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I. Application of 1,10-phenanthroline to the spectrophotofluorometric analysis of zinc and cadmium; II. Partially sulfonated, macroreticular ion exchange resins in the separation of metal ions

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I. Application of 1,10-phenanthroline to the spectrophotofluorometric analysis of zinc and cadmium

II. Partially sulfonated, macroreticular ion exchange resins in the separation of metal ions

by

James Nathan Story

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

Department: Chemistry
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For the Graduate College

Iowa State University
Ames, Iowa

1973

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PART I. APPLICATION OF 1,10-PHENANTHROLINE TO THE SPECTROPHOTOFLUOROMETRIC ANALYSIS OF ZINC AND CADMIUM

INTRODUCTION AND PURPOSE

For many years the fluorometer has been one of the major tools of the clinical chemist (1). The simple, inexpensive, filter fluorometer has been a major factor in bringing very sensitive analytical methods to laboratories to which sensitivity, simplicity and expense are major considerations.

The advent of highly sophisticated research spectrophotofluorimeters which can record both absolute excitation and emission spectra of fluorescent species directly, has brought the level of instrumentation available for spectrophotofluorometry up to a par with that commonly used in spectrophotometry. These instruments have both simplified and promoted interest in the study of fundamental physical and chemical processes involved in fluorescence. Several excellent books on the subject are available including those of Hercules (2), Becker (3), and Pringsheim (4).

Most analytical chemists are probably familiar with the important colorimetric reagent 1,10-phenanthroline and its more important relatives such as 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline). The large molar absorptivities and complex-forming powers of these ligands make them of extensive use analytically in the spectrophotometric analysis of metals. It is not generally as well known that all of these compounds also fluoresce in aqueous solution.

Analytical methods which measure absorption of light are useful generally only to about 0.1 ppm. Fluorometric methods can give useful analytical results at levels as low as 0.01 ppb. Fluorescence methods are inherently more sensitive than absorption methods because the signal resulting from the fluorescence can be amplified directly, while with absorption methods the difference or ratio of two signals must be amplified.

Besides the greater sensitivity inherent in the fluorometric technique, fluorescence methods may offer greater selectivity than absorption methods. In absorption spectroscopy only the frequency of incident light may be varied to obtain selectivity in a prepared sample. In fluorescence methods either the excitation or emission wavelength may be varied. However, the advantages of fluorometric techniques must be paid for by the greater environmental effects in fluorescence and by the greater problems of impurities in reagents.

Jones (5) pointed out that 1,10-phenanthroline was potentially an extremely sensitive fluorometric reagent for the determination of metal ions. Initial studies confirmed that zinc and cadmium form highly fluorescent 1,10-phenanthroline chelates in aqueous solution. The purpose of this work was to investigate the application of the complexes of zinc and cadmium with 1,10-phenanthroline (and similar nitrogen heterocycles) to the spectrophotofluorometric analysis of these metals.

LITERATURE SURVEY

History

In the discussion which follows, the abbreviation "phen" will be used in place of the name "1,10-phenanthroline", and "bipy" will represent 2,2'-bipyridine.

Phen is one of the most commonly used organic reagents in analytical chemistry. Its importance is demonstrated by the fact that almost every textbook of quantitative analysis makes some mention of it either as a colorimetric reagent for iron or as an oxidation-reduction indicator.

The discovery of phen and the first investigation of its properties can be traced to the work of Fritz Blau (6,7) in the years between 1888 and 1898. During this time Blau first prepared phen and bipy and he prepared and characterized their iron(II) and (III) complexes. He also noted the reversibility of the oxidation of the iron(II) complex and he synthesized nickel(II), cobalt(II) and copper(II) complex salts of phen and bipy.

In 1889 Gerdeissen (8) also reported the synthesis of 2-methyl-phen, although he did not notice any reaction with iron.

Analytical applications of these compounds were not immediately forthcoming. As Brandt et al. have pointed out, the resolution of tris-bipy iron(II) into its optically active forms by Werner in 1912 was probably the last important work on these compounds for almost 20 years (9).

The analytical possibilities of these compounds were initially exploited in the early 1930's. In 1931, Walden, Hammett and Chapman demonstrated that the iron(II) complexes could be used as reversible, high-potential redox indicators (10). The first direct application of compounds of this type to quantitative analysis was performed by Bode (11), who used bipy to determine iron in beer following digestion with sulfuric acid and hydrogen peroxide. The use of phen instead of bipy for the colorimetric determination of iron was begun in 1938 by several workers almost simultaneously.

Much of the work in exploiting these reagents for analytical uses was done by G. Frederick Smith and Francis Case in the 1940's and 1950's and has been described by Diehl and Smith (12). Smith and Case studied the effects of substitutents in the bipy and phen molecules on the absorption spectra of metal chelates and on the reduction potential of the iron couple. Smith and Case studied about 150 compounds which were closely related to bipy and phen. They produced a series of redox indicators in the range of 0.87 to 1.33 volts and several colorimetric reagents of extraordinary sensitivity and selectivity.

Phen and its relatives gained attention initially in analytical chemistry on the basis of their reactions with metal ions to form colored complexes. Although the ligands are used for a variety of other purposes, this discussion will be confined to those uses involving metal complexes.

Phen forms metal chelates with almost all metal ions under some conditions. Conditions of preparation will not be given since they have been outlined elsewhere (13). In most cases the phen molecule reacts with the metal ion to give the mono, bis, and tris complexes. It has also been shown to give poly-(phen)hydrogen(I) species in aqueous solution (14).

Among the most common colorimetric applications of phen type compounds are those in the determination of iron, copper, ruthenium, cobalt, nickel, palladium, and silver.

Nitrogen Heterocyclic Compounds as Fluorometric Reagents

The formation of a metal chelate compound is usually necessary if a metal ion is to be determined fluorometrically, since most metal ions and metal complexes with monodentate ligands are nonfluorescent. In many cases it may be possible to use the quenching of fluorescence of a fluorescing species to quantitate a metal ion or other species in solution.

Several factors combine to influence the amount of fluorescence obtained from a metal chelate. The presence of a paramagnetic species (e.g. the metal ion) often reduces or completely quenches fluorescence by facilitating intersystem crossing from the singlet to the triplet state. The extent of intersystem crossing is also increased by the presence of heavier metal ions. Heavier atoms increase spin-orbit coupling and thus cause an increase in the rate of intersystem crossing. The fluorescence of chelates of heavier metal ions is usually less than that of the lighter metals.

Collisional deactivation of the metal chelate also results in a decrease in fluorescence intensity. Collisional deactivation is increased by increasing solute-solvent interactions. This is done by introducing polar substituents onto the ligand or using a more polar solvent. Thus if the fluorescing species can be insulated from such interactions, the fluorescence intensity will usually increase.

Ligands which successfully form fluorescing metal chelates are usually large molecules, with a high degree of aromaticity and few polar substituents. They complex the central metal ion strongly, helping to prevent metal ion-solvent interactions.

The introduction of substituents onto the ligand structure usually produces only minor changes in the absorption maximum and intensity and in the position of the emission maximum. However, such substituents may have a great effect upon the intensity of fluorescence. Structural changes created by addition of substituents may greatly influence the efficiency of certain energy transfer steps such as intersystem crossing and solvent-solute interactions.

Although phen and its nitrogen heterocyclic relatives would appear to possess most of the characteristics of good fluorescent chelate ligands, many aspects of their spectroscopic behavior are unexplained. As Hercules (2) has stated, "the nature of the emission (if any) from nitrogen heterocyclic compounds cannot presently be predicted with certainty...This fact, together with the extreme sensitivity of the emission of nitrogen heterocyclic compounds to solvent effects, has retarded the use of fluorescence and phosphorescence for determinations of these compounds." Nevertheless, many fluorescent complexes of these ligands have been reported. But only one direct fluorometric method involving measurement of the fluorescence of a phen complex has been reported to date.

Phen has been used as a chelating agent in several cases to shield certain lanthanides from solvent interactions leading to collisional deactivation and thus permit the observation of their natural fluorescence. Several reported analytical methods have been based on this technique (15-18).

Wunschel and Ohnesorge (19) reported the luminescence of tris(bipy)iridium(III) and tris(phen)iridium(III) in dimethylformamide and ethyl alcohol. They measured the absorption and emission spectra of these species and assigned absorption bands. They reported that relative quantum yields in dimethylformamide were about an order of magnitude less than quinine fluorescence in 0.1 N H_2SO_4 and that the intensity of fluorescence of $Ir(phen)_3^{+3}$ was about twice that of $Ir(bipy)^{+3}$. An osmium-phen compound has also been reported to fluoresce (20).

Veening and Brandt (21) developed a fluorescimetric method for ruthenium using tris(5-methylphen)ruthenium(II). They found that the analysis of ruthenium at the 1 ppm level could be accomplished with an accuracy of 2% if osmium were present at a concentration of less than 28 ppm. They claimed the lack of the necessity of a separation of osmium and ruthenium as the main advantage of their method. It was found that platinum and nickel do not interfere while iron(II), palladium (II), cerium(IV), manganese(II), permanganate, silver(I), and dichromate are all serious interferences.

Veening and Brandt also compared the relative efficiencies of fluorescence emission of the ruthenium(II) complexes of several phen derivatives. They found that the greatest intensity of fluorescence was observed from the 5-methyl-phen complex. The ratio of fluorescence intensity of each of the other chelates to that of the 5-methyl derivative was measured and the ratios were found to be: bipy, 0.53; 4,4'-dimethyl-bipy, 0.21, phen 0.84; 5,6-dimethyl-phen, 0.87, 3,5,6,8-tetramethyl-phen, 0.47.

Jones (5) reported a fluorometric method for the determination of copper. The method relies on the quantitative quenching of the intense fluorescence of bathocuproine (2,9-dimethyl-4,7-diphenyl-phen) in acid solution upon formation of the bis(bathocuproine)copper(I) complex. The method can be used to determine copper(I) at the 2 ppb level. Silver(I), and iron(II) and (III) seriously interfere.

Bailey, Dagnall and West (22) also developed a spectrofluorometric method involving phen for the determination of small quantities of copper. In this method the complex copper $(phen)_2RBE$ (where RBE is tetrachloro(P)tetraiodo(R)fluorescein, also known as rose bengal extra) is extracted into chloroform, dissociated by the addition of ammonical acetone, and the fluorescence of RBE is measured at 570 nm. This procedure thus does not rely directly on any fluorescence of the phen chelate. The method is useful for copper in the range of 1-6 ppb.

Very similar methods for cadmium(II) (23), and silver(I) (24) were devised by Shcherbov *et al*. Both methods employed an extraction of the metal$(phen)_2$eosin complex into chloroform followed by addition of acetone and measurement of the eosin fluorescence. Results of the silver method indicated a standard deviation of 6% on samples containing $5\text{-}40\times10^{-4}\%$ silver.

Messier *et al*. (25) first reported the fluorescence of the $zinc(phen)_n^{+2}$ species about 3 months after it had been independently observed in this lab. These authors studied the fluorescence and phosphorescence of $zinc(phen)_n^{+2}$ at 77°K and noted several things. First the complexation by zinc shifts the fluorescence emission maximum to longer wavelengths by about 7 nm. Second, the intensity of fluorescence of the complex is an order of magnitude greater than that of the free phen. Third, the magnitudes of the quantum efficiencies of the three chelates are in the order tris > bis > mono. Since the experimental conditions under which the measurements were made were not described, and the authors were primarily interested in elucidating the electronic transitions occurring, this report was of limited use from an analytical viewpoint.

DEVELOPMENT OF THE METHOD

Principles and Instrumentation

Fluorescence in dilute solution may be expressed in terms of the following equation

$$F_m = K\ I_o\ e\ c\ \phi$$

where F_m is the magnitude of the fluorescence emission in arbitrary units; K is a constant for a particular instrument; I_o is the intensity of the exciting light; e is the molar extinction coefficient of the fluorescing compound at the wavelength of exciting light; ϕ is the quantum efficiency, i.e. the fraction of total photons absorbed which is reemitted as fluorescence; and c is the concentration of fluorescent species.

Thus it is evident that one can increase F_m at constant c by increasing the intensity of the exciting source, by choosing an absorber of greater e or environmental conditions so as to allow greater quantum efficiency, or simply by increasing the electronic amplification (which is included in K). The lower limit of sensitivity for a fluorescent species is determined by the electrical noise level and residual light sources in the sample cell compartment such as Rayleigh or Raman scatter of the solvent or sample cell.

The fluorometric process can also be viewed as providing a direct measurement of absorbed light rather than the indirect procedure of measuring the difference in intensity between the attenuated and reference beam, as in spectrophotometry.

The fluorescence measurements in this work were performed on an Aminco-Bowman Spectrophotofluorometer. This instrument, which has been described by Hercules (2), employs a high pressure xenon arc source powered by a D.C. power supply. The wavelengths of excitation and of emission are controlled by dual monochromators which may be automatically or manually varied. The emitted light from the sample cell is detected by a photomultiplier and the signal is amplified and displayed on a meter and on a recorder.

Spectra Correction

Fluorescence emission spectra recorded on most spectrophotofluorometers are not true spectra. As a result of the presence of many wavelength sensitive sources such as monochromators, light sources and photomultiplier tubes, the spectrum obtained directly from the instrument does not represent the true spectrum exactly. Thus, if spectra are to be compared in the literature and if accurate quantum efficiencies are to be determined, it is necessary that the spectra presented be independent of the instrument on which they are measured. Several methods of spectral correction have been discussed in the literature (26-27). The method of Melhuish (26) was used in this work.

The correction factors necessary for spectral correction on this instrument were previously determined by Hensler (14).

His correction factors were used directly in the correction of the spectral data obtained in this work.

Reagents

The zinc stock solution was prepared by dissolution of Baker Analyzed Reagent (99.9%) zinc in 50 ml of concentrated sulfuric acid. More dilute solutions were prepared by appropriate dilutions with distilled water.

The cadmium stock solution was prepared by dissolution of Baker Analyzed Reagent Cadmium Chloride in distilled water so as to produce a solution about 0.1 M in cadmium(II). The solution was then standardized by EDTA titration.

Phen was obtained from Aldrich Chemical Co. and recrystallized from water.

4,7-dimethylphen, 5-phenylphen, 4,7-diphenylphen, 5-methylphen, and 5,6-dimethylphen were obtained from the G. Frederick Smith Chemical Co. and used as received.

The 3- and 5-sulfonic acid derivatives of phen were prepared by John Richard of this laboratory.

Since fluorescence analysis is a very sensitive trace method, the water sources of the laboratory were checked periodically for a fluorescence blank. The water used for fluorescence measurements was taken from three sources: 1. The distilled water line of the laboratory; 2. Distilled water taken from the laboratory line and passed through an Illco-Way mixed-bed ion exchange column; 3. Distilled water from the laboratory line which had been placed in a plastic squeeze bottle. Figure 1 shows a sample of the fluorescence emission spectra of each of these solutions. Such day-to-day measurements of the blank contributed by these water sources indicated that the blank was highly variable. Figure 1 shows perhaps the highest blank for the first two distilled water sources recorded during this research. From the figure it can also be seen that plastic containers may contribute to the blank. The plastic squeeze bottle water whose emission spectrum is shown in Figure 1 had been left in the bottle for one week. Most of the time, however, the laboratory distilled water supply provided a fluorescence-free solvent. For these reasons the water used in this research was taken from the laboratory distilled water line on a "clean" day and stored in glass containers until used. No water was used which had been in contact with a plastic squeeze bottle for more than a few hours.

Measurement of Zinc Chelate Fluorescence

Ten solutions of increasing mole ratio of zinc to phen were prepared in the following way. 10 ml of 1.00×10^{-2} M phen was placed in each of 10, 100 ml volumetric flasks. Varying volumes of zinc stock solutions were added to the flasks to produce 10 solutions, with the ratio of zinc to phen ranging from 0.01 to 2.00. One solution had no metal. The solutions were diluted nearly to volume and adjusted to pH 3.1 ± 0.1 They were then diluted to the mark.

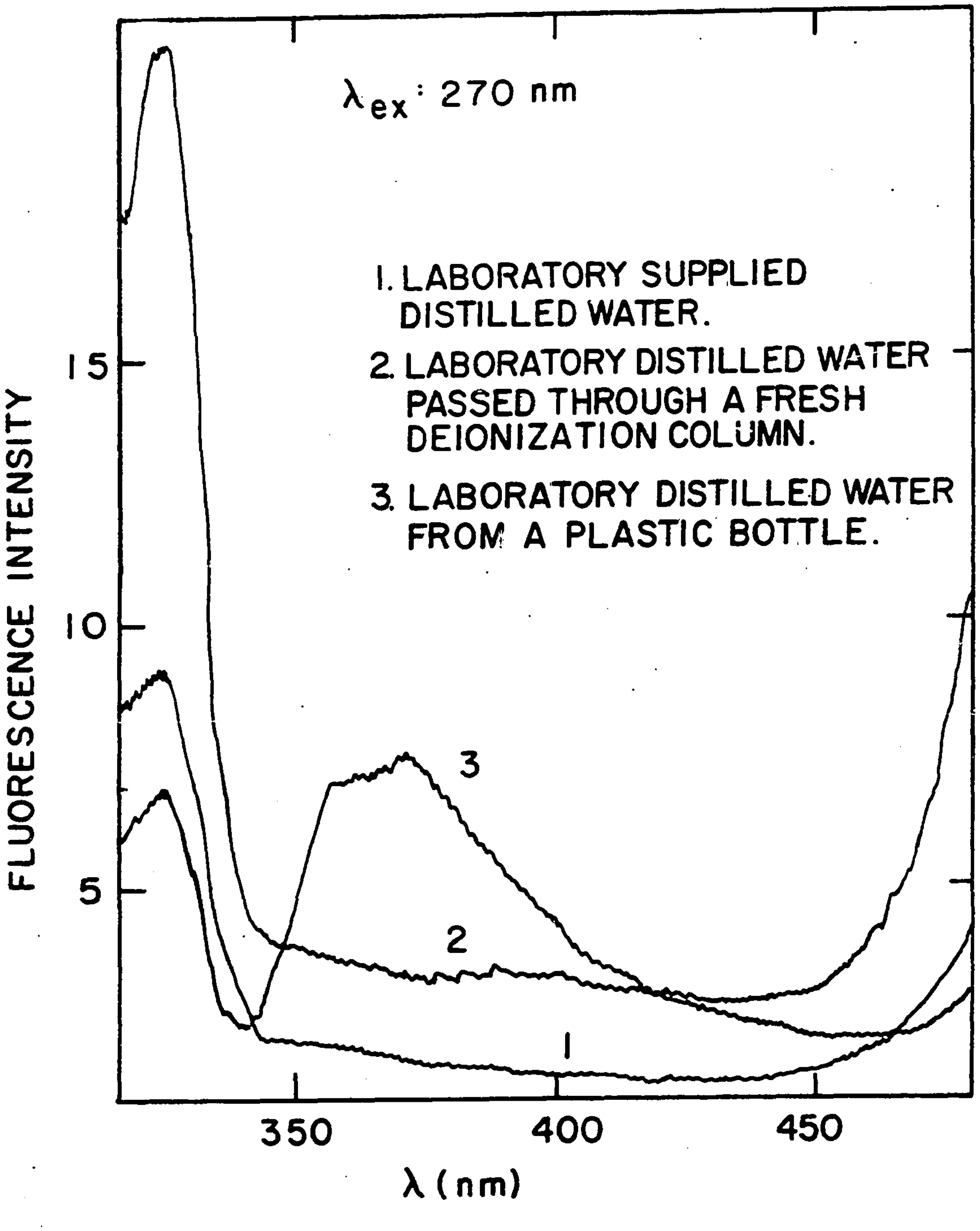


Fig. 1. Fluorescence spectra of three laboratory water sources

Absorption and fluorescence spectra were obtained for both the complex and the solution containing no metal. Absorption spectra of aqueous phen solutions have been presented by Hensler (14) and will not be presented here. The position and intensity of the absorption bands of the metal chelate are essentially the same as those of the protonated form of phen. The fluorescence emission spectra of these solutions are shown in Figure 2.

Figure 3 shows the chelate fluorescence of the zinc $(\mathrm{phen})_n^{+2}$ species in solution of pH 7.2. This figure allows comparison of the chelate fluorescence with that of the free base phen. The solutions were prepared in a manner analogous to that just described. The solutions were adjusted to pH 7.2 with NaOH solution.

From the fluorescence spectra of the $\mathrm{zinc(phen)}_n^{+2}$ chelates, several things should be noted. First, the wavelength of maximum emission of the chelate is shifted to 372 nm from the 415 nm peak of the protonated ligand.

Second, the intensity of fluorescence of the metal chelate is much larger than that of the protonated ligand. This has been attributed to the lowering in energy of the n states in the phen molecule such that the lowest energy transition is not $\pi^* \rightarrow n$, as in the ligand, but $\pi^* \rightarrow \pi$, which is a fully allowed transition (14).

Third, as the ratio of metal to ligand is increased, the fluorescence intensity of the complex increases to a maximum

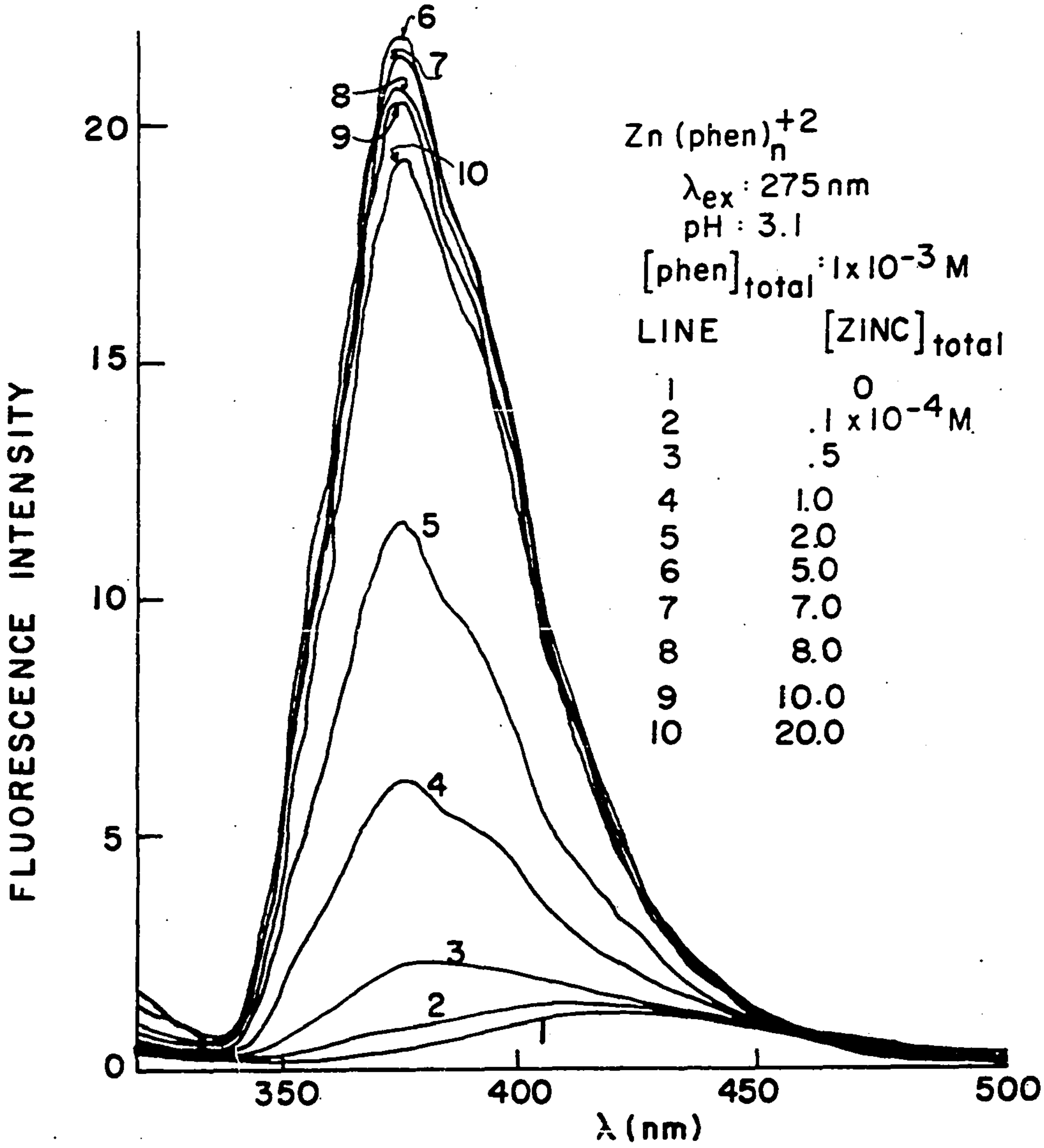


Fig. 2. Fluorescence spectra of zinc(phen)$_n^{+2}$ in acid

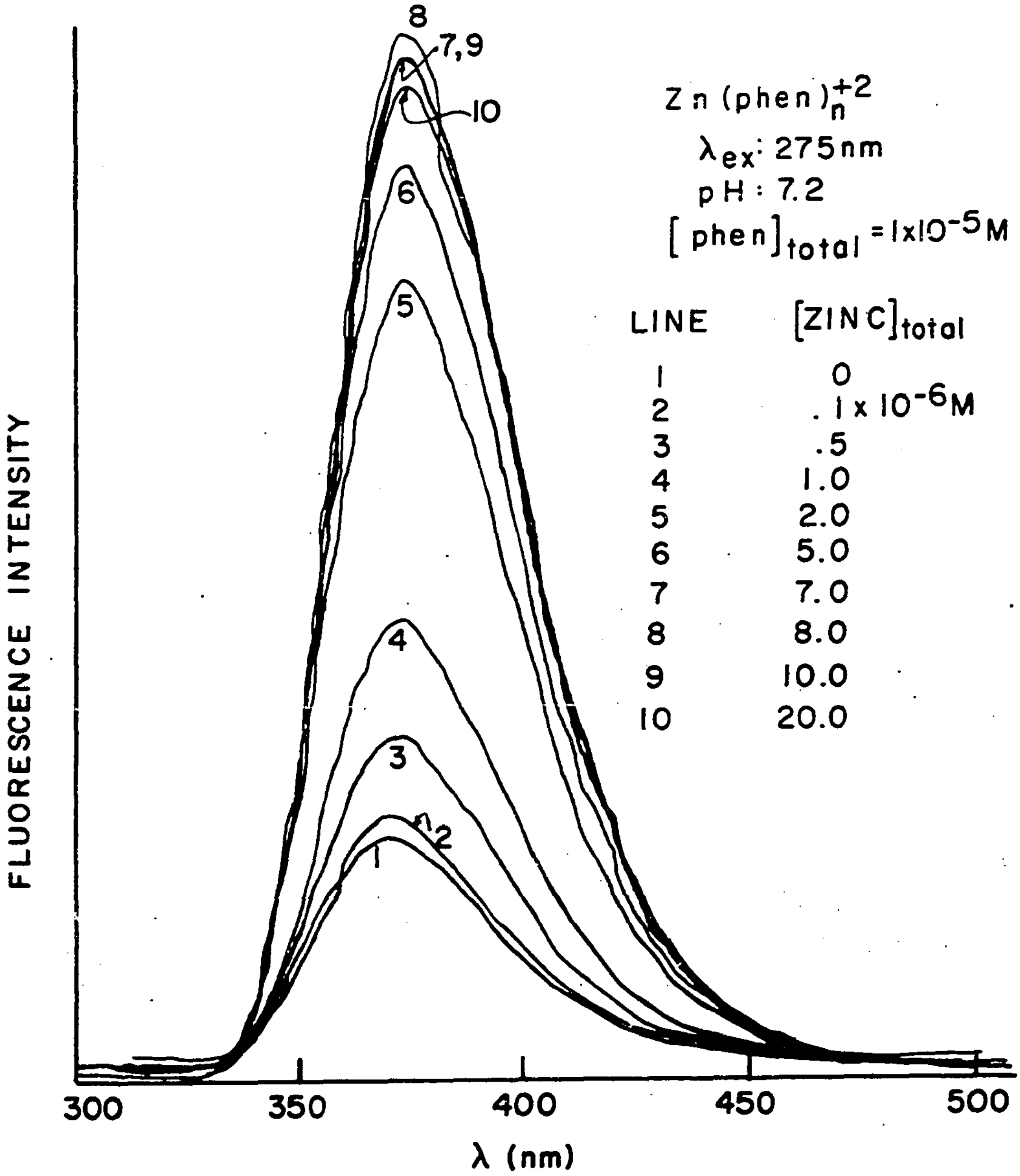


Fig. 3. Fluorescence spectra of zinc(phen)$_n^{+2}$ in base

and then decreases. This is a result of increasing solvent interaction effects with the chelates as the ratio of ligand to metal decreases from 3:1 to 1:1.

Measurement of Cadmium Chelate Fluorescence

Observation of the fluorescence of the zinc$(\text{phen})_n^{+2}$ species suggested that the analogous cadmium chelates might also fluoresce. Solutions of varying metal to ligand ratios were prepared as described previously and absorption and emission spectra measured. The fluorescence emission spectra are presented in Figures 4 and 5.

The fluorescence of the cadmium species is less intense than that of the corresponding zinc chelate. The decrease of fluorescence intensity with the metal/ligand ratio increasing beyond 0.5 is also not observed in the cadmium solutions. This is apparently due to the lower formation constants of the cadmium-phen chelates.

Determination of Quantum Efficiency of the Metal-phen Chelates

The fluorescence quantum efficiency is defined as the number of quanta emitted by a fluorescing species divided by the number of quanta absorbed. This number is a fundamental physical quantity analogous to the molar absorptivity in absorption spectroscopy. In analytical chemistry the quantum efficiency helps define the detection limit of a procedure.

The quantum efficiencies of the mono, bis, and tris zinc-phen chelates are different, however a quantum efficiency for

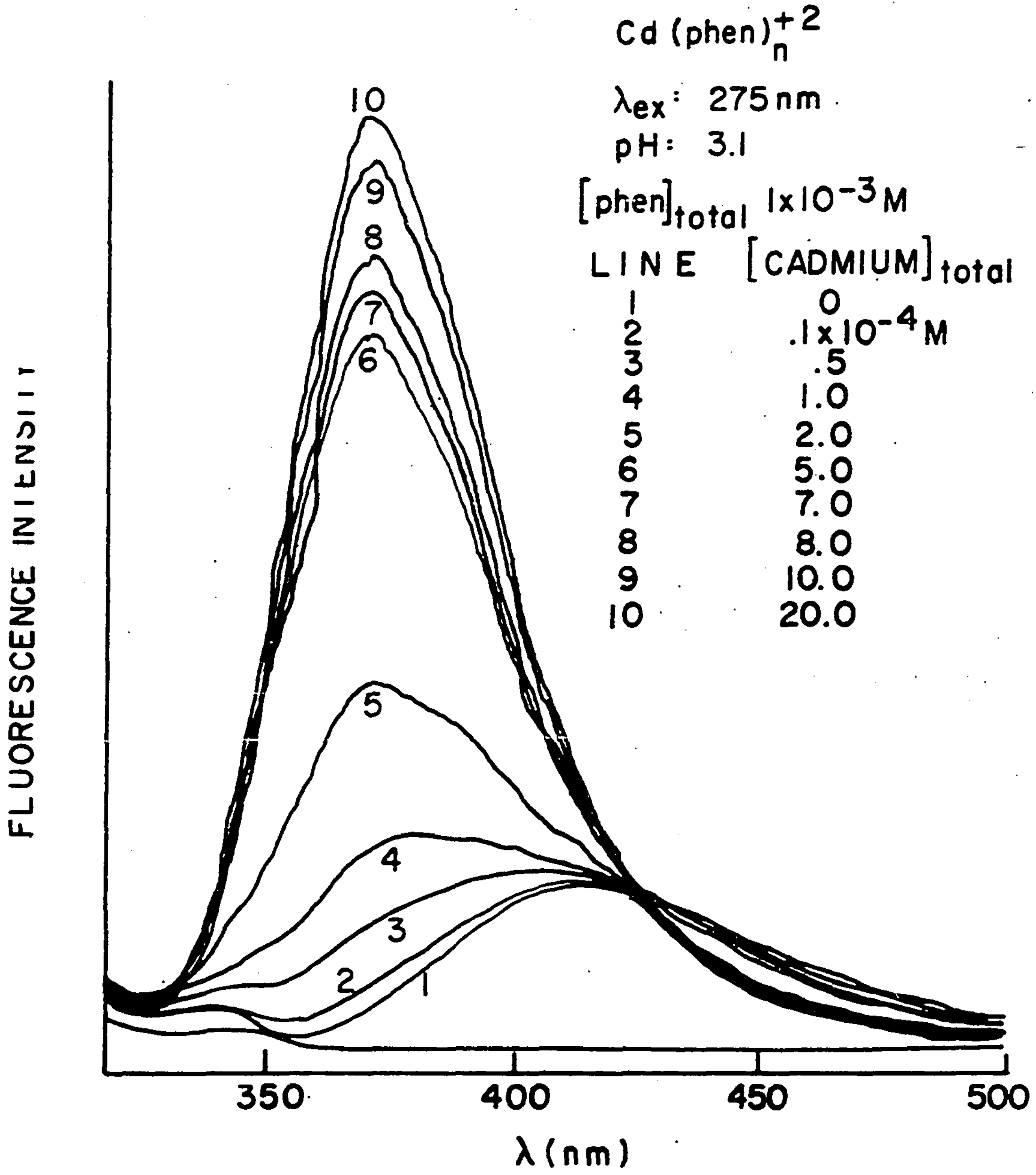


Fig. 4. Fluorescence spectra of cadmium(phen)$_n^{+2}$ in acid

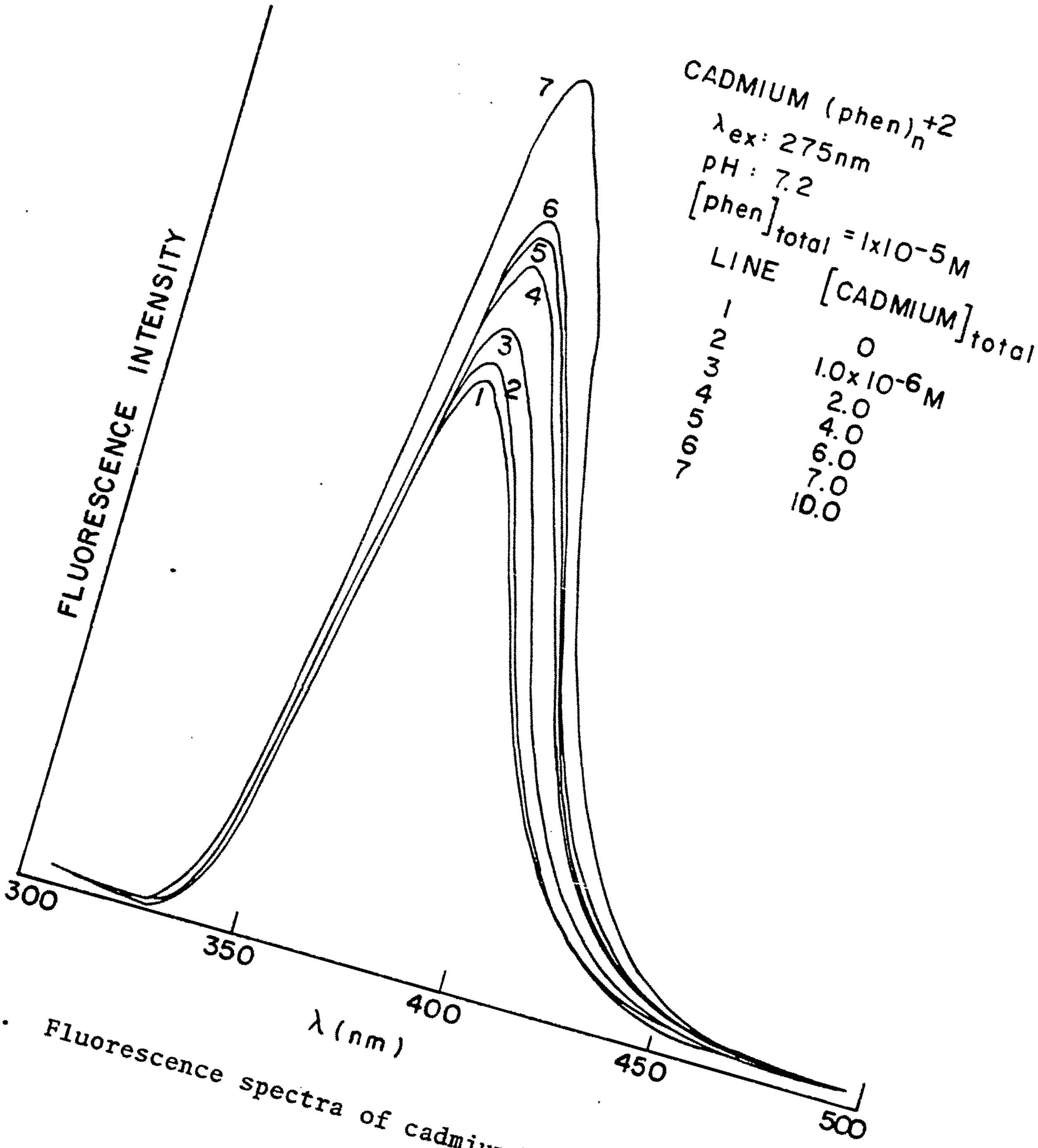


Fig. 5. Fluorescence spectra of cadmium$(phen)_n^{+2}$ in base

the solution containing a particular ratio of ligand to metal can be determined easily from the emission spectra. The quantum efficiency of the solution of [phen]/[zinc] = 2 was determined.

The integrated area of a corrected fluorescence emission spectrum is proportional to the total intensity of fluorescent light emitted by the solution, and hence to the product $I_0 \; e \; c \; \emptyset$ (28,29). Thus if the fluorescence emission spectra of two solutions are obtained with the same apparatus and with the same intensity of exciting light, I_0, then the ratio of their integrated areas can be used to determine the relative quantum efficiencies of the two solutions. Chen (30) evaluated quinine as a reference compound for the determination of quantum efficiencies of fluorescent species and suggested the formula

$$\frac{Q_1}{Q_2} = \frac{I_2}{I_1} \times \frac{F_1}{F_2} \times \frac{A_2}{A_1}$$

where Q is the quantum efficiency, F is the integrated area, A is the absorbance of the solution at the excitation wavelength, and I is the source intensity at the excitation wavelength. Thus the quantum efficiency of a solution may be determined by comparison with a solution whose quantum efficiency is known.

Hensler (14) originally determined the quantum efficiencies of the free and protonated forms of phen in aqueous solution to be 0.017 and 0.007 respectively. In this work the quantum efficiency of the solution of [zinc]/[phen] = 0.5 was determined by comparison with that of the protonated phen and found to be 0.110. This represents an increase in quantum efficiency of the chelate over the protonated ligand of a factor of about 16.

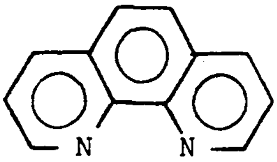
A similar determination of the quantum efficiency of the cadmium-phen solution, of ligand to metal ratio two, gave a value of 0.050 as the efficiency of the cadmium chelate.

Study of Substituted 1,10-phenanthrolines

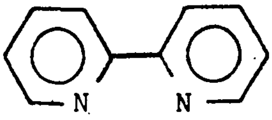
A number of substituted 1,10-phenanthrolines were investigated along with the unsubstituted phen as potential fluorometric reagents for zinc. Aqueous solutions of these compounds were prepared, and the fluorescence emission spectra were taken. The pH of the solutions was 3.0, and the excitation wavelength was 270 nm. The compounds, along with their measured emission maxima and relative intensities (ratios of peak area) are listed in Table 1. Their structures are shown in Figure 6.

Table 1. Fluorescence of substituted 1,10-phenanthrolines

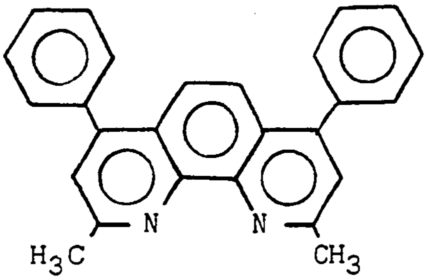
	Concentration	Emission maximum	Relative intensity
1,10-phenanthroline	1×10^{-5}M	415	1.00
3-sulfonic acid-1,10-phen	"	400	.65
5-sulfonic acid-1,10-phen	"	405	.72
5,6-dimethyl-1,10-phen	"	470	3.30
5-methyl-1,10-phen	"	450	5.90
4,7-dimethyl-1,10-phen	"	410	7.10
5-phenyl-1,10-phen	"	485	8.80
4,7-diphenyl-1,10-phen	"	450	122



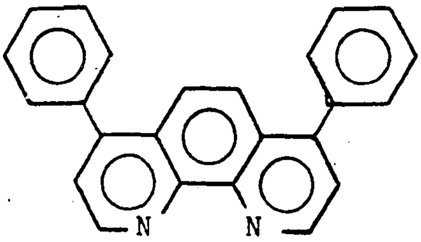
1,10-Phenanthroline



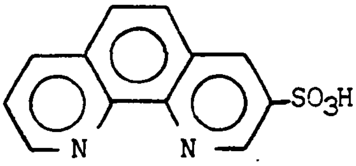
2,2'-Bipyridine



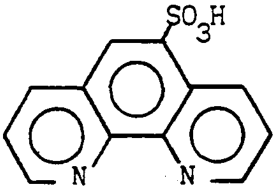
2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline



4,7-Diphenyl-1,10-phenanthroline

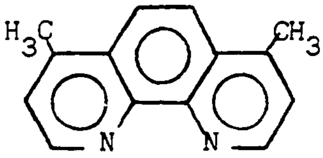


3-Sulfonic acid-1,10-phenanthroline

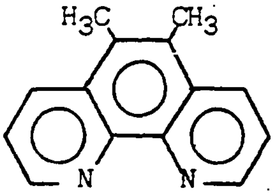


5-Sulfonic acid-1,10-phenanthroline

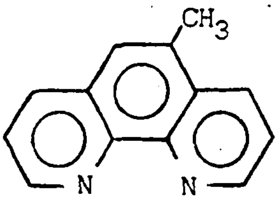
Fig. 6. Structures of 1,10-phenanthroline and related nitrogen heterocycles



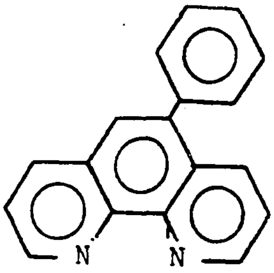
4,7-Dimethyl-
1,10-phenanthroline



5,6-Dimethyl-1,10-phenanthroline



5-Methyl-
1,10-phenanthroline



5-Phenyl-
1,10-phenanthroline

Fig. 6. (Continued)

The zinc chelates of these ligands were then prepared in aqueous solution and the corresponding emission spectra obtained. The solutions used were of pH 2.8, $[\text{metal}]_{\text{total}}$ = $3\text{x}10^{-4}$ M, and $[\text{phen}]_{\text{total}}$ = $1\text{x}10^{-3}$ M. The results are shown in Tables 2 and 3.

Table 2. Relative fluorescence of zinc phenanthrolates

Ligand	Solvent	λ(emission maximum) nm	Relative peak height
1,10-phenanthroline	H_2O	372	1.00
3-sulfonic acid-1,10-phen	"	380	.88
5-sulfonic acid-1,10-phen	"	365	1.22
5,6-dimethyl-1,10-phen	"	460	2.77
5-methyl-1,10-phen	"	440	1.40
4,7-dimethyl-1,10-phen	"	405	2.02
5-phenyl-1,10-phen	"	475	.91
4,7-diphenyl-1,10-phen	95% H_2O- 5% ethanol	446	6.40

Table 3. Relative fluorescence of cadmium phenanthrolates (compared to zinc-1,10-phen)

Ligand	Solvent	λ(emission maximum)	Relative peak height
1,10-phenanthroline	H_2O	375	.20
3-sulfonic acid-1,10-phen	"	393	.26
5-sulfonic acid-1,10-phen	"	390	.29
5,6-dimethyl-1,10-phen	"	465	2.83
5-methyl-1,10-phen	"	446	1.40
4,7-dimethyl-1,10-phen	"	408	1.85
5-phenyl-1,10-phen	"	480	.90
4,7-diphenyl-1,10-phen	95% H_2O 5% ethanol	450	6.43

From the data on Table 1 it is obvious that several ligands fluoresce more intensely than phen in aqueous solution. In particular, 4,7-diphenyl-phen(bathophenanthroline) fluoresces much more intensely than the unsubstituted phen. However, the data of Tables 2 and 3 indicate that the fluorescence of the chelates, in terms of peak height, is not as great relative to the phen chelates as are the ligand fluorescences compared to uncomplexed phen. Thus in most cases the chelate fluorescence of the substituted ligands is more intense than that of the unsubstituted phen, and the even stronger fluorescence of the free ligand creates a larger background. Only the spectra of the sulfonic acid ligands indicated a significantly greater fluorescence intensity for the chelate than the free ligand. The sulfonic acid ligands were not as sensitive as phen, however. Thus none of the substituted ligands provided the combination of low base line and high sensitivity that would make it a significantly better fluorometric reagent than phen.

Choice of Conditions

The peak fluorescence intensity for the chelate species zinc$(\text{phen})_n^{+2}$ occurs at about 372 nm. Phen shows two fluorescence peaks in dilute aqueous solution, one at about 370 nm resulting from the presence of the free base form, and one at 415 nm resulting from the monoprotonated form. Thus there exist two immediately obvious ways in which to approach the measurement of a difference in fluorescence intensity in this system. The first is to measure the fluorescence in acid solution at 372 nm and subtract the fluorescence resulting from $Hphen^+$. The advantages in this are that it allows one to take full advantage of the fact that the quantum efficiency of the chelate is 20 times that of $Hphen^+$ and that it is possible, by proper choice of slits, to resolve the chelate band from the $Hphen^+$ fluorescence more completely. The disadvantages are that for low metal concentrations the measurement is taking place on a sharply sloping shoulder of what is predominantly a $Hphen^+$ fluorescence peak, and that in acid solutions of $pH < 3$, $[Hphen^+] \gg [phen]$. Thus the fluorescent chelate concentration and the average number of ligands per metal are reduced because of the low concentration of the free base form of phen.

The other alternative is to perform the determination in a solution of acidity such that $[phen] \gg [Hphen^+]$. The serious disadvantage to this is that the free base form has a quantum efficiency almost 3 times that of the acid form and this reduces the observable difference in fluorescence emission between ligand and chelate as a result of a higher background.

Results indicated that while for normal concentrations of metal either approach was satisfactory, at the lower levels of metal concentration the difficulty of complexing all the metal made the second approach more desirable.

Using these conditions, the detection limits for the analysis of zinc and cadmium in aqueous solution were found to be about 0.1 ppb (2×10^{-9} M) and 112 ppb (1×10^{-6} M) respectively using the definition of detection limit as that concentration giving a signal which is twice the rms noise.

Using this approach, a series of zinc solutions of pH 7.2 ranging in concentration from $1x10^{-7}$ M to $1x10^{-6}$ M were prepared and the relationship between fluorescence intensity and zinc concentration determined. The formal phen concentration was $3x10^{-5}$ M and each solution was made $1x10^{-3}$ M in phosphate buffer. The results are shown in Figure 7. From the plot it can be seen that the fluorescence of the complex is characterized by a linear increase in intensity and that the plot could be used as a calibration curve in analytical determinations. A similar experiment in acid solution yielded results of much poorer reproducibility, apparently because of the lower sensitivity with decreased complexation.

Interferences

A study of interferences in the determination of zinc at the 63 ppb level was performed by preparing solutions containing known amounts of particular anions and cations as well as the zinc-phen chelates and noting the effect upon fluorescence intensity. All measurements were performed at pH 7.2. The phen concentration was $3x10^{-5}$ M and the zinc concentration was $1x10^{-6}$ M. All solutions were $1x10^{-3}$ M in phosphate since this was added as a buffer. Phosphate had been previously shown to give no interference. The results of this investigation are shown in Table 4. The error inherent in the method and instru-

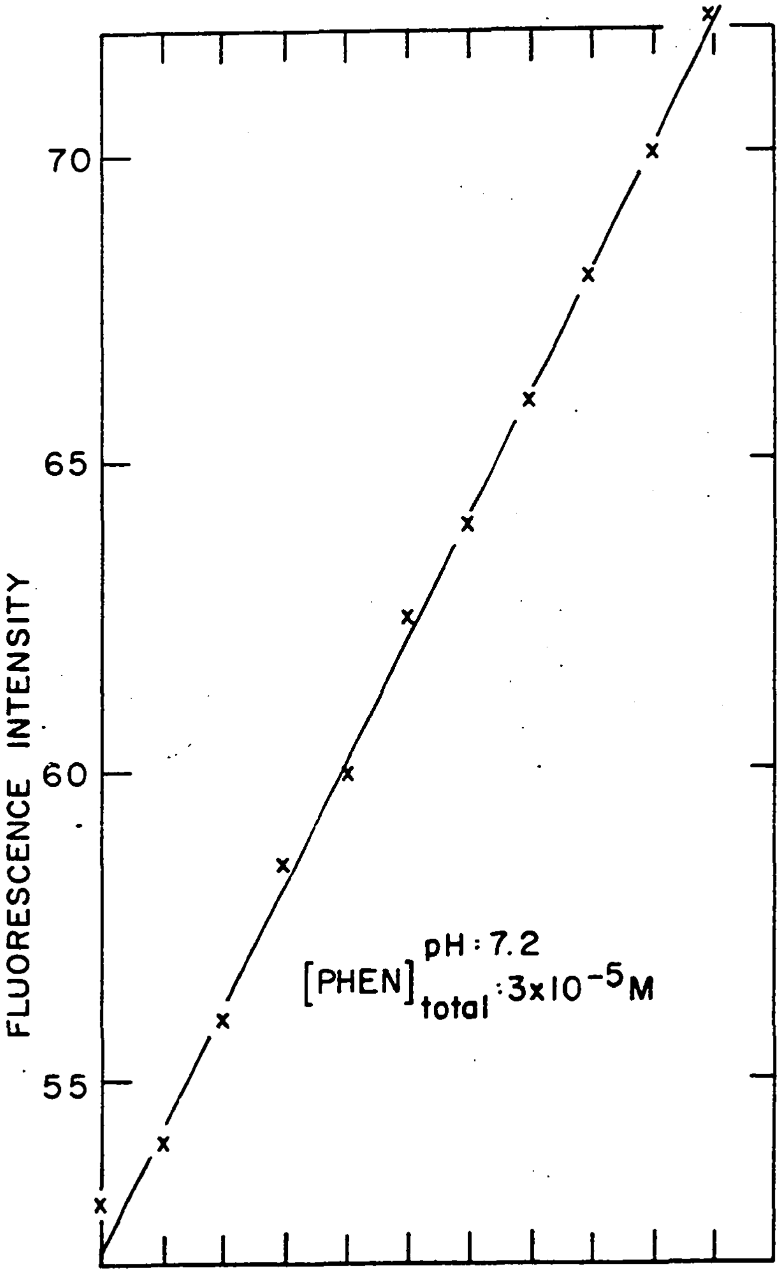


Fig. 7. Fluorescence intensity as a function of zinc concentration

Table 4. Interferences in the determination of $1\mathrm{x}10^{-6}$ M zinc

Species	Added as	Conc interference / Conc zinc	% error
Na^+	Na_2SO_4	2000	1.3
Li^+	$LiNO_3$	100	1.3
K^+	KNO_3	100	1.0
NH_4^+	NH_4NO_3	10	1.0
Mg^{+2}	$Mg(NO_3)_2$	10	2.5
Ca^{+2}	$Ca(NO_3)_2$	10	2.5
Sr^{+2}	$Sr(NO_3)_2$	10	1.0
Ba^{+2}	$Ba(NO_3)_2$	10	2.5
Pb^{+2}	$Pb(NO_3)_2$	10	1.1
Mn^{+2}	$MnSO_4$	10	2.5
Ni^{+2}	$Ni(NO_3)_2$	10	very high
Co^{+2}	$CoSO_4$	10	very high
Cu^{+2}	$CuSO_4$	10	very high
Hg^{+2}	$Hg(NO_3)_2$	10	very high
Cr^{+3}	$Cr_2(SO_4)_3$	10	17.5
VO^{+2}	$VOSO_4$	10	15.0
Fe^{+2}	$FeSO_4$	10	50.0
Fe^{+3}	$Fe_2(SO_4)_3$	10	2.5
Ti(IV)	$TiOSO_4$	10	17.5
U(VI)	$UO_2(NO_3)_2$	10	10.0
Ag^+	$AgNO_3$	10	very high
IO_3^-	$NaIO_3$	10	2.5
NO_3^-	$NaNO_3$	100	1.3
$C_2H_3O_2^-$	$KC_2H_3O_2$	10	2.5
CN^-	KCN	10	2.5
F^-	NaF	100	0.0
Cl^-	NaCl	100	0.0
I^-	KI	10	0.0
SO_4^{-2}	Na_2SO_4	1000	1.3
$Cr_2O_7^{-2}$	$K_2Cr_2O_7$	10	45

mentation is 2-3%.

From the results it can be seen that the major interferences are most of the first row transition metals as well as uranium(VI), silver(I), and mercury(II).

Since these interferences are rather common, it was decided to try to achieve a rapid, simple separation which would not hamper the sensitivity of the measuring technique. Anion exchange chromatography is such a separation method.

A batch of 150-200 mesh Dowex 1x8 was washed repeatedly with water and acetone. An ethanol wash was performed and the wash solution was used to check for the presence of fluorescent impurities. None were found. Nine columns of about 0.8x2.0 cm were prepared by slurrying the resin in water and pouring it into the column onto a small plug of firmly tamped glass wool.

A procedure for the separation of small amounts of zinc or cadmium in synthetic samples from the interferences listed above was tested and is as follows.

1. Place the sample, containing not less than 0.1 micromoles of zinc or cadmium in a beaker and add enough concentrated hydrochloric acid to make the solution 0.5 M in HCl. If silver precipitates, filter.

2. Pass the sample solution through the column. Air pressure may be applied to speed the process.

3. Pass 20-30 ml of 0.5 M HCl through the column.

4. Elute the zinc with 50 ml of 1 M Na_2SO_4 into a 100 ml volumetric flask.

5. Add enough 1 M sodium hydroxide to the collected effluent to bring the pH to about 7 and then add 1 ml of 0.1 M phosphate buffer. Dilute to volume.

Using this procedure it is possible to separate 0.1 micromole or more of zinc from 1000 times as much each of chromium (III), nickel(II), vanadium(IV), cobalt(II), copper(II), iron (II), titanium(IV), and uranium(VI). Analysis of zinc in three synthetic samples containing these interferences yielded the results listed in Table 5.

Table 5. Fluorometric determination of 0.1 micromole of zinc after separation

Amount of zinc present	0.1000 micromole
Amount found	0.0966 micromole
Error	3.4%
Relative standard deviation	6.0 pph

CONCLUSION

Complexation of zinc and cadmium with 1,10-phenanthroline provides a highly sensitive direct fluorescence method of analysis for zinc and a moderately sensitive method for cadmium. When interfering metals are present, a separation is necessary, but is easily accomplished. This method might be most appropriate to samples in which there are few other metal ions in high concentration, e.g. biological samples. In these cases the method could either be applied directly or, in case of other difficult-to-remove fluorescing materials, after digestion of the organic material. The procedure might also be easily applied to determinations of traces of zinc or cadmium in noninterfering metals. Or the separation procedure could be used to make the method more widely applicable.

PART II. PARTIALLY SULFONATED, MACRORETICULAR ION EXCHANGE RESINS IN THE SEPARATION OF METAL IONS

INTRODUCTION

Purpose

Most common chemical analytical methods are not specific enough to determine the amount of one substance accurately in the presence of often massive quantities of other, interfering substances. Thus most analytical procedures incorporate a separation step to remove unwanted material and to permit the quantification of the sought-for-substance.

Ion exchange separations by column chromatography have long been one of the principal methods of separation of metal ions in solution. Such separations usually required a minimum of 20-30 minutes for a separation and in rare cases the time necessary for separation reached months. Even when the separation was complete one often still had to measure the amount of the desired component by another method. Long separation and analysis times resulted in expensive analyses.

Recently the application of high pressure chromatographic techniques to ion exchange separation of metals has resulted in greatly reduced analysis times (31-35). High mobile phase velocities, small particle sizes and in-stream detection and quantitation have combined to permit the separation and analysis of many different metal ions in five minutes or less.

The ion exchange resins used in these separations have usually been strong acid or strong base type poly(styrene-divinylbenzene) copolymers. They may be either gel-type beads (microporous), or macroreticular (macroporous). With few exceptions they are usually full-capacity resins, i.e. resins containing as many ionic sites on the matrix as it is possible to obtain without destroying the polymer network.

The rapid separations obtainable by high pressure chromatography require that the sought-for-substance be removed from the resin easily under simple conditions. There are some metal ions which are very strongly retained on a cation exchange resin in strong acid media and require very concentrated acid or complexing agent for removal (36).

Simple consideration of a typical ion exchange equilibrium

$$A + BR = AR + B$$

shows that under conditions of low loading of A, (i.e. A ions occupy less than 1% of the available resin sites) one way to lower the distribution ratio of A, where the distribution ratio D is defined as

$$D = \frac{(AR)}{(A)}$$

would be to reduce (BR), that is to reduce the resin capacity. This would allow the removal of ionic species from the resin under simpler conditions, e.g. lower concentrations of strong acids. Correspondingly, this would allow shorter elution times for the same eluent.

The purpose of this study was threefold. 1. To produce separations of those metals which are strongly retained on fully sulfonated cation exchangers in a short period of time using simple and inexpensive eluents. 2. To study the effect of lowered capacity on selectivity of macroreticular sulfonic-acid type exchangers. 3. To verify that the kinetics of metal ion-hydrogen ion exchange on partially sulfonated, macroreticular resins are fast enough to permit rapid, separations with high resolution and good peak shape.

Literature Survey

Ion exchange

Probably the first application of ion exchange has been attributed to Moses, who, in 3000 B.C. "rendered the saline water of the spring of Marah potable" by throwing a dead log into the spring (37). It has been suggested that carboxyl groups present in the log as a result of cellulose oxidation could have resulted in removal of metal ions by ion exchange.

The discovery of the ion exchange process itself is more generally credited to Way (38) and Thompson (39) who, in 1850, found that calcium was replaced by ammonium ion in soils. Way traced the cause of this exchange in soils to the presence of double silicates of aluminum with sodium, potassium or calcium (40).

For many years ion exchange was performed almost exclusively with such natural and synthetic aluminum silicates. However, in 1935 Adams and Holmes (41) synthesized the first organic ion exchange resins. They prepared cation exchange resins with phenolic functional groups and anion exchange resins with aromatic amine groups. The synthesis of sulfonic acid, poly(styrene-divinylbenzene) copolymers by D'Alelio (42) in 1945 gave the first strong acid, monofunctional cation exchange resin.

Most of the research with ion exchange resins has been with the sulfonated poly(styrene-divinylbenzene) type. A great deal of published material on full capacity resins of this type is available in the literature. Most of this material is summarized by Rieman and Walton (37), Helfferich (43), and others.

The first attempt to investigate partially sulfonated cation exchange resins was made by Boyd, Soldano and Bonner (44). They employed acid hydrolysis of fully sulfonated resins at 180-220 C to desulfonate the resin. By this means they hoped to achieve a random distribution of remaining functional groups. There was, however, considerable evidence of side reactions leading to reduction of the sulfonate and breakdown of the polymer.

Freeman and Aiyar (45) found that a dilute solution of sulfuric acid in nitrobenzene provides homogeneous sulfonation at room temperature for gel type beads of 1 to 4% divinylbenzene content and ranging in size from 250 to 700 microns in diameter.

Reichenberg (46) attempted a random sulfonation by a similar process of swelling the beads in nitrobenzene, allowing sulfuric acid to diffuse into the cold mixture, and then raising the temperature to effect the sulfonation. However, he found that this technique could not be applied to resins containing more than about 15% divinylbenzene.

Fricke (47) studied partially sulfonated ion exchange resin beads which had been prepared by the method of Freeman. Homogeneity, size distributions in various electrolyte solutions, density, capacity and selectivity for univalent cation exchange systems were studied. All beads were sulfonated in the same reaction vessel concurrently and the partially sulfonated beads were obtained by removing batches of copolymer beads from the reaction vessel at periodic time intervals. It was found that even using this technique individual fractions removed from the vessel could be separated into density fractions, each of which had different capacities and which were therefore heterogeneously sulfonated. Fricke concluded that "sulfonation occurs to different extents at various sites within single beads and to different extents among beads which have been sulfonated concurrently".

Fricke also studied a series of superficially sulfonated resin beads. These were prepared by a method which was based on the explanation of the sulfonation process presented by Freeman and Aiyar (45), and Wiley and Venkalachalan (48). The model assumes that there is a core in the center of the bead, which is never reached by sulfuric acid in a polar solvent such as water, and a shell around the core which is 100% sulfonated. Thus the bead sulfonates successive layers from the outside in toward the core. The selectivity order for univalent cations was found to be the same as for fully sulfonated poly(styrene-divinylbenzene) exchangers. The distribution coefficients for both the surface sulfonated and the homogeneously partially sulfonated beads were less than those for the fully sulfonated beads.

Skafi and Lieser (49,50,51) also investigated a series of superficially sulfonated resin beads. They prepared their own beads by copolymerization of styrene with 25 weight per cent divinylbenzene according to the method of D'Alelio (42). Different batches of the beads were then sulfonated for various lengths of time. By using high crosslinking (i.e. high divinylbenzene content) and short sulfonation times they produced unsulfonated inert cores. The beads were 200 to 340 microns in size and had capacities of from 5.5×10^{-4} meq/g to 3×10^{-2} meq/g. Results of their studies indicated that their superficially sulfonated beads possessed selectivity similar to that of fully sulfonated conventional ion exchange resins, that the rates of exchange on superficially sulfonated beads are much faster than on conventional resins, and that very fast separations in dilute eluents can be achieved on superficially sulfonated resins. However, the kinetic and equilibrium studies and the separations were all performed under tracer conditions using radiochemical methods of detection. The very low capacity resins which they used are not easily adaptable for use on an analytical scale.

Forced flow chromatography

For several years, high pressure liquid chromatography has been used for the rapid separation and analysis of organic systems. The use of high pressures to force eluents through a packed column allows the use of fine particles as column packings which in turn permits more rapid equilibration of stationary and mobile phases and thus fast separations with high resolution. Application of high-speed techniques to inorganic separations has been slow in arriving (52).

Several recent papers have dealt with rapid separations of metal ions by forced flow or pressurized ion exchange chromatography. Seymour (31-33) *et al*. described a forced flow liquid chromatographic system employing gas pressurization of the mobile phase and spectrophotometric detection. The unique feature of the system is the absence of any but inert materials (Kel-F, Teflon, glass) from contact with the eluent. Analytical methods based on rapid separation by anion exchange and in-stream eluent analysis were presented for lead, iron, and several other metal ions.

Fritz and Sickafoose (34) described a similar method for the separation and analysis of chromium. Chromium was first oxidized from chromium(III) to chromium(VI) and collected on an anion exchange column. The chromium(VI) is then stripped from the column with a perchlorate solution and the chromium measured spectrophotometrically as it is removed. The entire analysis cycle required 10 minutes.

Kawazu and Fritz (35) separated a mixture of seven metal ions by forced flow cation exchange chromatography with eluents of hydrochloric acid in 2-propanol-water and HCl in acetone-water mixtures. Detection was achieved by addition of a color forming reagent to the column effluent stream. A mixture of seven metal ions could be separated and detected in about 25 minutes.

Campbell (53) separated the lanthanide ions by pressurized ion exchange chromatography. The separation was done in alpha-hydroxyisobutyric acid on a cation exchange column. Although the separation itself took 2-4 hours, detection was by fraction collecting and radiochemical techniques. The total separation and analysis required about a week.

PREPARATION OF PARTIALLY SULFONATED RESIN BEADS

Introduction

The difficulties encountered by previous workers in the preparation of homogeneously sulfonated poly(styrene-divinylbenzene) have been described in a previous section. Only one group of workers (45) have claimed the production of homogeneously sulfonated low capacity resin beads by direct sulfonation and Fricke was unable to duplicate that homogeneity using beads of only slightly higher crosslinking.

The previous discussion of superficially sulfonated ion exchange resin beads revealed that such beads have very rapid exchange rates. This is a highly desirable characteristic in an exchange material which is to be used in high speed separations.

It is known that poly(styrene-divinylbenzene) copolymer beads are highly hydrophobic. They can be penetrated only by a nonpolar solvent. When the beads are placed in sulfuric acid solution, the solution cannot initially penetrate and sulfonates the exterior. After the first layer is sulfonated, the polar sulfuric acid can penetrate since ionic groups have been introduced into the bead, and the bead surface has been wetted. This was the rationale behind Frickes preparation of superficially sulfonated resins.

Experimental

Beads

The copolymer materials used in this study for the preparation of partially sulfonated ion exchange resin beads was the commonly used poly(styrene-divinylbenzene) copolymer. However, these materials were different from all those partially and superficially sulfonated beads described previously. Most of the previous research into low capacity sulfonic acid type exchangers employed either solid copolymer beads (superficially sulfonated) or, more often, gel type exchangers (partially sulfonated). The beads used in this research were macroreticular.

Macroreticular copolymer beads differ from conventional beads in several important ways. Macroreticular beads are produced by a special polymerization process which produces large, discrete physical pores in the copolymer matrix. Pore size and internal surface area can be varied over a wide range depending on the polymerization conditions. The pore walls are made up of hard, impenetrable chains of copolymer microspheres. The crosslinking is very high and the beads themselves do not swell significantly.

Gel-type beads, on the other hand are loose networks of polystyrene with varying amounts of crosslinking. Unless the crosslinking is very great they swell to two to three times their dry volume when placed in an appropriate solvent. Such pores that do exist are very small (on the order of 10 Å) and vary in size depending on crosslinking and solvent.

Macroreticular ion exchange resins are more resistant to chemical attack and to alternate wetting and drying than are gel-type resins (54,55). Gel-type resins swell much more in a solvent of high dielectric constant such as water than in most organic solvents. This creates a mechanical strain on the resin network which often causes a breakage of the bead. It is also known that macroreticular resins reach equilibrium faster than gel-type resins in the exchange of ions of large size (56), and that in most cases selectivity coefficients for the exchange of metal ions ($M^{+n} + nHR = MR_n + nH^+$) on macroreticular resins are greater than on gel-type resins (57) if the fraction of resin in the metal form is small. Macroreticular resins were chosen for this work because of these properties. Their chemical and physical stability give them long column life under conditions of forced flow chromatography. Beads which shrink or swell substantially depending on the eluent passing through the column require constant readjustment of the column to eliminate column dead volume. Part of the purpose of the research was to obtain rapid separations of metal ions having high ionic charge and, hence, a large hydration sphere making it difficult to pass into a microporous gel exchanger.

It was believed, on the basis of the work discussed in the introduction, that one could prepare low capacity beads that were sulfonated primarily on those surfaces of the copolymer bead that would be easiest for a polar solvent such as sulfuric acid to reach. As the exterior surface of the bead was sulfonated, the sulfuric acid would penetrate into the pores of the bead, thus sulfonating the interior surfaces.

The beads used in this research were obtained in 300-150 micron size from Rohm and Haas Chemical Co. as XAD-2. This and other macroreticular copolymer beads marketed by the same company have been well characterized (58). XAD-2 resin beads have a surface area of 300 square meters per gram of copolymer and an average pore diameter of 90 Å. Their structure is shown in Figure 8 .

Reagents

The water used in this work was distilled and then deionized in an Illco-Way mixed-bed ion exchange column.

The sulfonating agent was DuPont Reagent Grade 95-97% Sulfuric Acid, used as received.

Reagent grade acetone and absolute methanol were obtained from Baker Chemical Co. and used as received.

Standard sodium hydroxide and hydrochloric acid solutions were prepared by dissolving appropriate amounts of Baker Analyzed Reagent Sodium Hydroxide and DuPont Reagent Hydrochloric Acid in water. The sodium hydroxide solution was standardized against Baker Primary Standard Potassium Biphthalate which had been dried at 100 C for 2 hours. The hydrochloric acid solution was then standardized against the standard NaOH.

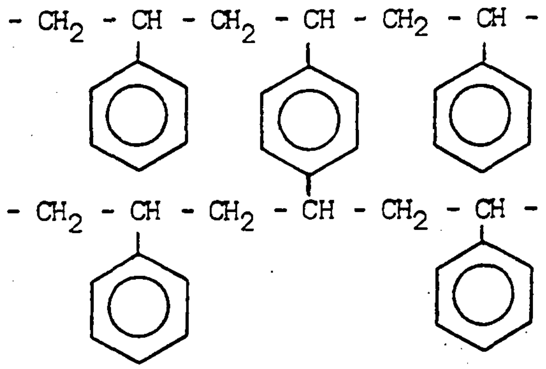


Fig. 8. Structure of poly(styrene-divinylbenzene)

Procedure

Batches of the beads were taken as received in the 300-150 micron size and placed in a Model 4-E Quaker City Mill and ground until they were all approximately 200-400 mesh. They were then dry-sieved and the portion not passing through the 250 mesh sieve was placed back in the mill for further grinding. The 250-325 mesh fraction (44-63 microns) size beads were removed for sulfonation.

The 250-325 mesh beads were washed with water, ethanol, and acetone and vacuum dried. 2-10 g portions of the copolymer were taken and placed in 50-100 ml of concentrated sulfuric acid. The beads were then sulfonated at various temperatures ranging from 2 to 170°C for varying lengths of time, as shown in Table 6 .

Table 6 . Sulfonation conditions

Resin capacity	Reaction temp C	Time hr	Resin capacity	Reaction temp C	Time hr
3.70	170	.5	1.72	62	.33
3.41	150	.5	1.35	57	.25
3.13	130	.5	1.14	35	.5
2.71	110	.5	1.05	25	.5
2.66	95	.5	1.01	50	.3
2.24	80	.5	0.84	30	.5
1.89	65	.5	0.23	2	.5

The reaction mixture was then placed in a suction filter and the liquid rapidly removed. The beads were then washed several times with water, methanol and acetone and air dried. Extreme fines were removed by flotation in methanol.

Measurements of the dry weight of a quantity of resin in the hydrogen form were made by two different methods.

1. Vacuum drying in a desiccator at 60°C overnight.
2. Air drying in a suction filter after washing with ethanol and acetone.

In no case did the weights obtained by the two methods differ by more than 0.5%. The second method, being the simpler, was then used for all subsequent weight determinations.

The dry hydrogen form weight capacity was determined by placing a weighed quantity of the dry, hydrogen form resin in a solution containing a stoichiometric excess of sodium hydroxide and back-titrating the excess with standard hydrochloric acid. Capacities of the twelve resins, determined

$$R\text{-}SO_3^-H^+ + Na^+ + OH^- = R\text{-}SO_3^-Na^+ + H_2O$$

by this method, ranged from .23 meq/g to 3.70 meq/g.

The capacity and monofunctionality of the resins were then checked by direct pH titration. The titrations of each of the resins were performed in 1 M KNO_3 using .1113 M NaOH as the titrant. Values of capacity determined by direct titration of the resins agreed within 1% of those obtained by the first method.

Results

Titration curves for two of the resins are shown in Figures 9 and 10. Both curves show the sharp break at the equivalence point which is characteristic of a strong acid

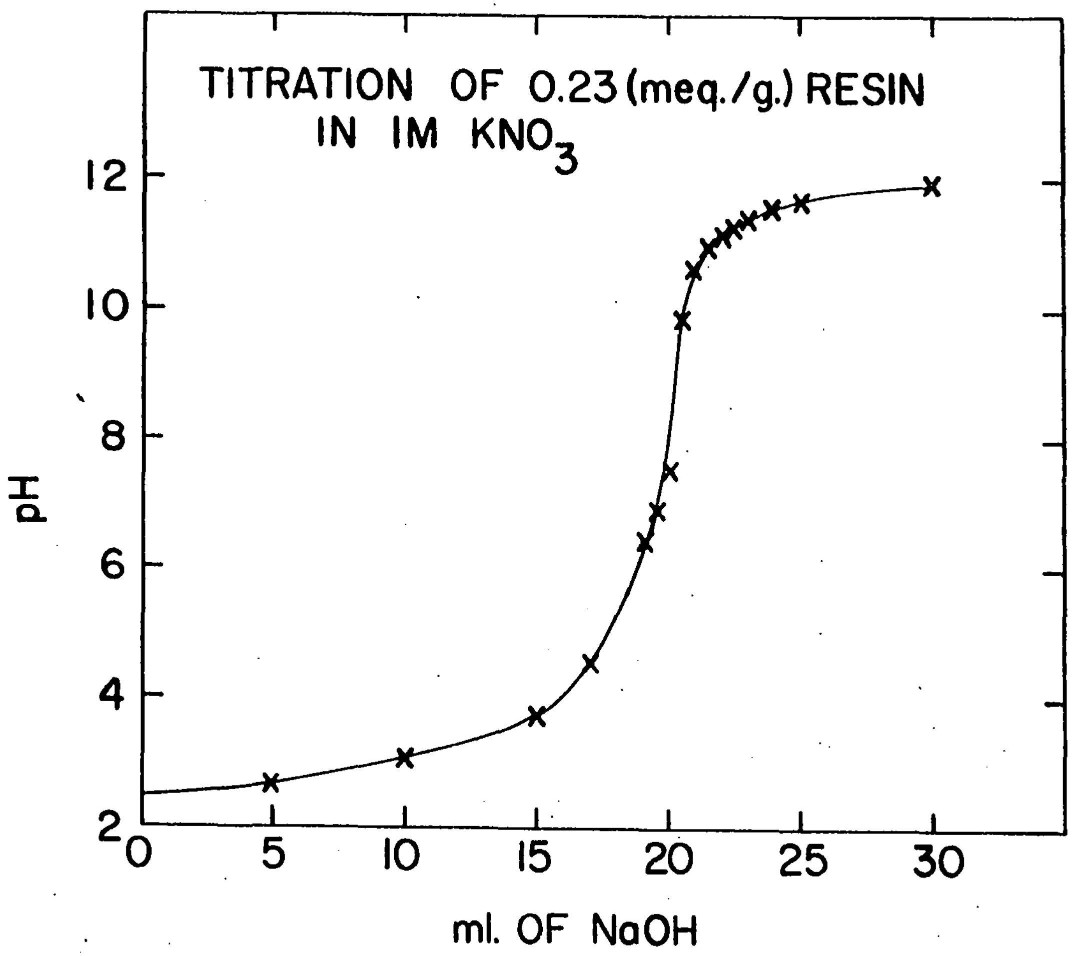


Fig. 9. Titration of 0.23 meq/g resin in 1 M KNO_3

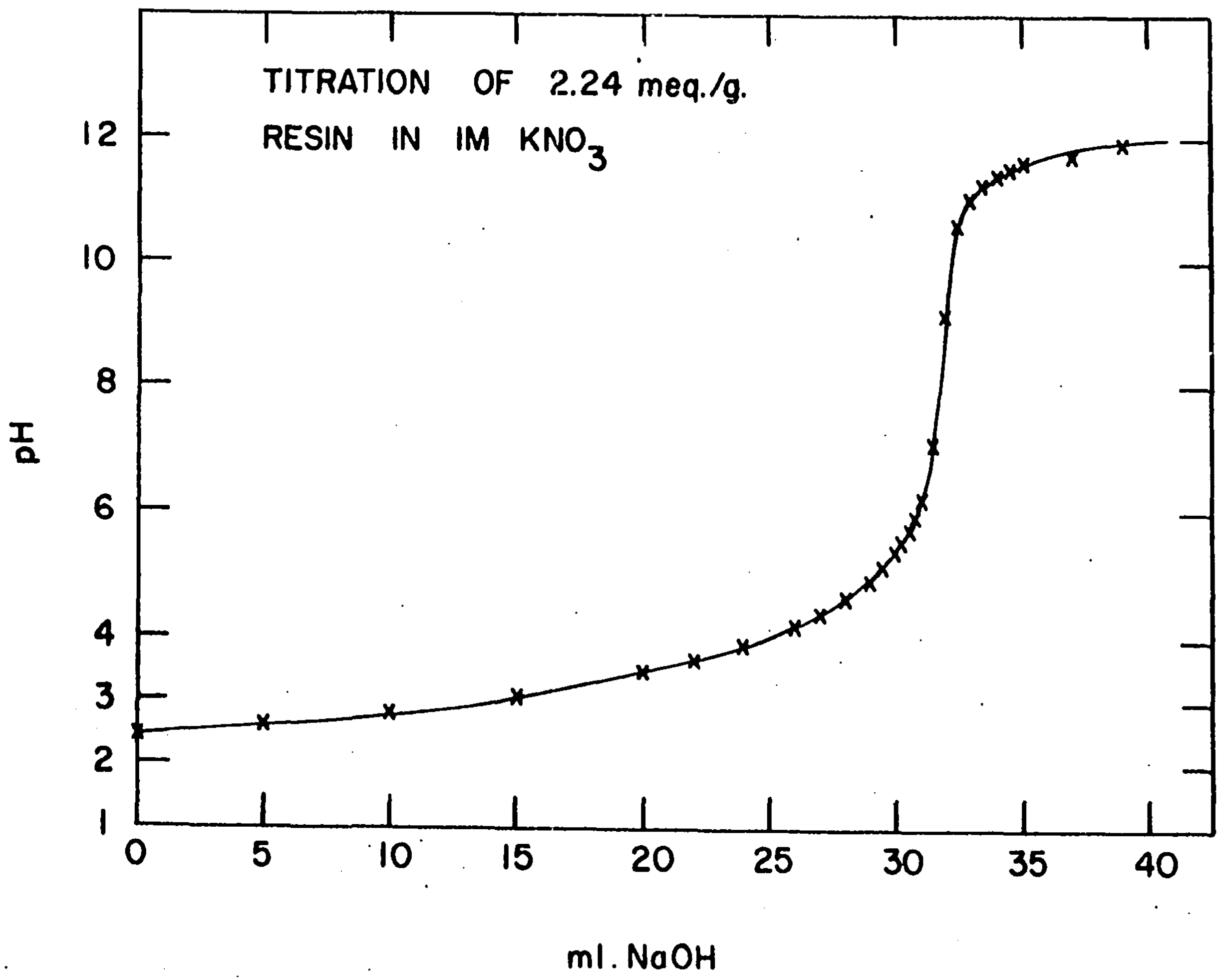


Fig. 10. Titration of 2.24 meq/g resin in 1 M KNO_3

resin with few or no weak acid resin sites (59).

Repetition of the direct titration capacity determination about one year after preparation of the resins gave results which agree within experimental error with the original determinations. This indicates little or no decomposition of the resins with storage in the hydrogen form. This is in contrast to the results of Fricke who noted severe decomposition of his gel-type beads with storage (47).

MEASUREMENT OF EQUILIBRIA

Introduction

Macroreticular cation exchange resins have been shown to have higher selectivity coefficients for a particular exchange in many cases than gel-type resins (57). However, loading has been shown to have a great effect on the selectivity coefficient for an exchange in resins of high crosslinking; the selectivity coefficient decreasing much faster with increased loading than for more lightly crosslinked resin (60,61). For these reasons, all determinations of the distribution ratio or selectivity coefficient were made at a loading of less than 1% of the total capacity of the resin.

Equilibrium for cations exchanging on a sulfonic acid cation exchanger is generally represented as

$$mA^{+n} + nBR_m = nB^{+m} + mAR_n$$

where R represents the organic exchanger. The thermodynamic equilibrium constant for the equilibrium is defined as

$$K_{eq} = \frac{a_{AR}^{m} \, a_{B}^{n}}{a_{BR}^{n} \, a_{A}^{m}}$$

where "a" is the activity of the species, whether it be in the resin phase or in solution. K_{eq} should depend only on the temperature, the nature of the resin and of the ions, A and B. The exact evaluation of K_{eq} depends on the choice of standard states in the exchanger and in the solution and, depending on these choices, is either one by definition, or is rather tedious to calculate. No experimental evaluations of K_{eq} were done in this work.

K_{eq} is related to two other useful quantities, E, the selectivity coefficient, and K', the corrected selectivity coefficient

$$E = \frac{[AR_n]^m \ [B]^n}{[BR]_m^n \ [A]^m}$$

$$K' = E \frac{\gamma_B^n}{\gamma_A^m}$$

The selectivity coefficient, E, represents the net result of all interactions which result in the preference of the resin for one ion over another.

K' takes into account the activity coefficients of the exchanging species in the solution. Thus K' reflects the preferences which occur in the resin phase alone.

In practical applications of ion exchange, equilibrium is most easily expressed in terms of the distribution coefficient D, defined by

$$D = \frac{[AR]}{[A^{+n}]}$$

or

$$D = \frac{\text{amount of A/g of dry resin}}{\text{amount of A/ml of mobile phase}}$$

In this work the terms distribution ratio and distribution coefficient will be used interchangeably. The relative distribution coefficients of two or more species in a particular solvent provides an indication of the possibility of achieving a separation in liquid chromatography. The larger the distribution coefficient, the larger will be the retention time or volume. The greater the difference in D values for two ions in a particular eluent, the easier is their separation. Conversely, if their D values are equal, separation cannot be achieved in that solvent.

In this work the fraction of the total resin anionic sites taken up by the metal ion in the equilibrium studies was maintained at less than 1%, and figures in this section compare data for exchangers only where $[H^+] = 1$, and the concentration of metal ion in solution is $<10^{-3}M$. The significance of these choices can now be seen.

K' was defined as $E(\gamma_B^n/\gamma_A^m)$. This quantity reflects preferences in the resin phase alone. For this work it can be rewritten

$$K' = \frac{[MR_n]\ [H^+]^n\ \gamma_H^n}{[M^{+n}]\ [HR]^n\ \gamma_M}$$

now $[H^+] = 1$ and $[HR]$ = meq/g resin = the resin capacity. In constant media of high ionic strength where $[H^+] >> [M^{+n}]$, γ_H^n/γ_M will be nearly constant. Thus K' can be reduced to

$$K' = \frac{D}{C^n}\,(\text{constant})$$

$$= E(\text{constant})$$

Thus in figures where E is plotted against capacity, the shape of the plot may be taken to indicate the relative preference of the resin phase only for a particular ion at a particular capacity since multiplying E by a constant would only shift the plot up or down, but would not change its shape.

Experimental

Distribution ratios of metal ions on the various resins were determined by two different methods. The first of these was batch equilibration. Batch equilibration has the advantage that a wider range of distribution ratios can be easily measured than in the second method, which will be discussed.

In the batch method about 3 g of dry, hydrogen form resin was weighed and added to 20 ml of a dilute solution of the metal ion. The mixtures were then shaken on a Burrell Wrist Action Shaker for at least 1.5 hours. The mixtures were then centrifuged and part of the supernatant liquid was removed by pipet for analysis. The amount of metal ion remaining in the supernatent was then determined by the appropriate EDTA titration method listed in Table 7 .

The stock metal ion solutions used in these determinations were prepared by dissolving reagent grade nitrates, or perchlorates in water to produce stock solutions which were approximately 0.1 M and were then standardized by titration with EDTA. The solutions used in the batch equilibrations

Table 7. EDTA titration conditions

Metal	pH	Buffer	Indicator	Note
Cu	6	pyridine	NAS	
Zn	6	"	"	
Pb	6	"	"	
Ni	6	"	"	
Al	6	"	"	Back titrate with Cu
Mg	10	NH_4OH-NH_3Cl	Calmagite	
Ca	10	"	"	Back titrate with Mg
Th	3		Xylenol Orange	

were then prepared by diluting aliquots of the stock solutions and of the desired background acid to volume in a volumetric flask to produce a solution approximately $1x10^{-3}M$ in the desired metal.

The second method of obtaining distribution ratios for metal ions on the resins involved measurement of the recorded column elution volumes. The elution volume is related to the weight distribution ratio by the expression

$$\bar{v} = D(W) + V_m$$

where $\bar{v}$ is the elution volume to the peak maximum, D is the weight distribution ratio, W is the weight of resin in the column, and V_m is the total volume of mobile phase between injection and detection points. Thus the distribution ratio is expressed as:

$$D = \frac{\bar{v}-V_m}{W}$$

Five columns were prepared for use in these determinations. They are listed in Table 8.

Table 8. Columns used in determination of distribution ratios

Column #	Weight of resin	Column dimensions	Resin capacity	V_m
1	2.25 g	6.3x200 mm	0.23 meq/g	4.3 ml
2	.67	4x130	0.84	1.9
3	.40	4x73	1.01	1.4
4	.37	4x70	1.89	1.3
5	.41	2.8x130	2.66	1.4

Column 1 was a Chromatronix Model LC-6M-13 column fitted with adjustable outlet plungers to fit closely against the bed in order to minimize dead volume. The column was dry packed with 2.25 g of resin and the plungers inserted. The column was then placed in position in the chromatograph and water was pumped through. This was followed by 1 M HCl. The plungers were readjusted after the entire bed had been wetted.

Columns 2-4 were prepared by dry packing the resin phase directly into the column with the aid of a funnel while tapping the sides. The bed was then lightly tamped. This process was repeated until the columns were nearly full. A small plug of glass wool was placed against each end of the bed to hold it in place and to reduce dead volume. The columns were then placed in the eluent line and wetted as before.

Column 5 was slurry-packed. The resin was slurried in water and poured into a funnel attached to the column with tygon tubing. Suction was applied at the lower end of the column to increase the rate of packing and reduce capacity segregation by settling. A plug of glass wool was placed at each end of the column as before.

In all cases the application of the pressurized eluent to the resin bed resulted in some shrinkage of the bed with use. This tended to produce an undesirable dead volume in the column. This was initially eliminated by adding a weighed quantity of the same resin to the bed, i.e. additional packing. In cases where the increase in column dead volume was small, the space could be eliminated by the addition of a small amount of glass wool. The bed shrinkage problem has been discussed by Seymour (62).

Columns 2-5 were simple glass tubes which were adapted to allow use of Chromatronix type MB-3 Cheminert Column Fittings.

The mobile phase volume for each column was determined in the following way. The column was placed in position in the eluent stream of the chromatograph and 1 M HCl was equilibrated with the column. (See the section on instrumentation.) A solution of Arsenazo III was then added to the column effluent by means of the mixing chamber. The rate of flow of the Arsenazo III solution, which also contained 2 M NH_3, was adjusted to produce a pH of about 5 in the effluent stream. The spectrophotometer was then adjusted to read zero for this solution. A 56.3-microliter volume of water was then injected onto the column by means of the sample injection valve and the recorder trace begun. When the water sample had passed through the column and reached the mixing chamber the eluent became suddenly basic, due to the unneutralized NH_3, and the Arsenazo III took on a blue color which could be measured at 650 nm. The mobile phase volume was thus taken to be the elution volume for water, which is not retained.

The distribution ratios of several different ions were then determined by a procedure similar to that just described. A 56.3-μl sample of the metal ion solution was injected into the flowing eluent stream with the strip chart recorder running. The injection time was marked. The eluent was then passed through the column at a flow rate of 1 ml/min until the detection system indicated that the sample had eluted. The elution volume was then measured and D calculated.

For a description of the reagents used in this part of the work, see the section on separations.

Results

The measured distribution ratios in various media as determined by both batch and column methods are summarized in Tables 9, 10, 11, and 12. Note the very large distribution ratio of thorium compared to other ions even on resins of low capacity. Zirconium and the lanthanides also exhibit relatively large distribution ratios. A clear correlation between cationic charge and D can be observed at all capacities.

Most metal ions show lower distribution ratios on cation exchangers in HCl than in HNO_3 or $HClO_4$. This is shown to be the case for these resins. The few exceptions of this general-

Table 9. Distribution ratios of metal ions on 0.84 Meq/g resin

	HCl						$HClO_4$		HNO_3	
	.5M	1M	2M	3M	4M	8M	1M	2M	1M	2M
Th(IV)		63	11.5	4.2	1.6	.4		40		7.6
Zr(IV)			6.1	1.95	.8					9.1
La(III)		11.2	1.65	.9	.2		22.3	3.3	7.6	1.3
Eu(III)		9.1	1.5	.6	0		16.6	2.8	6.0	1.0
Ho(III)		7.6	1.0	.2	0		11.2	1.5	5.5	1.0
Al(III)			.15	0	0					
Ti(IV)			.15	0	0					
Ba(II)		2.1	0	0						
Sr(II)		.9	0	0						
Ca(II)			0					0		0
Pb(II)	5.1	.75					9.9		1.9	
Cu(II)	3.3	0					1.2		.9	
Zn(II)	3.1	0					.9		.9	
Ni(II)	3.0	0					.9		.9	

Table 10. Distribution Ratios of Metal Ions on 1.01 Meq/g resin

		HCl				$HClO_4$	
	.2M	.5M	1M	.3M	.5M	1M	2M
Th(IV)			82				
Pb(II)		8.0	1.0			27.5	5.5
Cu(II)	25.9	3.5	.5	12.2	5.1	1.8	
Zn(II)	23.5	3.25	.2	8.3	4.6	1.3	
Ni(II)	23.5	3.5	.8	9.5	4.4	1.3	
Ba(II)						5.0	
Sr(II)						3.0	
Ca(II)						2.5	
Mg(II)						1.4	
Al(III)						3.5	

Table 11. Distribution ratios of metal ions on 1.89 Meq/g resin

		HCl		$HClO_4$
	.5M	1M	2M	1M
Th(IV)		>125	>50	
La(III)		>125		
Al(III)			2.0	19
Ca(III)	34	16.3	3.0	22
Mg(II)	12.4	2.4		4.6
Pb(II)		6.5		>100
Cu(II)		3.2		5.3
Zn(II)		1.8		4.3
Ni(II)		4.0		4.0

Table 12. Distribution ratios of metal ions on 2.66 Meq/g resin

	HCl			$HClO_4$
	1M	4M	8M	1M
Al(III)				55
Pb(II)	24			
Ca(II)		6.5	2.2	140
Mg(II)	11.3			
Cu(II)	10			15.5
Zn(II)	9.5			14.2
Ni(II)	8.9			15

ization are thorium and the lanthanides, which form moderately strong nitrate complexes. The influence of complexation on the distribution ratio is especially evident in the case of lead.

Figures 11 and 12 show a plot of distribution ratio versus capacity for a variety of metal ions. The most obvious feature of the graphs is the sharp increase in D with increasing capacity. This was to be expected purely from mass action effects. However, consideration of mass action effects, ignoring activity coefficients, indicates that a plot of the logarithm of the distribution ratio as a function of the logarithm of the capacity should yield a straight line of slope n, where n is the valence of the metal ion. Clearly this is not the case. The slopes increase with increasing capacity. It is not obvious whether the slopes continue to increase at the very high capacity end or whether the plot becomes linear.

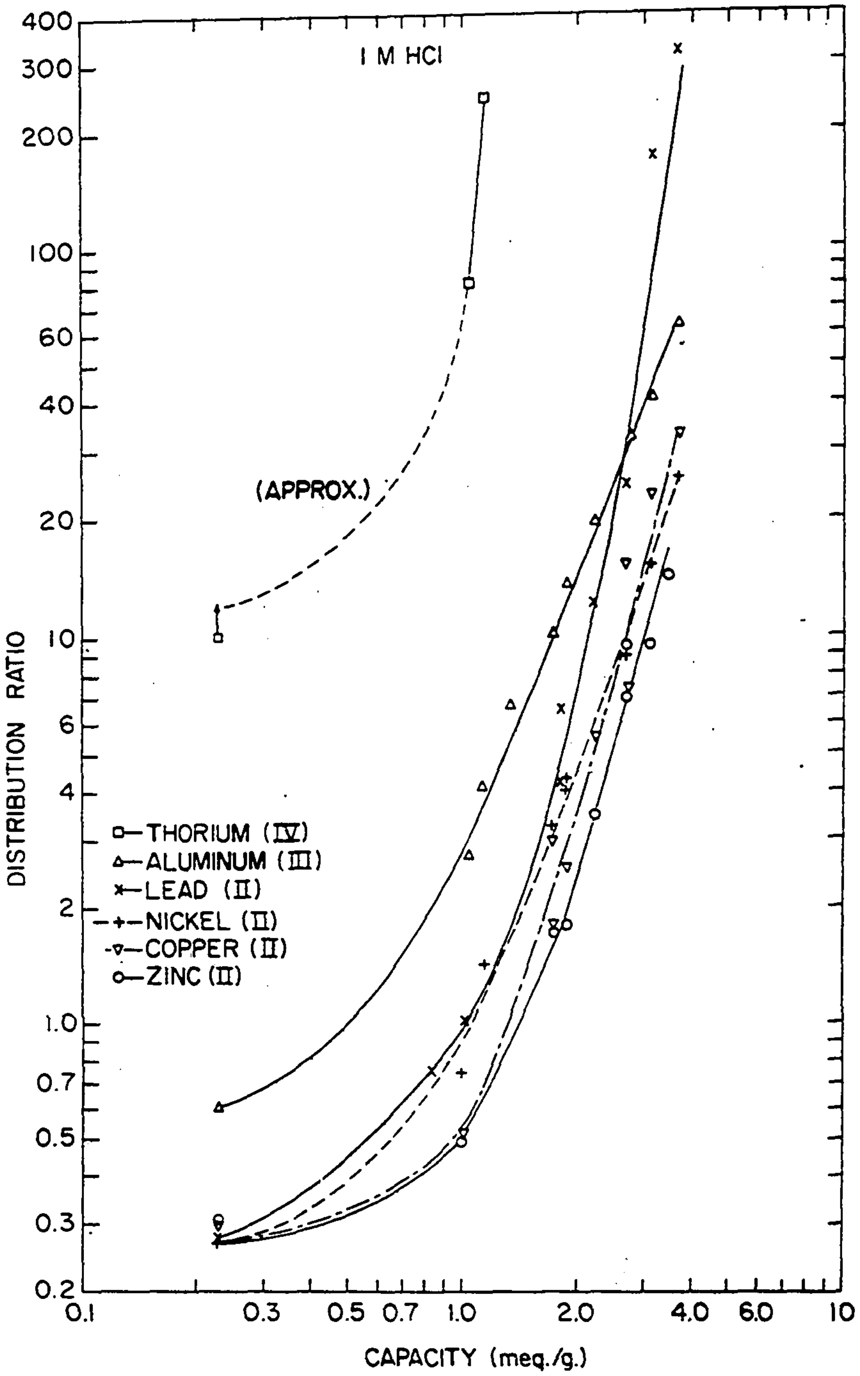


Fig. 11. Distribution ratios of metal ions in 1 M HCl on low capacity resins

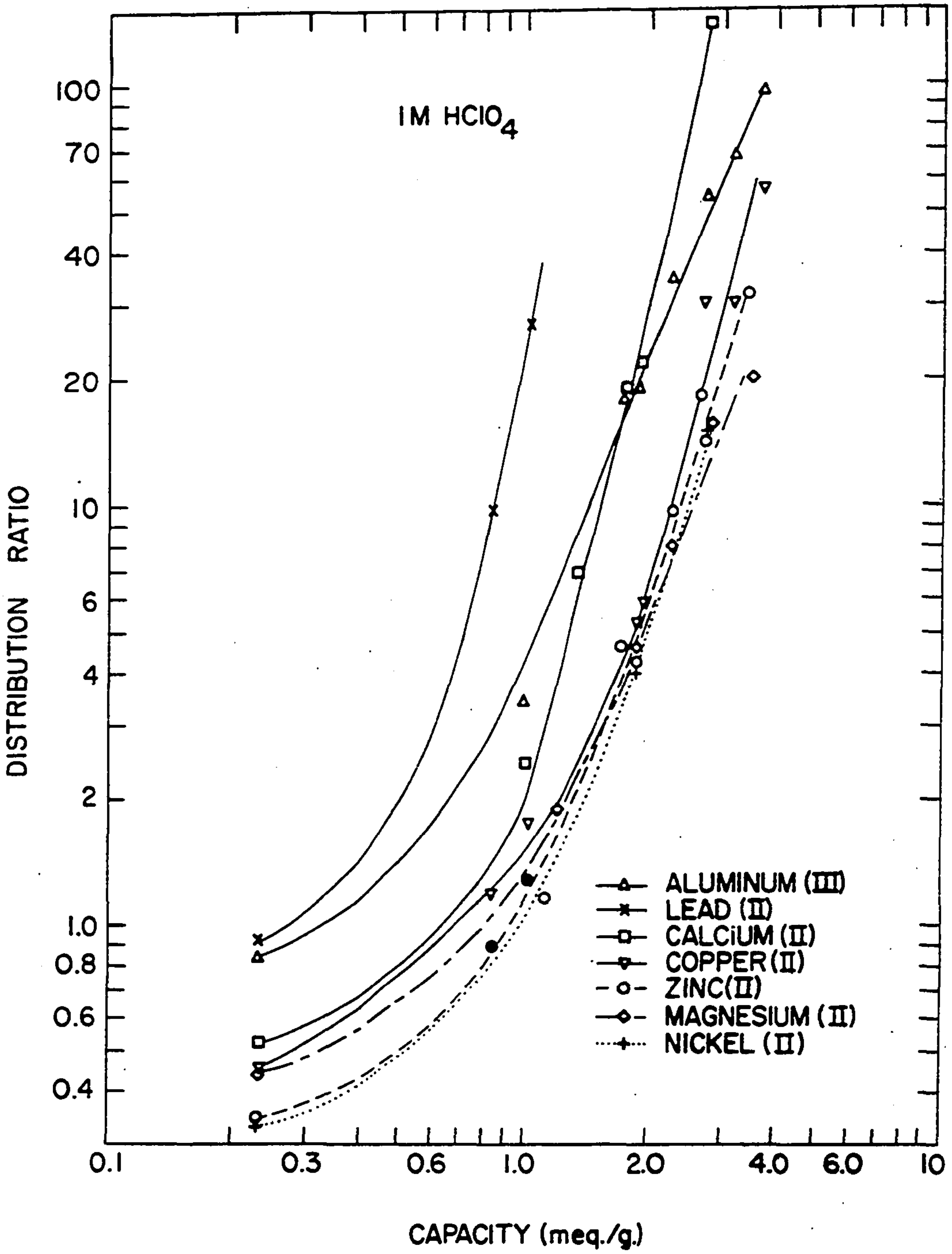


Fig. 12. Distribution ratios of metal ions in 1 M $HClO_4$ on low capacity resins

If the abscissa is made linear in capacity, several of the plots become linear. The reason for this is not obvious.

Rates of Exchange

It was considered desirable to obtain some indication of the rates of exchange of metal ions on the partially sulfonated resins. To this end a measurement of the rate of exchange was conducted by the batch equilibration method. A weighed quantity of resin was placed in a flask containing a dilute metal ion solution and the solution was shaken for a measured length of time. This was the same procedure used in the batch equilibria work. The liquid was then rapidly filtered off and the solution analyzed. The results indicated that the reaction was too fast to be measured accurately by this method. This is undoubtedly due to the relatively small particle sizes employed throughout this work.

Discussion and Theory

An ion exchange resin is a very complex system and any attempt to explain processes occurring in such a system in a limited amount of time must do so by making what may appear to be drastic assumptions in order to achieve simplification. As has been shown, completely homogeneous ion exchange beads are very difficult to prepare. As Freeman and Aiyar have pointed out; "The significance of ion exchange measurements depends on the nature and definition of the ion exchange material. ...ion exchange resin beads are seriously heterogeneous and therefore, incapable of being precisely defined" (45).

As if to complicate matters, most attempts to explain the behavior of ion exchangers have been concerned with models based on gel-type, poly(styrene-divinylbenzene) exchangers. In most cases the accuracy of the model declines with increasing crosslinking. The ideal state is considered to be the zero crosslinked resin and data for this state can only be obtained by extrapolation from data obtained using resins of finite crosslinking.

One of the first attempts to explain ion exchange selectivities was that proposed by Gregor (63,64). Gregor considered selectivity to arise primarily as a result of differences in the partial volumes of counterions in the resin. He assumed the ions to be hydrated in the exchanger and that the selectivity could be expressed by

$$RT\ln K'_{B/A} = \pi(\bar{V}_A - \bar{V}_B)$$

where $\bar{V}_A$ and $\bar{V}_B$ are values taken to be the hydrated volumes of counterions A and B in the resin and π is the "elastic counterpressure" exerted by the resin matrix. This model leads to several conclusions regarding ion exchange resin behavior. First, it predicts that the counterion with the smaller hydrated radius will always have the greater distribution ratio, i.e. if $\bar{V}_A > \bar{V}_B$ then $K'_{B/A} > 1$. This correctly predicts the order of affinity for alkali metal ions, Cs > K > Na > Li, at low crosslinkings. Another aspect of the theory is that if $\bar{V}_A > \bar{V}_B$, then as X_B, the equivalent fraction of total ionic sites on the resin occupied by B, increases, the resin will be expected to contract. This is what is known to occur. Also, if $\bar{V}_A - \bar{V}_B$ does not change sign, then ln K' should not change sign since π is positive. Finally, if the crosslinking is increased, then π increases and the binding strength increases, but the selectivity order should not change. This is not the case for resins of medium crosslinking involving Cs or Na as a counterion and this aspect of the theory fails entirely at higher crosslinkings. Thus the model of Gregor fails to account for selectivity inversions and requires that one explain why $\bar{V}_A - \bar{V}_B$ should change in sign in such cases.

The theory of Rice and Harris (65) assumes that the selectivity of an ion exchanger is a result of primarily two factors, the strength of ion-pair formation between counter ions and fixed ionic groups on the resin, and the electrostatic repulsion between adjacent resin sites. This repulsion is considered to be the only force responsible for swelling of the resin. This theory does not directly concern itself with hydrated volumes of particular ions involved in the exchange. This theory, like that of Gregor, does not easily explain affinity reversals since this would require that the so-called "binding constants" vary.

The theory of Eisenman as described by Rieman and Walton (37) considers electrostatic interactions to be the primary cause of selectivity and affinite reversals. Hydration of ions is considered important as a result of the energies of hydration involved rather than because of hydrated ionic radius or volume. In its simplest form this theory treats both the counterion and the resin site as non-polarizable point charges at the center of an incompressible sphere. The electrostatic energy is given directly by Coulomb's law as (for univalent exchange)

$$E = \frac{e^2}{r_R+r_A}$$

where r_R and r_A are the radii of anionic resin site and cation respectively and e is the electrostatic charge. The free energy required to dehydrate or rearrange water molecules around the anionic site and cation is also considered. The total energy involved in these two processes for each of two ions determines selectivity.

Eisenman's theory has been successful primarily in predicting the different affinity order of carboxylic acid (weak acid) resins, for univalent cations, as compared to sulfonic acid resins. The carboxylate group is of small size and the sulfonic acid group of larger size. The theory predicts that if r_R is large, then the difference in electrostatic interaction energy between the two competing counterions will be small, and the selectivity will be determined by the ease of dehydration of the cation. Thus for sulfonic acid exchangers

(r_R large), the order of selectivity should be Cs > Rb > K Na > Li which is the case. When r_R is small, as for the carboxylate group the electrostatic term will be predominate and the smaller ion will be preferred. Thus the order of affinity is Li > Na > K. Reversals in affinity can thus be accounted for by variations in r_R.

Pauley (66) preceded Eisenman's electrostatic approach by eight years with an extremely simple one of his own. Pauley's theory considers the resin to be a series of negative point charges randomly distributed with cations bound to the anionic sites an average distance, r, away. This average distance may be considered to be the sum of the radii of the resin anion, r_R, and the bound cation, r_A. The free energy change involved in the exchange of cations, and thus K_{eq}, may be determined from calculations of the electrostatic work necessary to remove each of the two types of cations from their distance of closest approach to infinity. Swelling pressure effects are ignored and assumed to be negligible. On the basis of this theory it is necessary to make certain assumptions about r_A and r_R. Pauley assumes that the resin anion is relatively unhydrated and that r_R is the crystallographic radius of oxygen. The assumptions are also made, based on data from the literature, that: 1. cations in solution are hydrated; 2. cations are also solvated inside the resin; and 3. that the a^o term in the derived Debye-Huckel equations for concentrated electrolytes represent a true "distance of closest approach" of oppositely charged ions in solution and that these a values may be taken as equal to r_A, r_B, etc. Thus the free energy of exchange may be expressed by

$$-\Delta G_o = \frac{Ne^2}{D}\left(\frac{z_B z_R}{r_R + r_B} - \frac{z_A z_R}{r_R + r_A} \right)$$

and

$$+\log K_{eq} = \frac{Ne^2}{2.3RTD}\left(\frac{z_B z_R}{r_R + r_B} - \frac{z_A z_R}{r_R + r_A} \right)$$

where N is Avogadros number, e is the electrostatic unit charge, z represents the charge of the subscripted ion, and D is the dielectric constant of the medium.

Pauley used literature "a^o" values for cations and obtained good agreement between calculated and experimentally determined equilibrium constants. This explanation ignores second order coulombic attractions and swelling pressure effects.

The primary objection to Pauley's theory, besides its extreme simplicity, is that the calculations are heavily dependent upon the values assumed for r_R and a^o. The values of a^o obtained from the literature show significant disagreement.

Most theories of ion exchange selectivity assume a high degree of uniformity within the resin in order to simplify the model. However, Reichenberg (46) has shown that heterogeneities produced by the sulfonation process can cause substantial differences in selectivity. He found that the rate of sulfonation of cross-linked polystyrene decreases with increasing crosslinking; however the rate of desulfonation by a method similar to that of Boyd et al. (44), but less destructive of crosslinks, is independent of crosslinking. He prepared partially sulfonated resins of identical capacity and crosslinking by each process. The two resins were found to have very different selectivity properties. These differences can be explained if one assumes that: 1. crosslinking is not uniform and the sulfonation process preferentially sulfonates those areas of lower crosslinking first, whereas the desulfonation process is uniform; 2. the local differences in crosslinking affect the selectivity.

There are many other factors involved in selectivity which have not been considered in the theoretical models discussed above. Among the more important are ion-induced dipole effects resulting from the polarizability of cation and anion; sieve action by the resin in excluding larger ions; secondary columbic attractions; and deviations of both cation and anionic sites from the point charge ideality assumed in Pauley's and Eisenman's theories.

In choosing a model or combination of models for use in explaining the selectivity behavior of multivalent ions on partially sulfonated XAD-2, it is necessary to first consider the nature of the resin.

The copolymer beads from which the resins were made have been described in a previous section. Essentially the resin consists of pores or channels averaging 90 Å in diameter. The pore walls are sulfonated to varying extents with the concentration of sites decreasing toward the center. The resin is very highly crosslinked and undergoes little or no swelling. Liquid take up is the same no matter what the liquid (55). This appears to rule out the swelling pressure model of Gregor.

The theory of Rice and Harris gives little indication of the factors which determine the values of the binding constants. The values they used were determined empirically. This theory does not predict affinity reversals such as that shown on Figure 12.

The theory of Eisenman has been perhaps the most successful at predicting experimentally observed selectivity orders for univalent cations. However, it ignores the well-documented data indicating that counterions in the resin phase remain hydrated (at least partially) even when associated with a resin site (43). Eisenman's theory predicts that the purely electrostatic attractions will be greatest for those ions having the smallest crystal ionic radius and that the decisive factor for sulfonic acid exchangers will be the ease of dehydration of the cation. His theory is not easily applicable to multivalent cations.

Pauley's model is attractive in its simplicity. It predicts directly that exchangers will prefer ions of small hydrated size and large valence. Although no quantitative calculations as such using this model will be presented, it is Pauley's model along with other qualitative considerations which will be relied upon.

Consider a poly(styrene-divinylbenzene) copolymer bead, rather inflexible and containing open pores or channels throughout its structure with walls which are impenetrable to solvent or solute. As the first few anionic sites are produced on the resin the sites remain, on an average, distant from one another. A cation can be bound to the sulfonic acid site at its distance of closest approach, a^o, while remaining comfortably hydrated. A multivalent cation associated with one anionic site will require a larger hydration sphere, generally, than a proton associated with the same site. Thus as the capacity continues to be increased, the fixed metal ions come closer together. Solvation shells become crowded and repulsions of "unneutralized" counterions become more significant. This results in an increasing preference by the resin sites for protons. The sites are not yet close enough together to allow the multivalent metal ions to associate, at their distance of closest approach, with more than one anionic site.

When the site density becomes large enough to allow more than one site to interact with a multivalent cation at the distance of closest approach, the resin will begin to prefer the metal ion once again. As the site density at the pore wall increases, the attractive effects of nearby sites will, to an ever increasing extent, reinforce the selectivity of the resin for the metal. These attractions will be greatest for those ions which have the smaller hydrated size since they will have a shorter distance of closest approach to the anionic sites and thus to the pore wall and all other anionic sites. The more acidic cations, (those with a smaller crystal ionic radius or larger z) will have a more densely packed hydration sphere, greater shielding from negative sites and a greater distance from secondary sites.

Figure 13 shows a plot of the selectivity coefficient as a function of resin capacity for a number of ions. In each case E initially decreases sharply. However, between 0.5 and 1.5 meq/g it can be seen that the influence of capacity on the resin selectivity changes sharply. Above 1.5 meq/g, the influence of increasing capacity remains relatively constant.

Figure 14 shows a plot of the average distance between anionic sites as a function of capacity, assuming a pore surface area of 300 m^2/g and random sulfonation. The surface area of the resin was obtained from reference (57).

According to published data (67), the a^o values for most multivalent ions are in the range 3-4 Å. If a value of 1 Å is used to approximate r_R, then a metal ion should be able, on the average, to approach more than one site within its distance of closest approach when the average distances between sites reaches 8-10 Å. From Figure 14 it is evident that this point is reached at a capacity of about 0.5-1.0 meq/g. This

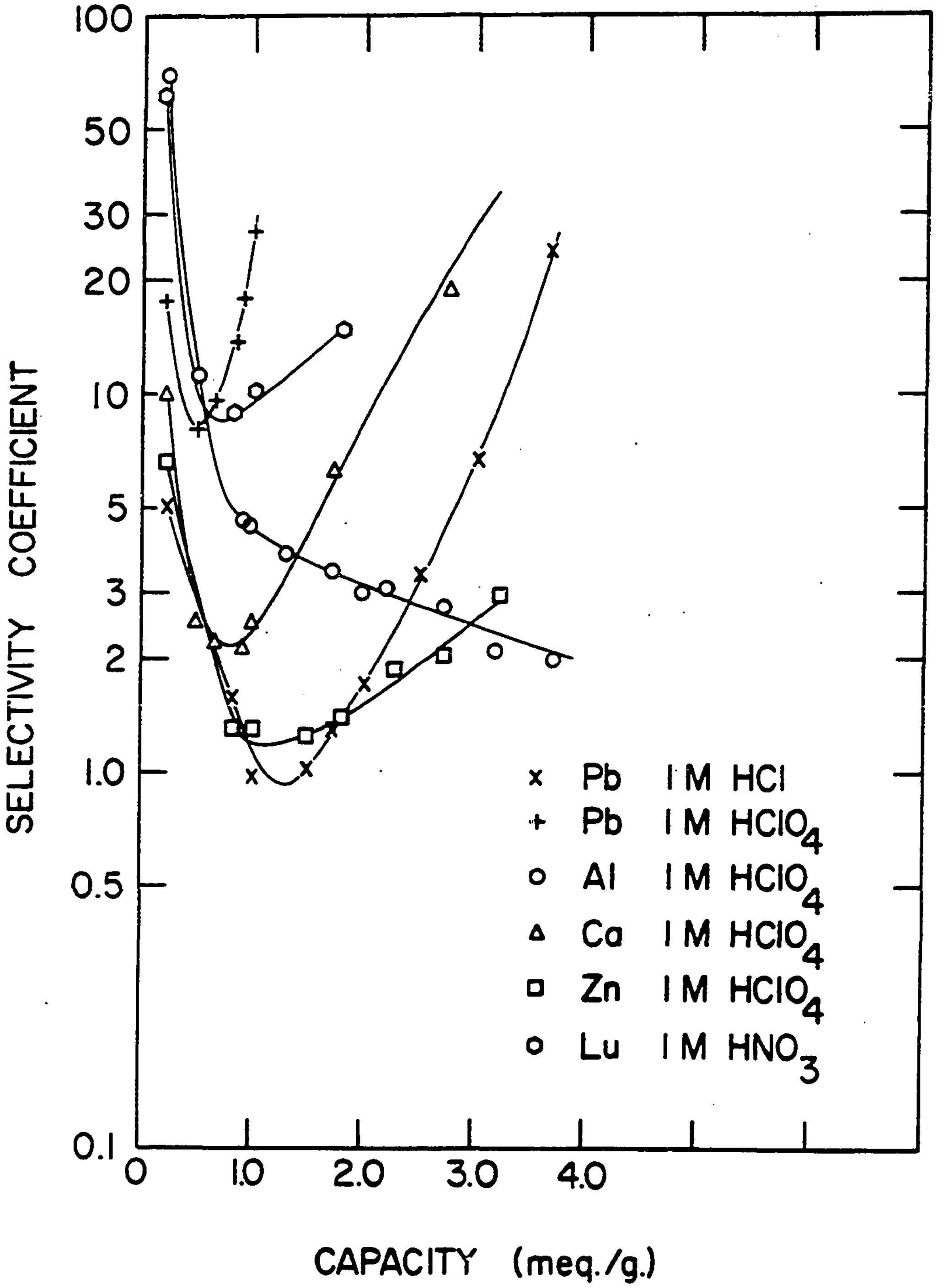


Fig. 13. Selectivity coefficients of metal ions on low capacity resins

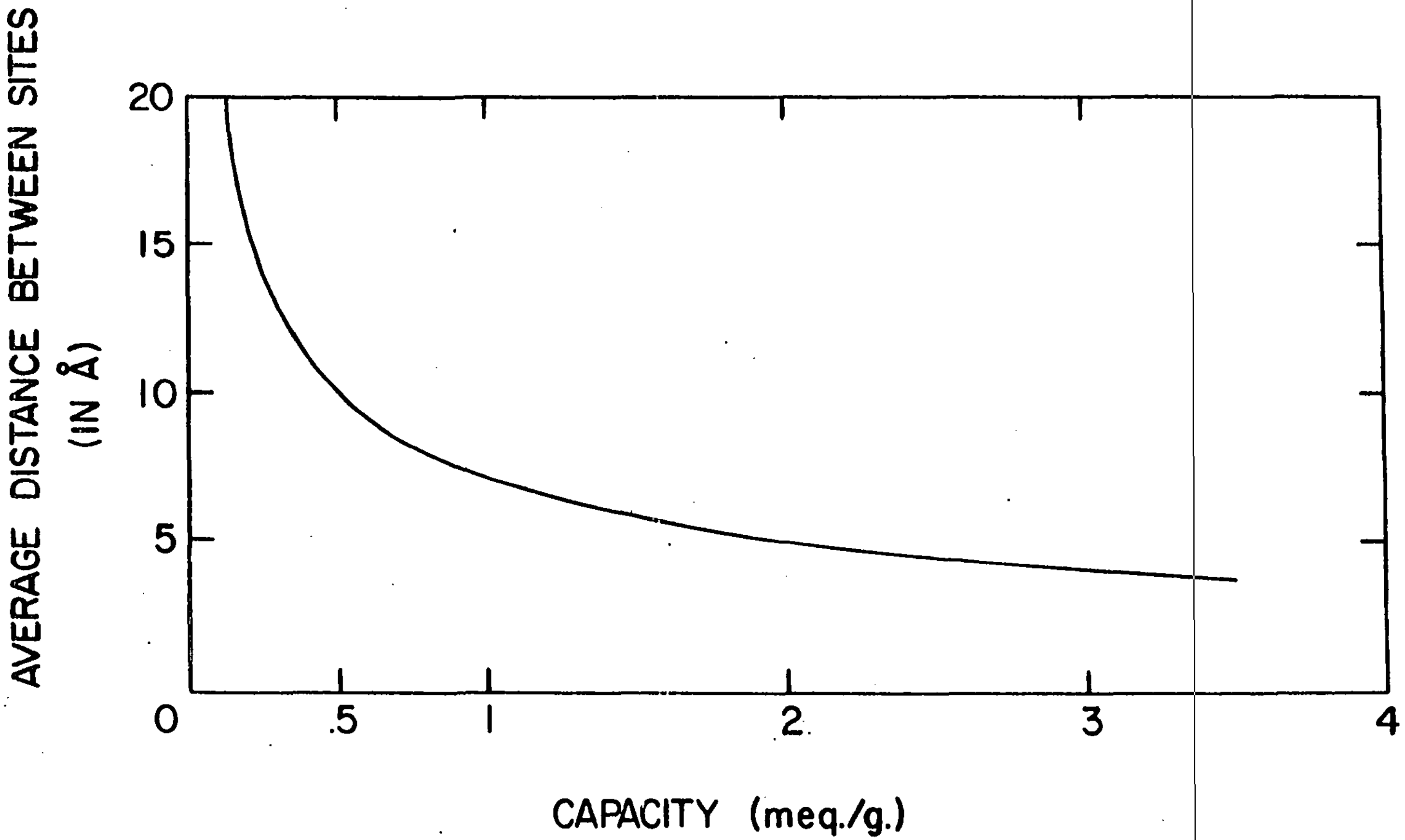


Fig. 14. The average distance between adjacent sites

corresponds to the sharp changes in slope shown on Figure 13, indicating the sharp change in effect of increasing capacity on resin selectivity.

There are a number of other factors which might be of varying importance in explaining the selectivity of these resins. First, it is known that partitioning of the metal ions sometimes occurs on the resin matrix itself (37,43). If such partitioning occurred in this case, it might account for part of the high selectivities noted at very low capacities. This was checked by a column measurement of the distribution ratio of zinc on unsulfonated XAD-2 and D was found to be nearly zero.

Second, mutual polarization is known to be extremely important in determining selectivity in some cases involving highly polarizable cations such as silver(I) or copper(I) (43). This effect increases the selectivity of the resin for the cation of larger crystal radius.

It is also possible that, as Reichenberg suggested, the sulfonation process sulfonates first the weakly crosslinked areas. This would also produce an increasing selectivity for cations of high valence and small a^o.

SEPARATIONS

Introduction

The primary purpose of this work was to produce rapid, simple separations and analyses of solutions of various metal ions. The separations which were attempted were aimed either at separating ions having high distribution ratios on conventional resins or at effecting those separations which published information on ion exchange, or the general scan of distribution behavior from the previous section, indicated were feasible. All possible separations were not attempted, rather the intent was to demonstrate the utility of the partially sulfonated resins in the separation and quantitative analysis of metal ions by forced flow chromatography.

Instrumentation

The chromatograph used in this work is outlined in Figure 15. All valves, fittings and columns were either purchased from Chromatronix, Inc. of Berkeley, Calif., or were machined from raw materials. All connecting tubing in the eluent system was .031" i.d. Teflon tubing.

Commercially available equipment is generally made available on the assumption that the user will be using only organic solvents or noncorrosive inorganic solvents as eluents. These instruments are intended for high pressure organic liquid chromatography. Valves, columns, pumps, e.g. generally contain a large amount of stainless steel in contact with the

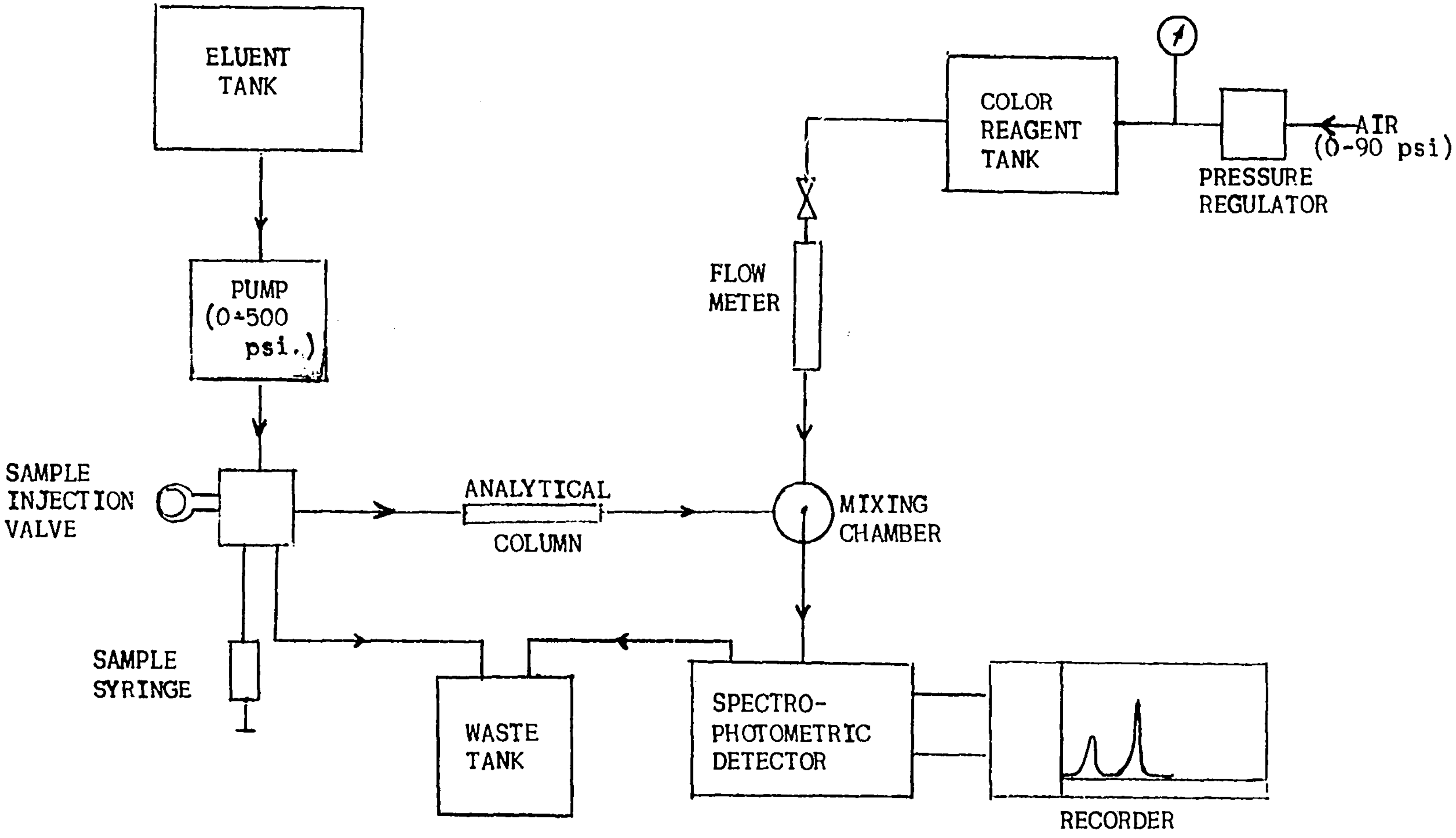


Fig. 15. Schematic diagram of the liquid chromatograph

eluting solvent. Few commercial liquid chromatographs are suitable for use with the highly corrosive solvents employed in inorganic analysis. For this reason the chromatograph shown was designed to allow only glass, Teflon or Kel-F plastic to come into contact with the mobile phase.

The eluent tank was a simple glass bottle or beaker containing the eluent.

The mobile phase was pumped from the eluent tank by a CMP-2 "Cheminert" metering pump purchased from Chromatronix. This pump is rated at 0-500 psi. It employs a three-piston design to reduce pressure and flow pulsation and to provide very accurate control of flow rate. The pump can be adjusted for flow rates of 2.4 to 120 ml/hr. Flow control is provided by pneumatically actuated three-way valves which control the filling and draining of each piston.

The sample injection valve was an SV-8081 Chromatronix valve fitted with a calibrated sample loop which allowed injection of a sample volume ranging from 50 μl to 10 ml. Most of this work was performed using a 56.3 μl sample loop.

From the sample injection valve the solvent passed on to the analytical column which has been previously described. In addition to the five columns prepared for the determination of distribution ratios, 4 additional columns were used in the separation study. Of these none were extensively used except for a 1 m x 2.5 mm column of Dowex 50 x 8 which will be described in a later section.

The mixing chamber was the whirlpool, divided tangential entry chamber developed by Sickafoose (68). This chamber has a volume of 17 µl.

Color reagent addition to the mixing chamber was achieved by means of a series of three pressurized bottles of indicator solution. The color reagent tanks were pressurized by a connection to the laboratory air line (0-90 psi) via 1/4" Dacron pressure tubing. A Johnson Service Co. R-130 pressure regulator and Koby air filter were placed in the air line to control the color reagent flow rate. A pressure gauge allowed continuous monitoring of the reservoir pressure (which never exceeded 5 psi). The three color reagent tanks were connected to a 4-position Teflon valve which allowed any one or none of the three tanks to be connected to the mixing chamber. A Roger Gilmont size #1, F-1100 flow meter was placed in the color reagent stream to allow continuous monitoring of the reagent flow rate.

The column effluent, mixed with color-forming reagent, then passed to the detector, a Beckman Model B variable wavelength, visible range, spectrophotometer. The sample compartment was altered to accommodate a flow-through cell of light path 1.0 cm and internal volume of 32 µl.

The spectrophotometer output, which was linear in percent transmittance, was amplified and converted to read linearly in absorbance by means of a logarithmic amplifier.

The amplifier output was fed to an E. H. Sargent S-72150 recorder which provided a display of input voltage (absorbance) as a function of time. The Sargent recorder was fitted with a Disc Instruments Model E-4 integrator.

Operational Notes

Dissolved gases in the eluents used in high pressure chromatography often form bubbles in the chromatograph, especially at the low pressure end of the analytical column (69, 70). Bubbles passing through the flow-through absorbance cell may create a noise level which swamps the signal. Bubbles became especially troublesome in this system when they became trapped in the pump pistons. This resulted in periodic compression and expansion which produced distinct pulses in the eluent flow. These pulses manifest themselves in the form of periodic, very sharp peaks or minima in the recorder trace. It was also found that the flow-through detection cell acted like a bubble trap, collecting bubbles as they passed in. This created an unstable baseline or plateau in the chromatogram.

Degassing of the mobile phase by heating or vacuum prior to use is the most obvious way to reduce bubble formation, and it was employed. However, complete degassing of some solvents was difficult, and although the difficulties caused by bubble formation were reduced, they were not completely eliminated. It was found, however, that entrapment of the bubbles in the eluent system, which was the crux of the problem, could be eliminated simply by tilting the pump and spectrophotometer such that the exit port was 10-20 degrees higher than the inlet port. This was done by placing a shim under the appropriate end of the instrument. This effectively eliminated bubble entrapment in the pump and detection cell, allowing the few bubbles remaining after degassing to pass quickly through the system, showing up as only a small "blip" on the chromatogram.

It is appropriate here to discuss some of the advantages of the type of chromatograph herein described as compared to those used in other work (62,68). The difference lies mainly in the method of propelling the solvent through the column. The chromatograph described by Sickafoose and Seymour employs helium pressurization for solvent propulsion. The advantages obtained by using the metering pump are: 1. The metering pump provides an accurate and constant flow rate regardless of solvent viscosity. The flow rate can be controlled easily by a switch on the front panel. With a constant pressure system it is necessary to determine the relationship between flowmeter reading and flow rate for the eluent. This can only be done by experiment. A change in solvent to a solvent of different viscosity causes a change in flow rate. This is significant because peak height and peak area of an elution curve are both flow-rate dependent. Any difficulty in accurately reproducing the flow rate for a determination will result in error. 2. The pressurized reservoir system allows almost instantaneous change of eluent passing through the column. This produces so-called "solvent change peaks" which appear as an absorption peak accompanying the passage of 2-eluent interface through the detection cell. This peak may be superimposed on a baseline shift. If the second eluent is directed into the column, it may be in order to strip a sample component. If this is the case, the solvent change peak may exactly coincide with a sample component peak and make the analysis complicated or impossible. This problem is not encountered using the CMP-2 pump because the pump volume produces sufficient mixing to eliminate any solvent interface.

Color Reagents

In-stream addition of a color-forming reagent to permit photometric detection of sample components has been used for a number of years in automatic analyzers. However, these instruments employ mixing procedures which cannot be used in forced flow chromatography because of the large amount of band spreading and loss of resolution which they produce.

Sickafoose (68) first attacked the problem of efficient mixing in very short times and small volumes. The in-stream colorimetric detection of sample components by reagent addition done in this work is essentially based on that of Sickafoose. The whirlpool, divided tangential entry mixing chamber is the same.

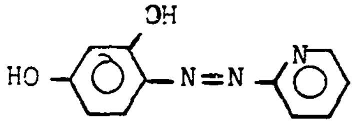
It was considered desirable that the colorimetric reagents chosen react quickly, and that their formation constant be high. They should form a complex of high molar absorptivity and the free indicator should have a low absorptivity at the wavelength of the maximum absorbance of the complex. The complexing reaction should take place in solutions in which close pH control is not necessary, so as to eliminate the need for the presence of large amounts of buffer or close control of reagent flow rate.

The colorimetric reagents chosen were 4-(2-pyridylazo)-resorcinol (PAR), and Arsenazo III, whose selection was based on the studies of Sickafoose, and Arsenazo I, which has been found to give a colored species with calcium, magnesium and several ions (71-73). Figure 16 shows the structure of these reagents.

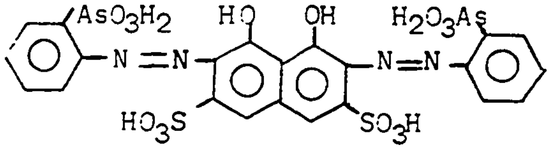
In addition to those colorimetric reagents just listed, Chlorophosphonazo III was also examined for suitability, especially in the detection of the alkaline earths and aluminum. Both the data of Sickafoose and others, and laboratory spot tests indicated that this reagent might give more satisfactory detection for these metals than Arsenazo III. This was not found to be the case. Although Chlorophosphonazo III was found to give a color-forming reaction at lower pH with aluminum, barium, strontium, and calcium than Arsenazo III, its overall sensitivity was less.

Each of the three color reagent solutions were prepared differently because of the general types of ions each was

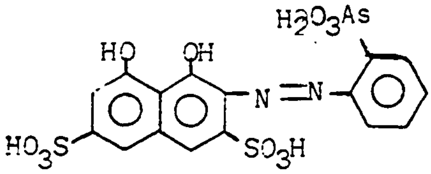
4-(2-pyridylazo)resorcinol (PAR)



Arsenazo III



Arsenazo I



Chlorophosphonazo III

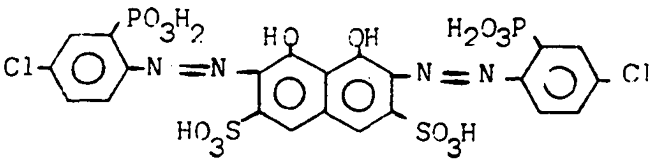


Fig. 16. Structures of color-forming reagents

expected to detect. The PAR solution was prepared by placing 0.25 g of Eastman 7714 PAR in a 2-liter volumetric flask and adding about 1 liter of water. 670 ml of B&A (28-30% NH_3) Reagent ACS concentrated NH_4OH was then added to the flask. The solution was then stirred with a magnetic stirrer until all solids had dissolved, and the solution was diluted to volume. The NH_3 served to neutralize the effluent acid solutions and provide a basic medium in which detection was most sensitive.

Arsenazo III gave a useful color change for several ions only in a narrow pH range. It was thus necessary to add an appropriate buffer to the solution in addition to the NH_4OH present to neutralize the acid. The buffer should buffer the mixed solutions in the pH range in which the analysis is to be done.

A simple study was done to investigate the pH at which some ions of interest gave a useful color change with Arsenazo III. The study was performed by placing a small amount of the particular ion in a solution of pH about 1. A few milliliters of Arsenazo III solution was added and titration was begun with dilute NaOH. pH was monitored with a Beckman Zeromatic pH meter and the pH at which the color change occurred was noted. A blank was run with no metal ion present and the color change due to conversion to the basic form was measured. The results of this study are shown in Table 13.

Table 13. Conditions for in-stream detection of metal ions using Arsenazo III

Metal	Effluent pH range for maximum sensitivity
Thorium, Zirconium	<1
Aluminum, the Lanthanides, and Chromium	2-3
Calcium, Copper, Strontium, and Lead	3.5-4.5
Barium	5.5-6.5
Maximum pH for useful analysis - 6.5	

Since the results indicate that the solutions should be buffered in the pH 3-6 range, ammonium acetate was chosen. It does not interfere with the analysis.

The Arsenazo III solution was prepared by placing .075 g of Aldrich A-9277-5 Arsenazo III in a 2 liter flask and adding about 1 liter of water as in the preparation of the PAR solutions. 154 g of Baker Analyzed Reagent NH_4OOCCH_3 and 270 ml of concentrated NH_4OH were added to the Arsenazo III and the solution stirred until all solids had dissolved. The solution was then diluted to a volume of 2 liters.

Arsenazo I was used only for the determination of calcium and magnesium. This analysis was done in basic medium. 0.18 g of Aldrich 10,798-0 Arsenazo I was combined with 402 ml of concentrated NH_4OH and the solution made up to 2 liters with water and stirring, as before.

Table 14 lists the three colorimetric reagents used and those metal ions with which color-forming reactions were shown to occur. Only Arsenazo III required acid solution or any close control of pH. The list is not comprehensive. All combinations of color reagent and metal ion were not tested. However, the data of Sickafoose indicates that only a few other color forming reactions might have occurred.

Table 14. Observed metal-indicator reactions

	Arsenzao		
	I	III	PAR
Thorium(IV)		x	x
Zirconium(IV)		x	x
Hafnium(IV)		x	x
Aluminum(III)		x	
Chromium(III)		x	
Lanthanides(III)		x	x
Bismuth(III)			x
Iron(III)			x
Iron(II)			x
Vanadium(IV)			x
Manganese(II)			x
Cobalt(II)			x
Nickel(II)			x
Copper(II)		x	x
Zinc(II)		x	x
Cadmium(II)			x
Mercury(II)			x
Lead(II)		x	x
Magnesium(II)	x		
Calcium(II)	x	x	
Barium(II)		x	
Strontium(II)		x	

From the table and the previous discussion it can be seen that PAR was by far the most versatile and convenient of the photometric reagents to use. It was also the most sensitive. These were also the findings of Sickafoose.

Arsenazo III was relatively versatile, but insensitivity and the necessity for close control of pH for the analysis of a number of ions made this reagent somewhat less useful than PAR.

Arsenazo I provided a simple and sensitive determination of calcium and magnesium.

Principles

A large volume of literature on ion exchange chromatographic separations has been summarized in several books, (37, 74,75) and the volume of literature is constantly expanding (52). A recent scheme for the separation and analysis of 27 different metal ions employed selective elution from an ion exchange column to achieve the separation of 17 of the ions (76).

Any procedure for the separation and determination of substances by forced flow chromatography should meet several criteria. First, the method should, of course, be selective, otherwise the separation will not be achieved. The best way to accomplish this is to employ stepwise or selective elution. In this technique an eluting medium is chosen which allows selective retention of the sought-for-components with as many other ions passing through the column as possible. The eluent is then changed so as to allow a desired component to elute. This process is repeated for each component until all desired components have been eluted.

Second, the separation procedure should be rapid. Each component should be eluted in as short a time as possible in order to avoid bandspreading and loss of resolution and to keep the analysis time short.

The eluting media should not react with any component of the color forming solution in any way that impedes detection. For example, the reaction should not produce a gas (bubble) or an insoluble salt.

In each of the separations described in the following pages, preliminary work was performed to determine which partially sulfonated resin gave the best resolution and shortest separation time. The procedures described were found to be the most efficient for the particular sample involved.

Solvent changes indicated on the chromatograms are corrected to compensate for the volume of liquid in the pump and lines up to the column entrance. This volume was measured at 2.6 ml.

Reagents

The following reagents were used both in the separation and in measurement of equilibria of exchange.

Hydrochloric acid was DuPont Reagent 37-38%. Solutions were prepared by appropriate dilution.

Perchloric acid was Mallincrodt Analytical Reagent 70%, diluted to the appropriate volume.

Nitric acid was B&A 70-71% Reagent ACS diluted appropriately.

Ammonium hydroxide was B&A 28-30% (NH_3), diluted appropriately.

Ammonium acetate was Baker Analyzed Reagent CH_3COONH_4 98.4%, formula weight 77.08.

Citric acid was Baker Analyzed Reagent Monohydrate $HOC(COOH)(CH_2COOH)_2H_2O$ 99.9%, used as received.

Lactic acid was obtained from Baker and used as received.

Acetonitrile was Baker Analyzed Reagent 99.8%, used as received.

The sample metal solutions were prepared by dilution of the stock metal solutions whose preparation is described on page

2-Hydroxyisobutyric acid was obtained from Aldrich Chemical Co. as Catalog number 16,497-6, 98+%. 41.6 g of the solid crystals were weighed and placed into a one liter beaker along with a magnetic stirring bar. The solution was stirred until the soluble solids appeared to have dissolved and the solution was then filtered into a 1 liter volumetric flask and diluted to volume.

Ethylenediamine was Fisher Certified Reagent Anhydrous, formula weight 60.102.

Separations in acetonitrile-aqueous hydrochloric acid

Kawazu and Fritz (35) and Fritz and Rettig (77) have described cation exchange separations of several metal ions in mixed acetone-water-HCl media and in isopropyl alcohol-aqueous HCl solutions. An attempt to duplicate their results in acetone-aqueous HCl failed because of the extreme volatility of acetone.

Acetonitrile, however, has a higher boiling point than acetone, is inexpensive, and a good ligand (78).

Acetonitrile solutions were used to separate each of five very common divalent metal ions on the 1.89 meq/g resin as shown in Figure 17. The column was equilibrated at a flow rate of 2 ml/min with 50% acetonitrile-0.5 M HCl and the sample was injected in this medium via the sample injection valve. Zinc elutes easily in the initial medium. The pump, recorder, and color reagent were then switched off and the inlet line of the pump was placed in the 70% acetonitrile-0.3 M HCl solution and lead was then eluted. Copper was removed with 90% acetonitrile-0.2 M HCl and manganese with 94% acetonitrile-0.5 M HCl. Finally nickel, which does not form chloride complexes, was stripped with 2 M HCl. Detection was performed with PAR as the color forming reagent.

Separation of calcium and magnesium

Calcium and magnesium are two of the most abundant elements in the earths crust. They are primary constituents of

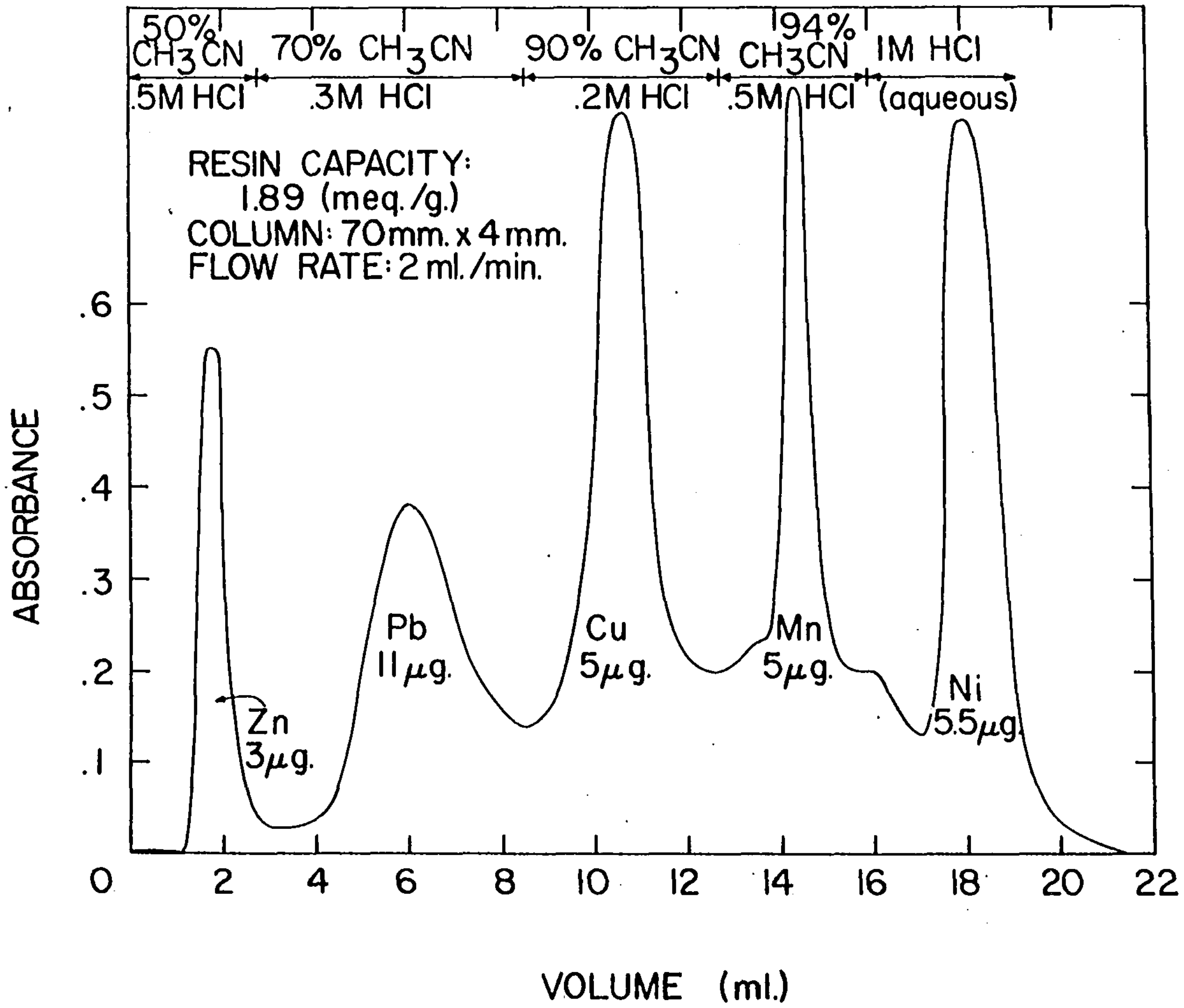


Fig. 17. Separation of divalent ions in acetonitrile-HCl

hard water and as such have inspired substantial effort at the development of methods for the determination of each in the presence of the other (79).

Two procedures were developed in this work which achieved the separation of calcium and magnesium by utilizing the greater ionic radius and hence greater distribution ratio of calcium.

Figure 18 shows the separation of magnesium and calcium in hydrochloric acid. The sample is placed on the column (capacity 1.89 meq/g) and magnesium is eluted with 0.6-1.0 M HCl. Calcium is then removed with 4 M HCl. The entire separation can be accomplished at a flow rate of 1 ml/min in 7 minutes or in about 4 minutes at 2 ml/min. The figure shows the magnesium peak to be broader than the calcium peak because the magnesium is not initially sorbed in as tight a band as is calcium.

Figure 19 shows a very similar separation of calcium and magnesium. In this figure the sample is injected into 1 M HCl as before, and magnesium is eluted. The eluent is then changed to 0.5 M ethylenediamine-1 M $HClO_4$ and calcium is removed in this solution. The purpose of the perchloric acid in the second eluent is to prevent a large baseline shift upon switching to the complexing eluent.

Separation of lead

Lead is strongly retained on the partially sulfonated resins in perchloric acid media, while most other common

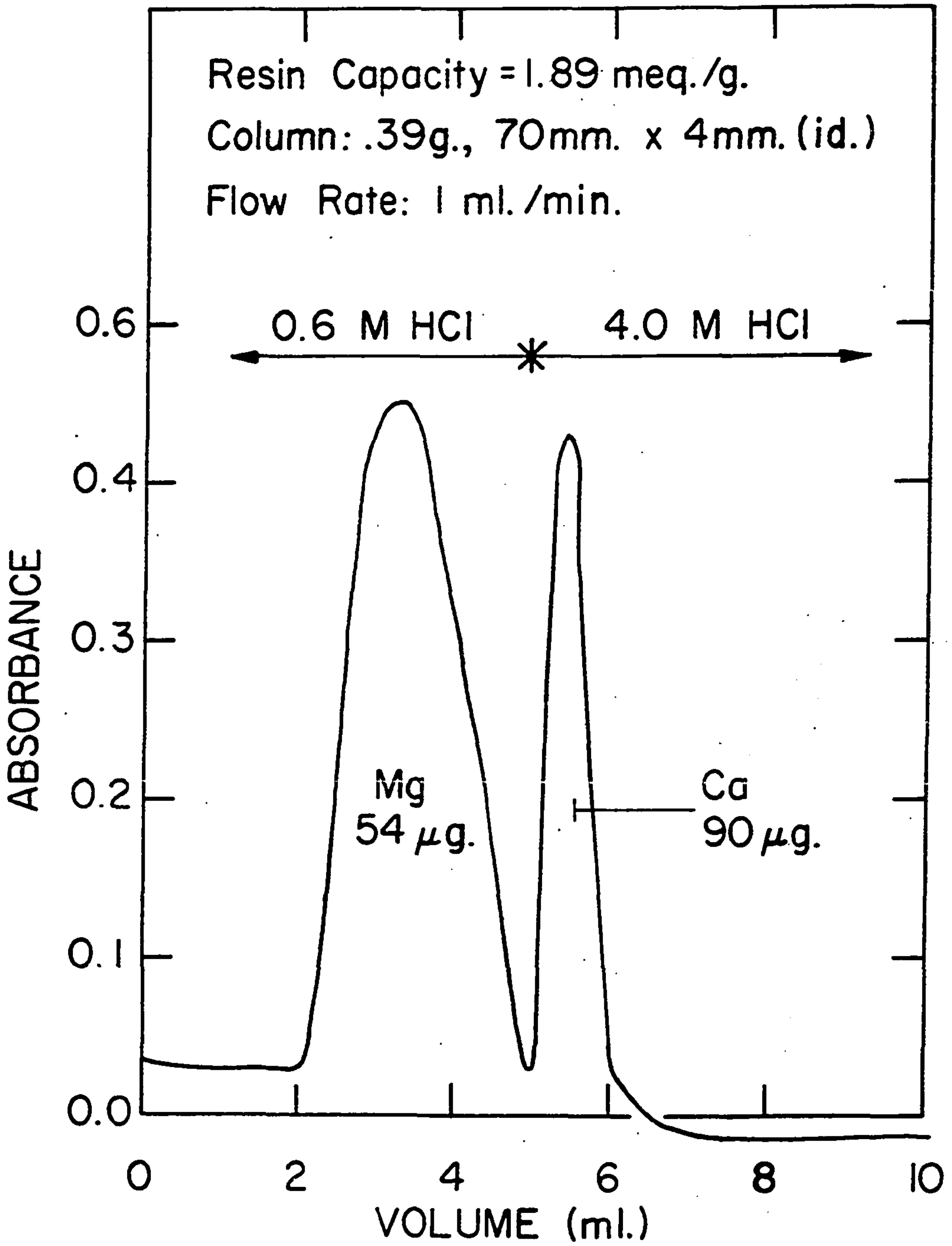


Fig. 18. Separation of calcium and magnesium in HCl

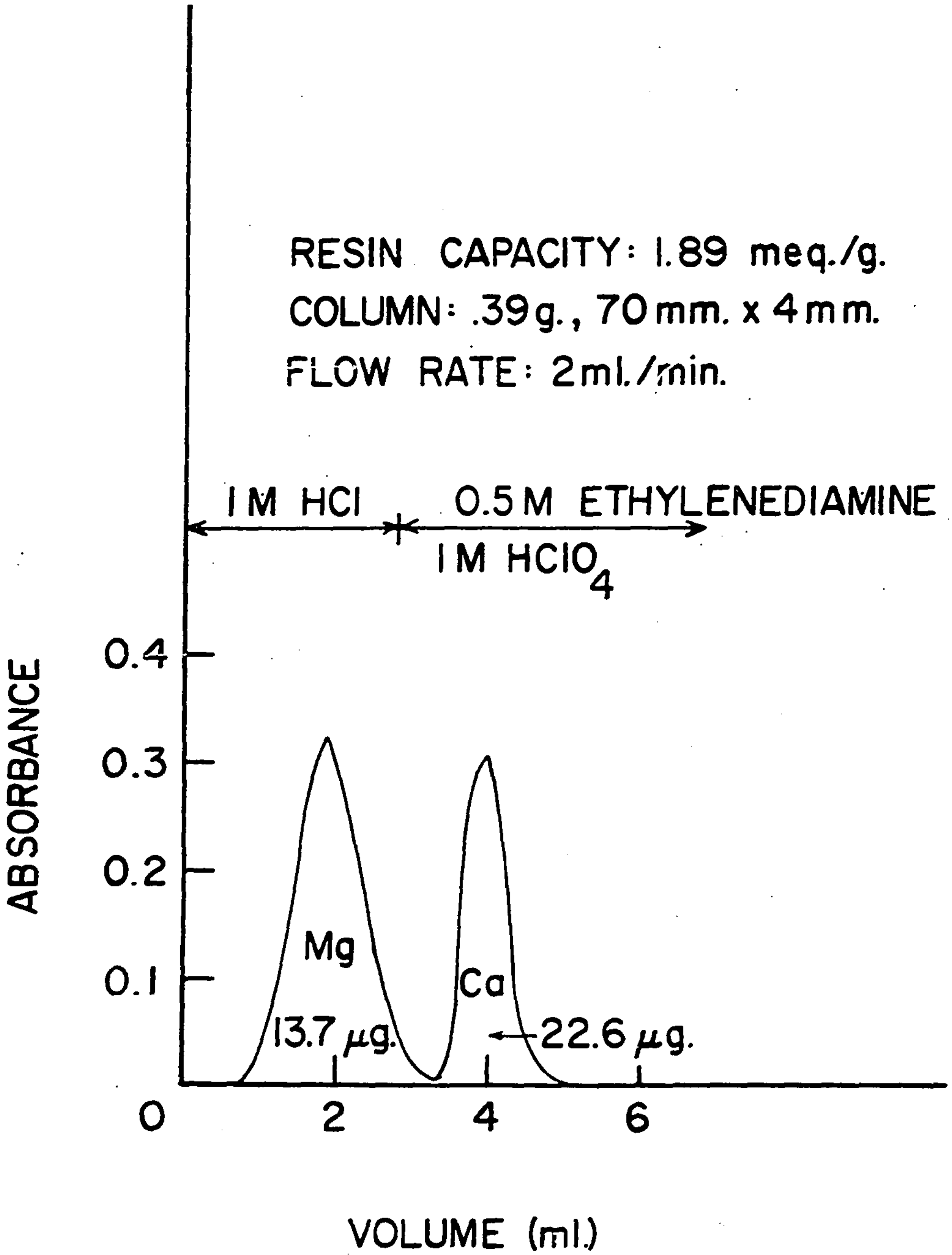


Fig. 19. Separation of calcium and magnesium

divalent ions are not. This fact permits an easy separation of lead from many other metal ions.

Methods for the determination of lead usually employ a separation of some kind, usually an extraction (80). A dithizone extraction and colorimetric determination of lead in the organic phase is widely used. But extraction procedures often require extensive purification of reagents and thus much laboratory time.

A forced flow chromatographic method for lead on an anion exchanger has been reported (32). However, there are many common metal ions which could sorb to the resin under the conditions employed and cause column overloading (81).

In the chromatogram shown in Figure 20, lead is separated from a much larger amount of four divalent ions. In this method the sample is injected into the column in 1-2 M $HClO_4$. Vanadium(IV), iron(II), iron(III), copper(II), zinc(II), nickel(II), manganese(II), aluminum(III), magnesium(II), and calcium(II) pass through the column unretained. Lead is then eluted with 1-2 M HCl.

Separation of zirconium and thorium

Zirconium and thorium are two elements which exist most commonly in the quadrivalent state and are among those most strongly taken up sulfonic acid cation exchangers. Strelow (82) found that zirconium and thorium could be separated on Dowex 50 by eluting zirconium with 5 M HCl while thorium is retained. Thorium could then be eluted in oxalic or sulfuric

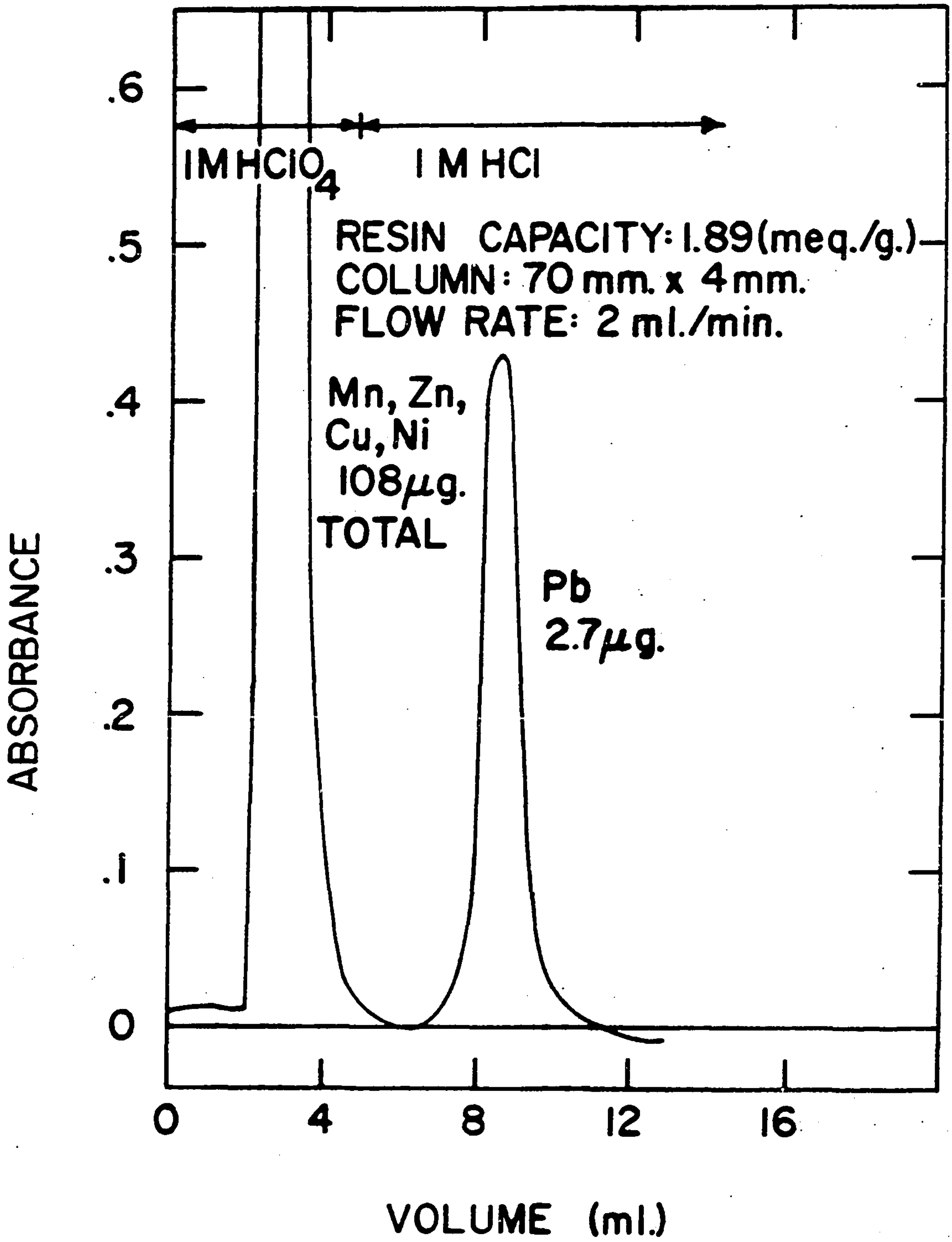


Fig. 20. Separation of lead(II) from maganese(II), zinc(II), copper(II), and nickel(II)

acid (83). A number of other cation exchange methods have been reported (84,85).

Figure 21 shows a separation of 25.6 μg of zirconium from 13.1 μg of thorium. The separation was done on the relatively low capacity resin 0.84 meq/g. The sample was injected into the 0.1 M ammonium citrate solution at a flow rate of 2 ml/min and zirconium was eluted almost immediately. After the zirconium had eluted the eluent was changed to 4 M HCl and thorium eluted from the column.

Separation of lanthanum and thorium

Lanthanum is the member of the lanthanide series which is generally the most difficult to remove from sulfonic acid type cation exchangers. Thorium is perhaps the most difficult metal ion to remove in the periodic chart in inorganic acid media.

The separation of lanthanum and thorium is also important because the mineral monazite is the primary natural source of both metals. A gravity flow cation exchange method for the separation of thorium from monazite concentrates has been reported (86), however this procedure is somewhat complicated.

The separation shown in Figure 22 uses only two different concentrations of one eluent to obtain a complete separation on an analytical scale in about four minutes.

The column chosen for the separation was of capacity 0.84 meq/g. The sample solution was injected into a flowing eluent stream of 1 M HCl. The greater part of the actual separation

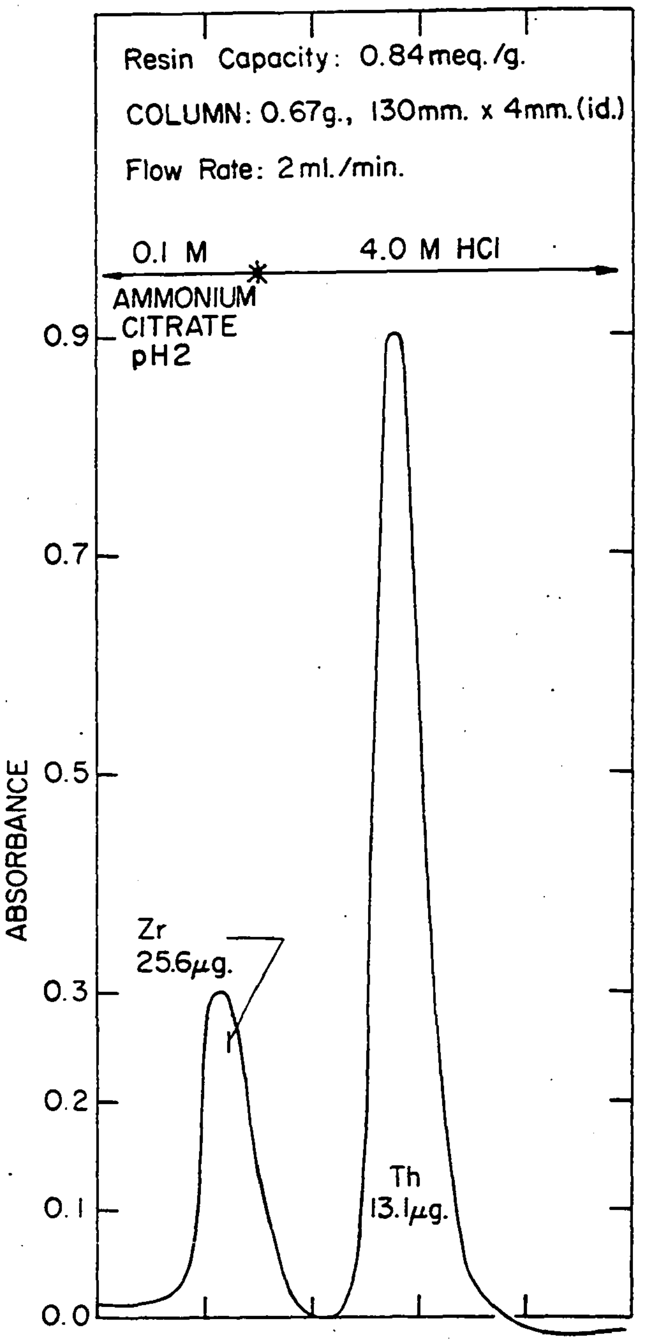


Fig. 21. Separation of zirconium(IV) and thorium(IV)

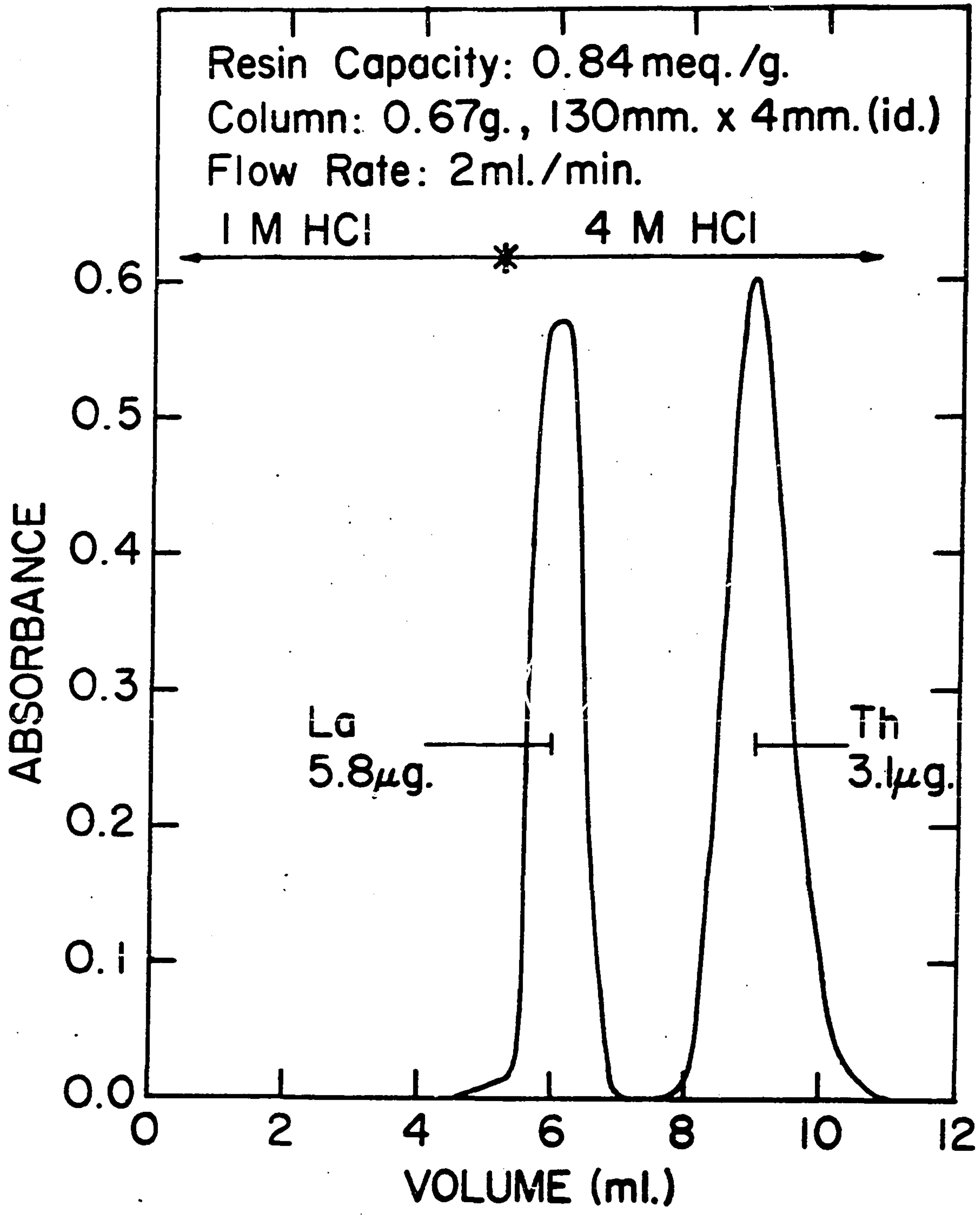


Fig. 22. Separation of lanthanum(III) and thorium(IV)

takes place during the time that the first solvent is passing through the column. At a measured time, just before the lanthanum peak is to elute, the eluent is switched to 4 M HCl. Lanthanum then immediately elutes and thorium follows. The same procedure can be used for the separation of any or all of the lanthanides from thorium. However, if more than one lanthanide ion is eluted, the resolution will permit only thorium to be quantified.

Separation of the lanthanide rare earths

For many years the only method for the separation of the rare earths was fractional crystallization or precipitation. These methods often required as many as 5000 recrystallizations involving several years for complete separation. The development of ion exchange chromatography has vastly simplified this problem.

Although the chemical properties of the lanthanides are almost identical, an ion exchange separation can utilize the very slight decrease in ionic radius from lanthanum to lutetium to obtain a separation. This separation can be enhanced by the use of a complexing eluent at an appropriate pH. The complexing agents complex lutetium most strongly and the formation constants decrease smoothly to those of lanthanum. Thus the slightly increasing acidity of the heavier lanthanides relative to the lighter ones can be used to obtain a separation.

Citrate, lactate, ethylenediaminetetracetate, and 2-

hydroxyisobutyrate have been found to be the most useful in effecting rare earth separations (74). All were tried in this work.

The potentialities of ion exchange chromatography were perhaps made most obvious in 1947 when Spedding, Powell, et al. performed the first successful separation of the rare earths and subsequent preparative scale separations (87-90). The initial work by these workers was done using citrate solutions. However, better eluents have been found since that initial work.

Lactate solutions have also found wide use in the separation of the lanthanides (74). Analytical scale separations of some of the rare earths are better in lactate solutions than in citrate. However, some problems have been reported in reproducing elution volumes with lactate eluents apparently because lactic acid solutions contain varying amounts of dimers. It is also often necessary to employ heated columns when using lactate in order to facilitate the separation.

Ethylenediaminetetracetate (EDTA) as well as other polyacetic acids have been shown to give very good rare-earth separations (91-93). But if the pH is too high the separation requires a very long time and if it is too low, EDTA precipitates. In this work EDTA could not be used because the metal-EDTA complexes are so much stronger than the complexes formed by the metallochromic indicator, that the eluted metal is not detected.

2-Hydroxyisobutyrate (HIBA) is one of the best complexing agents developed for the ion exchange separation of the rare earths (53,94,95). It gives better separation factors than lactic or citric acid and can be used with faster flow rates than EDTA (95). HIBA does not require high temperatures to achieve maximum separation factors.

A pressurized ion exchange separation of the lanthanides has been reported by Campbell (53). The separation was done in HIBA on a column 1 ft x 9 mm using 25-60 micron particles at 500 psi. The separation required only 2 hours. However, detection and construction of the chromatographic curve was performed by collecting fractions and radiochemical analysis. The entire process required about a week.

Figure 23 shows a separation of 13 lanthanide rare earths on Dowex 50x8 in about 4 hours. The eluent employed in this separation was a pH gradient (2.9 to 4.5) of 0.4 M HIBA. The separation was performed on 44-63 micron particles in a column 93.5 cm x 2.8 mm. Cerium is not included in the separation because it is easily extracted in the Ce(IV) state. Promethium is also not included since it is not naturally occurring. The sample, containing about 5 μg of each of the 13 lanthanides was injected onto the column in HIBA of pH approximately 2.9. The pH of the eluent was then raised slowly by means of the apparatus shown in Figure 24.

Two beakers were placed on a level surface and equivalent amounts (75 ml) of HIBA were added to each. The connecting

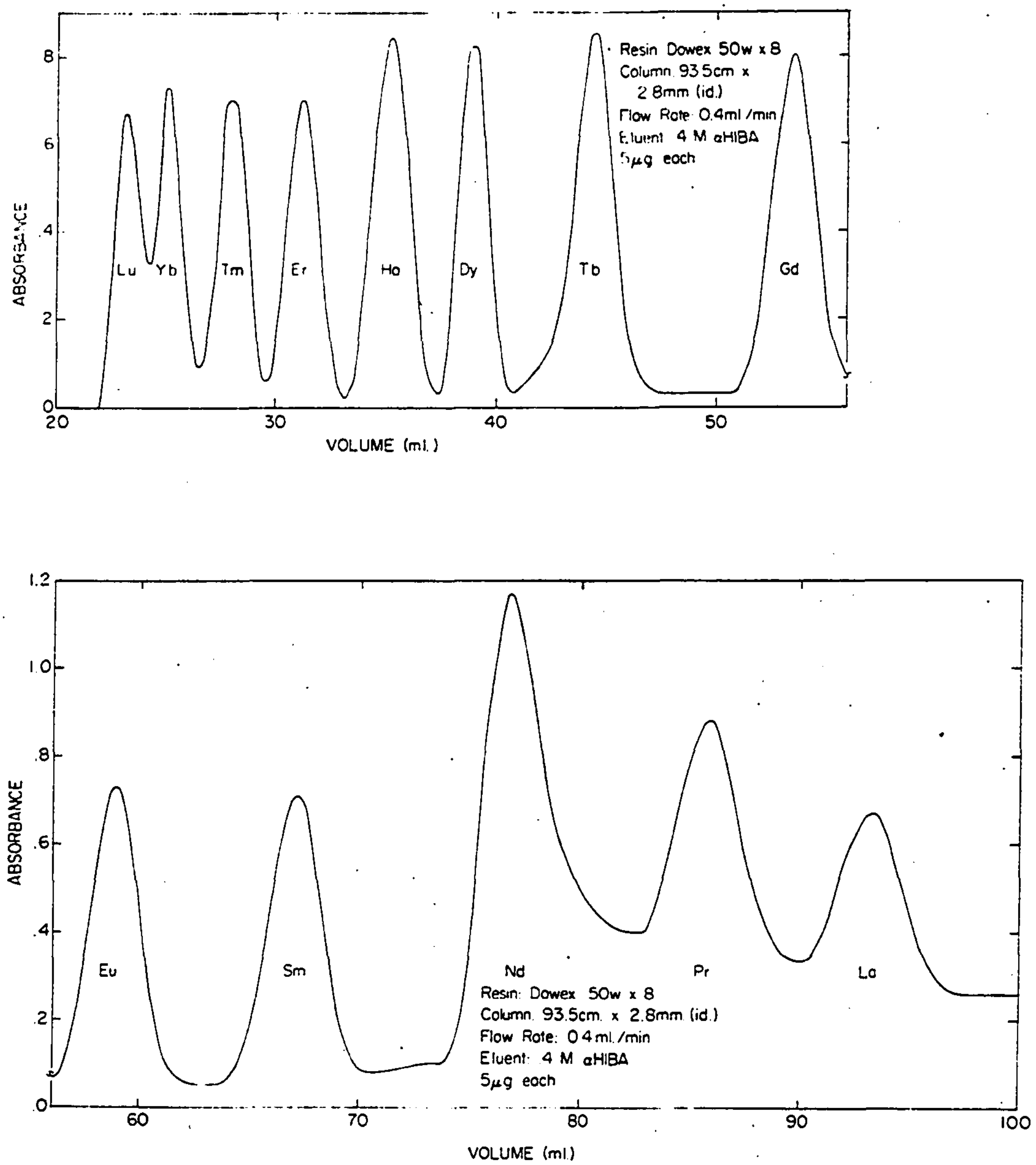


Fig. 23. Separation of trivalent lanthanide rare earth metal ions

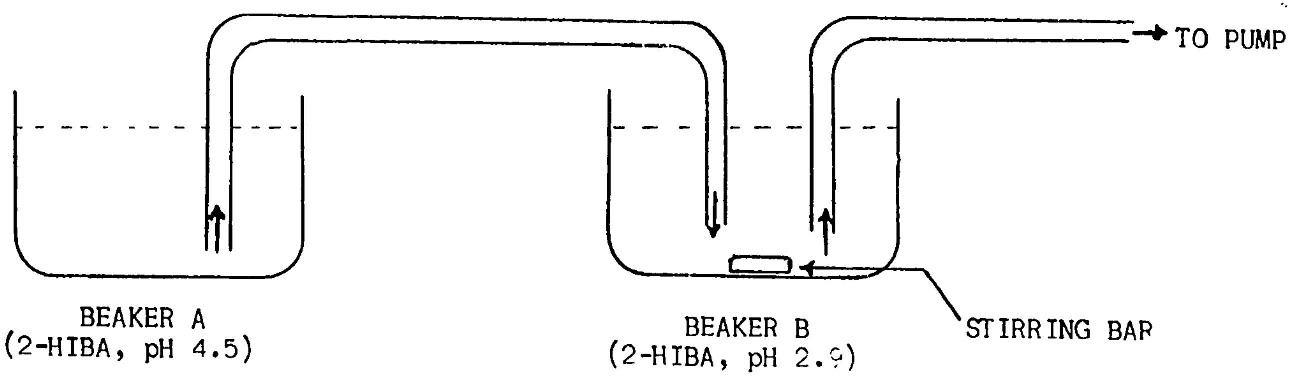


Fig. 24. Gradient apparatus used in rare earth separation

tube was filled and a stirring bar placed in beaker B. The pH of beakers A and B was adjusted to 4.5 and 2.9 respectively and the pump inlet was placed in beaker B and elutions begun. The sample was then injected. As the liquid level in B was lowered, the solution of higher pH (beaker A) passed into B, raising the pH of the eluent solution.

After injection the entire separation could be left unattended until all ions had eluted. The order of elution is the same as that usually observed. Resolution on the first and last pairs of ions is barely complete.

The tailing observed with the last few ions was found to be even more pronounced on the partially sulfonated resins used for all other separations. However, the partially sulfonated resin of capacity 0.84 meq/g gave a better separation for the first seven elements than Dowex 50.

The detection could easily be used to give quantitative results if desired.

Analyses

Analyses were performed on two NBS standard samples in order to give some indication of the versatility of the separations developed.

Analysis of NBS 37d

2-3 g of NBS Standard Sample 37d sheet brass was washed in acetone, dried, and about 0.5 g was weighed accurately into each of three 250 ml beakers. About 10 ml of concentrated

HNO_3 was added and the mixtures were heated until the original solids had dissolved. The solutions were filtered through Schleicher and Schnell red ribbon filter paper to remove precipitated tin and the filtrates were collected in a 100 ml volumetric flask. The solutions were then diluted to volume with water.

A 56.3 μl sample from each of the sample solutions was injected directly onto the 1.89 meq/g column in 2 M $HClO_4$. The matrix, consisting of copper, zinc, and nickel was unretained and passed directly through the column as shown in Figure 25. The eluent was then switched to 2 M HCl and lead eluted.

Peak height and peak area were measured for each of the three sample solutions and compared with calibration curves. The calibration curves, shown in Figures 26 and 27, were prepared from measured peak heights and areas obtained from chromatograms of lead injections in 2 M HCl. The lead stock solution was standardized by titration with standard EDTA solution.

Results of this and other analyses are listed on Table 15. Relative standard deviations are given for triplicate analyses of each solution. It was found that peak area gave a much better quantitative measure of the amount of sample component present than peak height. This was apparently due to the greater band spreading in the sample chromatograms and hence lower peak heights than in the chromatograms used for

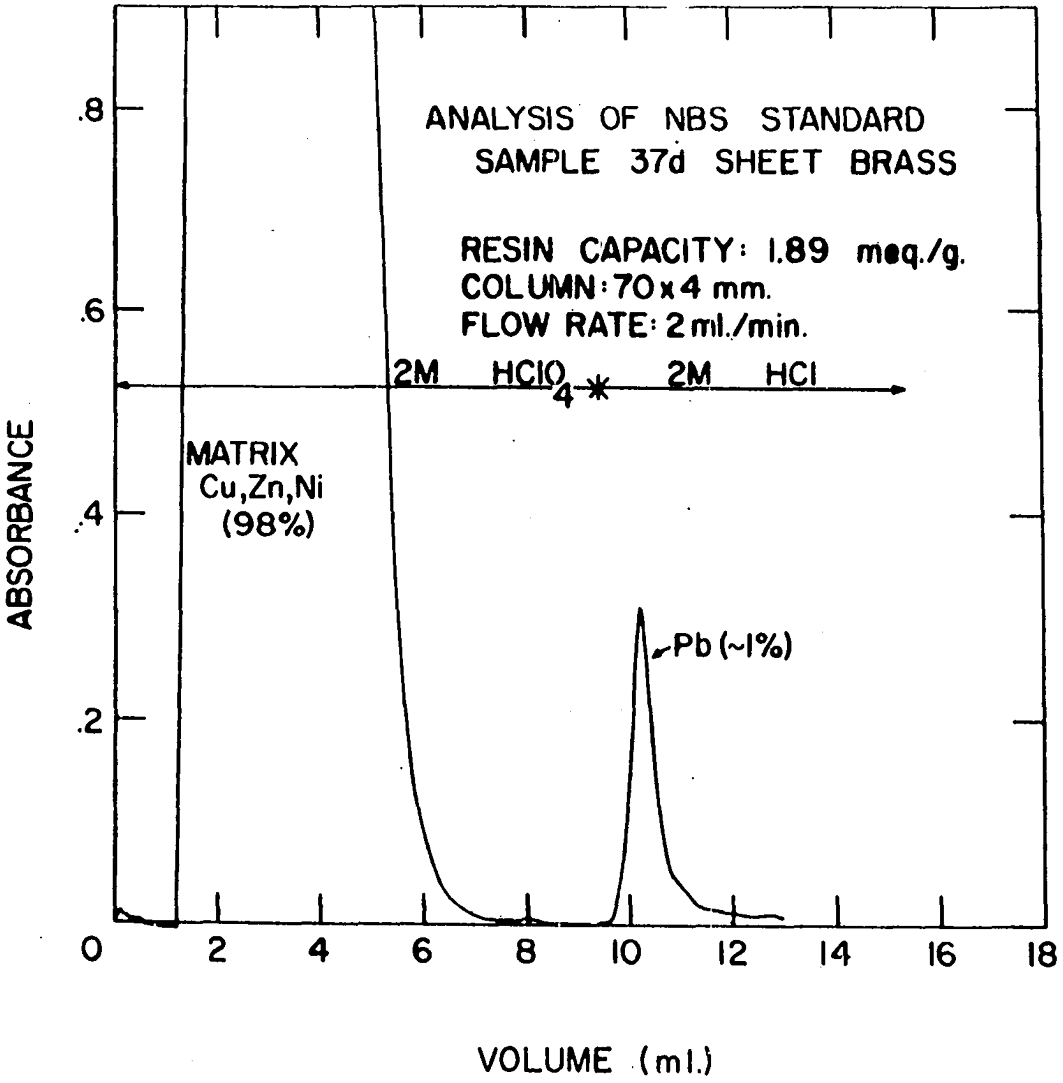


Fig. 25. Analysis of NBS standard sample 37d sheet brass

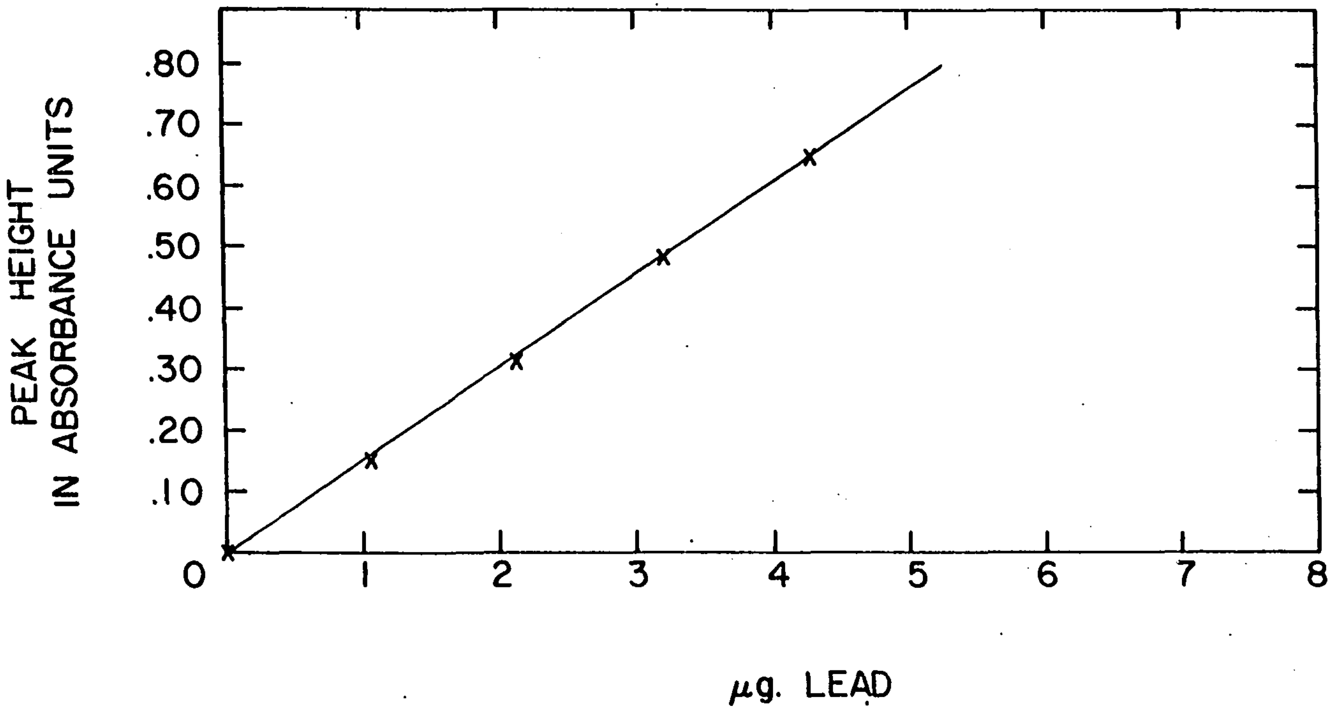


Fig. 26. Calibration plot for lead determination in terms of peak height

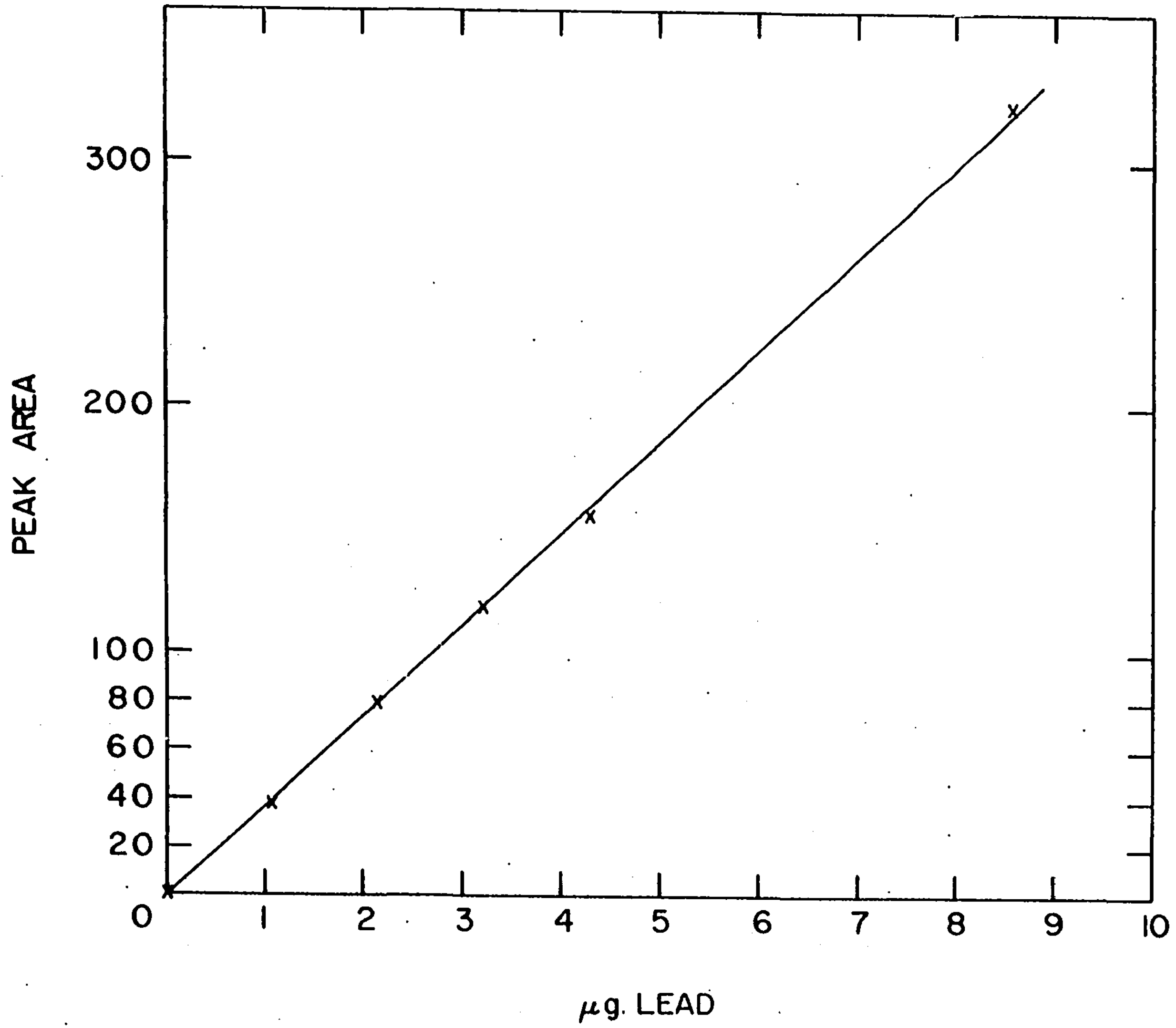


Fig. 27. Calibration plot for lead determination in terms of peak area

Table 15. Results of NBS analyses

Sample	Metal ion	Our analysis	NBS analysis	Relative standard deviation
37d	Pb	0.96%	0.94%	1.7 pph
124d	Pb	5.16	5.20	0.3
124d	Zn	5.10	5.06	1.6
124d	Ni	0.97	0.99	1.8

the preparation of the calibration curves.

Analysis of NBS 124d

Three solutions of NBS Standard Sample 124d ounce metal were prepared by dissolution in HNO_3 and filtration as for 37d. Five ml aliquots of the stock sample solution were then diluted to 25 ml and these solutions were analyzed for lead content by the same procedure as that used for 37d and previously described. The same calibration curves were used to obtain the quantity of lead present. The results are listed on Table 15.

Zinc NBS 124d was analyzed for zinc by the acetonitrile-HCl separation method previously described. 56.3-µl portions of the 5:1 dilutions of stock sample solutions were injected into 50% acetonitrile-0.5 M HCl and zinc was eluted immediately. All other components were retained. This separation is shown in Figure 28.

Stock zinc solution, used for the preparation of the

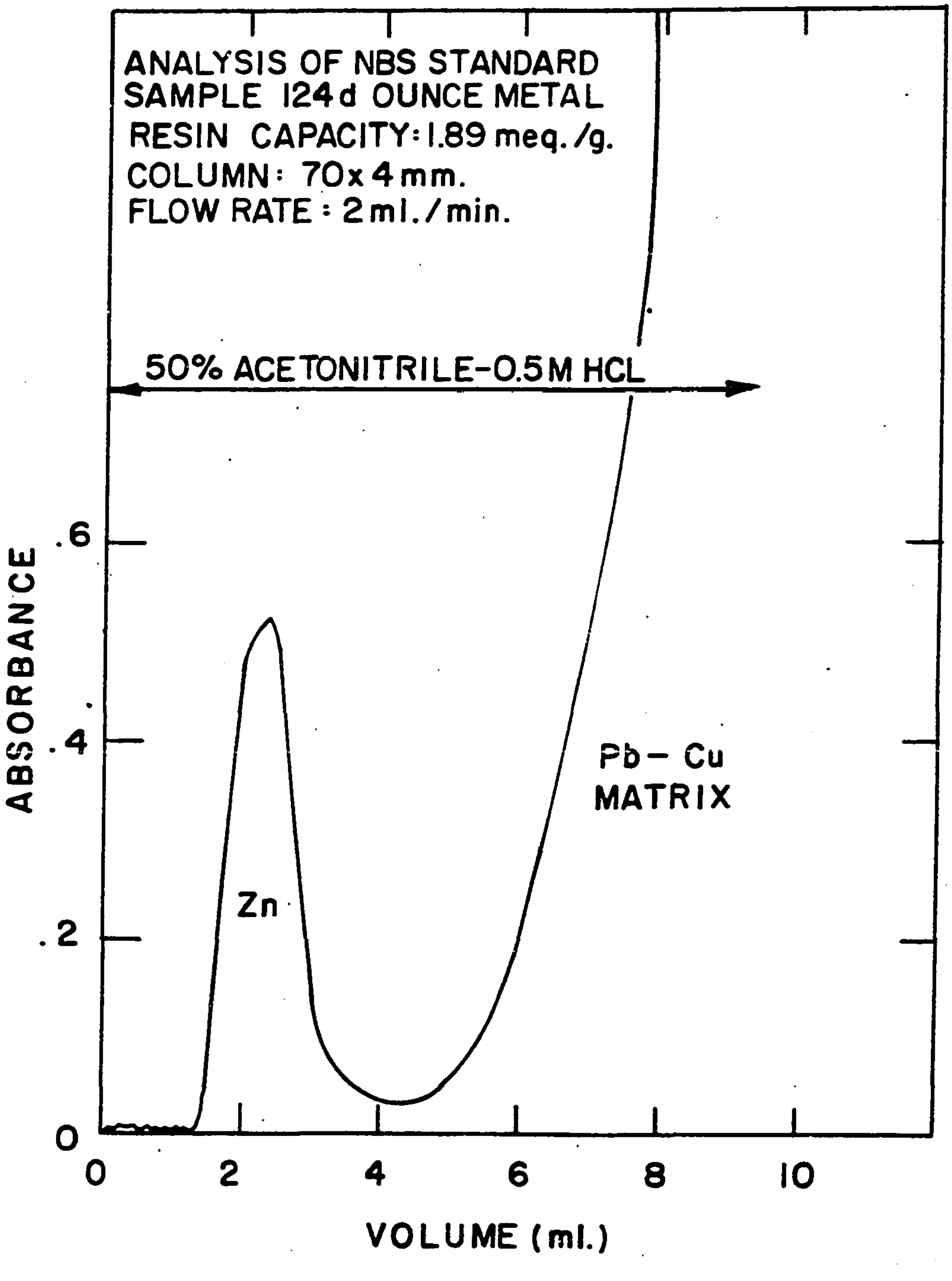


Fig. 28. Analysis of NBS standard sample 124d ounce metal for zinc

calibration curve, was prepared by dissolving Baker Analyzed Reagent zinc metal (99.9%) in HCl and diluting to volume. The calibration curves shown in Figures 29 and 30 were obtained from triplicate injections of dilutions of the stock solution in 50% acetonitrile-0.5 M HCl. Peak height or area can be used in this case but peak area gives better accuracy (0.8% error as compared to 2.2%) and precision (1.6 pph compared to 2.7 pph).

Nickel NBS 124d was also analyzed for nickel content by the acetonitrile-HCl procedure. 56.3 µl of the original, undiluted sample solutions were placed on the column in an eluent of 90% acetonitrile-0.2 M HCl, and zinc, lead, and copper were eluted. Nickel was retained from this medium and was then stripped with 2 M HCl (aqueous).

A calibration curve was prepared using dilutions of a stock nickel solution standardized by EDTA titration. The standard solutions were sorbed in 90% CH_3CN-0.2 M HCl and eluted in 2 M HCl. The amount of nickel in the sample was then determined by comparison of peak areas. The calibration plot is shown on Figure 31.

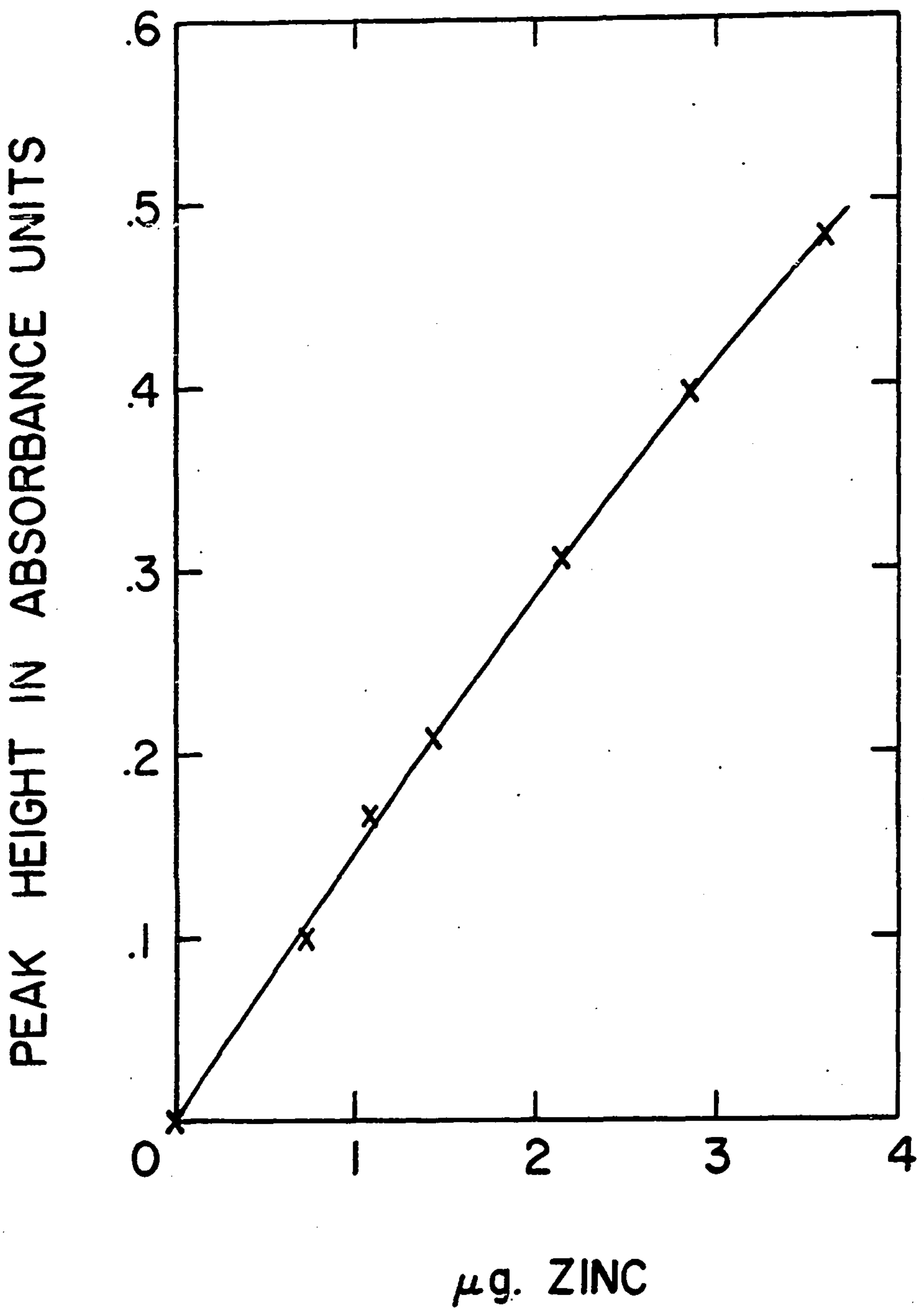


Fig. 29. Calibration plot for zinc determination in terms of peak height

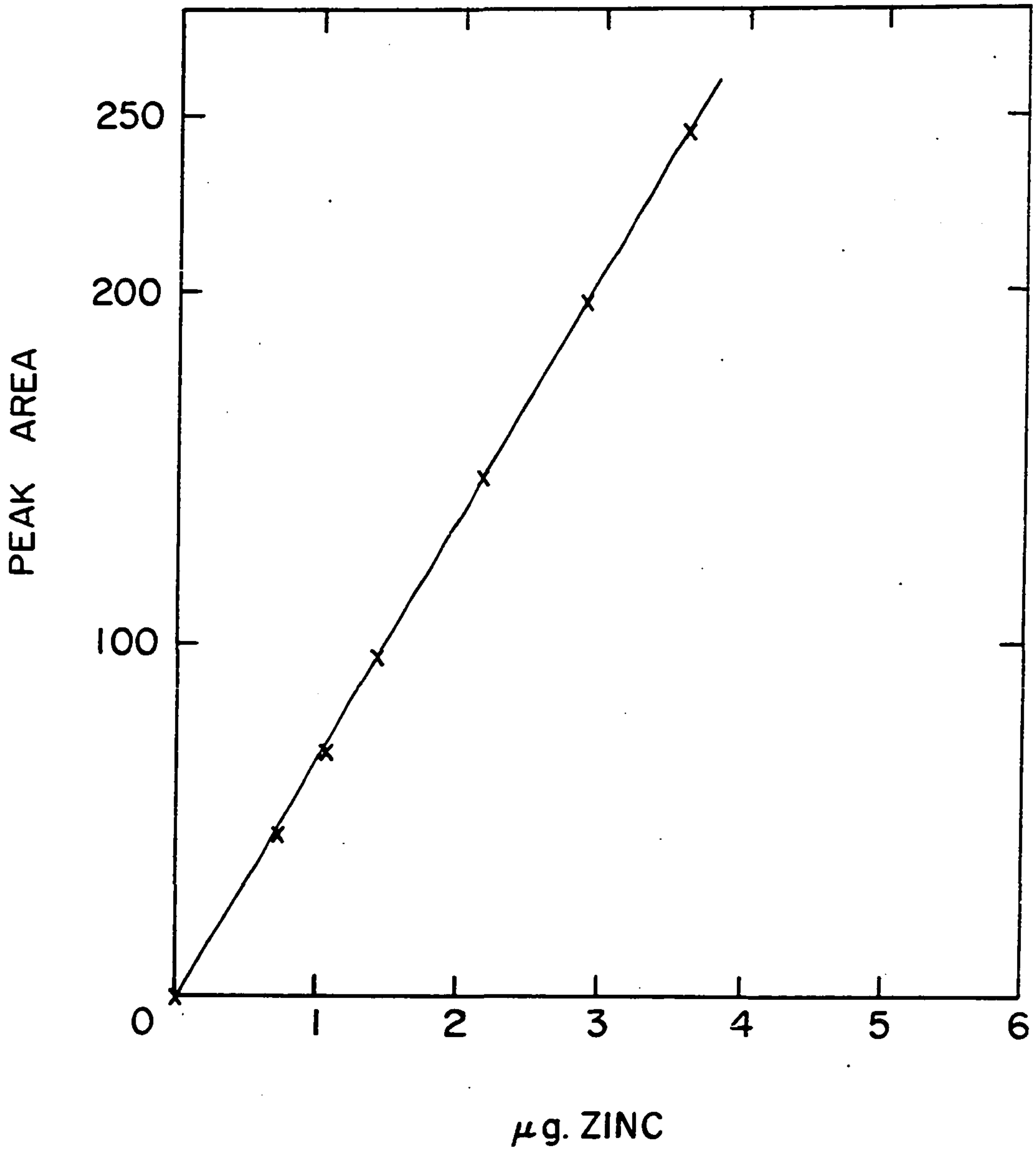


Fig. 30. Calibration plot for zinc determination in terms of peak area

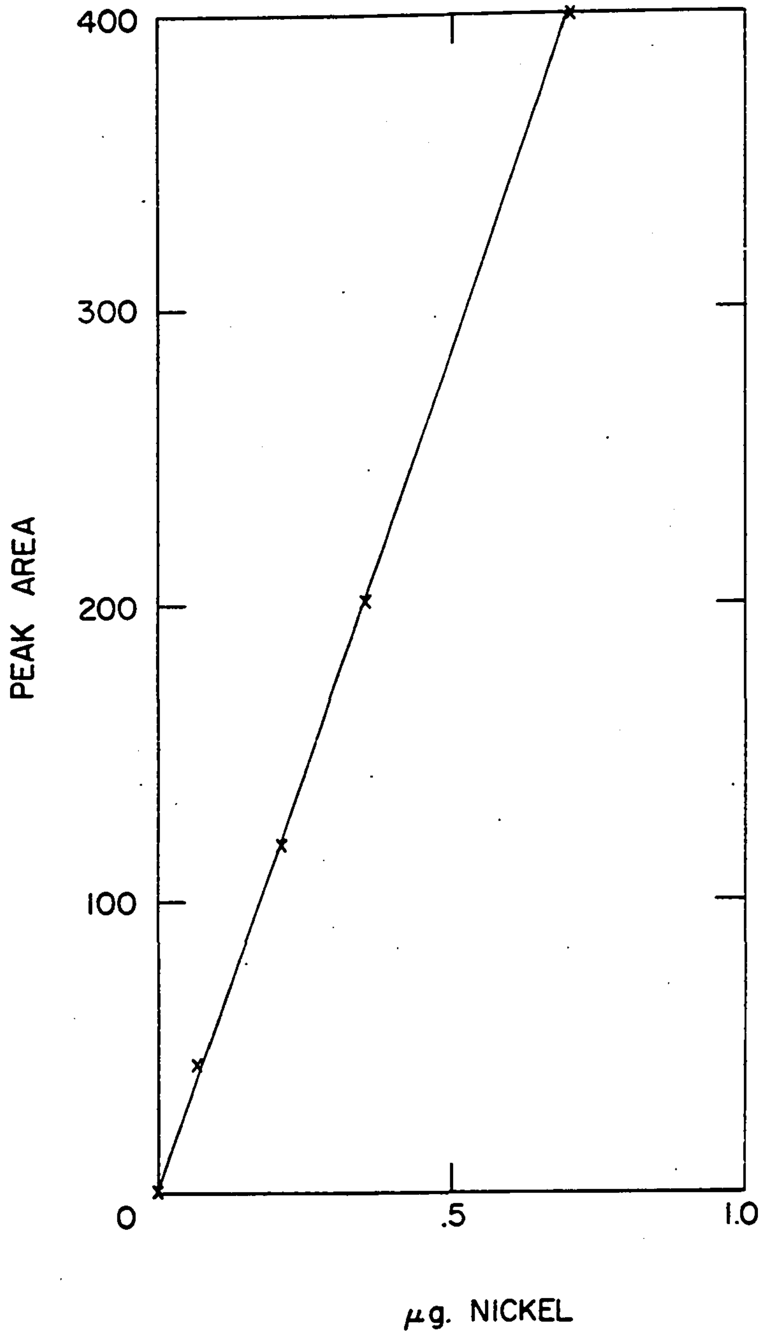


Fig. 31. Calibration plot for nickel determination in terms of peak area

CONCLUSION

Partially sulfonated, macroreticular, cation exchange resins are very simple to prepare. They are chemically stable and do not degrade easily. Their mechanical strength makes them well suited to forced flow chromatography.

Distribution ratios measured on the low capacity resins indicate that there are a large number of metal ions that can be separated easily on such resins. The separation factor for two multivalent metal ions usually increases with increasing capacity. But this is not always the case, as is demonstrated by the plots of distribution ratio versus capacity shown on Figures 11 and 12. Distribution ratios measured on the resins do increase continuously as capacity is increased. The rate of increase for a metal ion can be estimated from its crystal radius and ionic charge with distribution ratios increasing faster for those ions having large ionic radius and large ionic charge.

Selectivity coefficients for metal ion-hydrogen ion exchange are also shown to increase with increasing capacity when the anionic charge density on the pore walls becomes great enough to allow more than one anionic site to interact closely with a metal ion.

Partially sulfonated resins provide adequate resolution and fast exchange of ions in the separation of many strongly-held metal ions in dilute acid media. Separations of zirconium and thorium, and lanthanum and thorium can be accomplished in a few minutes. In addition, many more common ions can be easily separated on these resins. Calcium and magnesium can be separated and determined in various samples. Lead can be selectively sorbed in $HClO_4$ and separated from most other metal ions. Zinc, lead, copper, manganese, and nickel can be separated on aqueous-organic HCl solutions. Other separations could certainly be accomplished.

The low capacity resin of 0.84 meq/g gives better resolution of the lighter lanthanide ions than does Dowex 50x8 of the same particle size.

Forced flow chromatography and in-stream effluent detection can be applied to lanthanide separations. The entire group can be separated and quantitated in about 4 hours.

PAR provides a sensitive and versatile reagent for in-stream detection of metal ions in forced-flow chromatography. Components can be quantitated using peak area on the chromatogram, or, in some cases, peak height.

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