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The Response of the Micro-Adsorption Detector to Inorganic Cations

David P. McKay

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THE RESPONSE OF THE
MICRO-ADSORPTION DETECTOR
TO INORGANIC CATIONS

by

David P. McKay

A Thesis
Submitted to the
Faculty of the Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
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INTRODUCTION

Recent developments in the field of liquid chromatography include the use of high pressure, high efficiency narrow bore columns. These columns require sensitive, low dead volume detectors. While many detectors are now commercially available, the liquid chromatography of metal ions has been hampered by the lack of a detector of wide utility (20). Commercially available detectors include the ultraviolet spectrophotometric (14), the differential refractometric (2), the ultrasonic (17), the rotating chain flame ionizing (12), the microwave (1), the radioactivity monitoring (3), the electrolytic conductivity (4) and the micro-adsorption detectors (11).

The ultraviolet spectrophotometric detector has gained the widest acceptance. The application for which it is best known is the detection of amino acids separated on an ion exchange column (6,7,10,13). However, the ultraviolet spectrophotometric detector is essentially a functional group detector, the position of strong absorption spectra depending to a large extent on the functional groups present. Monitoring the absorbance of a single wavelength may seriously limit the variety of compounds which can be detected.

Recent detector designs have included two cells

that employ differential techniques. While this has solved some problems, there remains the problem of detecting a small change relative to the large carrier solvent background. For example, the differential refractometric detector depends on a large difference between the refractive index of eluate to be detected and that of the carrier solvent. This precludes the use of certain carrier solvent-eluate combinations. In the conductivity detector, if the electrolytic conductivity of the carrier solvent is high and the ions being detected yield only a very small increase then the signal to noise ratio becomes small. This has been particularly troublesome for separation of inorganic mixtures as the carrier solvents are usually acidic. Detectors which monitor a change in some other physical property of the effluent similarly require a large difference between the physical property of the carrier solvent and the eluate to be detected.

Detectors which largely avoid this limitation include the rotating chain flame ionizing, radioactivity monitoring, and the micro-adsorption detectors. The first two detectors are specific. The rotating chain flame ionization detector is a modified gas liquid chromatography detector for carbon-containing compounds. The second detector requires the eluates to be radioactive.

Hupe and Bayer (11) designed the micro-adsorption detector. The principle of the operation is simple. When an eluate enters the detector it is adsorbed on a packing. The process of adsorption releases energy in the form of heat. Two thermistors are placed in the detector. The reference thermistor senses the average temperature of the column effluent. The second thermistor is surrounded by a packing that will adsorb the eluate to be detected. The heat exchange in the adsorption process is measured as a change in the temperature of the packing. The compound is eventually desorbed bringing the temperature of the packing back to that of the reference thermistor. The differential signal of the two thermistors is amplified and displayed on a recorder. The peak shape observed is not necessarily symmetrical. Although the heat of adsorption and the heat of desorption are equal, the rates involved are rarely equal. Also the rate at which heat is exchanged with the carrier solvent and the detector walls is significant to peak shape (15). If the rate of desorption is slower than the rate of adsorption then the peak will have a positive deflection followed by a negative deflection. A return to baseline is made by heat exchange between packing, carrier solvent, and detector walls. Peak shapes will

vary from approximately Gaussian to the first derivative of a Gaussian peak.

The micro-adsorption detector is in dynamic equilibrium with the carrier solvent. Adsorption of the carrier solvent is equal to desorption. Background noise of carrier solvent interactions is not a limiting factor, rather the limiting background noise is attributed to the electronic components.

Hupe and Bayer claimed the micro-adsorption detector was universal (11). The detector has not gained the acceptance that seemed likely (20). Papers have reported the use of the micro-adsorption detector to follow ion exchange separations of biuret, dicyanide, urea and urea type compounds (18), the anion exchange of borate complexes of sugars and the cation exchange of nucleocides (20).

No application of the micro-adsorption detector to metal ion separations has appeared in the literature. The purpose of this work is to investigate systematically potential packings and operating parameters and to determine linearity and sensitivity of the micro-adsorption detector to metal ions.

INSTRUMENTATION

A Varian Aerograph Model 4000 Liquid Chromatograph equipped with a micro-adsorption detector was used throughout this study. The basic system consisted of the detector, a constant temperature bath, a power supply and amplifier module, a high pressure solvent reservoir, and a chromatographic column. A one millivolt recorder was added to the basic chromatograph. Each component is described in some detail.

Micro-Adsorption Detector

The micro-adsorption detector, Figure 1, is based on the design developed by Hupe and Bayer (11). The outer shell of the detector is composed of two stainless steel disks with one-eighth inch Swagelock type fittings for connection to the column and to a waste reservoir for the effluent. Two matched thermistors are used to detect components passing through the detector. The thermistors are embedded in two one-eighth inch thick Teflon disks such that the effluent will pass through the cavities containing the thermistors sequentially. The upper reference cavity is packed with an inert support, i.e., Teflon, glass beads, etc. and the lower cavity is packed with an interacting material, i.e., ion-exchange resin, silica gel,

Figure 1. Cross section of the micro-adsorption detector.

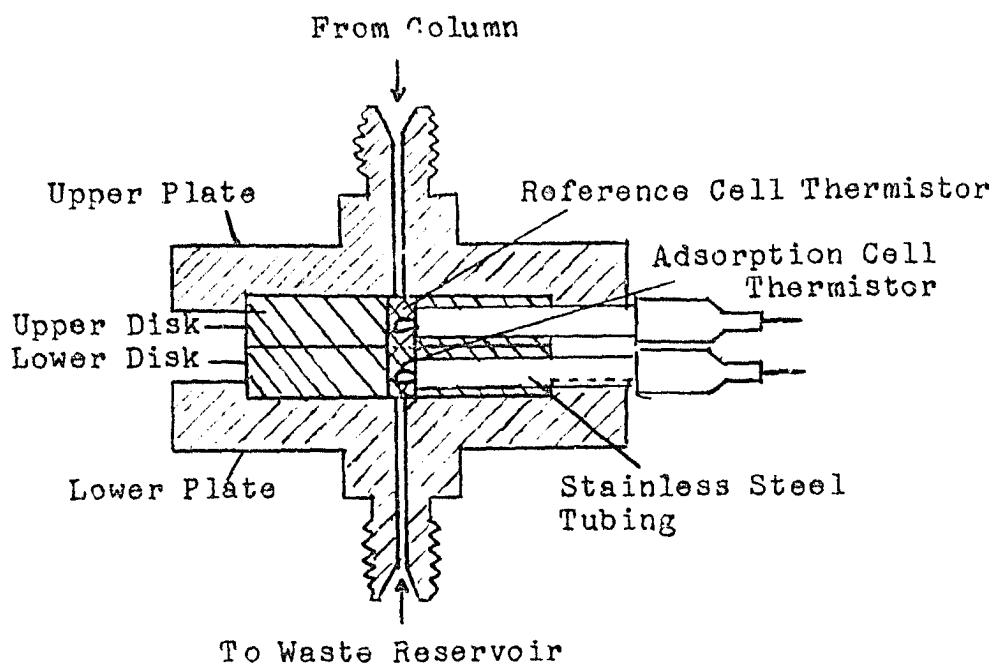
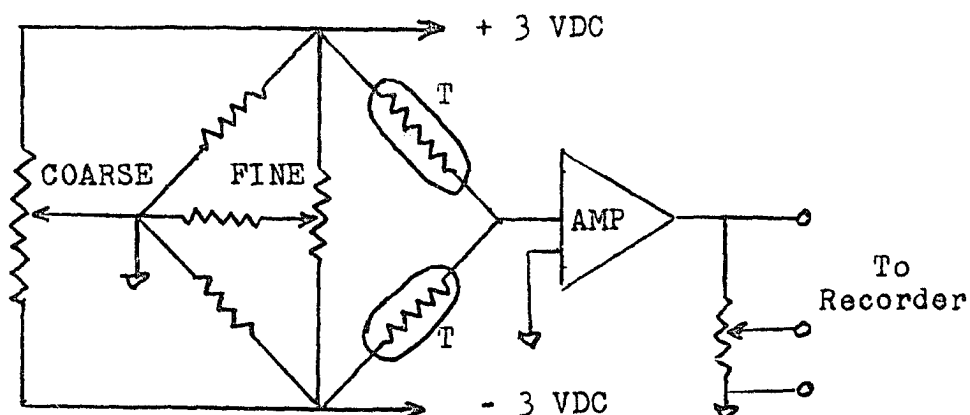


Figure 2. Schematic of thermistors in the micro-adsorption detector.



coated support, etc. A temperature difference of $6 \times 10^{-5}^{\circ}\text{C}$ (15) can be measured. To prevent loss or mixing of supports, one-half inch square stainless steel screens, 325 mesh, are placed before, between, and after the Teflon disks.

Water Bath

The micro-adsorption detector requires a very stable thermal environment to minimize baseline drift and changes in sensitivity. The water bath holds 5.8 liters of water. There is two inches of foam insulation between the inner and outer stainless steel shells. A stirring mechanism is included to minimize the temperature gradients. Heat loss corresponding to a temperature change on the order of one centigrade degree per two hours was observed at 55°C .

Power Supply and Amplifier Module

A solid state power supply and amplifier module is supplied with the basic chromatograph. The thermistors are connected as two arms of a Wheatstone bridge, Figure 2. The signal is amplified to drive a one millivolt recorder. Attenuation is available from the most sensitive setting of x1 to the least sensitive setting of x512 by nine powers of two.

Carrier Solvent Reservoir

A stainless steel carrier solvent reservoir with a capacity of 500 ml was used. A tank of compressed helium was connected to the carrier solvent reservoir to provide pressures up to 70 psi. An injector block is connected to the carrier solvent reservoir and has a one-eighth inch Swagelock type fitting to connect to the chromatographic column.

Chromatographic Columns

Two chromatographic columns were used. A restrictor column supplied with the instrument was used in the survey work. A six inch by one-eighth inch o.d. stainless steel restrictor column provided a back pressure of 8 psi at flow rates of 30 ml per hour. A Chromatronix Microbore Chromatographic Column Model number MB-2-150 packed with silanized glass beads was used in all other work. The glass column was 15.0 cm long by a 2.0 mm i.d. Chromatronix one-eighth inch stainless steel connectors, Model number 107A20, were used to connect the column to the injector and the detector. All other tubing was one-eighth inch Teflon.

Recorder

Two recorders were employed in this study. The first recorder, a Honeywell Electronik, Model number Y143X(57)-(VB)-II-III-(28)-NA, was a one millivolt recorder with a chart drive of two minutes per inch. It was used for survey work. The second recorder was a Honeywell Electronik Model number 194. It was set to one millivolt input and had a variable chart drive. Two chart drive speeds were used. A chart drive of 10 seconds per inch was used to adjust the flow rate and the data for other work was taken with a chart drive of 40 seconds per inch.

REAGENTS AND SOLUTIONS

All reagents used in this study were reagent grade except BeCl_2 , CsCl , InCl_3 and TlNO_3 . The CsCl and TlNO_3 were Fisher, purified. All solutions were prepared by direct weighing and appropriate dilutions. Carrier solvents, aqueous NaNO_3 and NaCl , were prepared by dilution of 5.0 M stock solutions. The pH was adjusted by the addition of 10^{-4} N HNO_3 or 10^{-4} N HCl .

Solutions of metal ions used in the survey work were 0.01 F prepared by direct weighing and made acidic or basic as necessary to keep them in solution. Metal ions used included Al(III) , Be(II) , Bi(III) , Ca(II) , Cd(II) , Co(II) , Cr(III) , Cs(I) , Cu(II) , Fe(III) , Hg(I) , Hg(II) , In(III) , Li(I) , Mg(II) , Mn(II) , Ni(II) , Pb(II) , Sn(II) , Sr(II) , Tl(I) , U(VI) , Zn(II) and Zr(IV) .

Solutions of metal ions used in the factorial experiment were 0.01 F nitrate or chloride salts of Ni(II) , Ca(II) and Cs(I) . Sodium chloride or nitrate and HCl or HNO_3 was added to give the same concentration as the carrier solvent. Solutions of metal ions used to determine linearity and sensitivity were prepared by dilution of appropriate aliquots of stock 1.0 F solutions of the metal ions and stock 5.0 M carrier solvent solutions.

Detector packings used in the reference cell were Haloport F, 60/80 mesh, F and M Scientific and Solid

Glass Beads, 200/340 mesh, Analabs. Detector packings investigated as potential supports were:

Amberlite CG-50, 200/400 Mesh, Baker

Dowex Chelating Resin, 50/100 Mesh, Sigma

Dowex 50, x-8, 200/400 Mesh, Sigma

Porasil B, 100/150 Mesh, Applied Science

Porous Glass Beads, 80/100 Mesh, Applied Science

Silica Gel, Chromatographic grade, Baker

The Dowex Chelating Resin was ground and sieved to a 200/340 mesh range.

Two reversed liquid phase packings were used. A di-n-octyl-phosphinic acid, H(DOP), exchanger was prepared by adding a solution of 5.0 ml 0.100 F H(DOP) in sym-tetrabromoethane and 5.0 ml acetone to 6.67 g of Celite, stirring to a uniform consistency, then letting the mixture stand in an open container until the observed weight loss had corresponded to the evaporation of the acetone. Porous glass beads were impregnated by placing them, covered with bis-(2-ethylhexyl)orthophosphoric acid, HDEHP, exchanger under vacuum for two days. Water was added and excess HDEHP was dispersed by shaking and decanting six times.

Solid glass beads were silanized with dimethyldichlorosilane, DMCS. A 50 ml beaker containing 15 ml of DMCS was placed in a desiccator along with a petri dish half full of solid glass beads. After one week,

the beads were removed, washed four times with 100 ml of methanol, and dried for three hours at 103°C.

SURVEY OF DETECTOR PACKINGS

Packings which have been successfully used with the micro-adsorption detector are the same as those used in columns to perform separations. Adsorbent materials used in liquid chromatography include silica gels, porous glass, alumina, and Florosil. Ion exchange resins have been used to detect several types of organic compounds. As potential packings porous glass and silica gel were chosen to represent strong adsorbents. The applications of ion exchange resins to metal ion separations indicate strong interactions. A strong acid resin, Dowex 50, a weak acid resin, Amberlite CG-50, and a chelating resin were included in the survey. The recent developments in reversed-liquid phase chromatography make it desirable to include two such materials, di-n-octyl phosphinic acid and bis(2-ethylhexyl)phosphoric acid. These ion exchangers have been used to separate rare earths.(3,19).

A reference material was sought that was inert or one that enhanced the observed response. Haloport F, solid glass beads, and silanized glass beads were investigated. The main function of the reference packing was to minimize dead volume in the detector.

Procedure

The apparatus used to pack the detector consisted of a one-liter vacuum flask fitted with a rubber stopper. A three inch piece of one-eighth inch O.D. stainless steel tubing was fitted with a Swagelock nut and ferrules. The tubing was inserted in the rubber stopper. As a vacuum source a water aspirator was used except in the case of Haloport F which required a vacuum pump. After filling, the detector was leak tested with the apparatus shown in Figure 3. The leak-testing apparatus consisted of one-eighth inch stainless steel tubing, two one-eighth inch Swagelock to one-eighth inch pipe unions, a 0-30 psi guage with one-eighth inch pipe fitting, and a stainless steel tee with one-eighth inch pipe threads. The detector was connected and the detector outlet was capped with a one-eighth inch Swagelock cap. The pressure was increased to 30 psi. The valve at the He tank was turned off. The detector maintained a pressure of 30 psi for 15 minutes. The liquid chromatograph was assembled with a restrictor column in place. If more than 40 psi was required to attain a flow rate of 20 ml/hr the detector was repacked. Flow rates were adjusted to 40 ml/hr for one half hour to wash the packing and allow it to equilibrate with the carrier solvent.

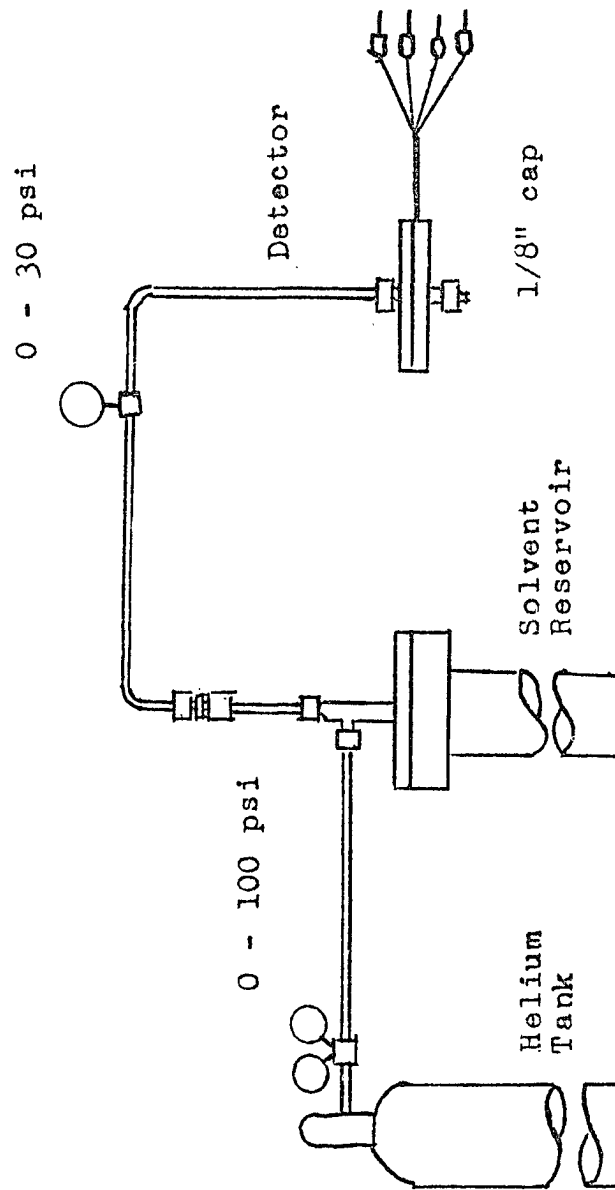


Figure 3. Leak testing apparatus.

Injectons of five microliter aliquots of 0.01 F metal ion solutions were made. At least eight different metal ions were injected. Packings which gave desirable responses were investigated further by injecting up to twenty different metal ions and five microliters of 0.1 N HCl. All solutions and equipment were at room temperature, $23 \pm 1^\circ\text{C}$.

Discussion

The reference cell was packed with Haloport F, solid glass beads, or silanized glass beads, Dowex 50, x-8 being used as the adsorbent. The observed response for Haloport F was greater than that for solid glass beads, and the shapes of the peaks, especially the trail-edges, suggested a slow interaction of some cations with the latter packing. Since the reference cell packing should be totally inert to the eluate, this is undesirable. Further investigation indicated the silanized glass beads gave a response equivalent to that of Haloport F. Silanized glass beads were easier to handle and to pack into the detector. Also, the Haloport material compacted under pressure, severely restricting flow rate and requiring frequent repacking. For these reasons, silanized glass beads were the preferred packing.

Table I lists the combinations of detector

DETECTOR PACKINGS	CARRIER SOLVENTS			
	0.1 <u>M</u> NaCl	0.01 N HNO ₃	0.001 N HNO ₃	H ₂ O
Dowex 50, x-8		X	X	X
Amberlite CG-50	X	X	X	X
Dowex Chelating Resin	X			
Porous Glass, 25 Å pore size			X	X
Porous Glass, 200 Å			X	
Silica Gel		X		
H(DOP) on Celite*				
HDEHP on Porous Glass, 200 Å		X	X	X

* Flow rates were unsatisfactory

TABLE I. COMBINATIONS OF SORBENT-CARRIER SOLVENTS SURVEYED

packings and carrier solvents tried. Porous glass beads gave a good response with distilled water. However, peak shapes and responses were not reproducible from injection to injection. Silica gel did not give a steady baseline and showed very little response. Reversed-phase packings gave unsteady baselines. Response was low with the largest peaks exhibiting tailing, severely in some instances.

Dowex 50, x-8, 200/400 mesh ion exchange resin exhibited reproducible peak shapes and an acceptable baseline, although sensitivity varied from one cation to another. Changing carrier solvent from 0.01 N HCl to .001 N HNO_3 increased sensitivity to several metal ions and decreased sensitivity to others. The response to an aliquot of 0.1 N HCl was greater than that for the metal ions. In general the trend was toward greater sensitivity at lower acid concentrations.

The weakly acidic ion exchange resin, Amberlite CG-50, 200/400 mesh, exhibited good response characteristics with 0.1 N HNO_3 . Peak shapes were well defined and narrow. Response equalled or exceeded that of Dowex 50, x-8.

The Dowex Chelating Resin exhibited good response characteristics. However, responses were equal for all cations except Fe(III). Further investigation by injecting distilled water and solutions of Ni(II)

at several concentrations showed that the observed peaks were dependent on volume injected and were independent of Ni(II) concentration. Apparently the response was simply due to change in the ionic strength of the carrier solvent, so this packing was not investigated further.

Equipment limitations were considered in choosing a packing for further study. The high acid concentrations used with Dowex 50, x-8, caused discoloration of packing. Apparently, the metal surfaces of the equipment were attacked and iron salts were adsorbed onto the resin. After several hours the detector had to be repacked. Thus Amberlite CG-50 was chosen for further study. The characteristics of the Amberlite CG-50 resin should be similar to the Dowex 50 resin except for the range of pH over which a weakly acidic resin will undergo cation exchange.

FACTORIAL STUDY OF OPERATING PARAMETERS

Several parameters are known to affect the response of the micro-adsorption detector. Utilization of the detector requires optimization of these parameters and determination of the limits within which they can be varied, without undue loss of response. The detector is flow sensitive, changes in flow rate giving rise to large deflections and unsteady baselines with recovery being fairly fast (16). The detector is temperature sensitive, hence the need for a stable thermal environment. Different carrier solvent-sorbent-eluate combinations give differing responses (15). Cation distribution on ion-exchange resins depends on pH, ionic strength, temperature and sometimes the anion present, so these parameters must be considered.

In order to study the significance and interdependence of parameters a factorial experiment was designed. Three metal cations were chosen to be independent variables. During the packing survey, some chosen cations, e.g. Al(III) and Fe(III) were strongly adsorbed on the Amberlite CG-50 ion exchange resin, their peaks showed considerable tailing and the peak areas were hard to reproduce. Others showed more desirable response characteristics. From this latter group Ni(II), Ca(II) and Cs(I) were chosen as representative of transition

metals, alkaline earths and alkali metals respectively.

Dependent variables used included those mentioned previously; namely temperature, pH of the carrier solvent, salt concentration of the carrier solvent, anion of the metal ions and carrier and the size of the injected sample. Table II lists the levels chosen for each dependent variable. Temperatures chosen were room temperature, 23°C, and 35°C. A higher temperature was the choice for level two, to provide an increase in diffusion and transfer rates. The useful pH range for weakly acidic ion exchange resins is 3 and higher. A choice of pH 5 and pH7 was made to keep well within these limits. Concentration levels of the carrier solvent were chosen as 1 M and 0.2 M to provide relatively strong eluting power. The salts used in the carrier solvent were NaCl and NaNO₃. These two anions provide a variety of metal ions having high solubilities, while representing a fairly good ligand and a very poor one. Lastly to investigate linearity of response with amount of cation injected and volume containing it, aliquots of 2, 4 and 8 microliters of 0.1 F metal ions were chosen. Three points allow for linearity measurements and provide good responses.

VARIABLE	LEVEL 1	LEVEL 2	LEVEL 3
Temperature, °C	23	35	
Anion of the Carrier Solvent	Nitrate	Chloride	
Concentration of the Carrier Solvent	1.0 <u>M</u>	0.20 <u>M</u>	
Carrier Solvent pH	7	5	
Injection Size, microliters	2.0	4.0	6.0
Metal Cations	Ni(II)	Ca(II)	Cs(I)

TABLE II. LEVELS OF MAIN VARIABLES

Procedure

The Amberlite CG-50, 200/400 Mesh, weak acid ion exchange resin was swollen with distilled water for two days. Approximately 100 ml of swollen resin was placed in a one liter beaker and 800 ml of distilled water was added. The resin was stirred vigorously and allowed to settle for 2.5 minutes. The resin was decanted into another one liter beaker. To the resin remaining in the beaker 800 ml of distilled water was added, stirred, settled and decanted as before. The process was repeated six times. Settling time was then increased to five minutes and repeated six times. Settling time was again increased to 7.5 minutes and finally to ten minutes. The 2.5 to five minute, five minute to 7.5 minute, and 7.5 to ten minute fractions were retained for further work. The detector was packed with the 2.5 to five minute size resin and the reference cell was packed with silanized glass beads.

Carrier solvents were made by diluting appropriate aliquots of stock salt solutions and aliquots of 10^{-3} N HCl or HNO₃ to 500 ml. Metal ion solutions were prepared from 1.0 F stock solutions by diluting appropriate aliquots of stock metal ion solutions, stock salt solutions, and 10^{-4} M HCl or HNO₃ to 50 ml. Temperature of the water bath was adjusted by adding hot or cold

tap water. Flow rates were adjusted to 30 ml/hr. The recorder speed was 40 sec/in. After allowing one half hour for stabilization of the baseline, 2, 4 and 8 microliter aliquots of metal ion solutions were injected with a ten microliter Hamilton syringe. Peaks were attenuated to give a 45-90% deflection where possible. All injections were made in triplicate.

Discussion

Both peak heights and peak areas, as calculated from height times width at half height, were measured. Generally peak areas proved to be more reproducible and so were used in analyzing the data. All peak areas were corrected for attenuation and normalized to a four microliter sample. The results were subjected to an analysis of variance using General Electric time share computer programs, MANV2\$ and MANV3\$ (9). The resulting F ratios are shown in Appendix I, and significant variables are listed in Table III in order of decreasing F.

Temperature was consistently the most important variable, the average area at 35°C being approximately 15% greater than at room temperature. Concentration of salt in the carrier solvent was very significant, the 1 M salt concentration giving approximately 20% greater average responses. This is not surprising as

VARIABLES	Ni(II) Rank Level		Ca(II) Rank Level		Cs(I) Rank Level.	
Temperature, °C (T)	1	35°C	1	35°C	1	35°C
Carrier Solvent Anion (A)	2	Cl ⁻	3	Cl ⁻	-	-
Carrier Solvent Concentration (C)	4	1.0 M	2	0.2 M	2	0.2 M
T x A	3	35°C-Cl ⁻	4	35°C-Cl ⁻	4	35°C-Cl ⁻
T x C	5	35°C-1.0 M	6	35°C-0.2 M	-	-
A x C	6	Cl ⁻ -0.2 M	5	Cl ⁻ -0.2 M	3	NO ₃ ⁻ -1.0 M
Carrier Solvent pH (P)	-	*	7	pH 7	-	-
Sample Size (S)	-	-	8	8 μl	-	-
T x P	8	35°C-pH 7	9	35°C-pH 7	5	35°C-pH 7
T x S	-	-	10	35°C-8 μl	-	-
A x P	7	Cl ⁻ -pH 7	-	-	6	Cl ⁻ -pH 7
* Insignificant Variable						

TABLE III. VARIABLES AND INTERACTIONS IN THE ORDER OF DECREASING SIGNIFICANT F RATIOS WITH THE LEVEL OF GREATEST AVERAGE RESPONSE

these two variables are known to affect separations performed on ion exchange resins. The anion used was significant for nickel and calcium but not for cesium, again, the chloride giving about 15% greater average response. This seems compatible with the complexing behavior of these systems. The interaction between variables correlated with the variables themselves, the more significant ones having the more significant interactions. In those cases where the interactions were not synergistic the interaction effects must have been smaller than the effects of the main variables.

Of the variables investigated, pH and size of sample injected were not significant by the F ratio test when the latter was normalized. Linearity of sample size for the range of microliters injected is thus established. The lack of a significant F ratio for the pH is surprising. Such a result seems inconsistent with literature on pH dependence of ion exchange resins. The values chosen may represent too small a range, or may lie on opposite sides of an optimum pH.

OPTIMIZATION OF OPERATING PARAMETERS

The parameters which were found to have significant F ratios in the factorial experiment were investigated further. The inclusion of pH was made to verify the small F ratio. The effect of the anion was not investigated. Practical considerations precluded the use of series of salts and carrier solvents based on other anions. Although chlorides gave a larger response than nitrates, as noted above, the nitrate salts of metal ions generally are more soluble. The advantage of preparing more concentrated stock and test solutions was thought to outweigh the loss of sensitivity, so nitrates were used in this part of the work.

Additionally, the reproducibility of injection technique and the influence of repacking the detector were investigated. General trends were established and regions of optimum performance were noted. Of the cations used in the factorial study, Ni(II) and Ca(II) were continued in this part. Cs(I) was not used as its response, small at best, was not great enough for the range of operating parameters to be covered. Zn(II) was used in those portions where a third cation was desired.

General Procedure

The detector cell was packed with Amberlite CG-50 ion exchange resin, settling time of 5 to 7.5 minutes, throughout these studies. Silanized glass beads were used in the reference cell of the detector and as a packing in the Chromatronix glass column. The carrier solvents were made by dilution of 5.00 M NaNO_3 . The temperature was ambient, 23°C, and adjusted by adding hot or cold tap water in the temperature study. Flow rate was optimized at 30 ml/hr, based on peak height and readjusted as necessary after every change in parameters. The retention time corresponding to the void volume of the system was 30 seconds. A recorder chart speed of 40 sec/in was used. Calcium and nickel solutions from the factorial study were used, and a 1.0 F $\text{Zn}(\text{NO}_3)_2$ stock solution was prepared for the study of pH and salt concentration effects. Differences in experimental procedure from one study to the next are elaborated in the discussion sections.

Discussion

Reproducibility of injection

Injections of eight and four microliter aliquots of 0.10 F $\text{Ni}(\text{II})$ and 0.10 F $\text{Ca}(\text{II})$ solutions were made in triplicate. The peak areas were measured by peak height

times width at half height. Attenuation was varied by a factor of two. Results of the reproducibility of injection technique are given in Table IV. Relative standard deviations of 3.3, 2.8, 2.3 and 1.8% were found for eight and four microliter aliquots of Ni(II) and Ca(II) respectively.

Reproducibility involves two critical measurements. The ten microliter Hamilton syringe is graduated to 0.1 microliter. Uncertainty in reading the syringe was 0.1 microliter due to parallax. Measurement of peak areas was limited by the width at half height, which could be read to about 0.1 mm. The peak width varied from 6.7 to 7.0 mm in this portion of the study. Relative uncertainties were thus 1.25 to 2.5% for syringe readings and 1.4 to 1.5% for peak areas. Thus the observed deviations are no larger than expected from the two sources.

Reproducibility of detector packing

Quadruplicate injections of eight microliter aliquots of Ni(II) and Ca(II) solutions were made. The detector was repacked with Amberlite CG-50 and the experiment repeated three times. The effect of repacking the detector is shown in Table V. Relative standard deviations of Ni(II) and Ca(II) were 10 and 12% for area and 16 and 20% for peak height respectively. These

	Ni(II)		Ca(II)	
	Area Deviation		Area Deviation	
Eight Microliters	1266	-13	1358	+10
	1247	-32	1313	-35
	<u>1326</u>	+47	<u>1372</u>	+24
Average	1279		1348	
Relative Standard Deviation	3.3%		2.3%	
Four Microliters	1289	+28	1410	+20
	1274	+13	1363	-28
	<u>1221</u>	-40	<u>1398</u>	+ 8
Average	1261		1390	
Relative Standard Deviation	2.8%		1.8%	

TABLE IV. REPRODUCIBILITY OF INJECTION

PACKING NUMBER	Ni(II)		Ca(II)	
	AREA	HEIGHT	AREA	HEIGHT
1	1700	190	1480	200
2	1920	240	1720	240
3	2060	265	1750	252
4	<u>1900</u>	<u>223</u>	<u>1370</u>	<u>175</u>
AVERAGE	1890	229	1590	217
RELATIVE STANDARD DEVIATION	10%	16%	12%	20%

TABLE V. REPRODUCIBILITY OF REPACKING THE DETECTOR

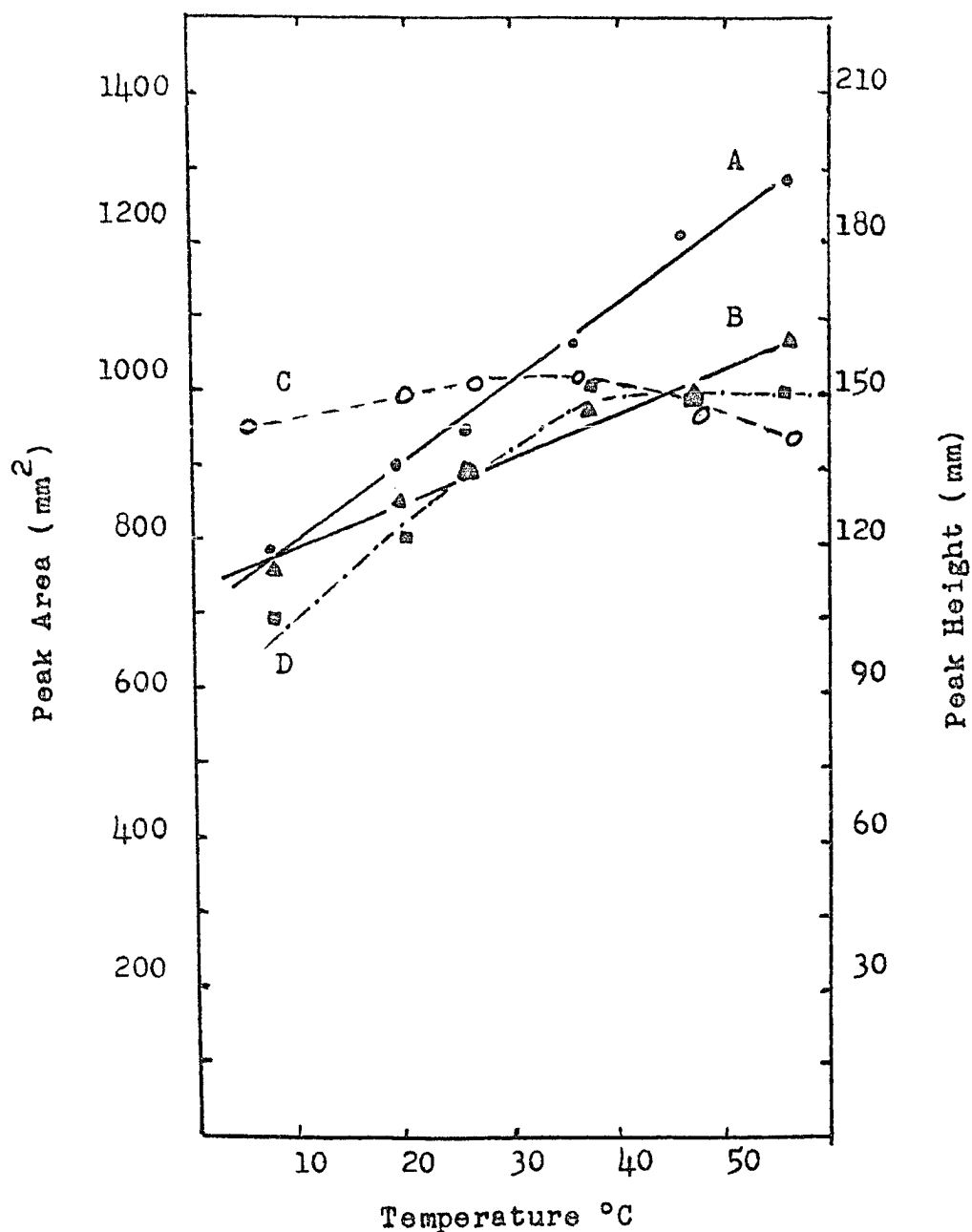
errors are too large to be attributed to area measurement and injection technique. Earlier work was done to determine the significance of resin size. It confirmed the variation of response upon repacking the detector. Any effect due to particle size of the resin was swamped by this latter effect. The peak shapes were not affected by repacking the detector, but the need to recalibrate the detector after each packing is obvious.

Temperature effects

The water bath temperature was adjusted to 7.5, 20, 25, 35, 45 and 55°C. The experiment was run in order of increasing temperatures and repeated in order of decreasing temperatures. Large baseline deflections were observed with each change, so the baseline was allowed to stabilize to a drift of 5% per hour at a sensitivity of X8 before proceeding. Duplicate injections of six microliter aliquots of Ni(II) and Ca(II) solutions were made.

The results of varying the temperature of the water bath are illustrated in Figure 4. An increase in temperature caused an increase in peak area. This was largely due to an increase of the peak width with temperature. This effect can be attributed to an increase in diffusion within the column of the metal ions. The peak heights varied about 20% for calcium with a maximum obtained near ambient temperature. Peak height was nearly

Figure 4. Effect of temperature on response: A. Peak area of Ni(II); B. Peak area of Ca(II); C. Peak height of Ni(II); D. Peak height of Ca(II).



constant for nickel. The optimum temperature was between ambient, 23°C, and 35°C. This region minimizes undesirable diffusion with a minimal loss in sensitivity.

Effect of pH

Carrier solvents were 1.00 M NaNO_3 made by dilution of 5.00 M NaNO_3 and the adjustment of the pH to the desired value with 0.2 N HNO_3 or 1.0×10^{-3} N NaOH . The pH values used were 3, 5, 7 and 11. Injections of eight microliter aliquots of 0.10 F $\text{Ni}(\text{NO}_3)_2$, 0.10 F $\text{Ca}(\text{NO}_3)_2$ and 1.0 F $\text{Zn}(\text{NO}_3)_2$ solutions were made in triplicate.

The effect of pH is depicted in Figure 5. A plateau is established above a pH of 5. As the acidity is increased the response drops drastically. The percent cation removal versus pH of a weak acid type ion exchange resin is given in Figure 5 (5). The contact time was one hour. The detector has a contact time of about five seconds. Even so the pH dependence of the two are strikingly similar. At pH 7 the detector response is optimum and many metal nitrates are soluble. The useful range for pH seems to be 5 to 11.

Effect of salt concentration.

The carrier solvents were made by dilution of 5.00 M NaNO_3 to obtain 1.00 M, 0.80 M, 0.50 M, 0.30 M,

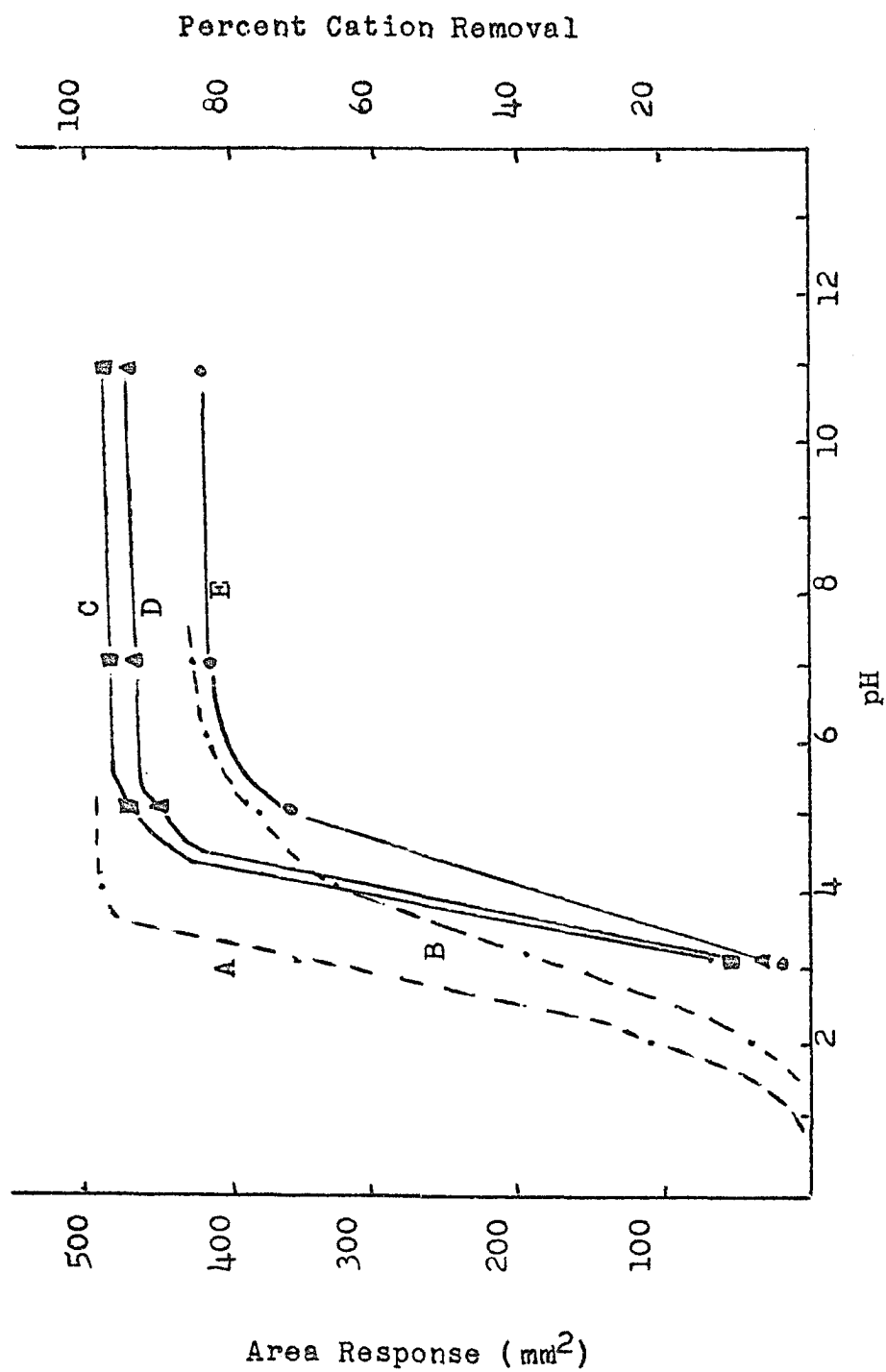


Figure 5. Effect of pH on response: A. Percent removal of Ni(II) by weak acid resin (5); B. Percent removal of Ca(II) by weak acid resin (5); C. Area response of Zn(II); D. Area response of Ni(II); E. Area response of Ca(II).

0.20 M and 0.10 M solutions. All solutions were neutral. Injections of six microliter aliquots of 0.10 F $\text{Ni}(\text{NO}_3)_2$, 0.10 F $\text{Ca}(\text{NO}_3)_2$ and 1.0 F $\text{Zn}(\text{NO}_3)_2$ solutions were made in triplicate.

The effect of salt concentration of the carrier solvent is illustrated in Figure 6. A plateau is established for concentration from 0.5 M to the highest level used, 1.0 M. Peak shapes were well defined throughout the range of concentrations. No tailing was observed.

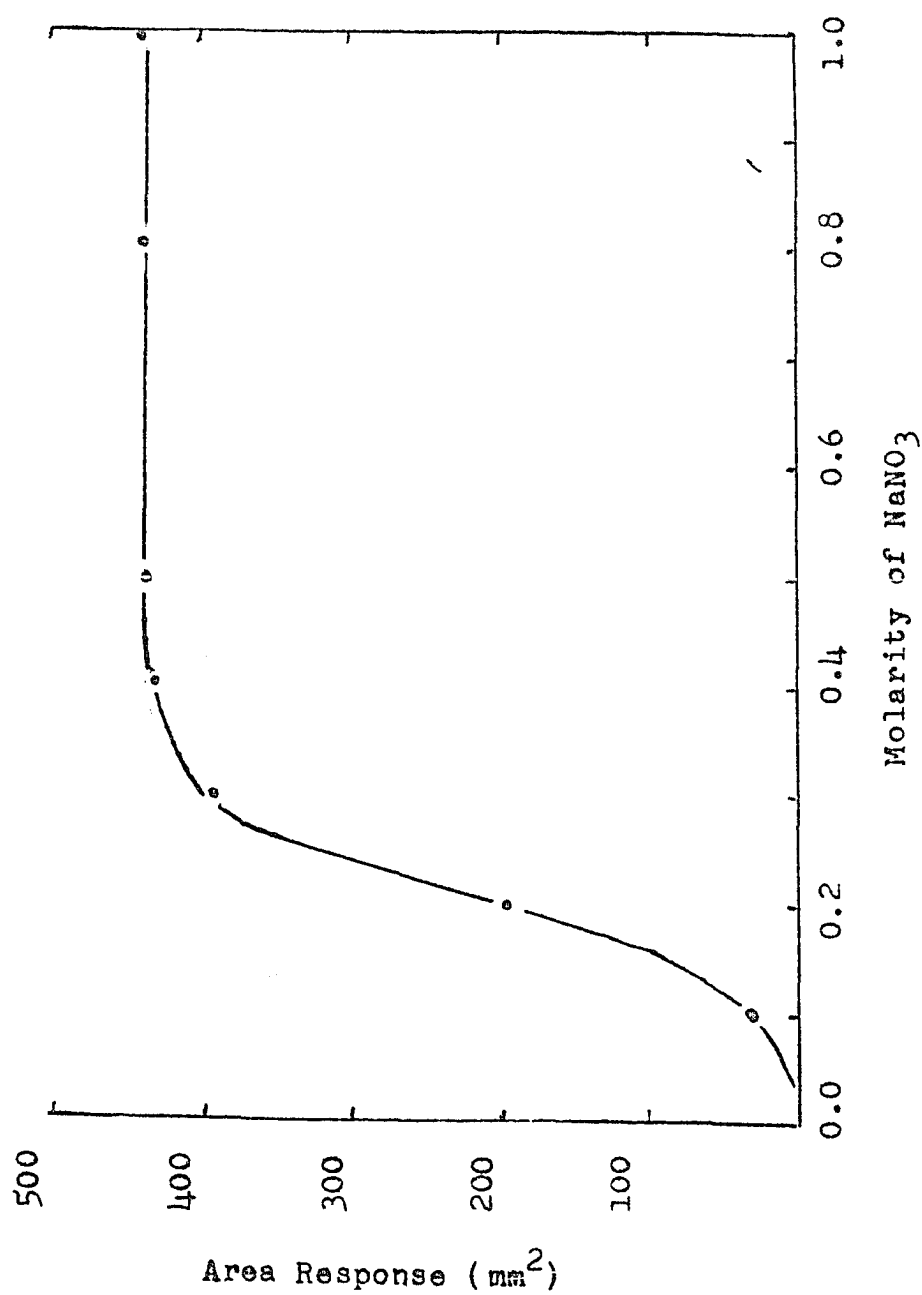


Figure 6. Effect of concentration of carrier solvent.

LINEARITY AND SENSITIVITY

The micro-adsorption detector was investigated to determine linearity and sensitivity of response for various metal ions. Using information from above, parameters were chosen to be convenient and to give nearly optimum response.

Experimental

The detector was packed with Amberlite CG-50, 200/340 mesh ion exchange resin, settling time of 5 to 7.5 minutes. Silanized glass beads were used as a reference material and as a packing for the Chromatronix glass column. Water bath temperature was ambient, 23°C. The carrier solvent was 1.00 M NaNO_3 . Flow rate was adjusted to 30 ml/hr. The nine metal ions used were Ca(II), Co(II), Cr(III), Cu(II), Mg(II), Mn(II), Ni(II), Sr(II) and Zn(II). Stock solutions each 1.00 F in a cation and in NaNO_3 were diluted to 0.010, 0.020, 0.050, and 0.10 F, with 1.0 M NaNO_3 . Injections of eight and four microliter aliquots were made in triplicate.

Discussion

The peak areas, heights, and rise times were measured. The last quantity approximates the elution

band half width. The rise time was 3.9 seconds and represented 32 microliters. Effluent concentrations were calculated by dividing the amount injected by 64 microliters. Sensitivities were estimated as a signal to noise ratio of two on the basis of peak height.

The response of the micro-adsorption detector to Ca(II), Co(II), Mg(II), Ni(II) and Sr(II) is shown in Figure 7. Minimum detectable concentrations and limits of linearity are given in Table VI. The plots are log-log to accomodate the wide range of response and concentration encountered. A response directly proportional to concentration should yield a log-log plot with unit slope. This was closely approximated for these five ions. These metal ions were well behaved, exhibiting no tailing. Response was more nearly linear for peak height than peak area due to the peaks becoming narrow at high concentrations.

Figure 8 depicts the response of the micro-adsorption detector to Cr(III), Cu(II) and Mn(II). Of these cations only Mn(II) was well behaved exhibiting linearity and sensitivity similar to Zn(II). The peaks were well defined and exhibited no tailing. As before linearity of response for peak height extended further than for peak area due to narrow peaks at high concentrations. In contrast, Cr(III) exhibited strong adsorption and tailing at low concentrations. Peak

Figure 7. Plot of response, peak area and peak height, versus formality of cation in the effluent:
 A. peak area of Zn(II); B. Peak height of Zn(II); C. Peak area of Ca(II), Co(II), Mg(II), Ni(II), and Sr(II); D. Peak height of Ca(II), Co(II), Mg(II), Ni(II), and Sr(II).

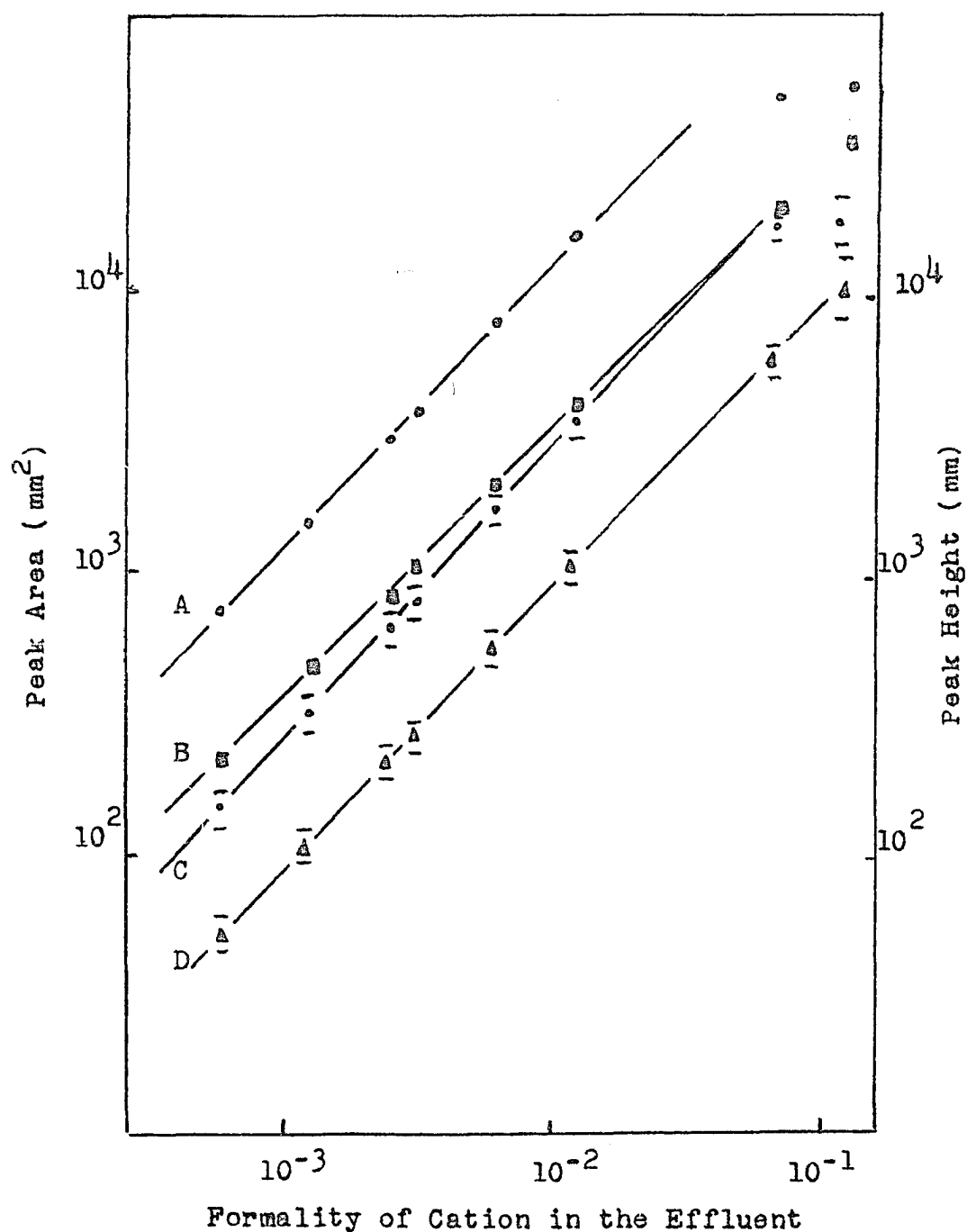
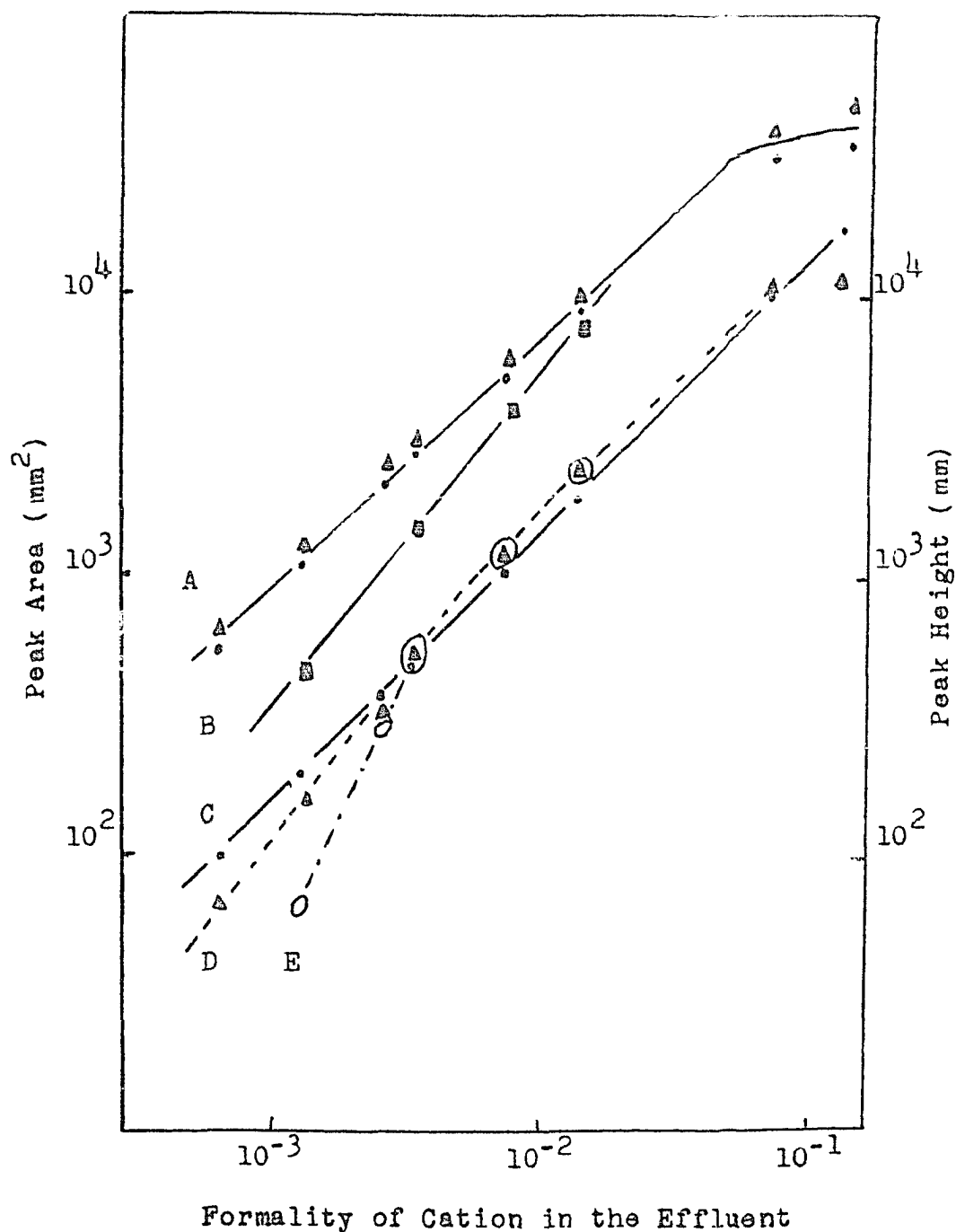


TABLE VI. LIMITS OF LINEARITY AND DETECTION

CATIONS	Lower Limit of Detection $\times 10^{-4} \underline{F}$	Upper Limit of Linearity $\times 10^{-2} \underline{F}$	
		Peak Area	Peak Height
Ca(II)	1.2	2.5	10
Co(II)	2.9	2.5	10
Cr(III)	4.0	2.5	-
Cu(II)	1.2	2.5	-
Mg(II)	1.8	2.5	10
Mn(II)	0.8	2.5	10
Ni(II)	1.4	2.5	10
Sr(II)	1.8	2.5	10
Zn(II)	0.7	2.5	10

Figure 8. Plot of response, peak area and peak height, versus formality of cation in the effluent: A. Peak area of Cu(II) and Mn(II); B. Peak area of Cr(III); C. Peak height of Mn(II); D. Peak height of Cu(II); E. Peak height of Cr(III).



widths of up to 11 μ m were observed. Based on peak height, the response for Cr(III) was not linear and the response based on peak area had a slope of 1.1. Based on peak height, the response for Cu(II) exhibited characteristics similar to Cr(III), but less pronounced. Tailing at lower concentrations was observed. The peak area response of Cu(II) was linear. The observed variance in peak area was greater than for all other cations observed.

CONCLUSIONS

Summary

The micro-adsorption detector was investigated to find if it was applicable to the monitoring of metal ions in the effluent of a liquid chromatographic separation. Two suitable packings were found, Dowex 50, x-8 and Amberlite CG-50 ion exchange resins. The Amberlite CG-50 resin was investigated to determine the parameters which affect response characteristics. Important parameters were found to be temperature, pH and salt concentration of the carrier solvent, anion of the carrier solvent, and repacking of the detector. Parameters were chosen that gave optimum performance and provided adequate solubilities for several metal ions.

An investigation of linearity and sensitivity of the micro-adsorption detector was made. Response was linear for a hundred-fold concentration range of Mn(II), Cr(III), and Cu(II) and a thousand-fold concentration range for Sr(II), Mg(II), Ca(II), Co(II), Ni(II) and Zn(II). Minimum detectable effluent concentrations of metal ions ranged from 1.4×10^{-4} F to 8×10^{-4} F.

The preferred method of quantification was the

measurement of peak areas. A precision of $\pm 3\%$ was established.

Recommendations

Further study of the Amberlite CG-50 resin should include the effects of temperature and pH with 1.00 M NaCl as a carrier solvent. Another anion which might be considered is perchlorate. The effect of anion could then be decided. In order to provide well buffered pH around five the use of sodium acetate solutions as a carrier solvent may be desirable.

Modification of the equipment must be made in order to investigate Dowex 50, x-8 ion exchange resin. Lining of the carrier solvent reservoir with Teflon and changing all tubing to Teflon would allow the use of more highly acidic solutions. Separations of metal ions on high efficiency columns have been performed using 1.0 M HNO_3 . To be applicable to monitoring the effluent of liquid chromatographic separations of metal ions the acid range of the equipment must be increased.

APPENDIX I. F RATIOS DETERMINED IN THE FACTORIAL STUDY

Source	F Ratio		
	Ni(II)	Ca(II)	Cs(I)
Temperature (T)	251	420	1326
Anion of Carrier Solvent (A)	200	256	1.2
Concentration of Carrier Solvent(S)	111	368	536
Carrier Solvent pH(P)	0.8	24	4.9
Injection Size (S)	0.5	20	1.9
T x A	146	240	34
T x C	107	47	6.3
T x P	0.6	9.2	20
T x S	5.6	9.2	0.6
A x C	56	67	452
A x P	7.4	1.2	11
A x S	0.4	0.2	0.9
C x P	2.6	1.4	1.3
C x S	0.2	0.1	0.1
P x S	2.6	4.4	2.2

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