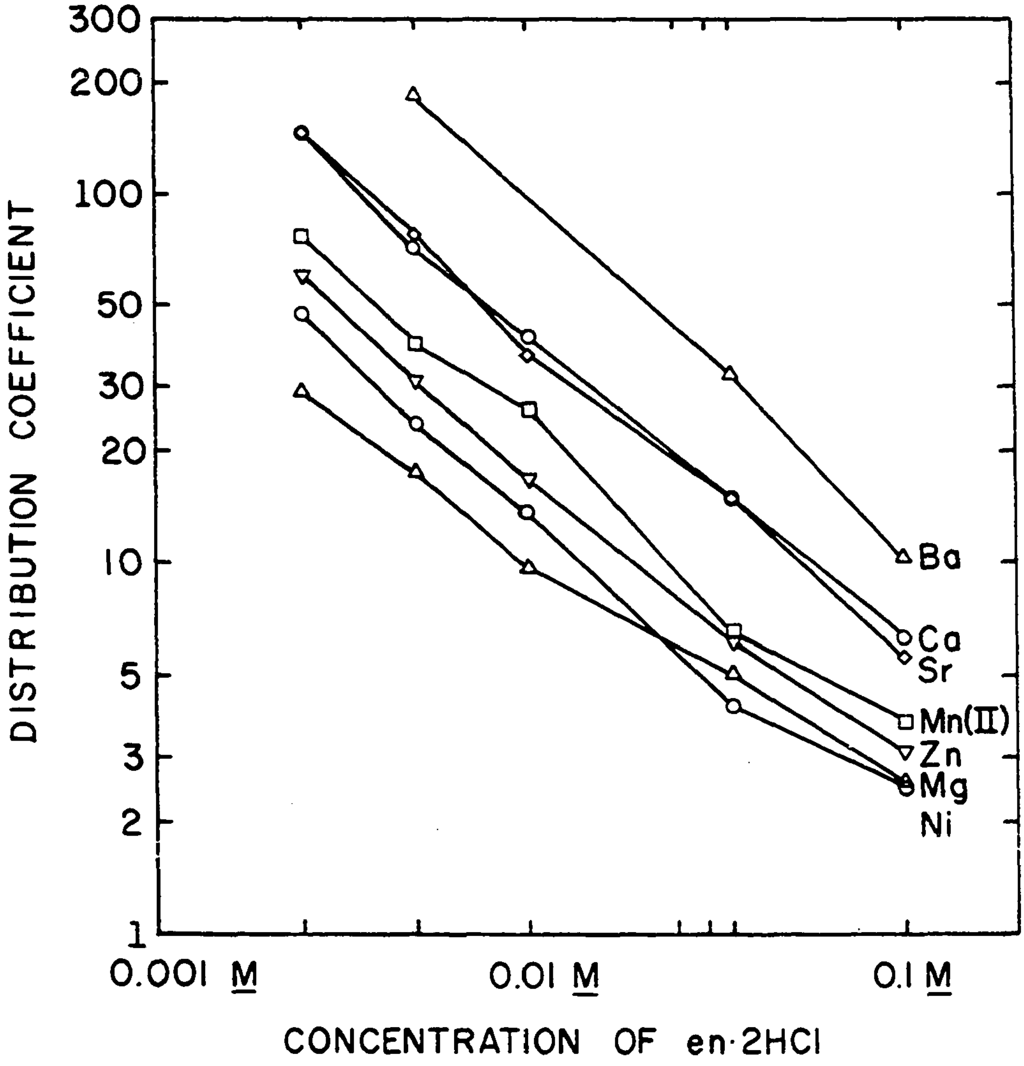


Figure 17. Distribution coefficients of several divalent metal ions on 2.0 meq/g low capacity catex as a function of ethylenediammonium chloride concentration

## B. Operating Conditions

The importance of various factors in establishing optimal operating conditions for liquid chromatography has been discussed by several workers.

Kirkland (70) reviewed preferred experimental conditions for trace analysis by liquid chromatography and emphasized the importance of sampling technique, detection method, and sample pretreatment. Martin *et al*. (71) discussed the current trend in liquid chromatography toward the use of shorter columns packed with fine particles and operated at low inlet pressures. They discussed optimization in terms of flow rate, particle diameter, and analysis time. Martin *et al*. (72) also discussed the importance of optimal equipment design and showed how peak broadening is affected by such factors as the volume of sample injected, the amount of connecting tubing, the detector cell volume, and the detector response time.

In this work, effort was directed towards optimizing both the time of analysis and the sensitivity of detection. Analysis time was optimized by using short columns and fast eluent flow rates. With a 7-cm column and elution at a flow rate of 2 ml/min, it was possible to cut the time of analysis for both calcium and magnesium to 10 minutes per sample. This was done using single-stage elution, *i.e.*, using a single eluent of fixed concentration chosen to elute both calcium and magnesium sequentially. Stepwise elution with dilute eluent to first remove the magnesium followed by more concentrated eluent to rapidly strip the calcium resulted in a further reduction of analysis time to less than 5 minutes per sample. A comparison of the two different methods of elution is shown in Figure 18. All quantitative analyses were performed using single-stage elution of calcium and magnesium since this method involved the least amount of instrumental manipulation and also provided the best reproducibility.

Stepwise elution is most useful in cases in which all ions are tightly sorbed to the upper end of a column because different stepwise changes can then be performed sequentially without disturbing the remaining ions. In such situations, the time of each stepwise change is not particularly important because it does not affect the elution behavior of the remaining ions. In the present case, however, both the calcium and magnesium ions move down the column from the moment of injection. As a result, the time of any stepwise change to a more concentrated eluent in order to rapidly strip the calcium becomes important since the position of calcium on the column and hence its elution behavior (*i.e.*, peak height) depend on the precise time when the change is performed. Too rapid a stepwise change results in a taller, more narrow peak whereas too slow a stepwise change results

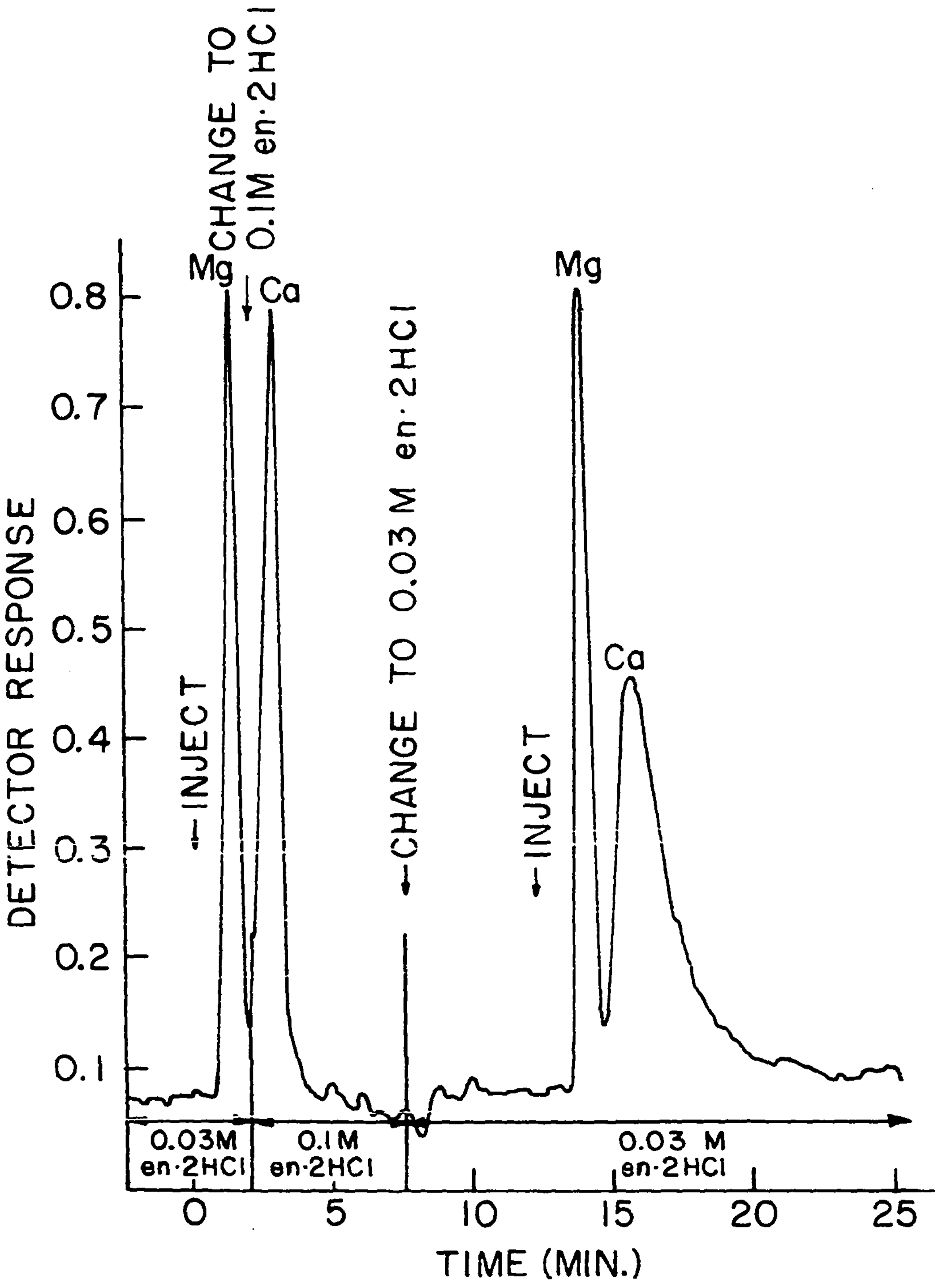
Figure 18. Comparison of stepwise and single stage elution of calcium and magnesium

Column: 2.0 meq/g low capacity catex;
7 cm x 0.4 cm

Eluents: 0.03 M en•2HCl; 2 ml/min
0.1 M en•2HCl; 2 ml/min

Detection: Arsenazo I color-forming reagent;
0.5 ml/min; 590 nm

Sample: 38.1 µl; 4.63 µg Mg and 30.5 µg Ca


DETECTOR RESPONSE
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
TIME (MIN.)
0
5
10
15
20
25
←INJECT
Mg
CHANGE TO 0.1M en·2HCl
Ca
←CHANGE TO 0.03 M en·2HCl
←INJECT
Mg
Ca
0.03M en·2HCl
0.1M en·2HCl
0.03 M en·2HCl

in a shorter, broader peak. It is difficult to reproduce stepwise changes manually to better than a few seconds either way and the reproducibility of peak heights decreases as a result.

This limitation of stepwise elution can, however, be overcome simply by employing a more automated approach. One of the chief advantages of any automated approach is that it oftentimes permits the reproducible use of conditions that are difficult or impossible to reproduce manually. Thus, by designing a chromatograph dedicated solely to the repetitive analysis of calcium and magnesium, the capability of automatically changing from one eluent to the next at a precise time after injection could be provided through the use of suitable timers and valves. Since the time of each stepwise change would be highly reproducible, the elution behavior and hence the peak height of calcium would also be reproducible.

In forced-flow chromatography with continuous spectrophotometric detection, the sensitivity and linearity of detection depend on many different factors (46). These factors include the flow rate of the eluent, the flow rate of the color-forming reagent, and the concentration of the color-forming reagent. Because of the complex interactions between the various factors, the most practical approach is first to develop suitable conditions for the desired separation using any convenient conditions for detection.

Once suitable eluent flow rates and concentrations have been established, the flow rate and concentration of color-forming reagent can be optimized to give maximum sensitivity and linearity.

This approach was used in this work. It was found that Arsenazo I color-forming reagent at a flow rate of 0.5-0.9 ml/min and a concentration of 0.1 mg/ml gave good detection in conjunction with ammonium chloride eluents at a flow rate of 2 ml/min. PAR-ZnEDTA at a flow rate of 0.25 ml/min and a concentration of $5 \times 10^{-4}$ $\underline{M}$ gave excellent detection in conjunction with ethylenediammonium chloride eluents at a flow rate of 2 ml/min. The sort of sensitivity that could be obtained using PAR-ZnEDTA is shown in Figure 19.

Some operating hints are:

1) It is sometimes advantageous to keep the Heath spectrophotometric detector setup operating continuously since this eliminates otherwise lengthy warmup times and also makes for more stable operation.

2) Columns should occasionally be unpacked and the resin slurried in acetone. The columns can then be repacked with the same resin after decanting the fines. This procedure serves to prevent the buildup of fines which may otherwise lead to excessive column back pressures and also helps to counteract the tendency of the resin to pack down somewhat with continued use.

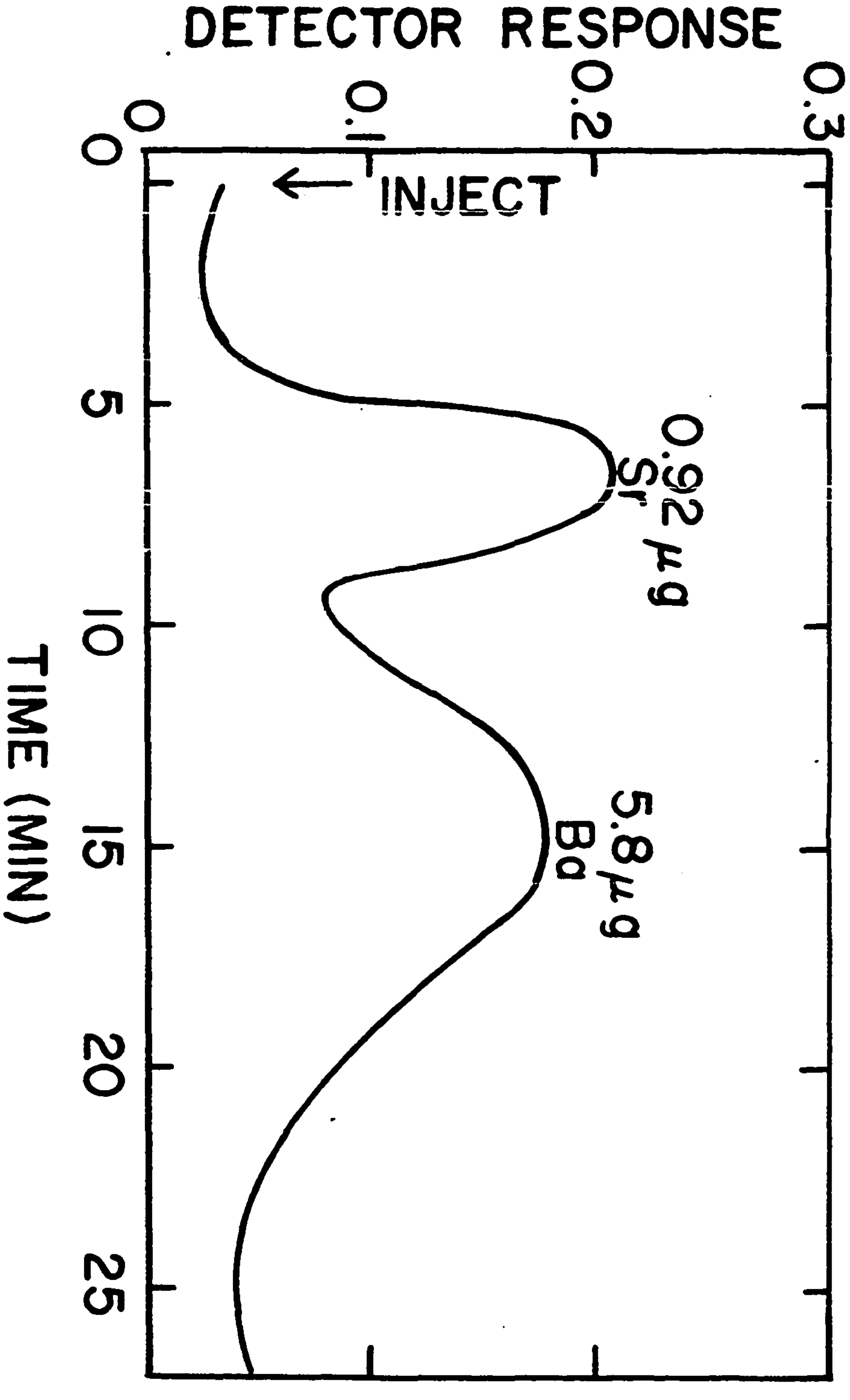
Figure 19. Partial separation of small amounts of strontium and barium

Column: 2.0 meq/g low capacity catex; 7 cm x 0.4 cm

Eluent: 0.035 $\underline{M}$ en·2HCl; 1 ml/min

Detection: PAR-ZnEDTA color-forming reagent; 0.3 ml/min

Sample: 42.4 μl


DETECTOR RESPONSE
0
0.1
0.2
0.3
INJECT
0.92 μg
Sr
5.8 μg
Ba
0
5
10
15
20
25
TIME (MIN)

3) Baseline shifts, such as sometimes occur when changing from one eluent to another, can often be corrected for by means of the Heath offset module. This is done by first changing from one eluent to another and then determining the correct amount of positive or negative offset voltage necessary to bring the baseline back to zero. Once the correct offset voltage for each eluent change has been determined, it can simply be switched in each time the eluent change is made.

## C. Analyses

### 1. Samples containing calcium and magnesium alone or in combination with noninterfering cations and anions

Alkali metal ions and ammonium ions are not detected using either Arsenazo I or PAR-ZnEDTA color-forming reagents. Virtual resolution of calcium and magnesium from these ions is therefore possible and mixtures of calcium and magnesium with these ions were analyzed directly.

7-cm x 0.4-cm columns of 1.8-1.9 meq/g low capacity cation exchanger were used for separation and the samples were eluted with 0.8-1.0 M ammonium chloride at a flow rate of 2 ml/min. Arsenazo I color-forming reagent at a flow rate of 0.5 ml/min was used for detection. Sample loop size was 51.4 µl. Time between injections was 10-12.5 minutes. Calibration was accomplished by means of calibration curves. The calibration curves were constructed by injecting standards containing known amounts of calcium (0-50 μg) and magnesium (0-30 μg) and plotting the resulting peak heights against the amounts of calcium and magnesium injected. Three or more standards were used to construct each calibration curve.

Those samples containing a ten-fold molar excess of calcium over magnesium or magnesium over calcium were first analyzed for the minor component using more dilute ammonium chloride solutions as eluents in order to obtain adequate resolution of the minor component from the major component. 0.4 M ammonium chloride at a flow rate of 2 ml/min was used to resolve calcium from a ten-fold molar excess of magnesium. Under these conditions, calcium was completely eluted in 25 minutes. 0.3 M ammonium chloride at a flow rate of 2 ml/min was used to resolve magnesium from a ten-fold molar excess of calcium. Under these conditions, magnesium was completely eluted in 5 minutes. The uneluted calcium could then be rapidly stripped from the resin using more concentrated ammonium chloride solutions. In order to analyze for the major component, it was only necessary to accurately dilute the samples by a factor of ten. The major component was then analyzed using a procedure similar to that first described.

### 2. Samples containing calcium and magnesium in combination with strontium and barium

Strontium and barium give quite weak detection using Arsenazo I color-forming reagent and ammonium chloride eluents. It was therefore possible to obtain "virtual" resolution of calcium and magnesium from small amounts of strontium and equimolar amounts of barium simply by eluting calcium and magnesium directly. A 7-cm x 0.4-cm column of 1.9 meq/g low capacity cation exchanger was used for separation and the samples were eluted with 0.8-1 M ammonium chloride at a flow rate of 2 ml/min. Arsenazo I color-forming reagent at a flow rate of 0.5 ml/min was used for detection. Sample loop size was 51.4 µl. Total analysis time was 10 minutes per sample. Calibration was by means of calibration curves. The calibration curves were constructed by injecting standards containing known amounts of calcium (0-50 µg) and magnesium (0-30 µg) and plotting the resulting peak heights against the amounts of calcium and magnesium injected. Three or more standards were used to construct each calibration curve.

### 3. Choice of eluents for the selective elution of interfering ions

Based on an examination of their distribution coefficients, it was apparent that certain metal ions would seriously interfere with the determination of calcium and magnesium due both to the similarity of their distribution coefficients to those of calcium and magnesium and the fact that they are strongly detected by both Arsenazo I and PAR-ZnEDTA. Nickel is one of the most serious interferences of this kind.

Accordingly, it was necessary to devise methods for the selective separation of interfering ions prior to the elution and determination of calcium and magnesium. This was relatively easy to do using forced-flow chromatography with continuous spectrophotometric detection since various methods which have been used or proposed for the selective separation of different ions can be rapidly evaluated and the best approach to take quickly determined.

Based on their known chemical behavior, the different interfering ions were divided into groups and conditions were found for the selective separation of each group prior to the elution and determination of calcium and magnesium.

The different groups and the methods used for their selective separation were as follows:

| IONS | METHOD |
|---|---|
| Be, Al | Selectively eluted with sulfosalicylic acid |
| Mn(II), Fe(III), Cu(II), Zn, etc. | Selectively eluted with HCl-acetone |
| Ni | Selectively eluted with HCl-acetone-DMG |

Batch distribution coefficients of calcium and magnesium were measured in the different solutions used for the selective elution of interfering ions. The results of this study are shown in Table VI together with distribution coefficients of some of the interfering ions.

The batch distribution coefficients were determined by equilibrating 1.00 g of air-dry resin (1.8 meq/g, 250-325 mesh) with 50 ml of solution containing 0.2 meq of calcium or magnesium. After shaking for several hours on a wrist action shaker, the resin was filtered off and the amount of ion remaining in solution was determined. Each solution was analyzed by standard atomic absorption methods after appropriate pretreatment. The sulfosalicylic acid solutions were diluted ten-fold and run directly versus standards containing the same concentration of sulfosalicylic acid. The HCl-acetone and HCl-acetone-DMG solutions were evaporated to dryness under heat lamps and the residue ashed by baking with a small amount of concentrated nitric acid. The remaining ash was taken up in distilled water and analyzed directly for calcium or magnesium. Distribution coefficients were then calculated by means of the formula:

$$D = \frac{\text{amount of ion/g of resin}}{\text{amount of ion/ml of solution}}$$

Table VI. Distribution coefficients of metal ions in eluents used for the separation of interfering ions

| Eluent | Distribution Coefficients | | |
|---|---|---|---|
| Sulfosalicylic acid pH 3.2-3.6 | $D_{Mg} = 64$ | $D_{Ca} = 260$ | $D_{Be}$ small<br>$D_{Al}$ small |
| 0.4 N HCl-95% acetone | $D_{Mg} = 135$ | $D_{Ca} = 1550$ | $D_{Zn} = 2.2$<br>$D_{Mn(II)} = 3.8$<br>$D_{Fe(III)} = 2$ |
| 0.4 N HCl-95% acetone -0.25 M DMG | $D_{Mg} = 73$ | $D_{Ca} = 930$ | $D_{Ni} = 4.3$ |

### 4. Samples containing calcium and magnesium in combination with beryllium and aluminum

Using 0.19 M sulfosalicylic acid solutions at pH 6 to 7, Fritz and Palmer (73) were able to elute quantitatively aluminum (III), iron (III), titanium (IV), uranium (VI), vanadium (IV), and zirconium (IV) from 4- to 8-cm columns of Dowex 50W-X8. Other bivalent metal ions and rare earths including cobalt (II), cadmium (II), lanthanum (III), lead (II), magnesium (II), nickel (II), ytterbium (III), yttrium (III), and zinc (II), were quantitatively retained under these conditions. A similar approach was used in this work for the selective elution of aluminum and beryllium prior to the elution of calcium and magnesium.

Various concentrations of sulfosalicylic acid in the pH range of 0.7 to 7.5 were tested for use as eluents and it was determined that 0.1 M sulfosalicylic acid neutralized to a pH of 3.2 to 3.6 with ammonia was suitable for separating beryllium (II) and aluminum (III) from equimolar amounts of calcium (II) and magnesium (II).

Samples containing calcium and magnesium in combination with beryllium and aluminum were analyzed using a 7 cm x 0.4 cm column of 1.9 meq/g low capacity cation exchanger. Beryllium and aluminum were selectively eluted with 0.1 M sulfosalicylic acid (pH 3.6) at a flow rate of 3.5 ml/min. 2.5 minutes after injection, the eluent was switched to 0.8 M ammonium chloride at a flow rate of 2 ml/min in order to elute the calcium and magnesium. Total analysis time, including 2.5 minutes equilibration with sulfosalicylic acid prior to injection, was 15 minutes per sample.

### 5. Samples containing calcium and magnesium in combination with those elements that form anionic chloride complexes

Owing to the pronounced enhancement of complex formation and the resulting decrease in distribution coefficients, hydrochloric acid-acetone mixtures have found considerable use in cation exchange chromatography for the selective elution of those metals that form anionic or neutral chloride complexes.

Several systematic surveys of metal ion distribution coefficients on various cation exchangers as a function of hydrochloric acid-acetone concentration have been published. These include studies by Fritz and Rettig (74), by Peterson *et al*. (75), by Peters and Del Fiore (76), and by Strelow *et al*. (77). Quantitative gravity-flow separations on cation exchangers with hydrochloric acid-acetone mixtures as eluents have been described by Fritz and Abbink (78). Rapid forced-flow separations on macroreticular cation exchangers with hydrochloric acid-acetone mixtures as eluents and continuous spectrophotometric detection have been described by Kawazu and Fritz (20) and by Kawazu *et al*. (58). Cation exchange separations in hydrochloric acid-acetone have been reviewed by Strelow and suitable conditions for the separation of various ions discussed (79).

Column distribution coefficients of several representative elements were measured as a function of hydrochloric acid-acetone concentration in order to establish suitable conditions for the selective elution of those elements forming anionic or neutral chloride complexes. Particular attention was given to manganese (II) since it is usually the most difficult element to selectively elute from cation exchangers with HCl-acetone mixtures (13, pp. 170-175). Distribution coefficients of manganese (II) as a function of hydrochloric acid-acetone concentration are shown in Figure 20 together with distribution coefficients of zinc. Zinc is one of the easiest elements to selectively elute from cation exchangers with hydrochloric acid-acetone mixtures (13, pp. 170-175) and its distribution coefficients are shown for the purpose of comparison. Based on these results, 0.4 M HCl-95% acetone was chosen to be used as an eluent for the selective prior separation of those metals that form chloride complexes.

Samples containing calcium and magnesium in combination with elements forming anionic chloride complexes such as cadmium, cobalt, copper (II), iron (III), manganese (II), uranium (VI), and zinc were analyzed using a 7-cm x 0.4-cm column of 1.8 meq/g low capacity cation exchanger. Elements

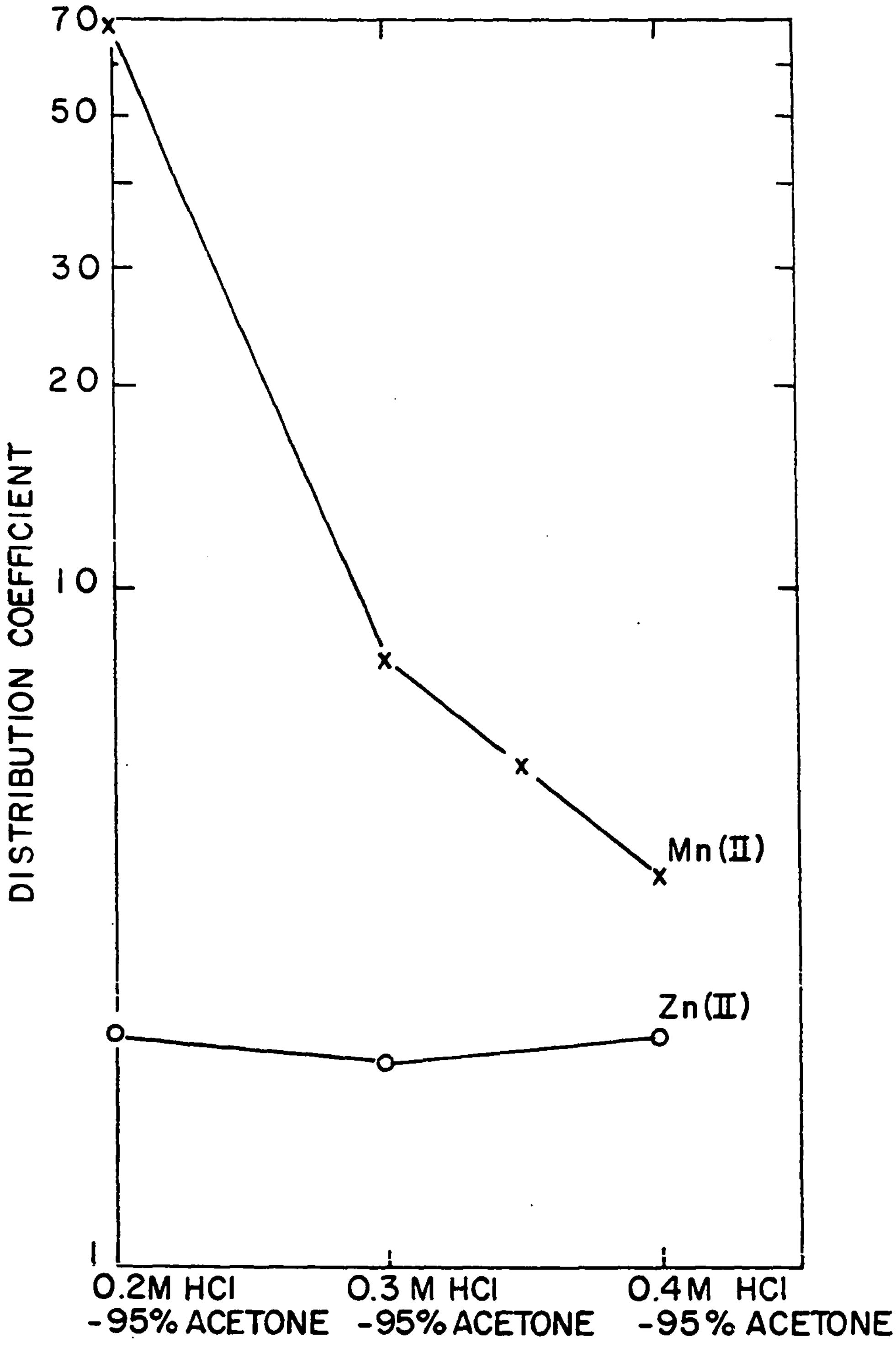


Figure 20. Distribution coefficients of manganese (II) and zinc on 1.9 meq/g low capacity catex as a function of HCl-acetone concentration

forming anionic chloride complexes were selectively eluted with 0.4 M HCl-95% acetone at flow rates of 1.6 - 2 ml/min. Sufficient time (7.5 - 15 minutes) was allowed for complete elution of the interfering ions. After the recorder trace had returned to baseline, the calcium and magnesium were eluted with 0.8 - 1 M ammonium chloride at a flow rate of 2 ml/min. Other elements known to form anionic chloride complexes such as mercury (II), gold (III), palladium (II), iridium (IV), rhenium (III), vanadium (V), molybdenum (VI), bismuth (III), germanium (IV), selenium (IV), tellurium (VI), arsenic (III), and antimony (III) could presumably be separated by similar means.

### 6. Samples containing calcium and magnesium in combination with nickel

Using hydrochloric acid-organic solvent-dimethylglyoxime mixtures, Wahlgren *et al*. (79) were able to separate selectively nickel from more than 30 elements on Dowex 50W-X8. Because of the specificity and effectiveness of the method described, it was decided to use a similar approach in this work.

Column distribution coefficients of nickel were measured at several different concentrations of hydrochloric acid-acetone-DMG and it was observed that the distribution coefficient of nickel decreased from a value greater than 75 in 0.1 M hydrochloric acid-0.25 M DMG-95% acetone to a value of 4.3 in 0.4 M hydrochloric acid-0.25 M DMG-95% acetone. Accordingly, a mixture of 0.4 M hydrochloric acid-0.25 M DMG-95% acetone was used for the selective elution of nickel prior to the elution of calcium and magnesium. As might be expected, this mixture was also effective in eluting any of the ions that could be eluted by 0.4 M hydrochloric acid-95% acetone alone. When measuring column distribution coefficients, it was necessary to use a methanolic solution of PAR saturated with THAM as a color-forming reagent rather than the usual aqueous solution so as to prevent problems with precipitation of DMG.

Samples containing calcium and magnesium in combination with nickel were analyzed using a 7 cm x 0.4 cm column of 1.8 meq/g low capacity cation exchanger. Nickel was selectively eluted with 0.4 M HCl-95% acetone-0.25 M dimethylglyoxime at a flow rate of 2 ml/min. Five minutes after injection, the eluent was switched to acetone at a flow rate of 1 ml/min in order to flush the column free of all traces of dimethylglyoxime. After a further five minutes, the eluent was switched to 1 M ammonium chloride at a flow rate of 2 ml/min in order to elute the calcium and magnesium.

Prior elution of interfering ions with HCl-acetone or HCl-acetone-dimethylglyoxime always resulted in partial elution of magnesium. Although calcium appeared to move somewhat on the column, it was sufficiently retained that it could be recovered quantitatively by elution with ammonium chloride. The reason for this behavior is not understood but it appeared to be related to the change from the hydrogen form of the resin to the ammonium form. Slight shrinkage of the resin was observed each time this change was made.

Various approaches were tried in an effort to obtain a quantitative recovery of magnesium. They included such things as putting a sample down in distilled water prior to eluting with HCl-acetone and also the use of more dilute HCl-acetone solutions as eluents. No approach tried, however, was completely successful. For example, the use of more dilute HCl-acetone solutions such as 0.2 M HCl-95% acetone did not result in complete retention of magnesium. Although the batch distribution coefficient of magnesium in 0.2 M HCl-95% acetone was determined to be nearly 900, use of 0.2 M HCl-95% acetone as an eluent resulted in elution of 50 to 75% of the magnesium.

### 7. Samples containing calcium and magnesium in combination with strongly retained elements

Samples containing calcium and magnesium in combination with strongly retained elements such as the rare earths and cerium (III) were analyzed using a 7 cm x 0.4 cm column of 1.8 meq/g low capacity cation exchanger. Calcium and magnesium were eluted with 1 M ammonium chloride at a flow rate of 2 ml/min. After 10 minutes, the eluent was switched to 4 M ammonium chloride at a flow rate of 1.5-2 ml/min in order to strip the more strongly retained ions. This approach should also be valid for the analysis of samples containing calcium and magnesium together with such ions as the other rare earths, yttrium (III), scandium (III), lanthanum (III), or thorium (IV).

Using the selective separation methods described, elution curves for calcium and magnesium in the presence of other ions are shown in Figures 21-23.

## D. Other Separations

The methods developed for the selective removal of interfering ions prior to the elution of calcium and magnesium can also be used for the separation of some of these ions alone. This is shown in Figure 24 where manganese (II) is separated from nickel using 0.4 M HCl-95% acetone to selectively elute manganese (II) and then switching to 0.4 M HCl-95% acetone-0.25 M DMG to elute nickel.

## E. Results of Analyses

### 1. Synthetic mixtures

The results of quantitative analyses of known mixtures of calcium and magnesium, both alone and in combination with other elements, are shown in Tables VII and VIII. All

Figure 21. Separation of calcium and magnesium from beryllium and aluminum

Column: 1.9 meq/g low capacity catex; 7 cm x 0.4 cm

Eluents: 0.1 M sulfosalicylic acid; pH 3.6; 3.5 ml/min; 0.8 M $NH_4Cl$; 2 ml/min

Detection: Arsenazo I color-forming reagent; 0.5 ml/min

Sample: 51.4 µl; 41.2 µg Ca, 25.0 µg Mg; 9.3 µg Be, 28 µg Al

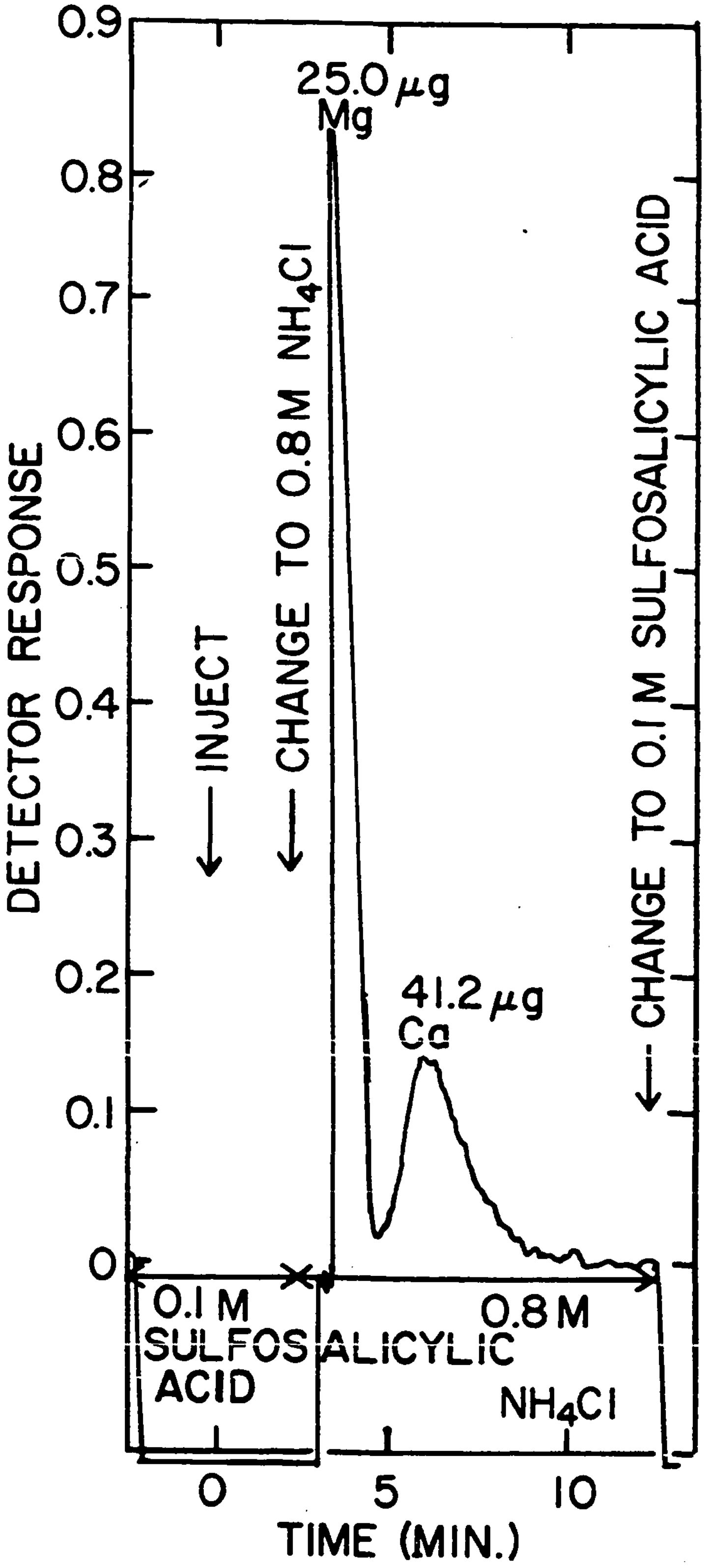

DETECTOR RESPONSE
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
TIME (MIN.)
0
5
10
← INJECT
← CHANGE TO 0.8 M NH$_4$Cl
← CHANGE TO 0.1 M SULFOSALICYLIC ACID
25.0 µg Mg
41.2 µg Ca
0.1 M SULFOSALICYLIC ACID
0.8 M NH$_4$Cl

Figure 22. Separation of calcium and magnesium from cadmium, cobalt, copper (II), and zinc

Column: 1.9 meq/g low capacity catex;
7 cm x 0.4 cm

Eluents: 0.4 M HCl-95% acetone; 1.6 ml/min;
1 M $NH_4Cl$; 2 ml/min

Sample: 51.4 µl; 41.2 µg Ca, 25.0 µg Mg,
120 µg Cd, 61 µg Co, 65 µg Cu(II),
67 µg Zn

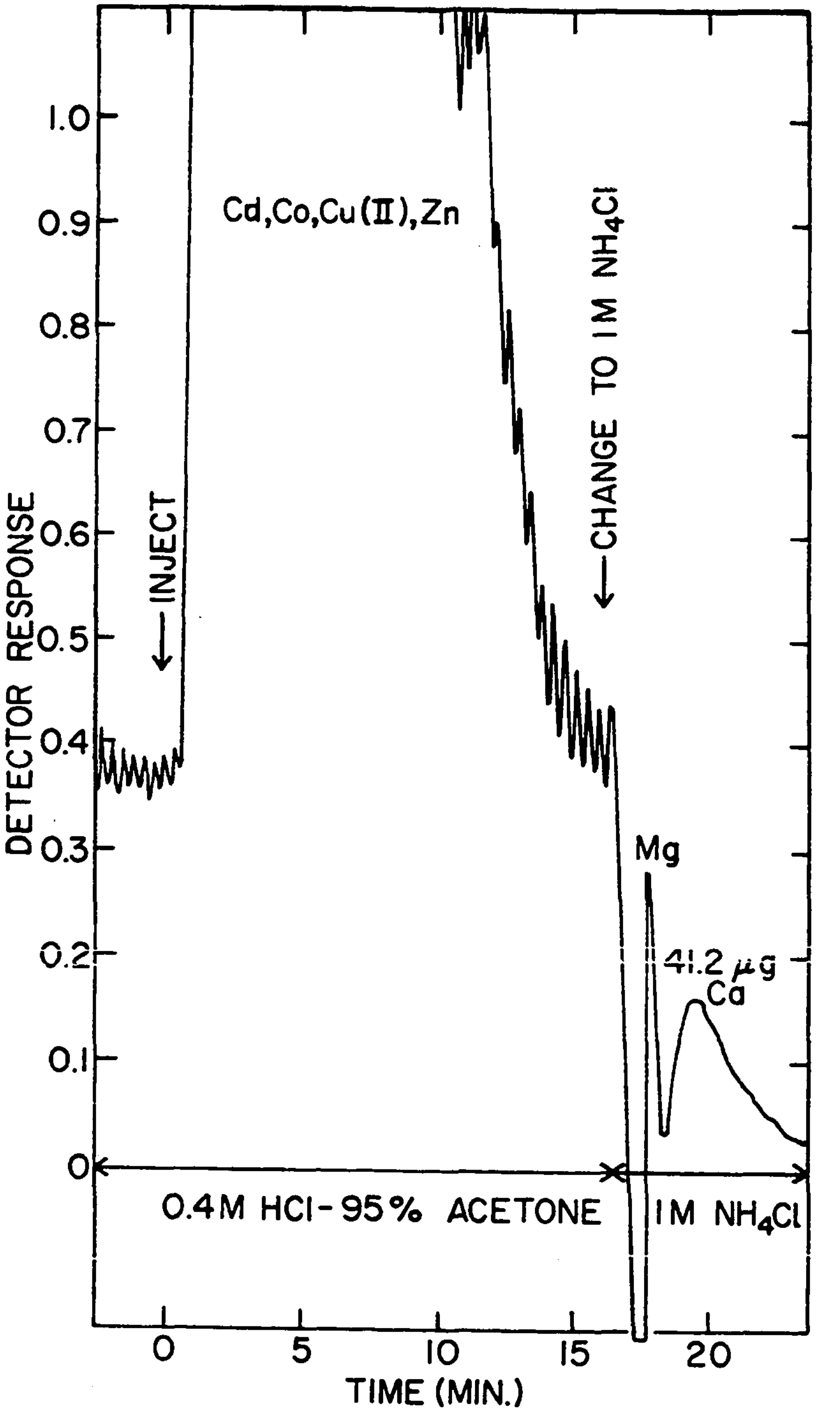

DETECTOR RESPONSE
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
← INJECT
Cd,Co,Cu(II),Zn
← CHANGE TO 1M $NH_4Cl$
Mg
41.2 μg Ca
0.4M HCl-95% ACETONE
1M $NH_4Cl$
0
5
10
15
20
TIME (MIN.)

Figure 23. Separation of calcium and magnesium from cerium (III)

Column: 1.9 meq/g low capacity catex; 7 cm x 0.4 cm

Eluents: 1 M $NH_4Cl$; 2 ml/min;
4 M $NH_4Cl$; 2 ml/min

Detection: Arsenazo I color-forming reagent; 0.5 ml/min

Sample: 51.4 µl; 41.2 µg Ca; 25.0 µg Mg; 144 µg Ce(III)

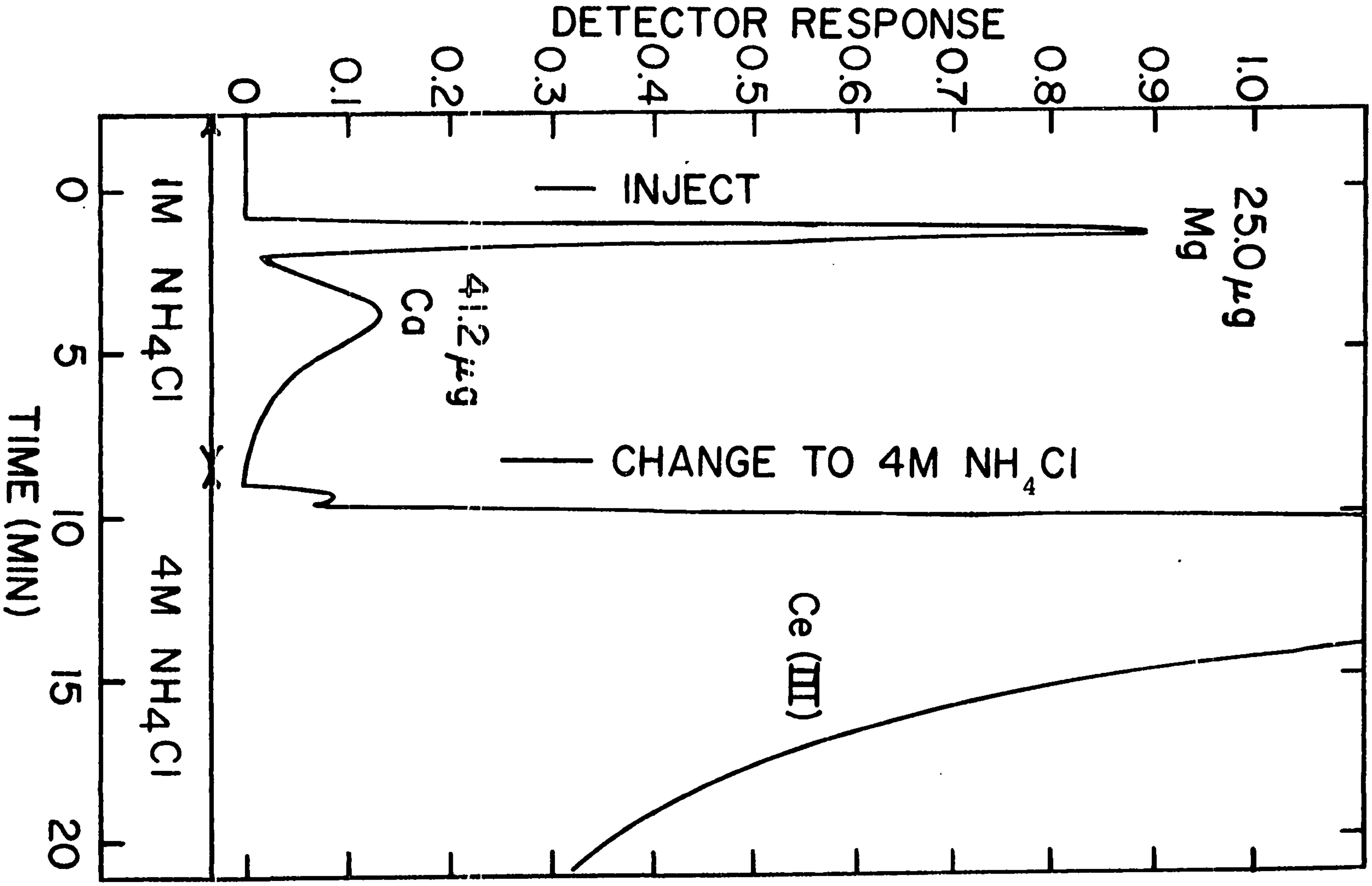

DETECTOR RESPONSE
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
INJECT
25.0 μg Mg
41.2 μg Ca
CHANGE TO 4M NH4Cl
Ce (III)
1M NH4Cl
4M NH4Cl
0
5
10
15
20
TIME (MIN)

Figure 24. Separation of manganese (II) and nickel

Column: 1.9 meq/g low capacity catex;
7 cm x 0.4 cm

Eluents: 0.4 M HCl-95% acetone; 1.5 ml/min;
0.4 M HCl-95% acetone-0.25 M DMG;
1.5 ml/min

Detection: Methanolic solution of PAR
saturated with THAM; 4 ml/min;
490 nm

Sample: 51.4 µl

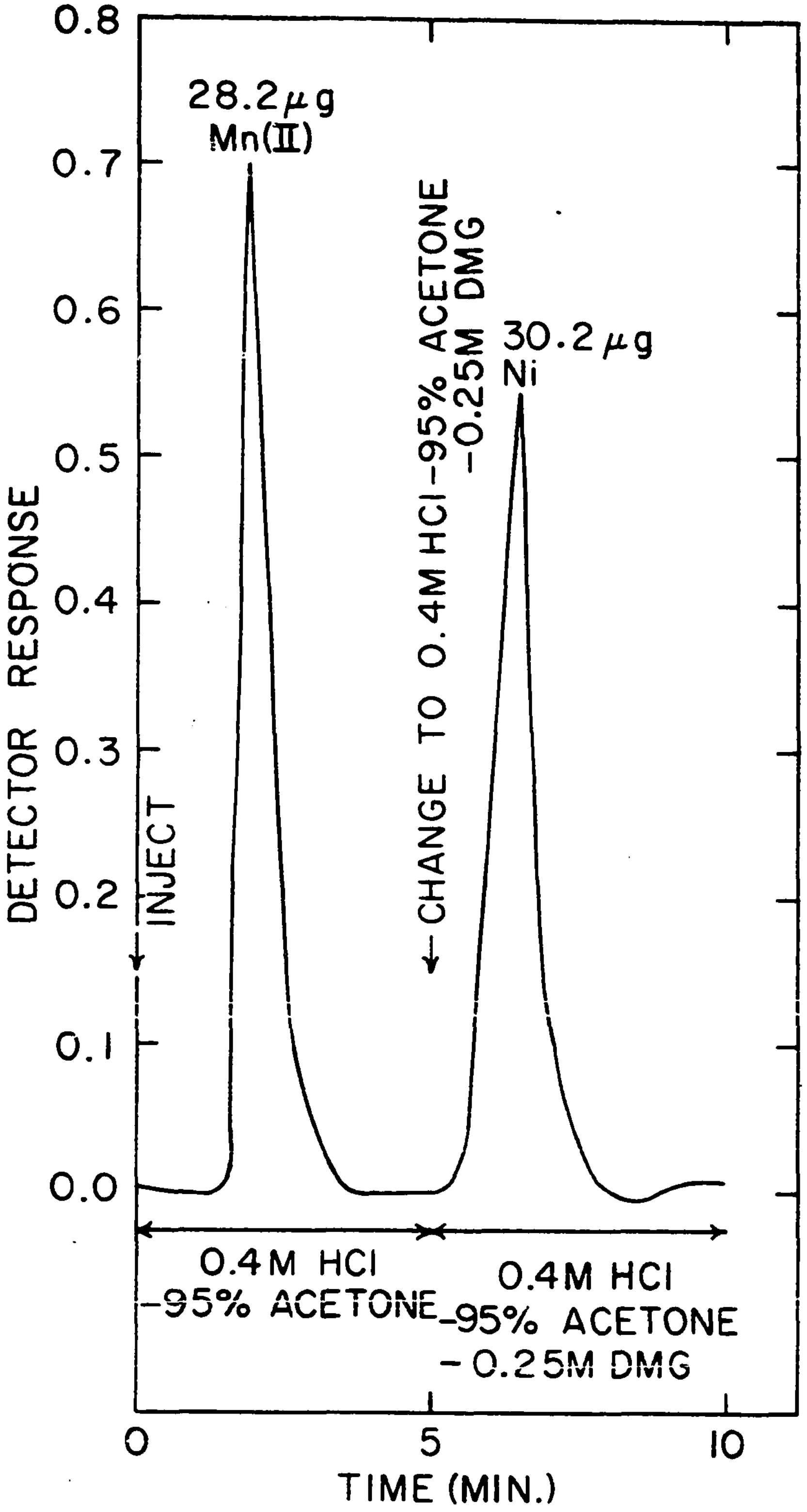

DETECTOR RESPONSE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
28.2 μg
Mn(II)
30.2 μg
Ni
INJECT
← CHANGE TO 0.4M HCl-95% ACETONE -0.25M DMG
0.4M HCl -95% ACETONE
0.4M HCl -95% ACETONE -0.25M DMG
0
5
10
TIME (MIN.)

Table VII. Analysis of known mixtures containing varying molar ratios of Ca and Mg

| meq Ca/meq Mg | Mg | | Ca | |
|---|---|---|---|---|
| | Taken (mg) | Found (mg) | Taken (mg) | Found (mg) |
| 10/1 | 0.0225 | 0.0238 | 0.37 | 0.36 |
| 1/1 | 0.0250 | 0.0253 | 0.041 | 0.042 |
| 1/10 | 0.225 | 0.225 | 0.037 | 0.037 |

Table VIII. Analysis of known mixtures containing equimolar amounts of Ca and Mg and varying amounts of other ions

| Other ion | $\frac{\text{meq other ion}}{\text{meq Ca, Mg}}$ | Mg | | Ca | |
|---|---|---|---|---|---|
| | | Taken (mg) | Found (mg) | Taken (mg) | Found (mg) |
| Li | 50/1 | 0.0250 | 0.0240 | 0.041 | 0.041 |
| Li | 5/1 | 0.0250 | 0.0248 | 0.041 | 0.041 |
| Na | 50/1 | 0.0250 | 0.0272 | 0.041 | 0.039 |
| Na | 5/1 | 0.0250 | 0.0244 | 0.041 | 0.042 |
| K | 50/1 | 0.0250 | 0.0226 | 0.041 | 0.040 |
| K | 5/1 | 0.0250 | 0.0247 | 0.041 | 0.040 |
| $NH_4^+$ | 50/1 | 0.0250 | 0.0246 | 0.041 | 0.038 |
| $NH_4^+$ | 5/1 | 0.0250 | 0.0248 | 0.041 | 0.043 |
| Sr | 1/1 | 0.0250 | 0.0246 | 0.041 | 0.049 |
| Sr | 0.1/1 | 0.0250 | 0.0246 | 0.041 | 0.042 |
| Ba | 1/1 | 0.0250 | 0.0238 | 0.041 | 0.041 |
| Be,Al | 1/1,1/1 | 0.0250 | 0.0238 | 0.041 | 0.041 |
| Cd,Co,Cu(II),Zn | 1/1,1/1,1/1,1/1 | 0.0250 | NQ | 0.041 | 0.040 |
| $UO_2$,Fe(III),Mn(II) | 1/1,1/1,1/1 | 0.0250 | NQ | 0.041 | 0.044 |
| Ni | 1/1 | 0.0250 | NQ | 0.041 | 0.042 |

Table VIII. (Continued)

| Other ion | $\frac{\text{meq other ion}}{\text{meq Ca, Mg}}$ | Mg | | Ca | |
|---|---|---|---|---|---|
| | | Taken (mg) | Found (mg) | Taken (mg) | Found (mg) |
| Er,Yb,Nd | 0.075/1,0.075/1<br>0.075/1 | 0.0250 | 0.0256 | 0.041 | 0.041 |
| Ce(III) | 1.5/1 | 0.0250 | 0.0250 | 0.041 | 0.042 |
| $H_2PO_4^-$ | 25/1 | 0.0250 | 0.0240 | 0.041 | 0.039 |
| $H_2PO_4^-$ | 2.5/1 | 0.0250 | 0.0255 | 0.041 | 0.041 |

NQ - not quantitated - recoveries low and variable.

results shown are the average of two or more determinations and the overall average relative error based on all samples analyzed was 3.0 pph in the determination of magnesium and 3.3 pph in the determination of calcium.

The precision of the method compared favorably with that of other instrumental methods used for the analysis of small amounts of calcium and magnesium. An estimate of precision was obtained using a 7-cm x 0.4-cm column of 1.8 meq/g low capacity cation exchanger. Elution was accomplished using 0.8 M ammonium chloride at a flow rate of 2 ml/min and Arsenazo I color-forming reagent at a flow rate of 0.5 ml/min was used for detection. A calibration curve was constructed in the usual manner by injecting standards containing known amounts of calcium and magnesium and plotting the resulting peak heights against the amounts of calcium and magnesium injected.

A single solution that contained 41.2 µg calcium and 25.0 µg magnesium per 51.4-ml injection was then analyzed. Based on five determinations, relative standard deviations of 2.0 pph for calcium and 1.44 pph for magnesium were obtained. These may be compared to relative standard deviations of 1.7-3.3 pph for calcium and 2.4-4.8 pph for magnesium obtained using atomic absorption spectroscopy (80).

### 2. Analysis of hard water samples

Samples of Iowa State University and Ames city tap water were collected using approved procedures (81). The samples were analyzed for calcium and magnesium using a 7-cm column containing 0.4 g of 1.8 meq/g resin with 1 M ammonium chloride as the eluent at a flow rate of 2 ml/min. Arsenazo I color-forming reagent at a flow rate of 24 ml/hr was used for detection. Sample loop size varied with a 0.969-ml loop used for analysis of the Iowa State University tap water and a 2.34-ml loop used for analysis of the Ames city tap water. Calibration curves were constructed by injecting standards containing known amounts of calcium and magnesium and plotting the resulting peak heights as a function of concentration.

The samples were also analyzed for calcium and magnesium by means of titration with EDTA, atomic absorption spectroscopy, and plasma emission spectroscopy. Results obtained by the different analytical methods are shown in Table IX and it can be seen that the different methods give comparable results.

The zinc, iron, and copper content of these samples was measured by plasma emission spectroscopy and found to be insignificant (low ppb levels). The samples could therefore be analyzed directly by forced-flow chromatography or EDTA titration without prior separation or masking of these

Table IX. Analysis of hard water samples

Iowa State University tap water

| Mg |  |  |  | Ca |  |  |  |
|---|---|---|---|---|---|---|---|
| Our analysis (ppm) | AA (ppm) | Plasma emission spectroscopy (ppm) | EDTA titration (ppm) | Our analysis (ppm) | AA (ppm) | Plasma emission spectroscopy (ppm) | EDTA titration (ppm) |
| 33.5 | 34.0 | 34.0 | 38.8 | 99.5 | 106 | 104 | 98.8 |

City of Ames tap water

| Mg |  |  |  | Ca |  |  |  |
|---|---|---|---|---|---|---|---|
| Our analysis (ppm) | AA (ppm) | Plasma emission spectroscopy (ppm) | EDTA titration (ppm) | Our analysis (ppm) | AA (ppm) | Plasma emission spectroscopy (ppm) | EDTA titration (ppm) |
| 10.5 | 10.1 | 10.5 | 10.5 | 38.0 | 36.9 | 36.0 | 35.9 |

elements. If it had been necessary to analyse samples containing interfering levels of copper and/or iron the procedure of Fritz and King (82) could have been used to remove the copper and/or iron prior to the determination of calcium and magnesium by forced-flow chromatography or EDTA titration.

Total hardness due to calcium and magnesium was determined using the EDTA titration procedure of Fritz _et al_. (36) and calcium hardness was determined by EDTA titration using the procedure of Patton and Reeder (83). Hardness due to magnesium was then calculated by difference. The samples were analysed for calcium and magnesium by atomic absorption spectroscopy using standard procedures (84). The plasma emission spectroscopy analyses were performed by Walter Sutherland on the inductively coupled plasma-optical emission spectroscopy analytical system located in the Ames Laboratory. The analytical use of such systems has been discussed by Fassel and Kniseley (85).

### 3. Analysis of serum samples

Most existing methods for the determination of calcium or magnesium in blood serum rely either on atomic absorption spectroscopy or on a colorimetric determination of one element in the presence of the other. Typical of the atomic absorption methods that have been described for the determination of calcium and magnesium are those of Willis (86,87).

Such methods, although quite specific for calcium or magnesium, suffer certain important limitations. These limitations include relatively complex equipment requirements and the necessity to determine each element separately. Additionally, such methods are not very amenable to automation because of the amount of manipulation and sample handling required.

Typical of the colorimetric methods that have been described for the analysis of calcium or magnesium in blood serum are those of Gitelman and coworkers. For the analysis of calcium in biological samples generally, Gitelman (88) described an automated spectrophotometric procedure using a Technicon AutoAnalyzer system. He detected calcium with cresolphthalein and masked magnesium with 8-hydroxyquinoline. For the analysis of magnesium in biological samples, Gitelman et al. (63) described an AutoAnalyzer procedure using Eriochrome Black T to detect magnesium and strontium-EGTA to mask calcium. They applied their procedure to the determination of magnesium in plasma, serum, urine, tissue, and diet samples after appropriate sample pretreatment. Lamkin and Williams (38) described a manual spectrophotometric procedure for the determination of calcium and magnesium in blood serum using simultaneous spectrophotometry. They determined the total concentration of calcium and magnesium using Arsenazo I and then masked the calcium with EGTA. The concentration of calcium was found by difference after determining the magnesium concentration. The chief limitations of such colorimetric methods as the above are lack of sensitivity and lack of specificity. Additionally, such methods necessitate a separate determination of each element. As a result, they involve considerable sample handling and increased probability of error.

Important considerations in the determination of calcium in blood serum have been outlined by Willis (86) and these considerations are equally applicable to the determination of magnesium in blood serum. Among others, these considerations include minimal sample pretreatment, rapid determination, accuracy, minimal sample size, and relatively inexpensive equipment requirements. Other considerations such as sensitivity and adaptability to automation might well be added to these.

### 4. Preparation and analysis of synthetic serum

The synthetic serum was prepared by spiking a solution containing potassium dihydrogen phosphate, potassium chloride, sodium chloride, and albumin with a known amount of calcium and magnesium. The albumin had been decalcified by passage through hydrogen form A-15 cation exchanger using a procedure similar to that described by Natelson (89).

The synthetic serum contained:

7 g albumin/100 ml

3.5 mg P/100 ml

20 mg K/100 ml

325 mg Na/100 ml

10 mg % Ca

2.5 mg % Mg

These levels of protein, cations, and anions were chosen to be in the range of normal human sera (90).

The synthetic serum was analyzed using a 7-cm x 0.4-cm column of 2.0 meq/g low capacity cation exchanger. 0.035 M ethylenediammonium chloride at a flow rate of 2 ml/min was used as the eluent and detection was with PAR-ZnEDTA at a flow rate of 0.3 ml/min. Sample loop size was 42.2 µl. Total analysis time was 12.5 minutes per sample. Calibration was accomplished by means of calibration curves. The calibration curves were constructed by injecting standards containing known amounts of calcium (0-6 µg) and magnesium (0-2 µg) and plotting the resulting peak heights against the amounts of calcium and magnesium injected.

Table X. Analysis of synthetic serum samples

| Taken | Found | Dilution Factor |
|---|---|---|
| 2.50 mg % Mg | 2.50 mg % Mg | 1 to 2 |
| 10.0 mg % Ca | 9.60 mg % Ca | 1 to 2 |
| 2.50 mg % Mg | 2.45 ± 0.0 mg % Mg | undiluted |
| 10.0 mg % Ca | 10.6 ± 0.4 mg % Ca | undiluted |

### 5. Analysis of clinical serum

Several clinical serum samples and also a control serum (Dade Diagnostics Labtrol) were obtained from Walter Hyde of Veterinary Medicine. The samples were refrigerated prior to analysis and were analyzed within several days of being received. All analyses were performed using a 7 cm x 0.4 cm column of 2.0 meq/g low capacity cation exchanger. 0.035 M ethylenediammonium chloride at a flow rate of 2 ml/min was used as the eluent and detection was with PAR-ZnEDTA at a flow rate of 0.25 - 0.3 ml/min. Sample loop size varied from 38.1 to 214 µl. Most analyses were performed using a 38.1- or 42.2-µl loop. Total analysis time was 12.5 minutes per sample. Calibration was by means of calibration curves. The calibration curves were constructed by injecting standards containing known amounts of calcium (0-6 µg) and magnesium (0-2 µg) and plotting the resulting peak heights against the amounts of calcium and magnesium injected. Results of analyses are shown in Figure 25 together with results obtained by Walter Hyde using atomic absorption spectroscopy.

Table XI. Comparison of atomic absorption and forced-flow chromatographic analyses of serum samples

| Sample | Results | | |
|---|---|---|---|
| | AA | LC | Dilution Factor |
| A | 3 mg % Mg | 3.0 ± 0.1 mg % Mg | 0.725 to 1.725 |
| | 5.5 mg % Ca | 5.35 ± 0.15 mg % Ca | 0.725 to 1.725 |
| B | 2.68 mg % Mg | 2.67 mg % Mg | 0.790 to 1.790 |
| | 4.95 mg % Ca | 4.81 mg % Ca | 0.790 to 1.790 |
| C | 2.9 mg % Mg | 3.05 ± 0.05 mg % Mg | 0.900 to 1.900 |
| | 9.8 mg % Ca | 9.5 ± 0.0 mg % Ca | |
| Control | 3 mg % Mg | 2.65 ± 0.05 mg % Mg | undiluted |
| | 10 mg % Ca | 9.4 ± 0.0 mg % Ca | undiluted |
| Bovine | 3.8 mg % Mg | 3.6 mg % Mg | undiluted |
| | 9.35 mg % Ca | 8.0 mg % Ca | undiluted |

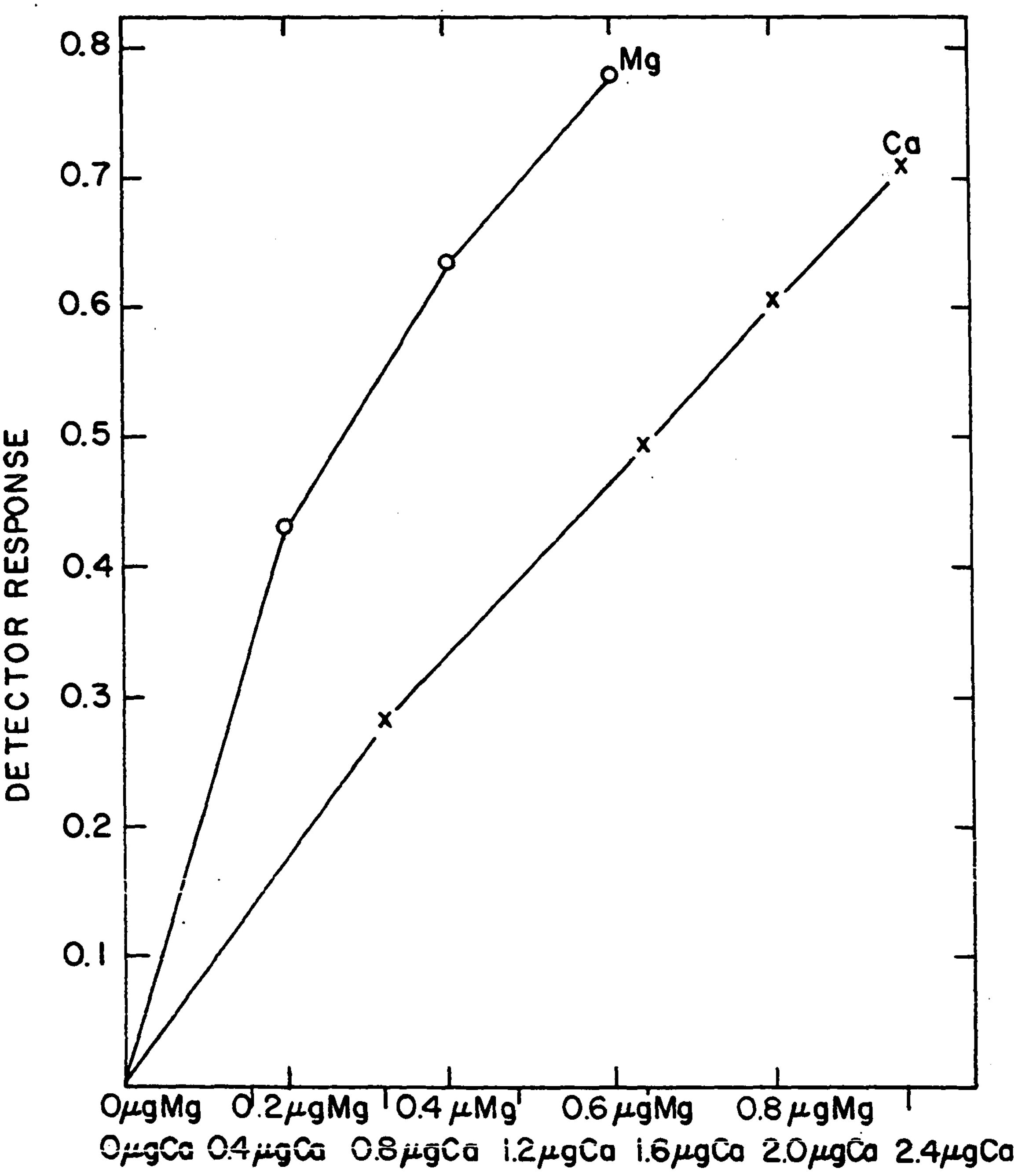
Figure 25. Calibration curves used for the analysis of diluted serum samples

Column: 2.0 meq/g low capacity catex; 7 cm x 0.4 cm

Eluent: 0.035 M en•2HCl; 2 ml/min

Detection: PAR-ZnEDTA color-forming reagent; 0.3 ml/min

Sample: 42.2 μl


DETECTOR RESPONSE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Mg
Ca
0μgMg
0.2μgMg
0.4 μMg
0.6 μgMg
0.8 μgMg
0μgCa
0.4μgCa
0.8μgCa
1.2μgCa
1.6μgCa
2.0μgCa
2.4μgCa

# V. CONCLUSIONS

Forced-flow cation exchange chromatography employing continuous spectrophotometric detection is seen to be applicable to the rapid, quantitative determination of calcium and magnesium in a variety of matrices. The separation and measurement of calcium and magnesium can be combined in one essentially continuous operation and analysis times of ten minutes or less per sample are possible.

Using simple salt solutions as eluents, low capacity cation exchangers derived from partially sulfonated XAD-2 exhibit calcium/magnesium separation factors comparable to those obtained on conventional cation exchangers using eluents containing complexing agents. The use of salt solutions as eluents results in easy, accurate detection of calcium and magnesium.

Arsenazo I color-forming reagent offers good sensitivity for the continuous spectrophotometric detection of calcium and magnesium in chromatographic effluents. PAR-ZnEDTA color-forming reagent offers excellent sensitivity for the continuous spectrophotometric detection of many different metal ions, including calcium and magnesium, in chromatographic effluents. Incorporation of an active low pass filter within the spectrophotometric detection system permits greater amplification of the spectrophotometer output and results in a considerable increase in detector sensitivity.

Certain ions that would otherwise interfere in the determination of calcium and magnesium may be readily separated by means of selective elution procedures.

The combination of highly efficient cation exchangers, mild eluents, sensitive color-forming reagents, and selective separation of interferences results in forced-flow chromatographic methods that rival and in some ways surpass other methods that have been used or proposed for the analysis of microgram amounts of calcium and magnesium. A distinguishing characteristic of forced-flow chromatographic methods employing continuous detection is the ability to accommodate very small samples containing very small amounts of metal ions. Using the methods described, a single sample only a few tens of microliters in volume and containing no more than a few micrograms of calcium and magnesium can be easily and accurately analyzed.

# VI. FUTURE WORK

## A. Apparatus

Consideration should be given to modifying the forced-flow chromatograph to extend and improve its capabilities. One possible modification would be to rebuild the system within one of the fume hoods so as to facilitate working with solutions that generate noxious or corrosive vapors. Moreover, by locating the various electronic components outside the hood, it would be possible to protect them from corrosion to a much greater extent than is now feasible. Lastly, use of a hood would minimize any fire hazard when using organic solvent mixtures as eluents.

A second possible modification would be the replacement of the Heath spectrophotometric detector setup with one of the newer commercial instruments. Although the Heath setup has given good service over several years of more or less continuous operation, it is now beginning to show its age and should probably be replaced.

A third possible modification would be to redesign the forced-flow chromatograph to achieve both greater control and greater convenience of operation. A number of new devices (electronic metering pumps, Teflow-lined solenoid valves, electronic integrators, etc.) have become commercially available within the last few years and some of these undoubtedly could be employed to great advantage in the construction of a new chromatograph.

Ideally, it should be possible to design and build a chromatograph with highly precise regulation of the flow rates of all solutions independently of one another. Such a chromatograph would need separate pumping systems for each solution and a series of electronic metering pumps might be used for that purpose.

## B. Use of Eluents Containing Multiply Charged Cations

Using forced-flow chromatography, several workers have shown that eluents containing perchlorate anions can be used to rapidly elute many otherwise strongly retained anions from anion exchangers. Thus, Fritz and Sickafoose (16) used 0.4 M sodium perchlorate to rapidly strip chromium (VI) from Amberlyst A-26 anion exchange resin. Similarly, Seymour and Fritz (17) used mixtures of hydrochloric and perchloric acids to selectively elute strongly retained anionic chloride complexes of certain metal ions from the same resin.

Analogously, it might be possible to rapidly elute strongly retained cations from cation exchangers by using eluents containing cationic species with large affinities for the resin. Solutions of protonated polyamine compounds might be the eluents of choice since they would not interfere with detection by means of color-forming reagents. Additionally, many such compounds are commonly used as buffers and are commercially available in pure form. An example of such a compound is hexamethylenetetramine.

## C. Development of Specific Methods

It should be possible to devise nearly specific forced-flow chromatographic methods for particular metal ions by utilizing some of the selective elution and detection procedures that have been described in the literature.

For example, it should be possible to devise a forced-flow chromatographic method for V (V) using the general procedure of Fritz and Abbink (91) who separated V (V) from 25 other metal ions on Dowex 50W-X8 by selectively eluting the V (V) with 0.01 M sulfuric or perchloric acid containing 1% hydrogen peroxide. Similarly, some of the selective elution procedures used in this work for the separation of interfering metal ions prior to the elution of calcium and magnesium might be used instead for the forced-flow chromatographic determination of those metal ions. Thus, it should be possible to devise a selective forced-flow chromatographic method for nickel using hydrochloric acid-acetone-dimethylglyoxime as an eluent.

Similarly, one might devise nearly specific forced-flow chromatographic methods of particular metal ions by utilizing selective detection of the ions of interest. Here one would use forced-flow chromatography to separate the ion of interest from the bulk of any interfering ions and then use selective detection to determine it in the presence of one or more overlapping ions.

There are two possible approaches to selective detection with systems employing continuous spectrophotometric detection. The first approach is the use of colorimetric reagents that are themselves rather selective as color-forming reagents. Thus one might devise a selective forced-flow chromatographic method for aluminum based on detection with Chromazural S. The second approach is to use masking agents to mask out interfering ions. Many possibilities exist using this approach. For example, in the course of this work it was observed that ethylenediamine effectively masked copper (II) against detection with PAR color-forming reagent. Consequently, one could base a method for an ion such as zinc in the presence of copper (II) on selective detection with PAR color-forming reagent containing ethylenediamine as a masking agent. Many other possibilities exist since many selective spectrophotometric methods employing masking of interferences have been described in the literature. Many methods of this sort have been reviewed by Perrin (59).

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