1971

Inorganic separation and analysis by high speed liquid chromatography

John Phillip Sickafoose
Iowa State University

Recommended Citation

Sickafoose, John Phillip, "Inorganic separation and analysis by high speed liquid chromatography " (1971). *Retrospective Theses and Dissertations*. 4584.
https://lib.dr.iastate.edu/rtd/4584

72-12,595

SICKAFOOSE, John Phillip, 1943-
INORGANIC SEPARATION AND ANALYSIS BY HIGH
SPEED LIQUID CHROMATOGRAPHY.

Iowa State University, Ph.D., 1971
Chemistry, analytical

University Microfilms, A XEROX Company, Ann Arbor, Michigan

Inorganic separation and analysis by high speed liquid chromatography

by

John Phillip Sickafoose

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

Major Subject: Analytical Chemistry

Approved:

In Charge of Major Work

For the Major Department

For the Graduate College

Iowa State University
Ames, Iowa

1971

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION AND STATEMENT OF THE PROBLEM

Virtually all chemical and electrochemical methods for determining small amounts of a metal ion are at times subject to interferences that necessitate a chemical separation. Methods such as atomic spectrometry are very selective but their dependency on excitation conditions and matrix composition necessitates frequent calibration and standardization. A separation or concentration is often carried out in conjunction with flame methods.

Extensive literature on chemical separations of metal ions by column chromatography exists and is continually expanding. Although some methods are unwieldy and sample solution conditions difficult to attain, virtually any separation can be done by ion exchange (1). Where a specific separation has not been done, survey work in a large number of systems is available to predict appropriate conditions.

These methods for separating metal ions from one another are quantitative and selective. However, most of the published methods are slow, often requiring 30 minutes or more to elute each element from the column. Recently a systematic scheme for 27 elements was published (2). By using samples of 25 to 50 mg and relatively small columns, the average elution time was reduced to 15-20 minutes per element. Subsequent analysis for the ion was then necessary by an appropriate chemical or instrumental method.

Pressurized chromatographs for rapid separations of organic components are available and rapidly becoming important. Their components are not resistant to extremely corrosive systems nor do their pumping systems provide instantaneous change of eluent. Each of these criteria is important to adapt an instrument to the majority of published metal ion separation methods.

Monitoring column eluate by continuously following a physical or chemical property of the analytical species or flowing stream is not new (1). Commercial instruments for organic analysis effectively use a variety of detectors. Each has limitations for monitoring organic separations and for metal ions the limitations become severe as to render most useless in all but a few specific cases.

The problem then is to design a simple, inexpensive chromatograph which can be used with published separation methods, corrosion resistant and adaptable to "go, no-go" chromatography. Monitoring the column eluate should use instrumentation generally available and easily adapted or simple and inexpensive to construct.

THE LIQUID CHROMATOGRAPH

Literature Survey and Background

Liquid chromatography has until recently been used as a last resort for separation and analysis of organic materials due to a misconception that it was inherently slow compared to gas chromatography. The latter has had wide acceptance and is well suited to rapid separations of compounds in the molecular weight range up to 200 with limited capability to perhaps 800. Of approximately 10^6 well characterized organic compounds however, only 15% or so are volatile (3). Analysis of the remaining 85% with molecular weights of 200 to 10^7 has been the impetus for high speed, high resolution liquid chromatography and the array of instruments now offered (4). The concept of slowness has been refuted by theoretical considerations (5) and previously impossible separations are now routine (6).

To date, inorganic separations by comparable instrumentation have been largely ignored. Lack of a suitable detector, the corrosive liquids necessary and the concurrent development of atomic absorption has contributed to this.

All liquid column chromatographic separations include 1.) a moving liquid 2.) a means of producing solvent flow 3.) a device or method for sample introduction 4.) an analytical column for the appropriate mode of separation and 5.) a method of detection for components in the eluate.

As shown below, to optimize conditions for a chromatographic separation, the column packing must be greatly reduced in particle size. Pressure becomes necessary to force liquid through the column so a forced-flow system must replace classical gravity flow. Forced eluent flow may be accomplished with gas pressure, peristaltic pumps, syringe type pumps or piston pumps (7). Wide variations in mechanical design are available for each type. The appropriate pump depends on its use, i.e. pressure necessary for resolution, solvents to be used and consequently the materials of construction, importance of pulseless flow, reservoir capacity, mechanical reliability and cost and simplicity of operation.

Devices for sample introduction rely on stopped-flow injection at the top of the column, septum and syringe injection into the flowing stream and tubing loops taken in and out of the flowing stream by valves. The latter two are universally used with variations of design.

A universal detector for continuous eluate analysis has been the weakest link in liquid chromatography. Detectors based on chemical, physical and nuclear properties are available (4). The ideal detector should be as general and sensitive as possible, have extremely low dead volume for negligible bandspreading and respond rapidly over a wide range of temperatures and pressures. A comparison of available detectors has been made (8) and suggestions for use of other principles (9). The most reliable, stable and sensitive detector in current use is the photometer with micro flow cell and amplification. It is not as general as the refractometer for organics but more sensitive by as much as 10^3 (10).

Choice of the analytical separation mode permits a broad range of capabilities. Based on chemical or physical mechanisms, the four modes presently available on most instruments are exclusion chromatography (gel permeation or gel filtration), ion exchange, liquid-liquid partition, and liquid-solid adsorption chromatography. Each mode is broad enough that books and reviews are available. Choice of a single moving solvent, multiple solvents or a gradient permits selectivity within a mode by changing distribution coefficients and therefore the time and/or components retained.

Time required for a liquid chromatogram is a direct function of the resolution required and the separation factors available by changing mode or solvents. If these are favorable, a factor of 2 decrease in resolution required permits a time decrease by a factor of 8 (5). Optimizing the physical parameters of column length and diameter, solvent flow rate, particle size and sample size may increase resolution and speed up a separation significantly when a mode and solvent system have been chosen.

The considerations below are simply definitive rather than theoretically inclusive. A summary is available (11) or more extensive theoretical treatment (12). All aspects of high speed liquid chromatography are treated in a new volume edited by Kirkland (7).

Separation occurs in a chromatographic system by either a chemical or physical mechanism when components migrate at different velocities. Although other more general indices have been suggested (13), resolution is a common index of the extent of separation in liquid chromatography,

$$\text{Resolution, } R_s = \frac{\Delta X}{W}$$

where ΔX is the distance between band centers and W is the base width of the band of interest.

Distance between band centers, ΔX, is a function of the thermodynamic and/or physical distinguishability between two components. For each mode of liquid chromatography, an equivalent expression can be derived to calculate elution volume to a band center.

$$V_A = V_M + KV_s$$

where V_A = volume to the band center of component A
V_M = mobile phase or interstitial volume
V_s = stationary phase volume or capacity
and K = distribution coefficient

$$= \frac{\text{concentration of component in stationary phase}}{\text{concentration of component in mobile phase}}$$

so, for two bands,

$$\Delta X = V_A - V_B = \Delta K(V_s)$$

The distance between zone centers or selectivity for components then depends on the choice of separation mode, stationary phase and mobile phase as they determine the distribution coefficient and on the amount of stationary phase interacting with the components.

Band width, W, (zone or band spreading) is a complex function of a number of parameters:

$$1/4W = \sigma = (HL)^{1/2} \text{ or } H = \sigma^2/L$$

where σ is a standard deviation width based on Gaussian distribution, L is the distance traveled and H is an efficiency parameter called the height equivalent of a theoretical plate or plate height.

In general, plate height is a function of eddy diffusion, longitudinal diffusion and non-equilibrium as predicted for gas chromatography. Significant additional terms are necessary to account for coupling of these terms and turbulence (5) in liquid chromatography. Each term of the plate height equation is a function of one or more of the variables: particle size diameter, flow velocity, thickness of the stationary phase, diffusion coefficients in the mobile and stationary phases, shape of the column and its diameter, shape and configuration of the packing. Most important is particle size since $H \propto d^2$, where d is the particle size diameter, while other variables are first order. Optimum time can also be calculated (7) from these known parameters.

Table 1 from reference (11) relates the important parameters to resolution and speed. The arrow direction up or down indicates that the parameter must be increased or decreased to optimize resolution and/or speed.

Table 1. Increasing resolution and speed

Variable	Increased resolution	Increased speed
Column length	↑	↑
Flow rate	↓	↑
Particle size	↓	↓
Column diameter	↓	↓ (↑)
Sample size	↓	↓ (↑)

Pressure does not appear explicitly in these considerations but is implied by the relationship

$$\Delta P \propto Lv/d^2$$

where ΔP is the pressure drop, v the flow velocity and L and d as defined above. So

$$\Delta P \propto Lv/H$$

with a minimum plate height sought. High resolution and high speed liquid chromatography therefore demands increasing the pressure.

Instrumental Design

A schematic diagram of the liquid chromatograph is shown in Figure 1.

The eluent tanks are heavy walled, one gallon polyethylene screw cap bottles encased by 6" iron pipe welded to a 1/4" iron base plate. A circular metal plate with a hole for the neck holds the bottle firmly in place. All eluent tanks were hydrostatically tested to 200 psi. With a safety factor of three, this allows continuous use to 70 psi. The eluent tanks are pressurized with helium introduced by 1/4" Dacron pressure tubing through a Teflon plate cap. The necessary fitting was machined from one-half inch diameter Kel-F rod. Eluent flows out through the cap in Teflon tubing attached with Chromatronix fittings.

The gas pressure manifold employs Hoke number H4J71M2B helium-tested, packless bellows valves. Pressure to the pneumatic actuators is controlled by number 1511M2B snap valves, also from Hoke Incorporated.

"Cheminert" CAV-2031-K valves of Kel-F are used for eluent selection. An SV-8031-K sample injection valve was fitted with two PA-875-K pneumatic actuators, also of Kel-F. Teflon, 0.031" i.d. tubing for liquid flow is connected by polypropylene fittings. All of these parts are available from Chromatronix Incorporated.

A Chromatronix FT-1 flanging tool was used to attach

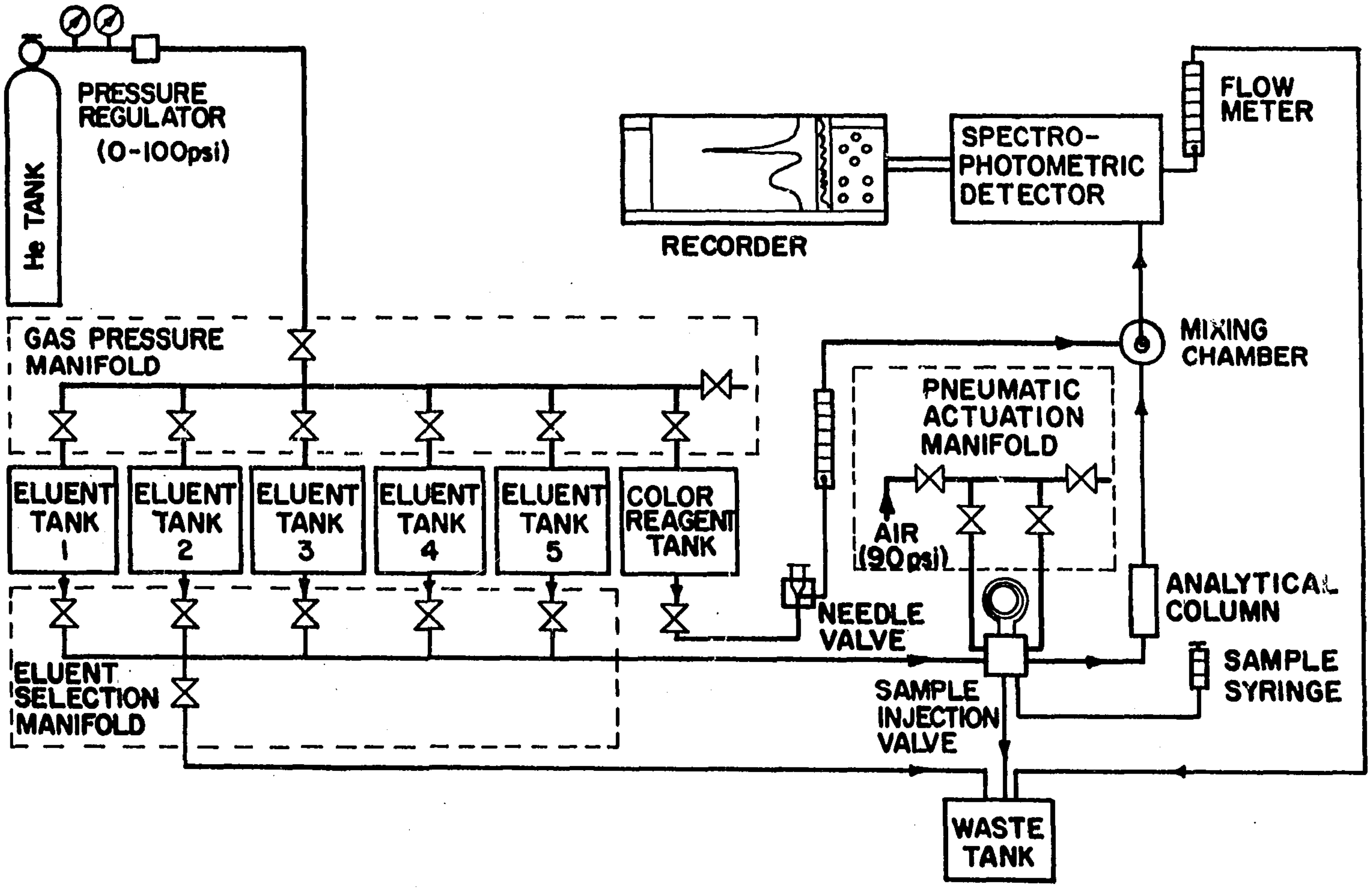


Figure 1. Schematic diagram of the liquid chromatograph

tube end fittings to appropriate lengths of Teflon tubing.

For metering the color forming reagent flow, a Fischer & Porter Company number 795-500, 1-1/4 mm glass needle valve with a Teflon stem was used. Chromatronix G1-C connectors were used to adapt the valve to Teflon tubing and fittings. To allow more precise control, a length of 0.012" i.d. Teflon tubing from Chromatronix was inserted between the needle valve and flow meter. This created a pressure drop approximating the analytical column pressure drop.

Sample loops of various smaller sizes (40 to 200 µl) were made and calibrated using a standard curve plot of absorbance vs. microliter pipet volumes of an indicator diluted to 50 ml. Larger volumes (0.5 to 10 ml) were calibrated by collecting the effluent from a loop filled with primary standard copper solution and titrating it with standard EDTA solution. Prepared loops are available from Chromatronix.

For determination of iron, a Chromatronix model LC-6M-13 analytical column, 6.4 mm i.d., was mounted with two outlet plungers for decreasing bed height. For subsequent determination of chromium and zinc and cadmium, the LC-6M-13 analytical column was cut to 15 cm, flanged to accommodate the appropriate fittings and mounted with two inlet plungers to decrease further the tubing dead volume.

A Coleman Model 101 Hitachi UV -visible spectrophotometer was fitted with a flow-through cell; a 2 mm i.d. by

3 cm long quartz tube mounted adjacent to the photocell for the determination of iron. The dead volume of the cell was approximately 100 µl, and the light path 2 mm. For further work, a high pressure "Z" flow-through cell was machined from Kel-F and Teflon after the studies of Conlon (10). Figure 2 shows the cell which has a 1 cm light path and 32 µl dead volume.

For a limited number of studies, a Chromatronix Model 200 UV detector was used. This has a 253.7 nm mercury line source and a 1 cm light path, 8 µl dead volume flow-through cell. It is a double-beam differential photometer with a photomultiplier which can be attenuated from 0.01 to 5.12 absorbance units full scale with a linear dynamic range from 0.0001 to 3.0 absorbance units.

Figure 3 shows the mixing chamber in detail as discussed later for in-stream reagent addition. The outlet is on top and inlets on the sides as seen in the cross section. The outlet hole is fitted with a Chromatronix CAV-K port face bushing of Kel-F and a Teflon port face to seal the chamber. Other Chromatronix tube end fittings adapt to the device. It was used only for detection of zinc and cadmium. For determination of iron and chromium, analytical column effluent passed directly to the spectrophotometer flow-through cell.

Roger Gilmont Instruments, Inc., catalog number 3201,

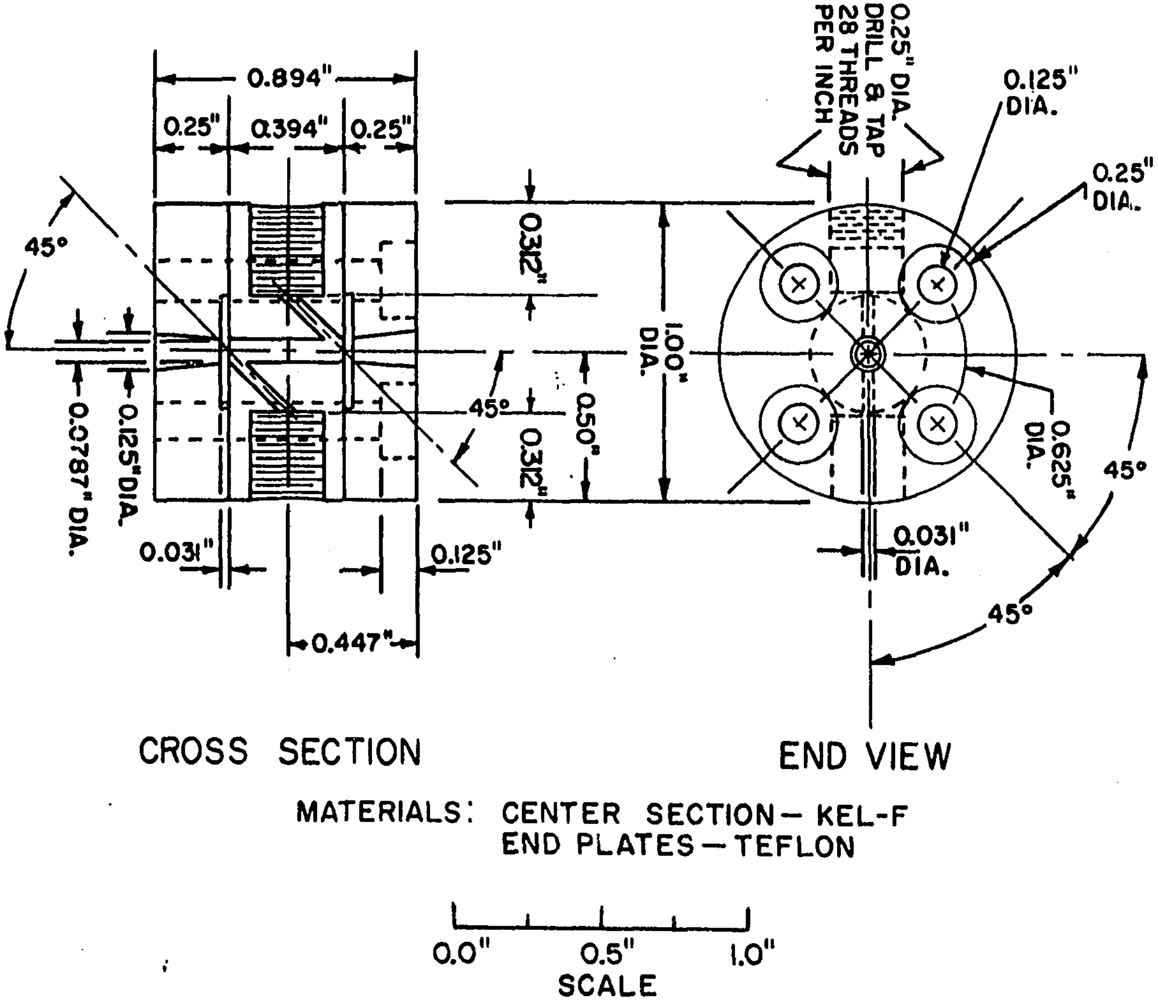


Figure 2. High pressure "Z" flow-through cell

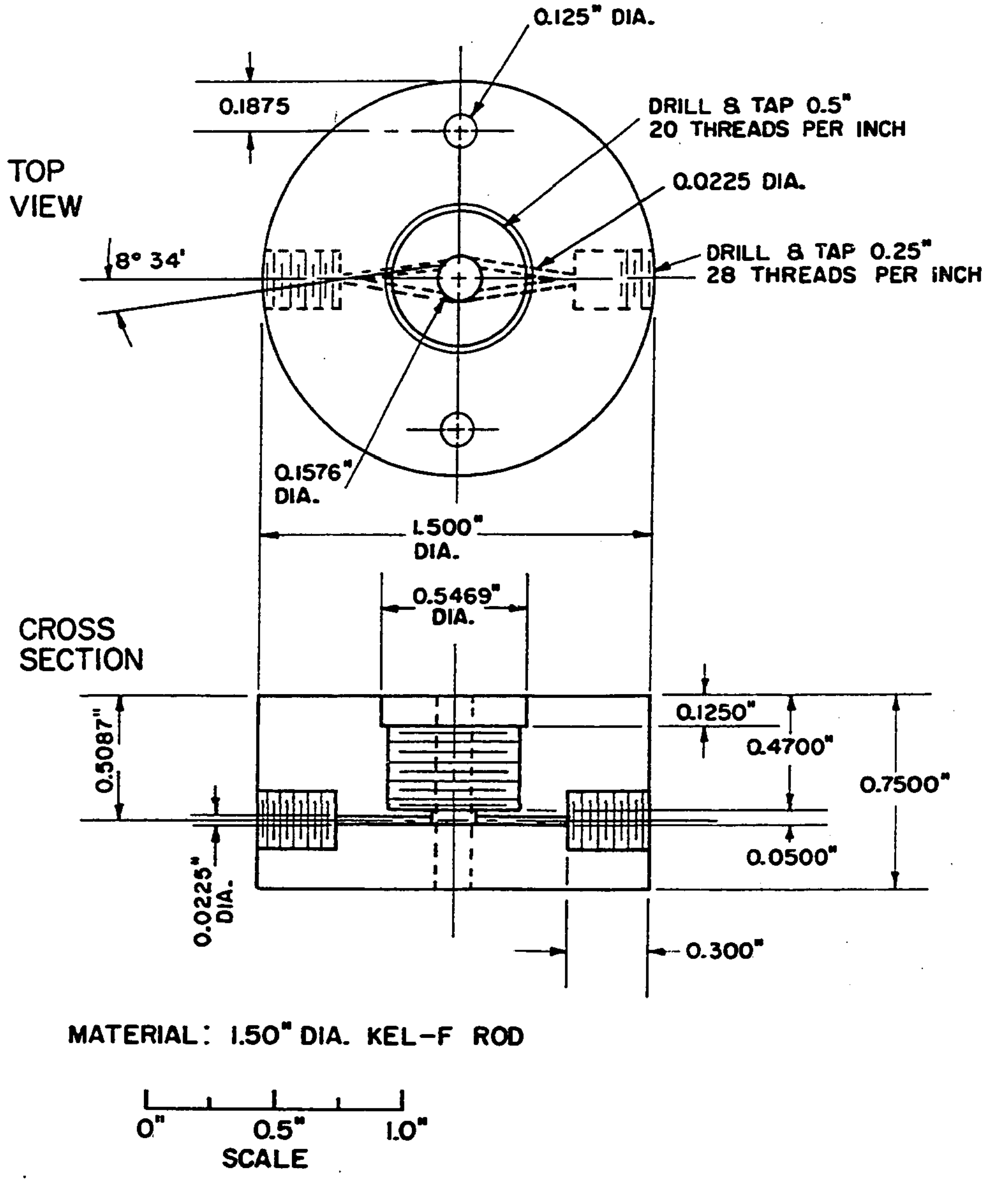


Figure 3. Whirlpool, divided tangential entry mixing chamber

size number 1 flowmeters are used. Adaptors were machined of Kel-F following the Chromatronix design for the G1-C glass connector to allow attachment of Chromatronix fittings.

The E. H. Sargent and Company recorder model SRLG has linear or logarithmic (absorbance) output and is equipped with a Disc Instruments, Inc. model 204DM integrator.

Discussion of Design

The construction materials for the chromatograph were chosen for resistance to corrosion. Only Kel-F, polyethylene Teflon and glass contact the liquid so the variety of acid systems which can be used is extensive.

This flow system has important advantages. The number of eluents in any separation is limited only by the number of valves and tanks available and cost per additional eluent is minimal. The volume of each tank is adequate for many separations.

Gas pressure, which is pulseless, is the conveniently controlled parameter for this chromatograph. The flow rate of a given eluent through the column is a linear function of pressure applied to the eluent tanks (see Figure 4). With less viscous eluents a faster flow rate is obtained at any given pressure.

Gas desolubilizing from the eluent on the column is not a difficulty at the pressures used. Initially, air pressure was used but after a month of operation, gas evolution on

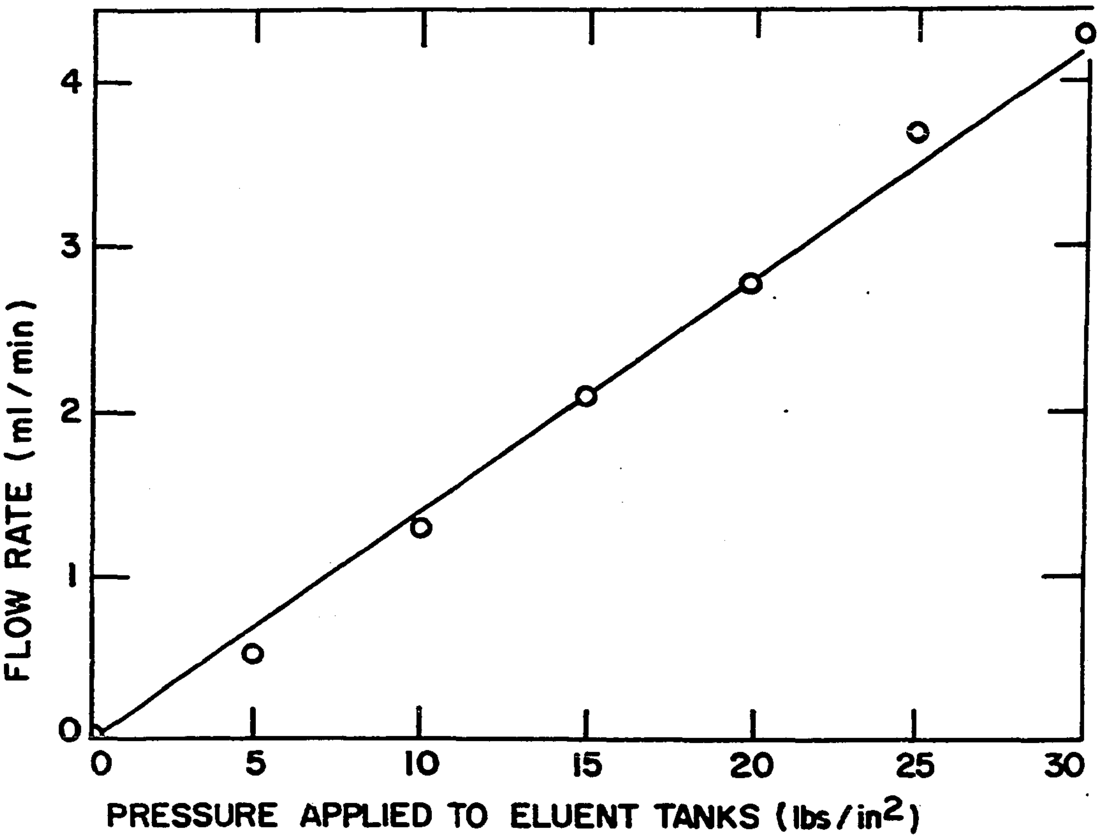


Figure 4. Flow rate of 6 M HCl vs. pressure applied to eluent tanks

the column caused detector noise. Helium was then chosen for its low solubility and availability. No further problem was encountered for the duration of studies.

A small dead volume is necessary for stepwise elution, particularly if mixing may occur. Commercial piston type pumps produce a gradient over approximately 1 to 8 ml. Syringe type pumps require a refill cycle after total expenditure of the first eluent. The commercial gas pressurization design requires disassembly. For the system described, the only dead volume is the tubing between the eluent selection valve manifold and the column. This includes the sample injection valve. With careful design this can be decreased to less than 200 µl. Gradient mixing is minimal in this length of tubing.

Since construction of this chromatograph, Chromatronix offers multi-port rotary valves which may be substituted for the entire eluent selection valve manifold at a lower cost and decrease of dead volume.

A disadvantage of the gas pressure system is the change in flow rate on changing eluents. This requires a flowmeter whereas with a pump the gear ratio of the piston drive unit determines the flow rate. Calibrating the flowmeter may be done by a plot of scale reading vs. ml/min determined with a stopwatch and buret. The scale reading of the flowmeter follows Stoke's Law for a body falling in a fluid (14). An alternate and more rapid method (15) includes calculation of empirical constants for an eluent, then using these on a programmable calculator to get flow rates in a few seconds.

The in-stream loop injection feature is convenient and useful for sample introduction. Because sorption on the resin is a concentrating step, sensitivity is a function of sample loop capacity. Sample volumes from 40 µl to 10 ml or larger can readily be used. The time required for sorption and matrix separation is proportional to sample size.

Use of a detector with monochrometer provides more flexibility than the commercial fixed-wavelength photometers. Selectivity for a given ion can be improved by the detection step as well as the separation. Sensitivity also increases as the wavelength of maximum absorbance is approached. Disadvantages of using a simple spectrophotometer are the absence of photomultiplier or electronic amplification of the signal and single beam detection.

The size (dead volume) and design of flow through cells has been discussed by Conlon (10). As the cross sectional area of the flow cell is reduced to eliminate bandspreading however, the light intensity passing through may become insufficient to produce full scale deflection on the detector.

DETECTION BY IN-STREAM ADDITION OF COLORIMETRIC REAGENTS

Introduction and Statement of the Problem

The use of in-stream reagent addition to produce colored species for subsequent photometric detection has been used for some time in automatic analyzers (16,17). These instruments incorporate the mixing and reaction step with a time delay for reaction. With such a time delay, mixing need not be extremely efficient. However, a large internal volume permits dilution and diffusion unacceptable for the micro amounts of ions in inorganic forced-flow liquid chromatography. Bandspreading and consequent loss of sensitivity and resolution would severely limit its use.

Experimental rapid reaction kinetics studies provided an impetus to solve the problem of efficient mixing in very short times and small volumes. An efficient device for mixing flowing streams has been sought since Hartridge and Roughton (18) examined various designs. Chance (19) gives details of a design. Several other approaches used are reviewed by Kletenik (20) to 1963 and compared with his own design. A colloquium in 1964 introduced other variations (21). Kletenik's device and a multicapillary tube (22) each achieved complete mixing in the microsecond time range. A countercurrent design has been described and patented for mixing a number of solutions simultaneously (23). All these devices used extremely high velocity flows compared to those used in liquid chromatography.

Toor and others have studied the mixing of solutions with chemical reactions. Both turbulent and non-turbulent (24,25) and first and second order reactions (26,27) have been discussed and mathematically described.

Here the problem differs from that of experimental kinetics and common automatic analyzers. Maximum flow rates of column effluent are in the range of 2 to 10 ml/min through 0.031" i.d. tubing (i.e. 6.8 to 34.2 cm sec^{-1}). Time from mixing to detection is easily varied by changing the length of tubing between the mixing device and flow cell but seldom would be less than 50 msec. In addition, the color species would be maintained in excess and could be chosen for its favorable kinetic rate and order. Mixing efficiency could therefore be less than complete if all of the analytical species had reacted or at worst if a reproducible majority of it had reacted. The most severe limitation was that of dead volume and therefore bandspreading between mixing and detection.

The 16 µl mixing chamber described is based on the tangential entry of divided streams of two solutions following from the discussion of Kletenik (20) and simplification and decreased volume of the designs suggested by Berger (21) and Chance (19). The parameters for its analytical use were experimentally determined with the rapid reaction of thorium

(IV) and Arsenazo III in pH 1 hydrochloric acid.

Experimental

A Durrum-Gibson Stopped Flow Spectrophotometer was used for studying time of the thorium(IV), Arsenazo III reaction.

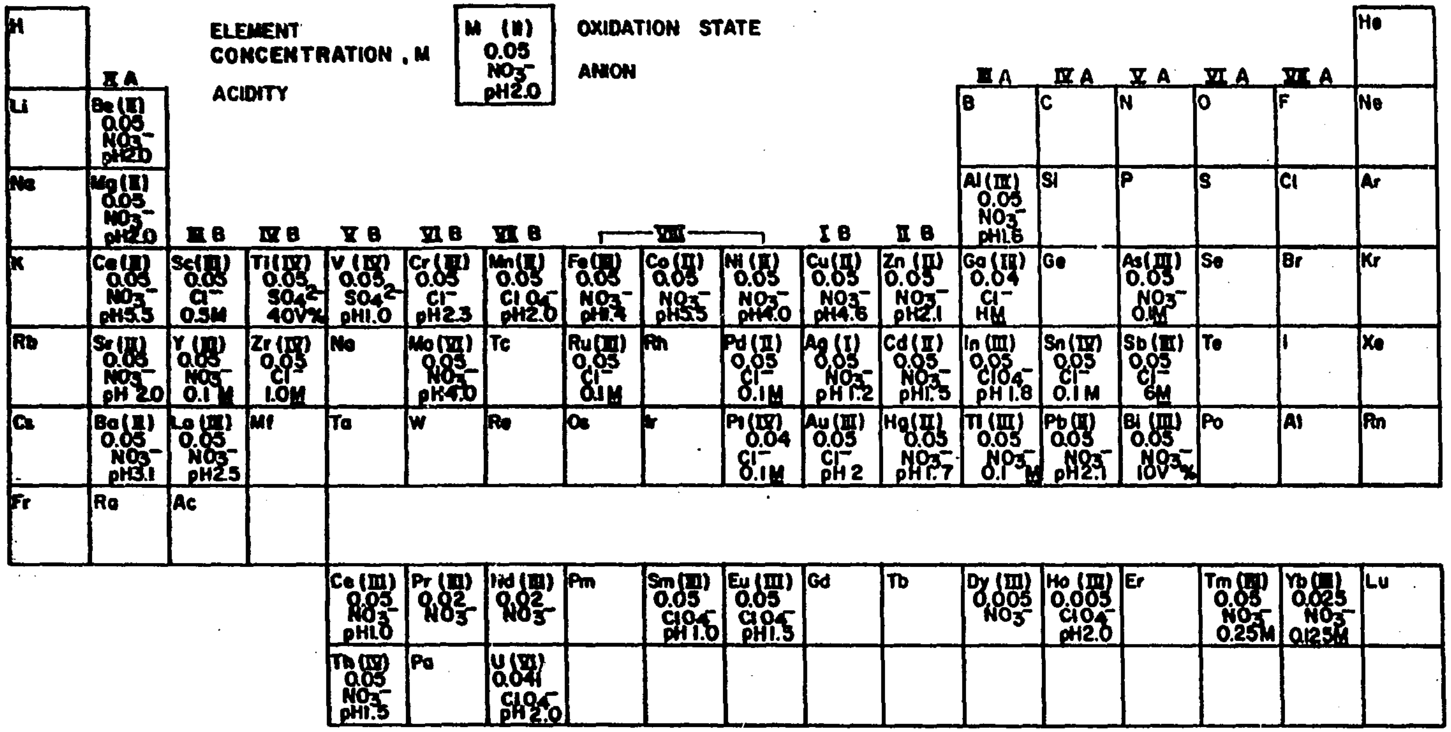
Schematic diagrams for the experimental set-ups used are given with appropriate parts of the results and discussion section.

A stock solution of 0.05 M thorium(IV) was prepared by dissolving Baker Analyzed Reagent thorium nitrate in acid and adjusting the pH to 1.0 with hydrochloric acid. Other solutions were prepared by diluting the stock.

Other metal ion solutions were prepared from analytical reagent salts with final conditions as shown in Figure 5.

Acetate buffer solution was prepared by dissolving Baker Analyzed Reagent sodium acetate and DuPont glacial acetic acid in water to give a final solution which was 0.5 M sodium acetate, 1.0 M acetic acid.

A stock solution of 1.24×10^{-3} M Arsenazo III was prepared from Aldrich Chemical Company number A 9277 indicator. A 2.0 g sample was weighed into a beaker (cleaned with an alkaline EDTA wash), 0.5 g sodium hydroxide and 50 ml of water added. This was mixed and indicator lumps crushed. One liter of pH 1 hydrochloric acid was mixed with this and the solution readjusted to pH 1 with hydrochloric acid. After stirring



ELEMENT — M (N) — OXIDATION STATE
CONCENTRATION, M — 0.05
ANION — NO_3^-
ACIDITY — pH2.0

	II A	III B	IV B	V B	VI B	VII B	VIII			I B	II B	III A	IV A	V A	VI A	VII A	
H																	He
Li	Be (II) 0.05 NO_3^- pH2.0											B	C	N	O	F	Ne
Na	Mg (II) 0.05 NO_3^- pH2.0											Al (III) 0.05 NO_3^- pH1.6	Si	P	S	Cl	Ar
K	Ca (II) 0.05 NO_3^- pH5.5	Sc (III) 0.05 Cl^- 0.5M	Ti (IV) 0.05 SO_4^{2-} 40V%	V (IV) 0.05 SO_4^{2-} pH1.0	Cr (III) 0.05 Cl^- pH2.3	Mn (II) 0.05 ClO_4^- pH2.0	Fe (III) 0.05 NO_3^- pH1.4	Co (II) 0.05 NO_3^- pH5.5	Ni (II) 0.05 NO_3^- pH4.0	Cu (II) 0.05 NO_3^- pH4.6	Zn (II) 0.05 NO_3^- pH2.1	Ga (III) 0.04 Cl^- HM	Ge	As (III) 0.05 NO_3^- 0.1M	Se	Br	Kr
Rb	Sr (II) 0.05 NO_3^- pH 2.0	Y (III) 0.05 NO_3^- 0.1 M	Zr (IV) 0.05 Cl^- 1.0M	Nb	Mo (VI) 0.05 NO_3^- pH4.0	Tc	Ru (III) 0.05 Cl^- 0.1M	Rh	Pd (II) 0.05 Cl^- 0.1M	Ag (I) 0.05 NO_3^- pH 1.2	Cd (II) 0.05 NO_3^- pH1.5	In (III) 0.05 ClO_4^- pH 1.8	Sn (IV) 0.05 Cl^- 0.1 M	Sb (III) 0.05 Cl^- 6M	Te	I	Xe
Cs	Ba (II) 0.05 NO_3^- pH3.1	La (III) 0.05 NO_3^- pH2.5	Hf	Ta	W	Re	Os	Ir	Pt (IV) 0.04 Cl^- 0.1M	Au (III) 0.05 Cl^- pH 2	Hg (II) 0.05 NO_3^- pH1.7	Tl (III) 0.05 NO_3^- 0.1 M	Pb (II) 0.05 NO_3^- pH2.1	Bi (III) 0.05 NO_3^- 10V%	Po	At	Rn
Fr	Ra	Ac															
				Ce (III) 0.05 NO_3^- pH1.0	Pr (III) 0.02 NO_3^-	Nd (III) 0.02 NO_3^-	Pm	Sm (III) 0.05 ClO_4^- pH 1.0	Eu (III) 0.05 ClO_4^- pH1.5	Gd	Tb	Dy (III) 0.005 NO_3^-	Ho (III) 0.005 ClO_4^- pH2.0	Er	Tm (III) 0.05 NO_3^- 0.25M	Yb (III) 0.025 NO_3^- 0.125M	Lu
				Th (IV) 0.05 NO_3^- pH1.5	Pa	U (VI) 0.041 ClO_4^- pH 2.0											

Figure 5. Stock metal ion solutions

continuously overnight, the solution was poured into a graduated cylinder and solids allowed to settle. The clear supernatant was siphoned off using a high intensity light to observe. The remaining solution was filtered through a tared filter paper, the residue weighed and filtrate added to the supernatant. The solution was diluted to 2 ℓ with pH 1 hydrochloric acid and approximate concentration calculated. Other concentrations were prepared by diluting this stock with pH 1 hydrochloric acid.

Indicator test solutions were prepared by dissolving approximately 25 mg of solid indicator in 25 ml water with a few drops of ammonium hydroxide, then diluting 1 to 5 ml of this solution to 200 ml with acetate buffer. The resulting solution was lightly colored so the complexed color change would be easily seen when compared to a blank. Indicators used and their sources were as follows. Alizarin red S, Baker Analyzed alizarin sodium sulfanate was used as received. Chlorophosphonazo III was received from John J. Richard as prepared for previous work (28). Chrome azurol S from Hach Chemical Company was used as received. Quinalizarin was C.P. grade from an unknown source. PAR, 4-(2-pyridylazo)resorcinol was number 7714 from Eastman Organic Chemicals and used as received. TAR, 4-(2-thiazolylazo)resorcinol was number 12,734-5 from Aldrich Chemical Company and used as received. The tetrasodium salt of xylenol orange was number 9964 from

Eastman Organic Chemicals and used as received.

Color tests were carried out in test tubes. A 1 ml automatic pipettor was used to add indicator-buffer solution to a test tube for each metal ion and one blank. Metal ion solutions were added dropwise with disposable droppers. Only positive tests were recorded.

Mixing devices were all machined from Kel-F to adapt to Chromatronix fittings. The series of tees had 0.031" i.d. holes with these exceptions. The color reagent entered through a 0.0135" i.d. side arm with the impinging jet tee. Off center tees had the side arm displaced by half the diameter and for spiral flow this side arm had grooving added by one turn of an 0.035" drill. The whirlpool chamber designs all followed closely that of Figure 3 with these modifications. The whirlpool tangential entry chamber had 0.031" i.d. holes leading to a chamber 0.050" deep by 0.0785" i.d. The whirlpool, divided tangential entry, conical taper exit chamber had 0.0225" i.d. holes leading to a chamber 0.050" deep by 0.0785" i.d. Exit was through a Teflon port face in which a conical exit 0.0785" diameter at the base and 0.050" high had been cut. Schematics of these devices are in Figure 6 with the discussion.

Results and Discussion

For kinetic studies the time and mixing efficiency must be optimized. In liquid chromatography the dead volume and retention time must be minimized to prevent loss of resolution

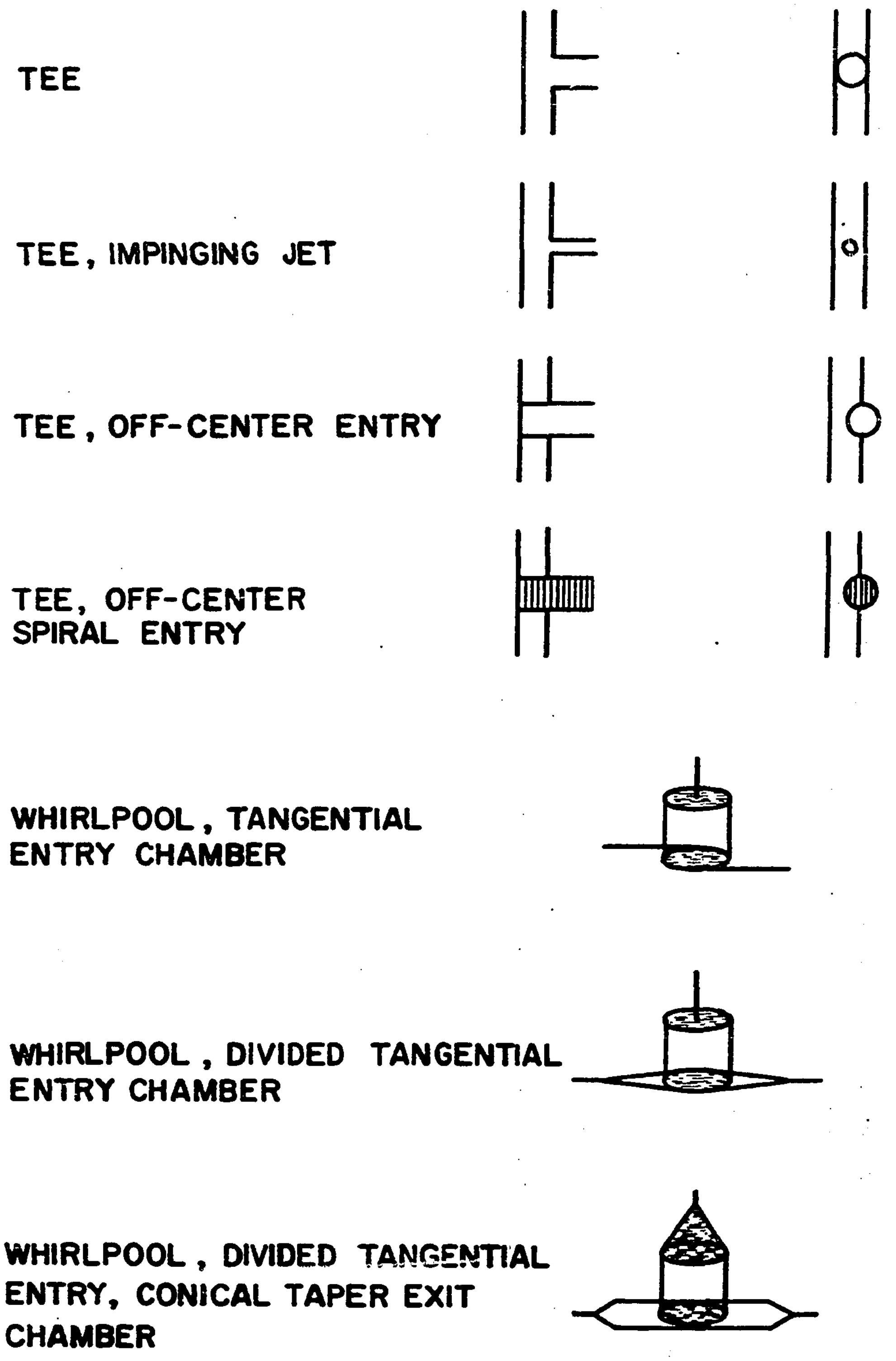


Figure 6. Schematic diagrams of mixing devices tested

between the separation and detection. Conlon (10) has discussed the important parameters for geometric design of a flow-through cell for spectrophotometric detection. Based on his own experimentation and other theoretical studies, he found this relationship to hold:

(geometric volume of cell) x (cell factor) = 1/2 peak volume,

where

$$\text{cell factor} = \frac{\text{ml to wash cell clear}}{\text{geometric volume of cell}}$$

From this, the milliliters to wash the cell clear must be equal to one-half the peak volume for less than 1% distortion of the peak. If the cell also is the entrance of a second stream, diluting the effluent 1:1 for example, then the peak height is decreased by two while at best the base remains a constant (i.e. a cell factor of 1).

The reaction of Arsenazo III with thorium(IV) was chosen to study mixing. End use of the system was to be with metallochromic indicator changes so this type reaction was sought rather than any of the diverse ones (20,22,27) used previously. The complex forms in pH 1 hydrochloric acid, minimizing any difficulty with greater viscosity of basic solutions. High molar absorptivity of greater than 10^5 (29) permits use at low concentrations of metal and indicator. The mechanism has been discussed and its complex shown to be a 1:1 metal indicator species (30).

The kinetics have not been described however, so an experiment was performed to show that the reaction was rapid enough under conditions used in these studies. Using the stopped-flow spectrophotometer, pH 1 solutions of Arsenazo III and thorium (IV) were injected into the cell at reagent to metal concentration ratios of 50:1, 5:1 and 1:1. Reaction was complete in 5 msec, 20 msec and 40 msec respectively. Also seen was an initial absorbance maximum which decayed slightly in the first 500 msec to a constant after 2.5 sec.

Initial trials were with a tee, the simplest possible device for joining two streams. The tee had the same i.d. as the tubing so a 3 µl volume increase was minimal. Tee variations as shown in Figure 6 improved the detection as tested experimentally with the schematic shown in Figure 7. Packing a short length of tubing with glass beads after the tee improved the peak height response by 10% but also increased the base width by 50%.

The improvement in mixing came from increased turbulence (as expected) so a 50 µl whirlpool chamber design with divided tangential entry was tried. Mixing and peak height were greatly improved but base width remained broad. The chamber height was decreased to give a total volume of 16 µl. Response improved while base width decreased. A simpler, single tube entry chamber was machined with a decrease in dead volume to 4 µl. See Figure 6.

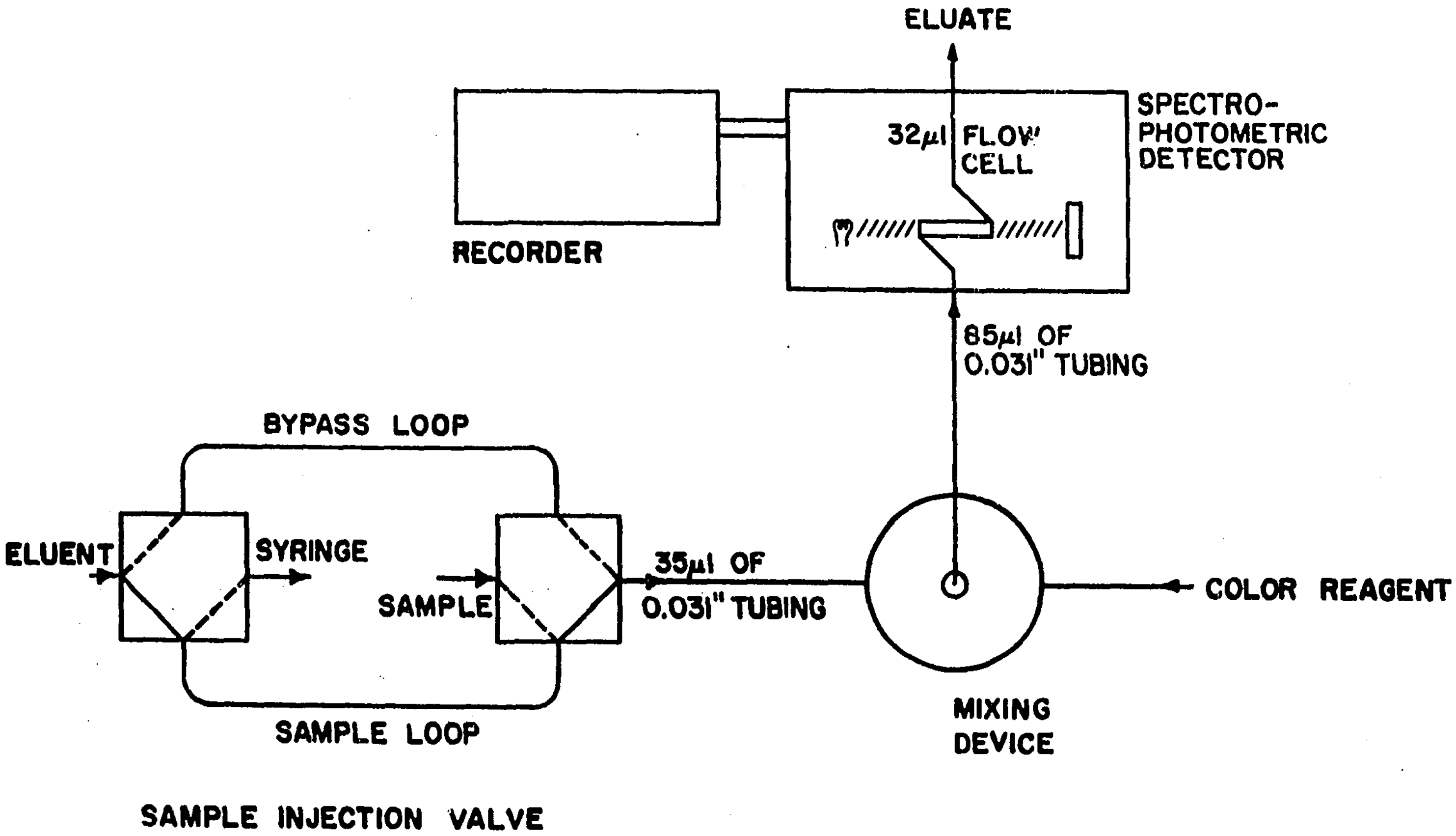


Figure 7. Schematic diagram for evaluation of mixing devices

A comparison of these devices became necessary. The same experimental schematic, Figure 7, was used. Thorium(IV) and Arsenazo III solutions were set to flow at equal rates with an excess of the color reagent. Constant absorbance was read above a baseline from pH 1 hydrochloric acid and Arsenazo III flowing. The two chambers were compared with a simple tee and a coupling standard. For the coupling standard, the reactants were premixed then injected into pH 1 acid flowing at a rate equal to the total of column effluent plus color reagent used in the device tests. The coupling joined the sample injection valve and detector cell directly.

The divided tangential entry chamber proved superior and efficient enough for use. The curves of Figure 8 show that with an excess of 5 times the stoichiometric amount of Arsenazo III, color formation is complete at 1 ml/min or greater total flow rate. The slight negative slope for the premixed solutions indicates decomposition of the complex over the time of the experiment.

A last design was machined to try the divided entry concept, combined with a conical taper exit suggested by Conlon's (10) work. Mixing was less satisfactory than with any of the other chambers.

An experiment was designed to compare the cell factors of these devices with a 3-way valve standard (see Figure 9 for the schematic). Here the device was filled with an indicator

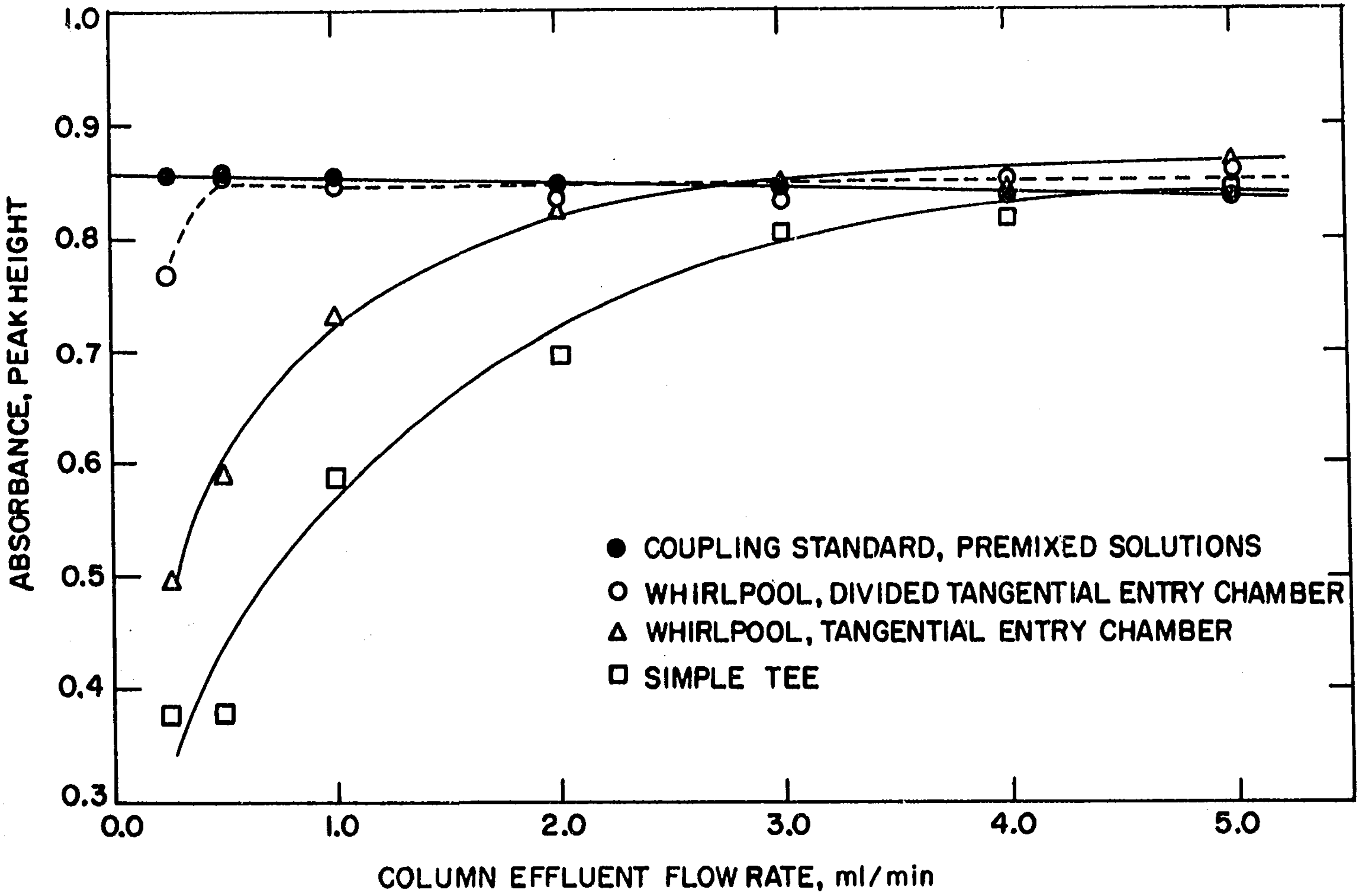


Figure 8. Comparison of mixing efficiency vs. a coupling standard Conditions: 6.2×10^{-4} M Arsenazo III in pH 1 HCl; 3.75×10^{-5} M Th(IV) in pH 1 HCl; flow rate of color reagent and metal solution equal; 5 ml sample loop; constant absorbance detected at 665 nm

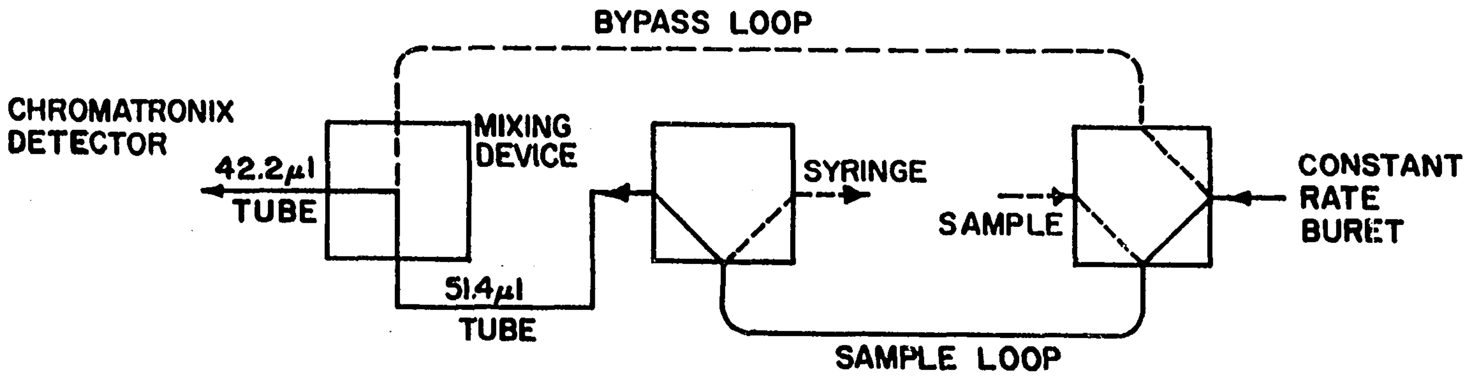


Figure 9. Schematic diagram for comparison of cell factors

solution from a flowing stream then the valves were changed pneumatically to pH 1 acid wash. The time was measured from the valve change to an absorbance of 1% of the maximum. The Chromatoronix detector, having 9 μl between the inlet and the 8 μl flow cell, was used. A 20"/min recorder speed was used and a flow rate of 1.00 ml/min from the buret.

Comparison is based on an arbitrary wash factor where

$$1\%\ \text{wash volume factor} = \frac{\text{volume required to reduce absorbance to 1\% of maximum}}{\text{geometric volume of device + tubing to detector}}$$

The results are in Table 2.

Table 2. A comparison of wash volume factors

Device	Chamber	Leads	1% wash volume factor	s
3-way valve, standard	0.0 μl	4.6μl	1.81	± 0.09
Simple tee	0.0	3.7	1.87	± 0.04
Whirlpool, tangential entry chamber	4.0	5.7	1.86	± 0.08
Whirlpool, divided Tangential entry chamber	17.0	5.9	1.77	± 0.06

The results are not conclusive because the recorder was slower than necessary for accuracy. Data indicate however, that retention of analytical species in the chamber would be no more than that in the three-way valve, i.e. negligible.

A bandspreading experimental comparison was run also.

Figure 10 is a schematic of the set up. Here a 214 µl sample was injected and the volume in which it was eluted calculated from the chromatogram. A spreading factor was arbitrarily defined as

$$1\% \text{ bandspreading factor} = \frac{\text{ml width at 1\% of peak height maximum}}{\text{ml of sample injected}}$$

where

1% of peak height maximum =

$$\frac{(\text{width at 1\%, in})(\text{ml/min flow rate})}{(\text{chart speed, in/min})}$$

Table 3 shows the results.

Table 3. A comparison of bandspreading factors

Device	1% bandspreading factor	s
3-way valve, standard	3.25	± 0.065
Simple Tee	3.22	± 0.047
Whirlpool, tangential entry chamber	3.09	± 0.075
Whirlpool, divided tangential entry chamber	3.08	± 0.079

Unequivocal conclusions cannot be drawn but the data indicate the WDTE chamber is as good or better than the 3-way valve standard.

The variable parameters of using the mixing device were found by simple tests, and then correlated by a series of experiments outlined in Table 4. Each primary variable of

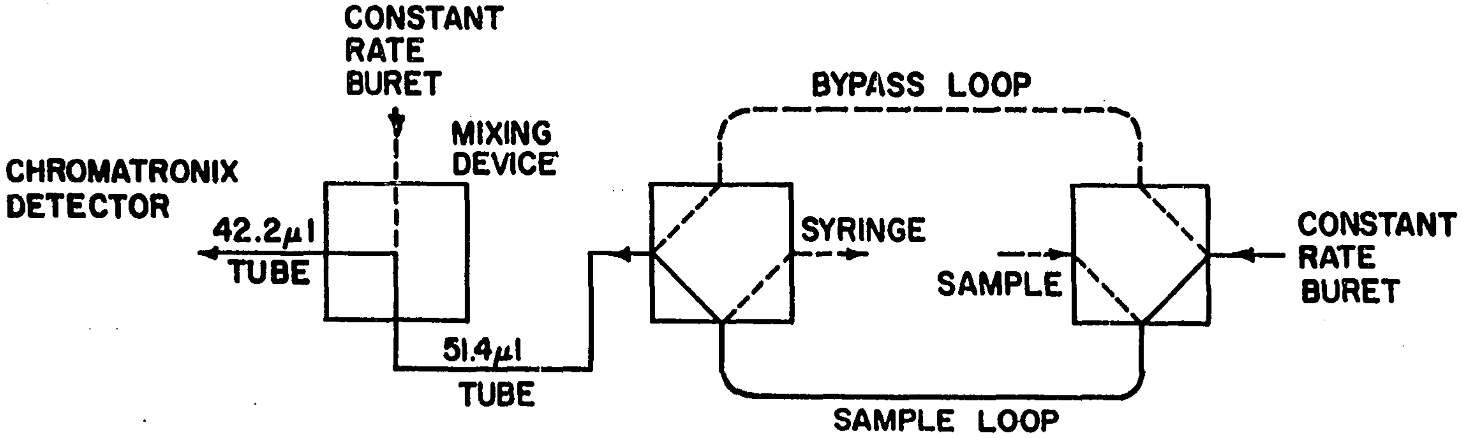


Figure 10. Schematic diagram for comparison of bandspreading factors

Table 4. Variable parameters of the mixing device

PRIMARY VARIABLE	INDEPENDENT VARIABLE	EXPERIMENT NO. 1	2	3	4	5	6	7	8	9	10
		I				D		D			
	a. CONCENTRATION OF COLOR REAGENT, mmole / ml	XS	I		D	XS	XS	XS	XS	XS	XS
1. FLOW RATE OF COLOR REAGENT, mmole / min	1 = a X b	V	V	V							
	b. FLOW RATE OF COLOR REAGENT, ml / min			I	I	I		D		D	
2. TOTAL FLOW RATE ml / min	2 = b + c				V	V	V				
	c. COLUMN EFFLUENT FLOW RATE, ml / min			D			I	I		D	
3. FLOW RATE OF ANALYTICAL SPECIES mmole / min	3 = c X d							V	V		
	d. CONCENTRATION OF ANALYTICAL SPECIES, mmole / ml		XS	XS	XS		D		I	I	
4. TOTAL ANALYTICAL SPECIES, mmole	4 = d X e									V	V
	e. SAMPLE VOLUME, ml										I

V = PRIMARY VARIABLE
I = INDEPENDENT VARIABLE
D = INTERDEPENDENT VARIABLE, RELATED TO V AND I
XS = EXCESS

BLANK SPACES INDICATE THE PARAMETER WAS HELD CONSTANT.

interest is a function of two independent variables. Experiments were designed to study only one primary variable and its independent variables in turn. Under conditions of use the color reagent would always be in excess, so most experiments were done accordingly.

The experimental schematic is the same as in Figure 7. Results of the experiments and conclusions for each follow.

Experiment 1. Figure 11 shows the incomplete color formation at too low a color reagent concentration. A more informative plot of molar ratios is shown in Figure 12. This may be used to show the concentration ratio below which an indicator does not completely complex the metal ion, or indicator purity if the combining ratio is known.

Experiments 2 and 3. Figures 13 and 14 show that, as expected, when the amount of color reagent is increased, the absorbance increases linearly when metal ion is in large excess.

Experiments 4 and 5. Figures 15 and 16 show that the peak absorbance response decreases as the total flow rate increases. This is a function of instrument response as seen later in the determination of iron (see Figure 25).

Experiment 6. Figure 17 shows the effect of diluting the same quantity of metal-indicator complex by increasing the flow rate of the column effluent. The absorbance decreases as a direct function of dilution as expected.

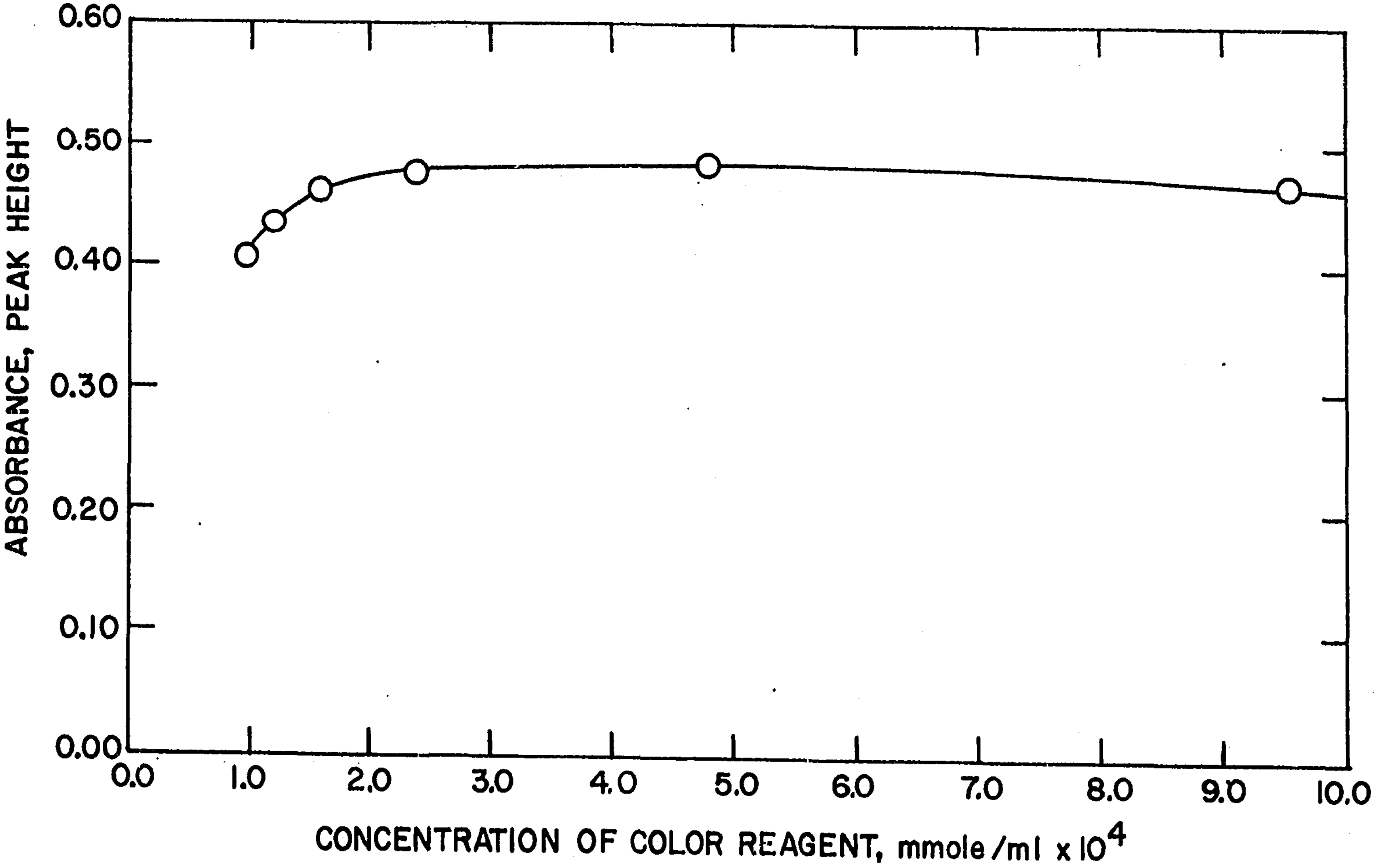


Figure 11. Variable parameters experiment 1
Conditions: a) varied; b) 2.0 ml/min; c) 3.0 ml/min; d) 3.75×10^{-5} $\underline{M}$; e) 0.0422 ml

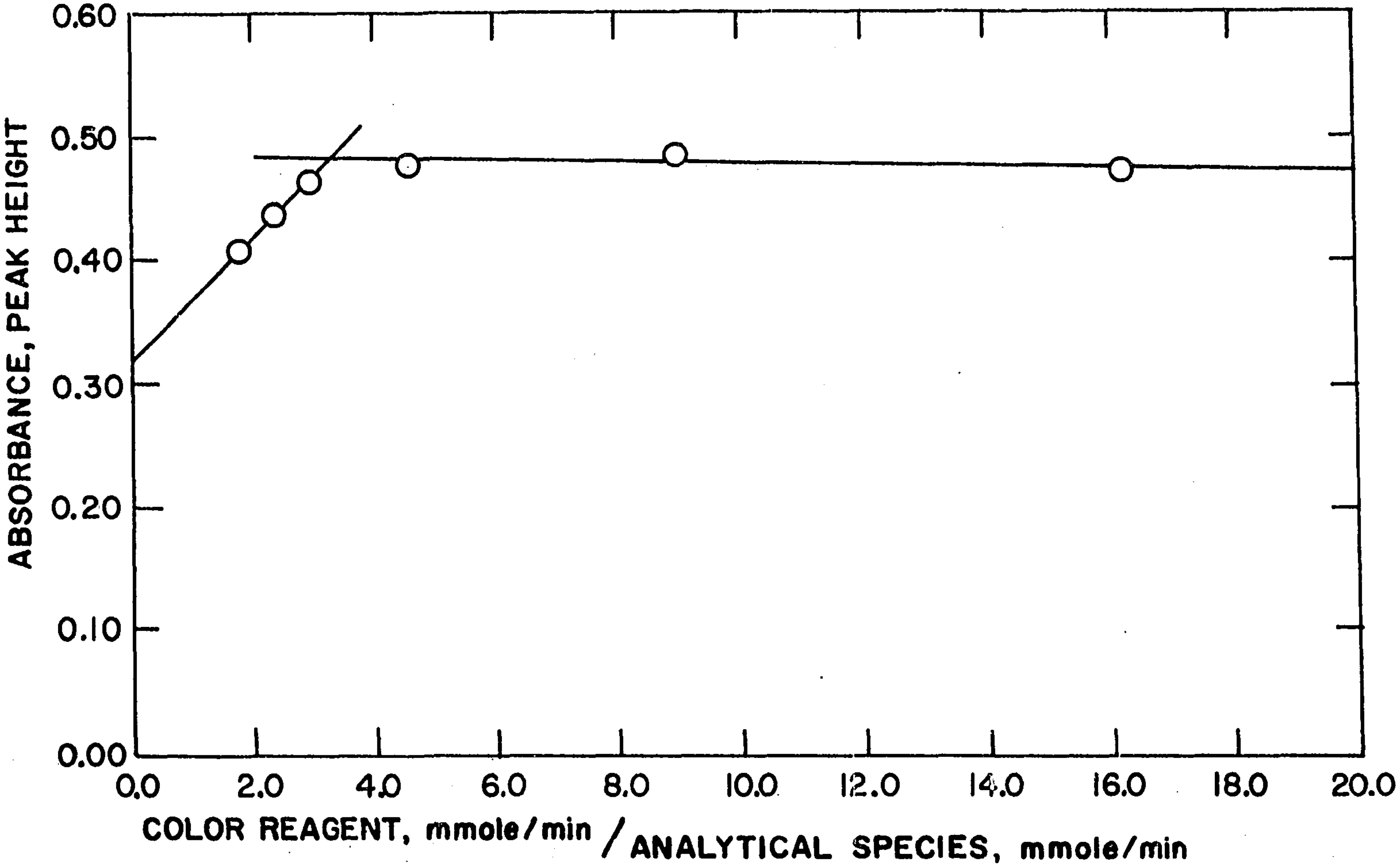


Figure 12. Variable parameters experiment 1; ratio plot
Conditions: a) varied; b) 2.0 ml/min; c) 3.0 ml/min; d) 3.75x10^{-5} $\underline{M}$; e) 0.0422 ml

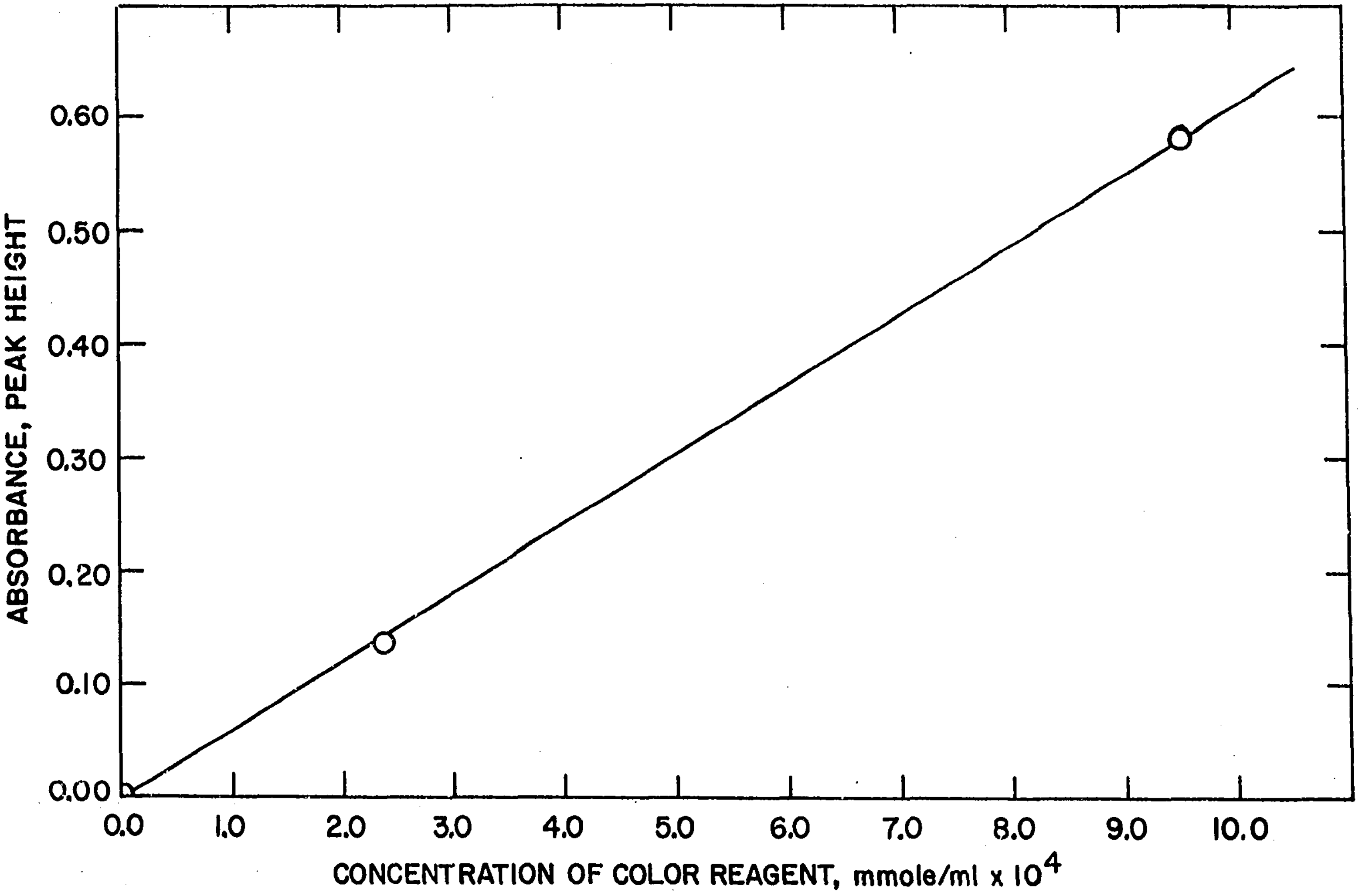


Figure 13. Variable parameters experiment 2
Conditions: a) variable; b) 1.0 ml/min; c) 3.0 ml/min; d) $5x10^{-2}$ M, excess; e) 0.0422 ml

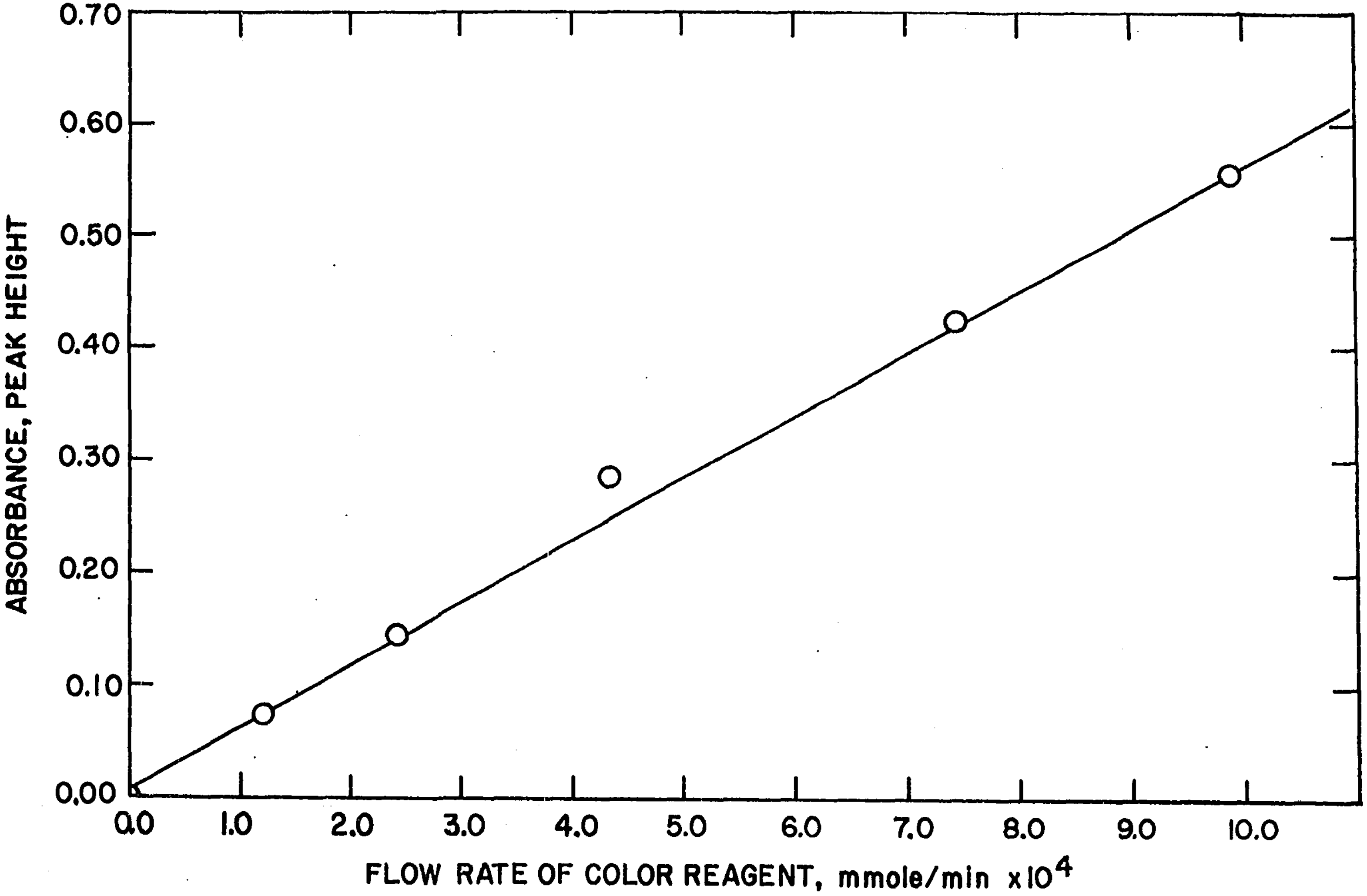


Figure 14. Variable parameters experiment 3
Conditions: a) 2.44×10^{-4} M; b) variable 2.50 ml/min; c) dependent variable; d) 5×10^{-2} M, excess; e) 0.0422 ml

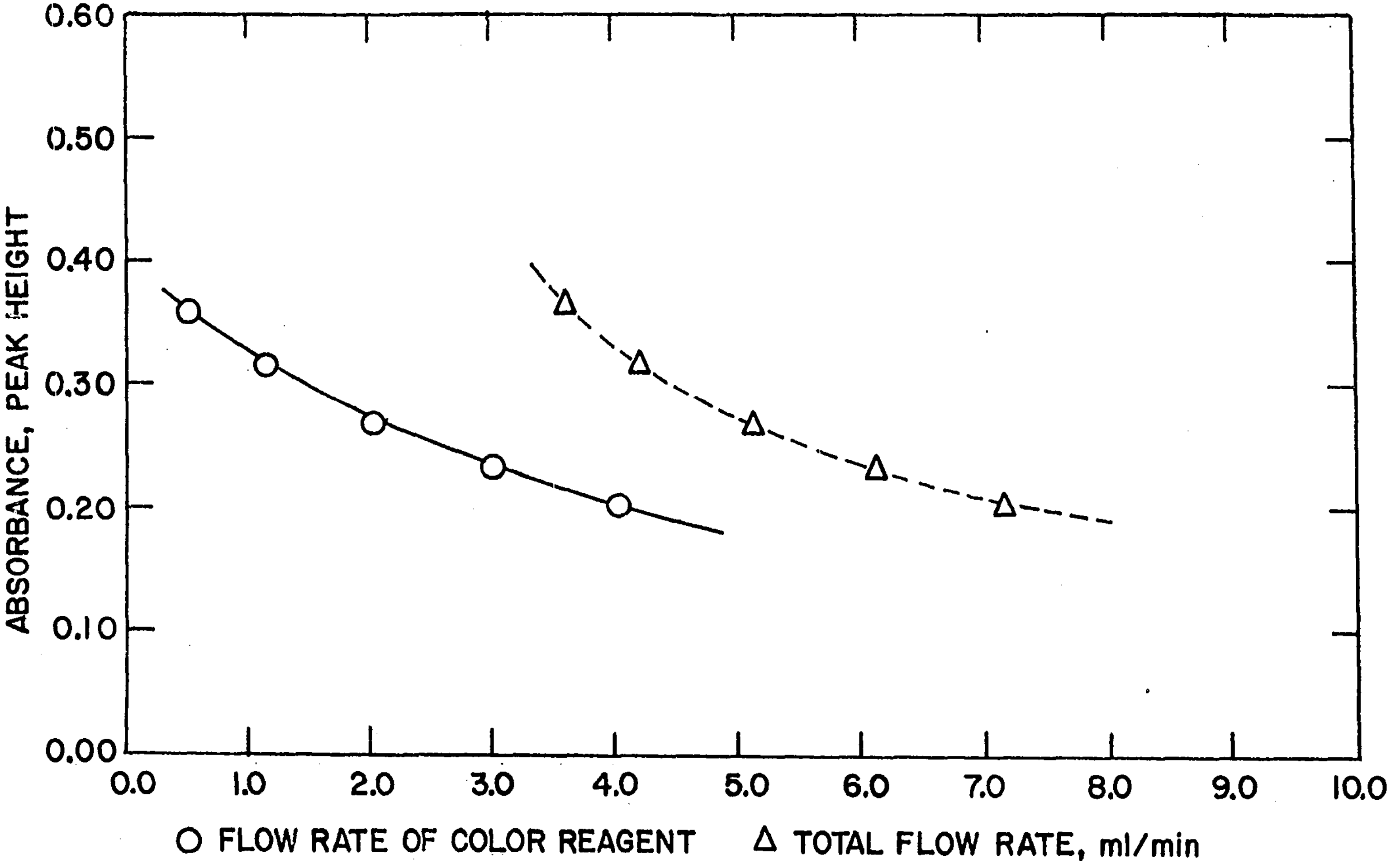


Figure 15. Variable parameters experiment 4
Conditions: a) dependent variable 1. 4.76×10^{-4} mmole/min; b) variable; c) 3.1 ml/min; d) 5×10^{-2} M, excess; e) 0.0422 ml

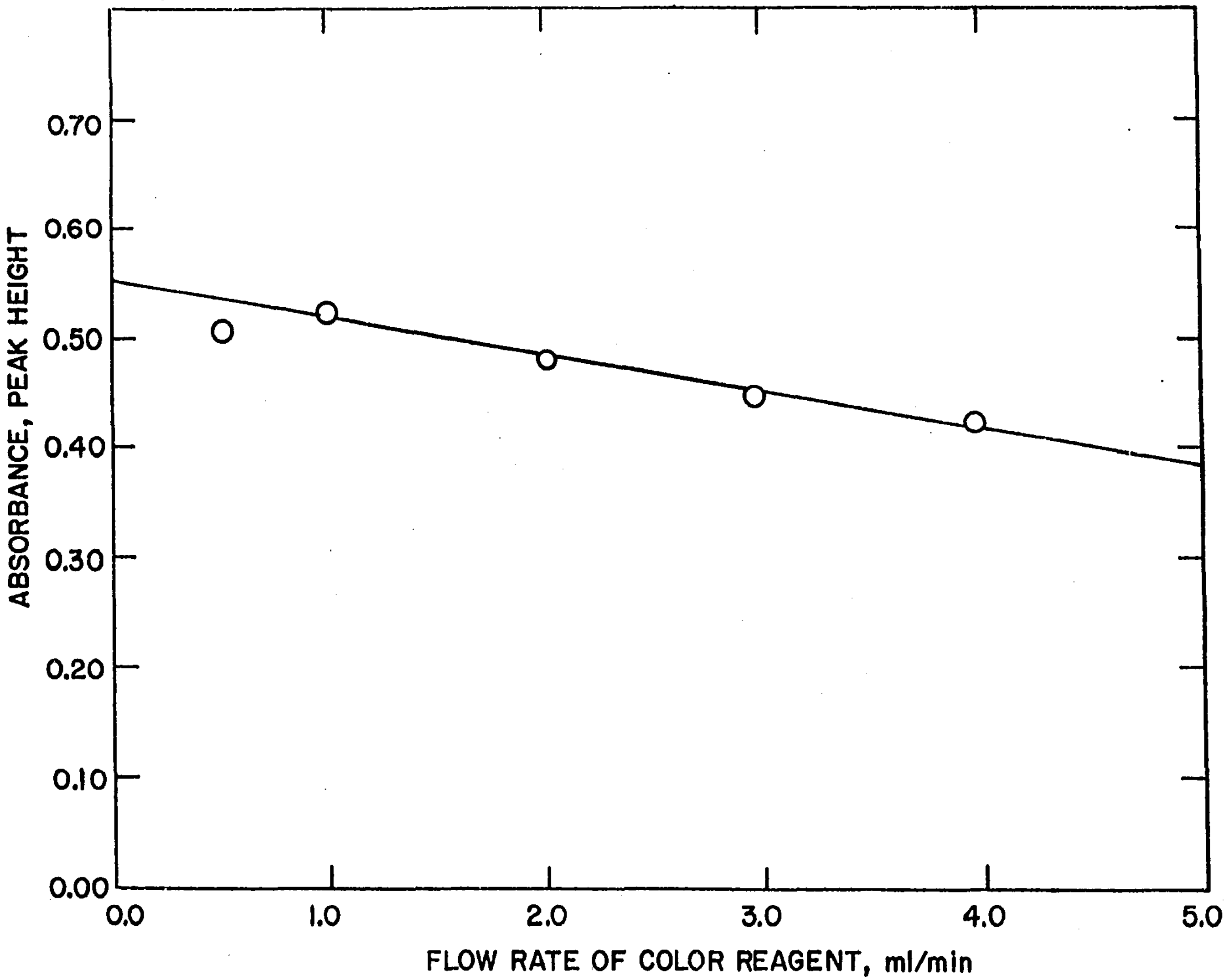


Figure 16. Variable parameters experiment 5
Conditions: a) dependent variable, excess 1. 4.76×10^{-4} mmole/min; b) variable; c) 3.0 ml/min; d) 3.75×10^{-5} $\underline{M}$; e) 0.0422 ml

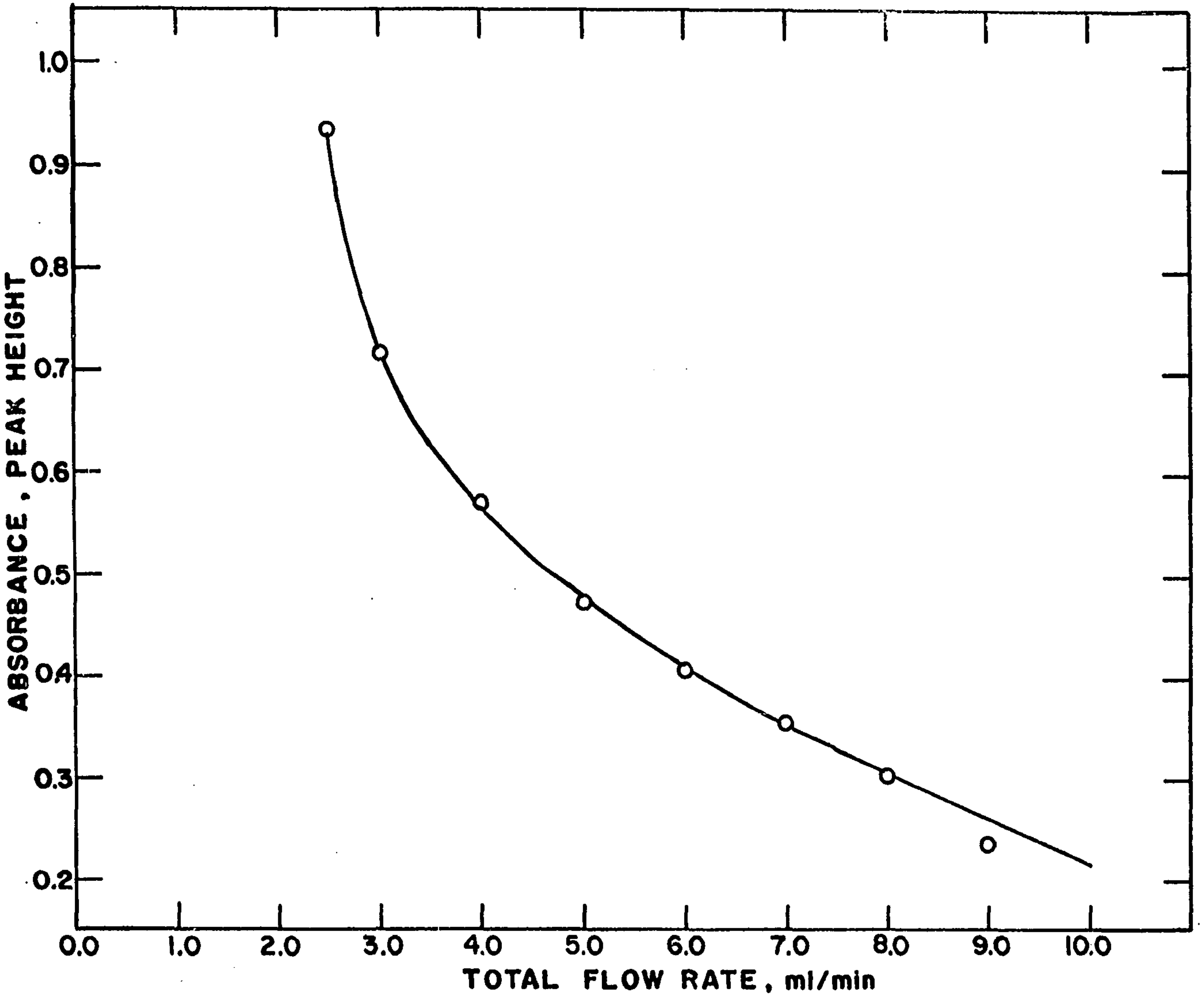


Figure 17. Variable parameters experiment 6
Conditions: a) 6.2×10^{-4} M, excess; b) 2.00 ml/min; c) variable 3. 5.0×10^{-5} mmole/min; d) dependent variable; e) 5.13 ml

Experiment 7. Figure 18 shows that as the column effluent rate is increased, linear response is seen until the effect of increased rate through the detector decreases response. The last point deviates because it fell below the molar ratio necessary for complete complex formation.

Experiment 8. Figure 19 shows the single most important experiment of the series for analytical purposes. This is a Beer's Law plot of peak height absorbance versus concentration of the analytical species.

Experiments 9 and 10. Figures 20 and 21 show that peak area is a function of the total mmole of analytical species passing through the detector with either concentration or sample volume varied.

Experiments 1 (concentration ratio plot), 6 and 8 are of particular importance for describing the mixing chamber use. The concentration ratio must be such that there is always excess reagent present. Flow rate of the column effluent must be held constant if response is to be precisely related to concentration of analytical species. Finally, a linear plot of concentration versus absorbance is possible using a mixing chamber for color reagent addition.

Other reagents for color formation were spot tested to find a few for general use. The ideal reagent would complex with a large number of metal ions in an acidic solution. Its formation constant and molar absorptivity would be high with

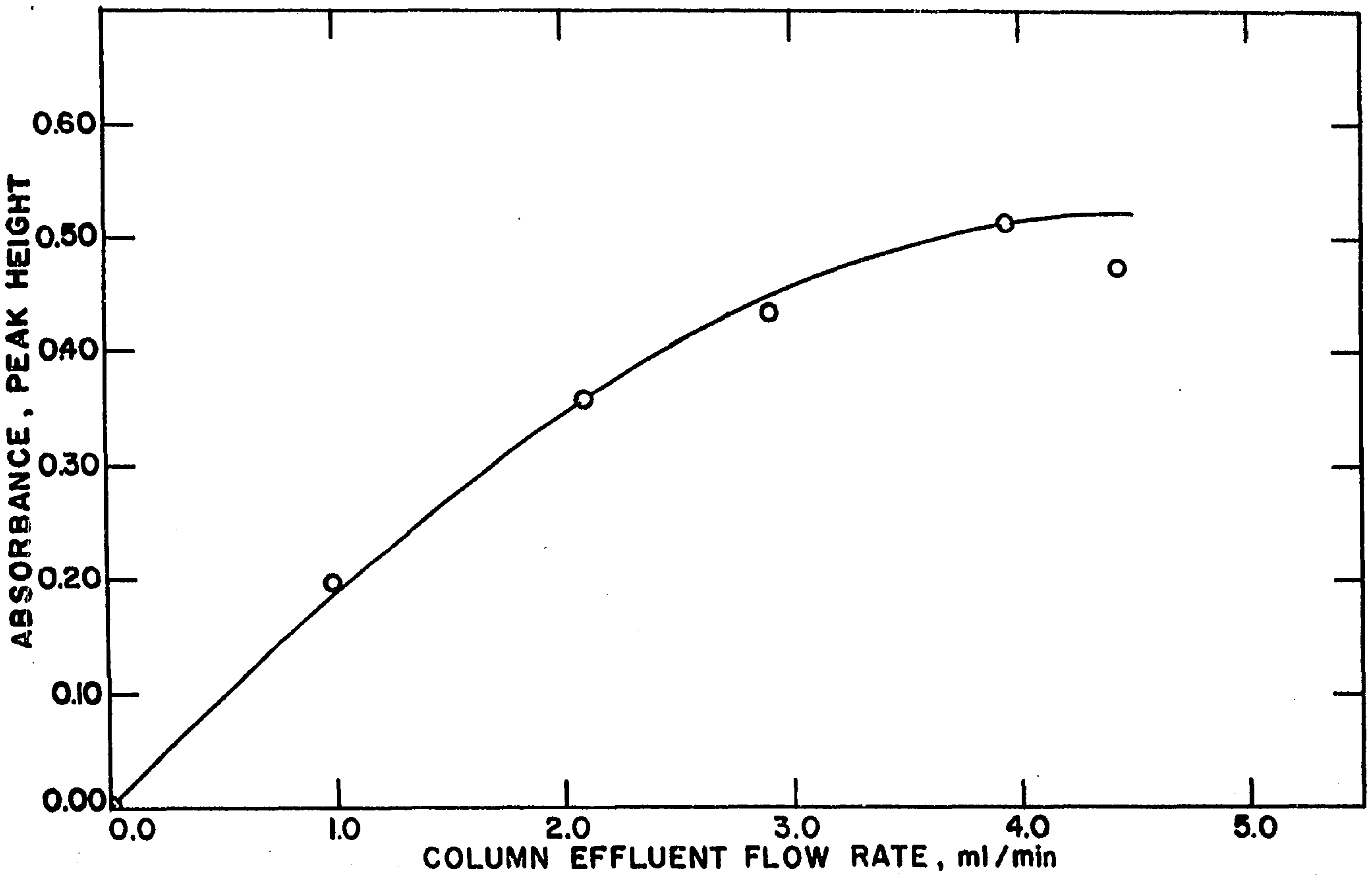


Figure 18. Variable parameters experiment 7
Conditions: a) dependent variable, excess 1. 4.76×10^{-4} mmole/min; b) dependent variable 2. 5.0 ml/min; c) variable d) 3.75×10^{-5} $\underline{M}$; e) 0.0422 ml

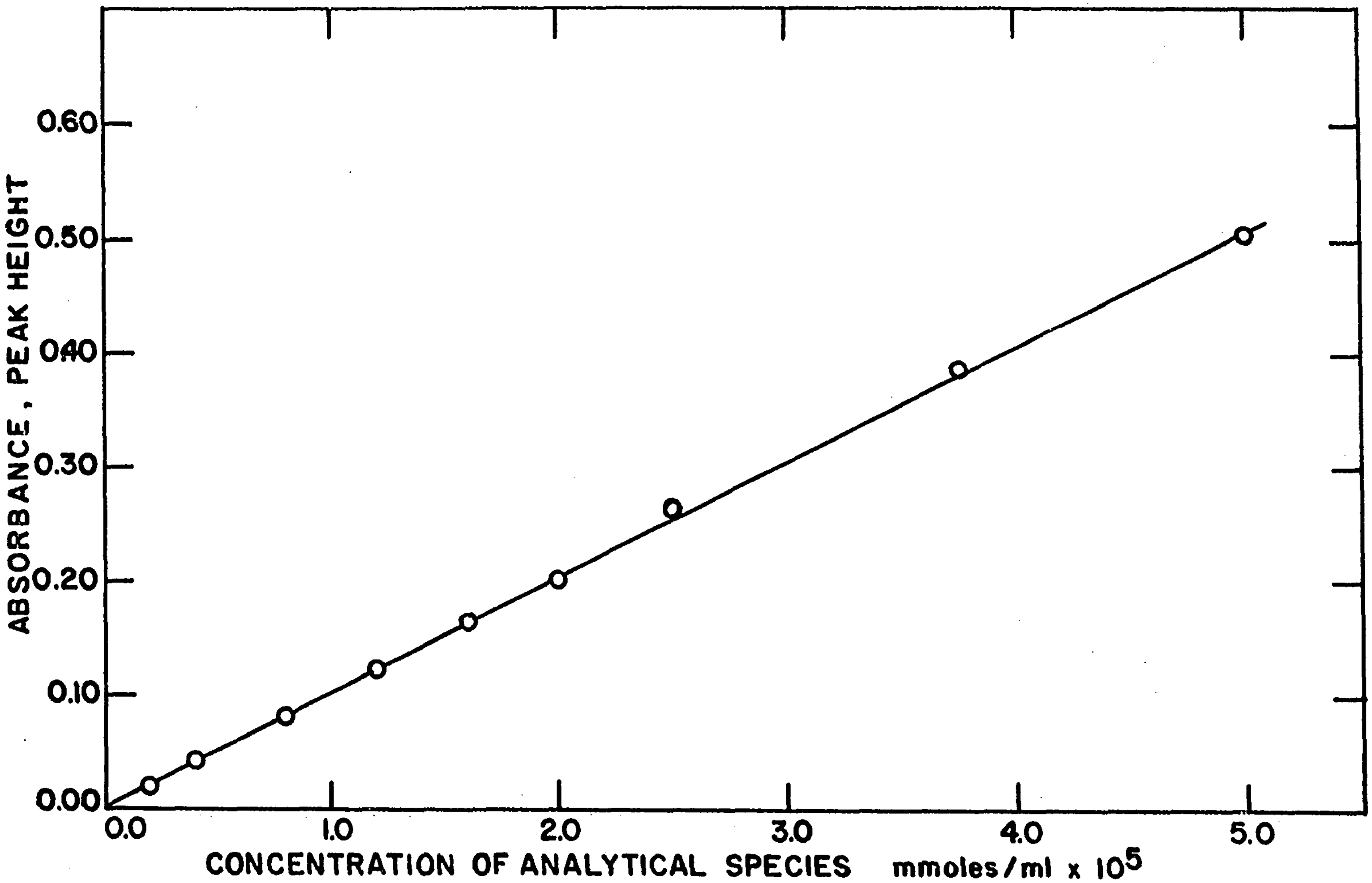


Figure 19. Variable parameters experiment 8
Conditions: a) 4.76×10^{-4} M, excess; b) 3.0 ml/min; c) 2.0 ml/min; d) variable; e) 0.0422 ml

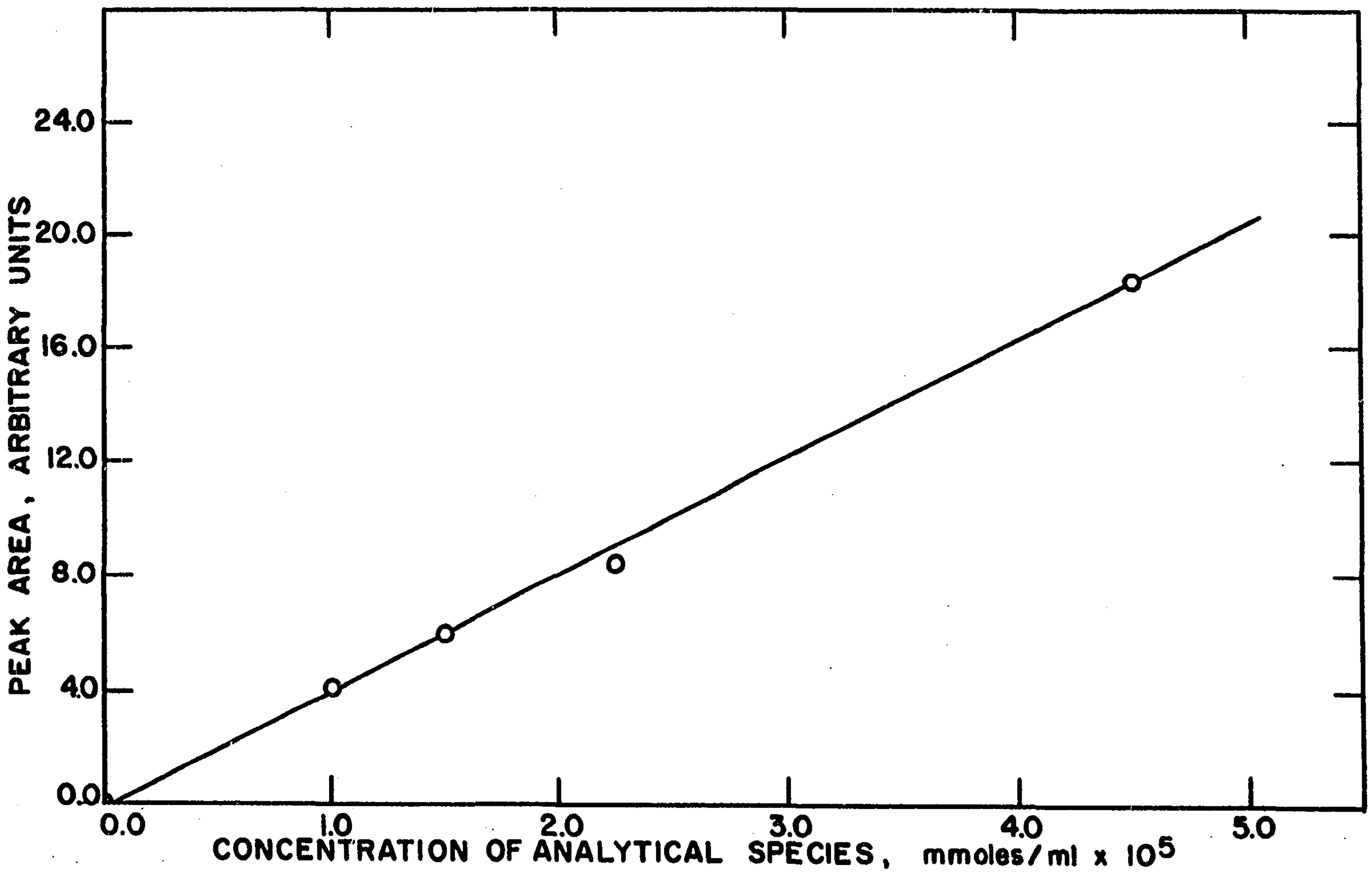


Figure 20. Variable parameters experiment 9
Conditions: a) 6.2×10^{-4} M, excess; b) dependent variable 2. 5.0 ml/min; c) dependent variable 3. $\overline{4}.5\times10^{-5}$ mmole/min; d) variable; e) 0.0514 ml

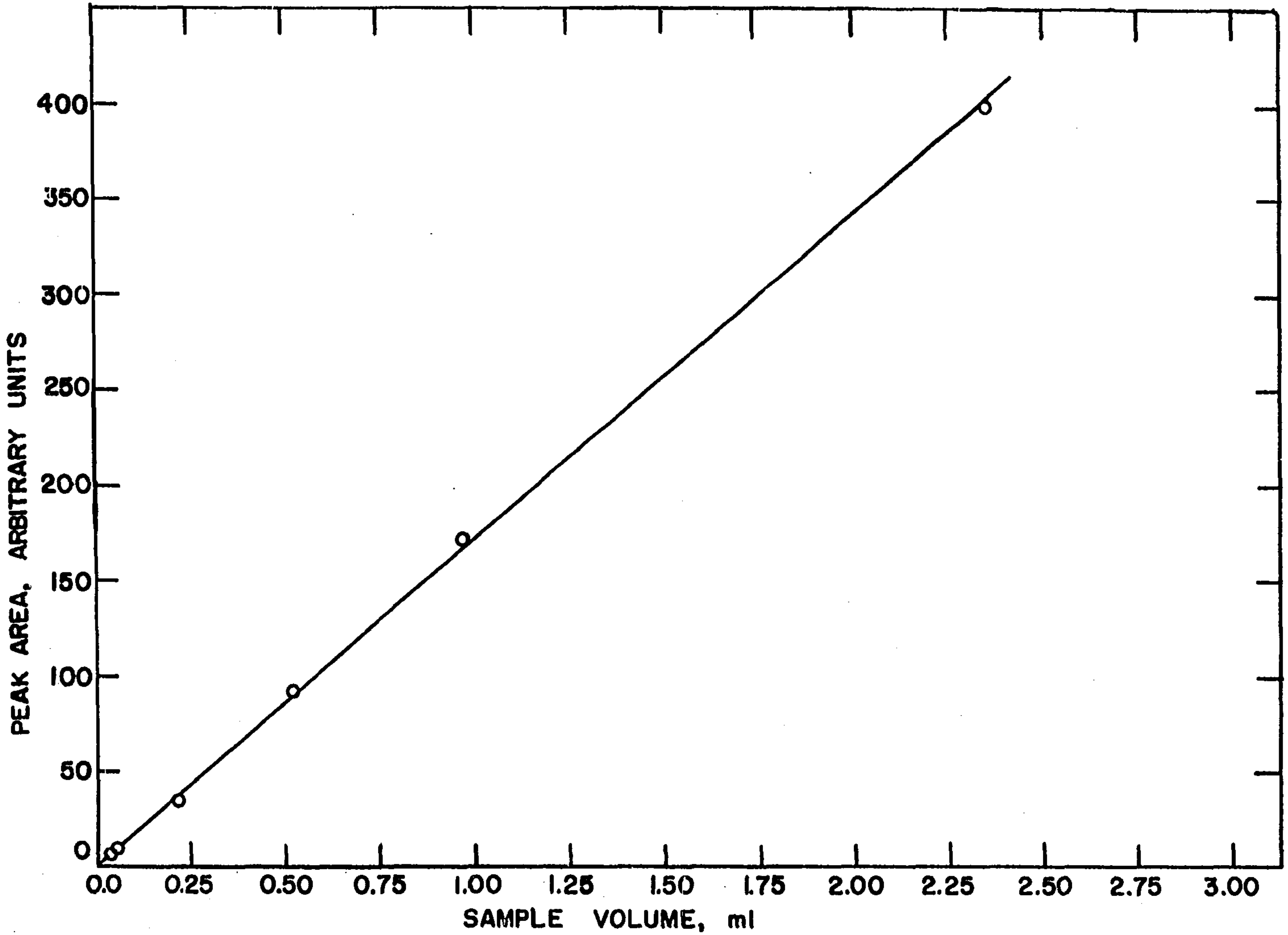


Figure 21. Variable parameters experiment 10
Conditions: a) 6.2×10^{-4} $\underline{M}$, excess; b) 2.0 ml/min; c) 3.0 ml/min; d) 2.5×10^{-5} $\underline{M}$; e) variable

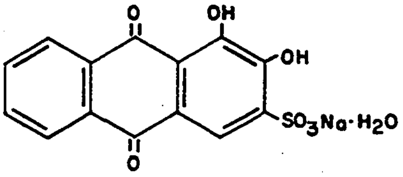
negligible free indicator absorbance at the complex λ max. The rate of reaction must give complete complexation in less than 100 msec with a reasonable excess of reagent present.

A few general reagents, Figure 22, were chosen and tested for reaction with metals of Figure 5. Figure 23 indicates positive tests, i.e. a color change, in acetate buffer. Of these PAR, 4-(2-pyridylazo)resorcinol is the most general, reacting with 34 metal ions. Chrome azurol S and quinalizarin are of limited value while the remaining ones react with 20 or more ions.

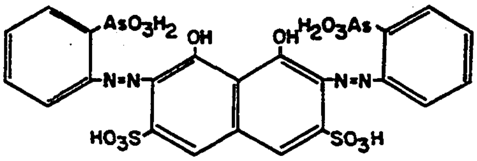
Acetate buffer was chosen to minimize hydrolysis problems from a large number of metal ions. Pyridine buffer at pH 5 was tested but hydrolysis interfered significantly. Ammonium hydroxide, ammonium chloride buffer was used with PAR in the determination of zinc and cadmium described later. Simple spot tests with the alkaline earths gave positive tests with this PAR solution as did a few others not reacting in acetate buffer.

Inorganic ions were not screened for use although hydrochloric acid (31,32) and thiocyanate (33) have been used previously in colorimetric methods for 15 and 17 metal ion respectively. Detection of As(III) with hydrobromic and hydriodic acids in the UV was shown but a column concentration and separation method was unsuccessful so it was not used.

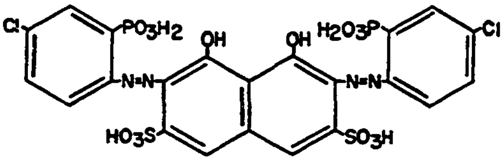
ALIZARIN RED S



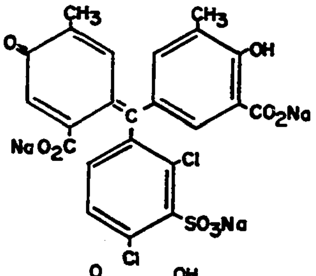
ARSENAZO III



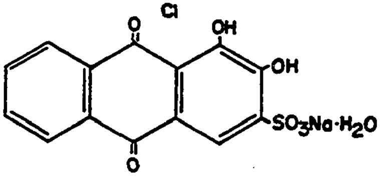
CHLOROPHOSPHONAZO III



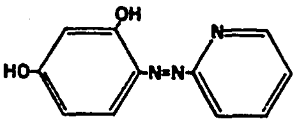
CHROME AZUROL S



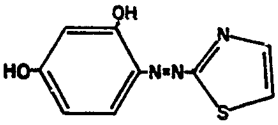
QUINALIZARIN



4-(2-PYRIDYLAZO)RESORCINOL (PAR)



4-(2-THIAZOLYLAZO)RESORCINOL (TAR)



XYLENOL ORANGE

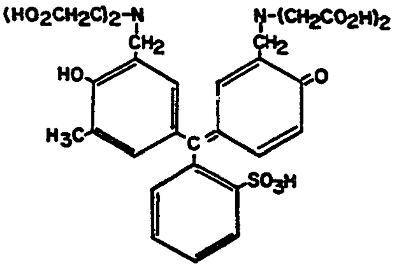


Figure 22. Color test indicator structures

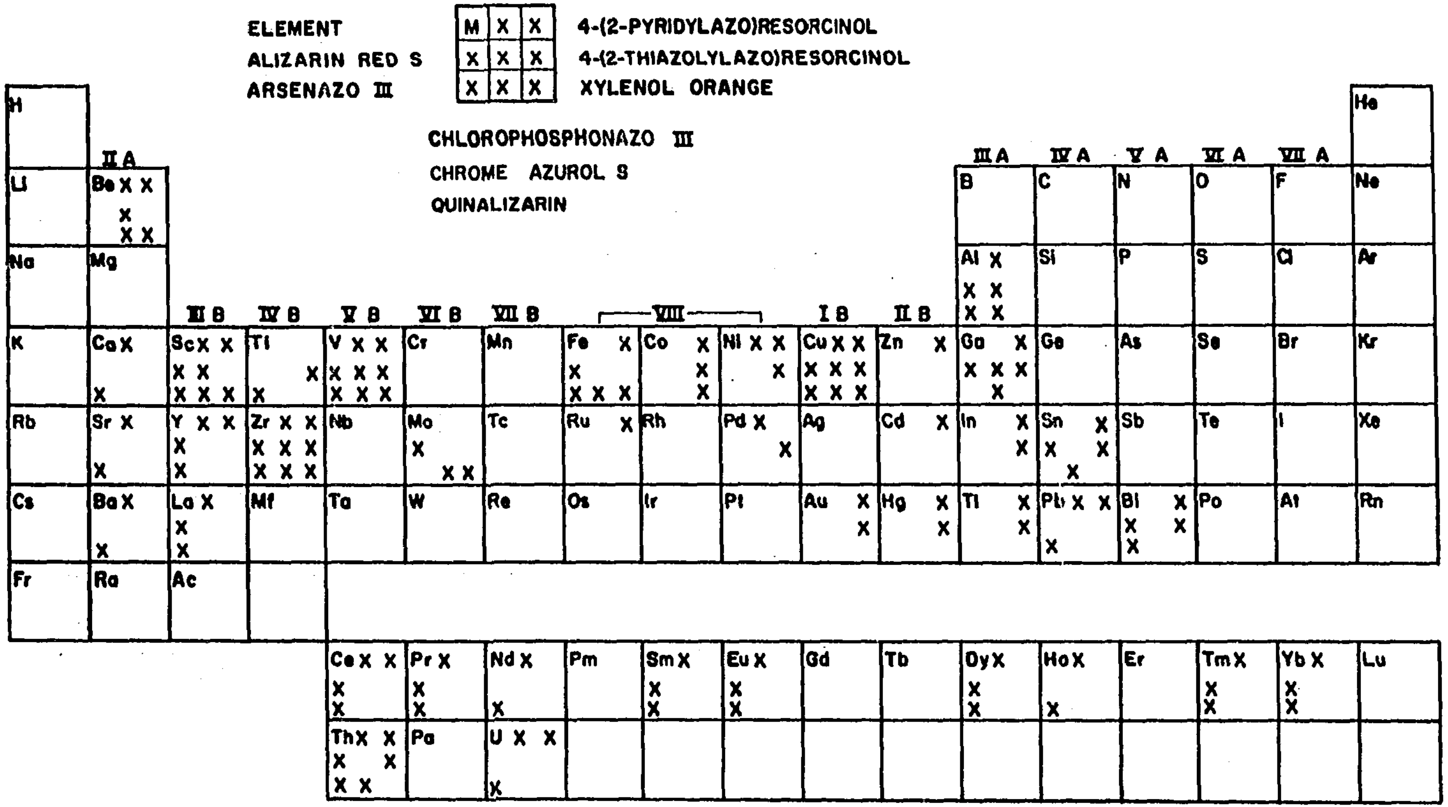


Figure 23. Positive color tests for metal ions in acetate buffer

ANALYSES

Introduction and Literature Survey

There is a vast and continually expanding body of literature on separation by ion exchange chromatography. Many books are available which include review sections and outlines of specific methods (1,34,35) for metal ions. Although the conditions necessary are at times unwieldy or difficult to achieve, virtually every element may be separated by either anion or cation exchange (1). Where the specific separation has not been done, survey work is published to guide selection of an appropriate method.

Not all chromatographic methods are at present easily adapted to the instrument used here. The complementary effects of ion exchange selectivity and complex ion equilibria in solution are most simply used in metal ion separations. All metal ions other than the alkali or alkaline earth metals form some type of complexes with common anions. The strength of many of these complexes in terms of formation constants has been reviewed (36).

Proceeding a step further, surveys of distribution coefficient on a resin versus acid concentration in the solution have been done for a large number of metal ions. Anion exchange separations in hydrofluoric (37), hydrochloric (38), sulfuric (39), and nitric (40) acids as well as mixed acid systems (38) have been surveyed. Cation exchange separations have been surveyed in perchloric (41), hydrochloric (41,42), hydrobromic (43), sulfuric and nitric (44) acids and various organic solvent-acid mixtures (35,45,46).

The simplest type of ion exchange separations uses selective sorption and selective elution (1); "go,no-go" or stepwise elution chromatography as it is frequently called. Here a limited number of elements are sorbed to the column by careful choice of the complexing ion and its concentration in the sample and sorbing eluent. The constituents of interest are concentrated and separated from the matrix. The eluent is then changed to a different concentration of the same complexing ion or a second complexing ion to strip the metal of interest from the resin. Illustrative of this is the sequential separation of first row transition elements, manganese (II) through zinc(II), by anion exchange in hydrochloric acid (38).

Determination of metal ions by their visible or ultraviolet absorbance or that of metal-inorganic ion complexes has been used frequently. Chloride (31,32), thiocyanate (33) and peroxide (47,48) among others have been used. Sandell (33) lists 22 elements for which inorganic reagents are important for photometric determination. Including organic reagents with these, virtually all except the alkali metals have sensitive photometric methods.

The ideal reagent for in-stream injection and color formation is readily soluble in an appropriate buffer solution, reacts rapidly and completely (high formation constant) with the metal ion of interest to give a highly colored complex absorbing at a wavelength where the free indicator does not. A general reagent reacting with a large number of metal ions is preferable. However, where separation cannot be easily achieved, a selective color reagent may be used to advantage.

The following analyses use "go,no-go" separations on anion exchange resins. These systems have been well characterized and large differences in selectivity coefficients compared to cation exchange (34) are helpful.

Automatic detection is based on the color of chromium(VI), the colored iron(III) chloro complex and the organic reagent PAR for zinc(II) and cadmium(II) color development.

Apparatus

pH Meter: A Corning Scientific Instruments Model 7 pH meter equipped with a Sargent combination electrode was used for pH measurements.

Spectrophotometer: A Bausch & Lomb Spectronic 600 UV visible recording spectrophotometer and Bausch & Lomb VOM 8 log, linear recorder were used with a matched set of 10 mm Spectrosil (Coleman Instruments Corporation) cells for recording spectra.

Constant rate buret: An E. H. Sargent & Company Model C automatic constant rate buret was used. It has a 10 ml delivery volume at 1.00 ml/min.

Determination of Iron

Choice of separation method

The determination of iron was first attempted by separating iron(III) and cobalt(II) on a short column of 100-150 mesh XAD-2 resin impregnated with isopropyl ether (49), using 8*M* hydrochloric acid as the eluent. Under these conditions iron is retained by the column, cobalt is eluted and detected at 610 nm. The wavelength is changed to 400 nm then iron is stripped from the column with aqueous 0.1 *M* hydrochloric acid. At a flow rate of 4.9 ml/min a complete separation of iron(III) and cobalt(II) required only 3.25 min including sample injection, wavelength change and elution of both iron and cobalt from the column. The flow rate used is approximately 5 times that recommended previously for similar separations using a 80-100 mesh XAD-2 reversed-phase system (2). This type of separation requires pre-equilibration of aqueous reagents with the stationary organic phase; even then the organic phase may bleed from the column at high flow rates.

Next the separation of iron from other metal ions was evaluated using a column containing Rohm & Haas A-26 anion exchange resin. This macroreticular resin was chosen because of its durability, the ease of particle size segregation, and its minimal shrinking or swelling on changing eluents. From 6 M hydrochloric acid iron(III) is strongly retained by a column containing 180-200 mesh A-26 resin; many other metal ions are not retained under these conditions (38). A 2 M solution of aqueous hydrochloric acid elutes iron(III) very quickly as a tight, compact band which is intensely colored. The peak height of the recorded elution curve is a linear function of the amount of iron in the sample. This was made the basis of a method for the rapid determination of small amounts of iron.

Experimental

Resin   Amberlyst A-26 macroreticular, strong-base anion exchange resin was received as water moist 20-40 mesh beads from Rhom and Haas Company. The capacity is approximately 4.1-4.4 meq/g of dry resin or 0.95-1.1 meq/ml as received. It was dried at 100°C, ground in a model 4-E Quaker City mill and sieved dry to obtain a 180-200 mesh fraction, was slurried repeatedly in water and the extremes fines decanted. The column bed was 2.0 cm long and contained 0.36 g of dry resin.

Standard reference materials   These are from the National Bureau of Standards. The treatment of each sample is described below; the final step was dissolution in iron free hydrochloric acid.

Argillaceous Limestone 1b. A sample was dried 2 hours at

110°C, weighed into a platinum dish and fired at 800°C for 30 minutes. It was then cooled, 3 ml water, 5 ml HCl, 5 ml HF added and taken to dryness. The acid treatment was repeated also adding 3 drops of $HClO_4$, then taken to fumes.

Glass Sand 81. A sample was dried 2 hours at 110°C and weighed into a platinum dish. 5 ml HF and 3 drops $HClO_4$ were added then taken to fumes. This treatment was repeated. It was taken up in 6 M HCl and filtered. The residue was fused with a minimum of potassium pyrosulfate, then dissolved and the filtrates combined.

Aluminum Alloy 85a. A sample was weighed into a platinum dish. 10 ml of 6 M HCl, 1 ml HF and 2 ml $HClO_4$ were added then taken to fumes.

Bronze Ounce Metal 124d. A sample was weighed, 10 ml of 6 M HCl and 3 ml of 30% hydrogen peroxide were added then evaporated to near dryness to remove excess peroxide.

Portland Cement 177. A sample was dried 2 hours at 110°C and weighed into a platinum dish. 5 ml HF and 2 ml $HClO_4$ were added then taken to fumes. The HF treatment was repeated.

Primary standard iron Standard of Reference Grade iron, electrolytic, ignited in moist hydrogen, assay 100.00% from G. Frederick Smith Chemical Company was used for standard calibration solutions. Dissolution was with 6 M iron free hydrochloric acid and a small amount of 30% hydrogen peroxide. After taking to near dryness to remove excess peroxide, the solution was diluted to volume with 6 M iron free hydrochloric acid.

Iron free hydrochloric acid DuPont reagent hydrochloric acid was diluted to 6 M and passed through a column of Amberlyst A-26 for removal of traces of iron. This was used only for sample and iron standards dissolution.

Procedure

The 6.3 mm i.d. analytical column was filled with 0.36 g (dry weight) of 180-200 mesh Amberlyst A-26 anion exchange resin slurried in water. This gave a column bed 2.0 cm long having a total capacity of approximately 1.5 meq. A typical iron sample would contain 3×10^{-3} meq of iron.

The detector was set at 355 nm using a straylight filter and a tungsten source. The pressure was set to give a flow rate of 3.0 ml/min for the eluent used to sorb iron to the solumn. The sorbing eluent (6 M hydrochloric acid) was allowed to run at this rate for one minute to pre-equilibriate the column. The sample (2.34 ml), dissolved in 6 M hydrochloric acid, was then injected. Elution with 6 M hydrochloric acid was continued for 3 minutes to allow removal of non-sorbing matrix elements from the column. The eluent was then changed to 2 M hydrochloric acid and the iron eluted; the peak was obtained within 6 minutes from the beginning of the analysis.

Using the peak height obtained, the micrograms of iron in the sample were read from a calibration curve prepared from standard solutions using the same procedure.

Results and discussion

A typical chromatogram is shown in Figure 24. This sample is an aluminum matrix containing about 2% copper and the first broad peak is due to the copper(II) chloro complex.

Although the anionic iron(III) chloro complex will be strongly sorbed to the resin from more concentrated hydrochloric acid, 6 M provides greater selectivity, faster flow rate and easier sample preparation. The 2 M hydrochloric acid desorbs iron in a tight band while maintaining the colored complex for detection.

The wavelength of detection was a compromise between maximum sensitivity, instrumental efficiency, and selectivity. Maximum absorbance lies at 250 nm for iron(III) chloro complex; however, at this wavelength a number of other metal chloro-complexes also absorb (31,32). The absorption spectrum of iron(III) in 2 M hydrochloric acid also has a maximum at 340 nm and here only a few elements which form yellow chloro complexes will interfere. A wavelength of 355 nm allows use of a tungsten source which provides radiant power sufficient to illuminate the flow-through cell and gives maximum detector output which is not possible with a deuterium source.

For reproducible results it is essential to control the flow rate used for elution of the iron. A plot of peak height against flow rate is shown in Figure 25. The elution flow

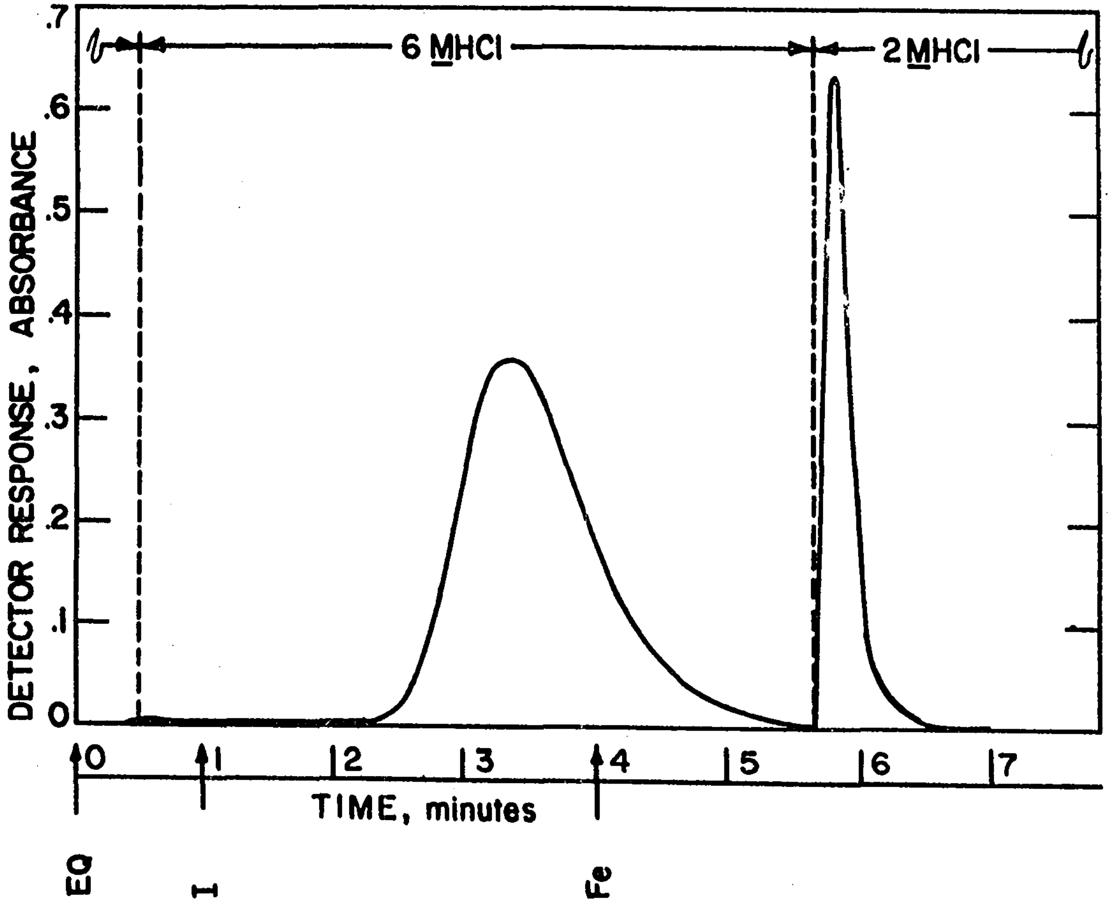


Figure 24. Typical chromatogram of iron analysis
Conditions: sample NBS 85A, 773 mg/50 ml; 2.34 ml loop; detection at 355 nm

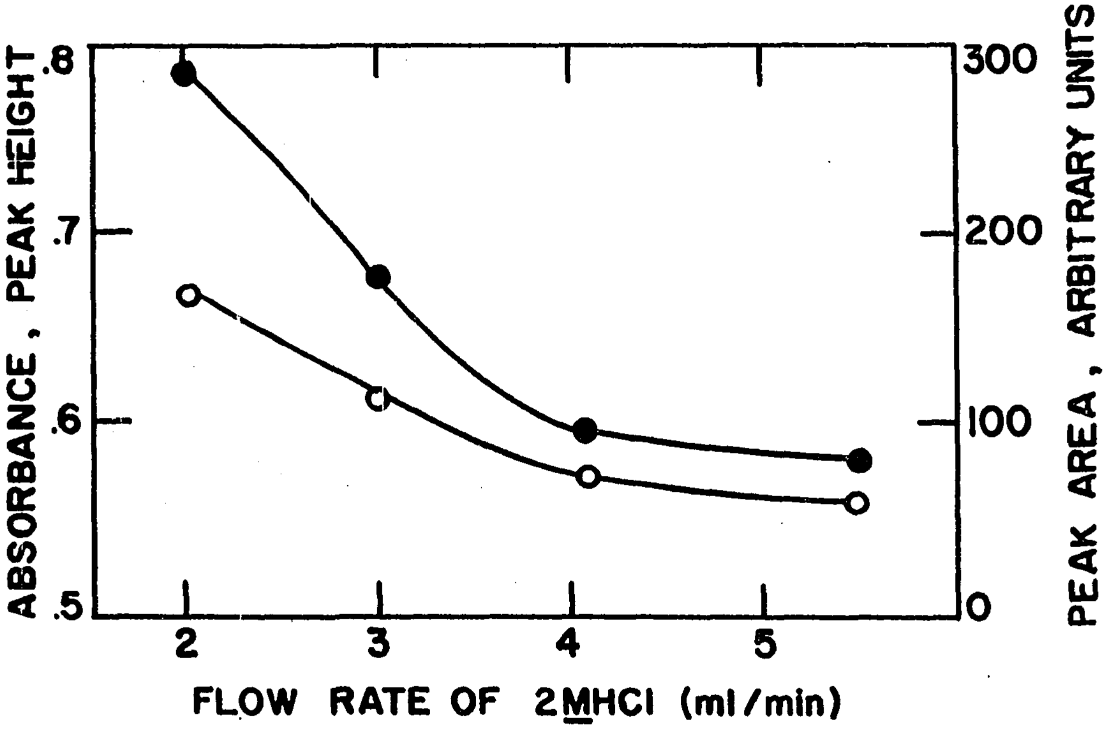


Figure 25. Detector response vs. elution flow rate
0 peak height; 0 peak area
Conditions: 2.34 ml loop; 5.49×10^{-4} M iron(III); sorbing eluent flow, 3.0 ml/min; detection at 355 nm

rate of 5 ml/min is on the flat part of the curve. The 3 ml/min flow rate at which the sample is added to the column in 6 M hydrochloric acid is not critical. The same pressure setting that provides a flow rate of 3.0 ml/min for 6 M hydrochloric acid gives a flow of 5.0 ml/min for 2 M hydrochloric acid.

The effect of varying the concentration of hydrochloric acid used to introduce the sample was studied. Figure 26 shows very little difference in the results when the hydrochloric acid is varied from 5 M to 12 M.

The linear calibration plot for iron is shown in Figure 27. For a standard sample giving a peak height of 0.602 absorbance units the reproducibility of peak area is ± 0.4% whereas the reproducibility of peak height is within instrumental error, ± 0.3%. Peak height was used for greater reproducibility and to simplify readout.

For the instrumentation described the optimum range for iron detection was 10 to 100 mg. It should be noted, however, that 10 mg does not represent the absolute detection limit in terms of a signal/noise ratio. The 2 mm path length cell also is a limitation of sensitivity.

It was not necessary to use iron-free hydrochloric acid in eluent preparation as use of the same elution sequence for both standards and samples would cancel a blank. In practice no blank was observed.

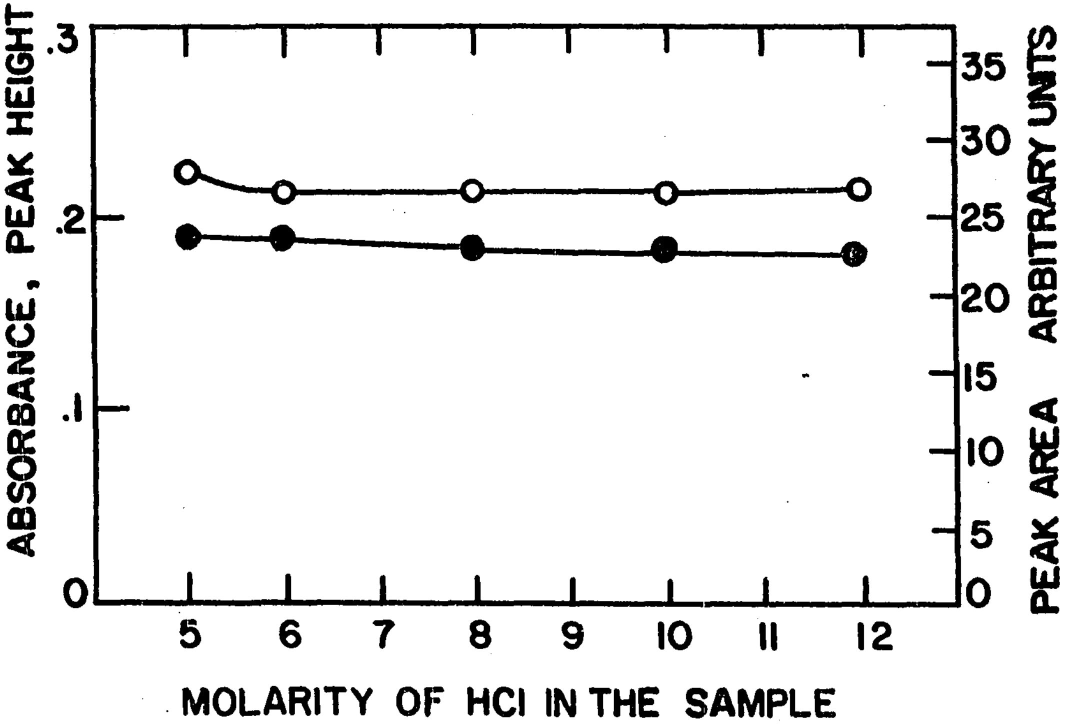


Figure 26. Detector response vs. concentration of hydrochloric acid in the sample
0 peak height; 0 peak area
Conditions: 2.34 ml loop; 2.02×10^{-4} $\underline{M}$ iron(III); sorbing eluent flow, 3.0 ml/min; detection at 355 nm

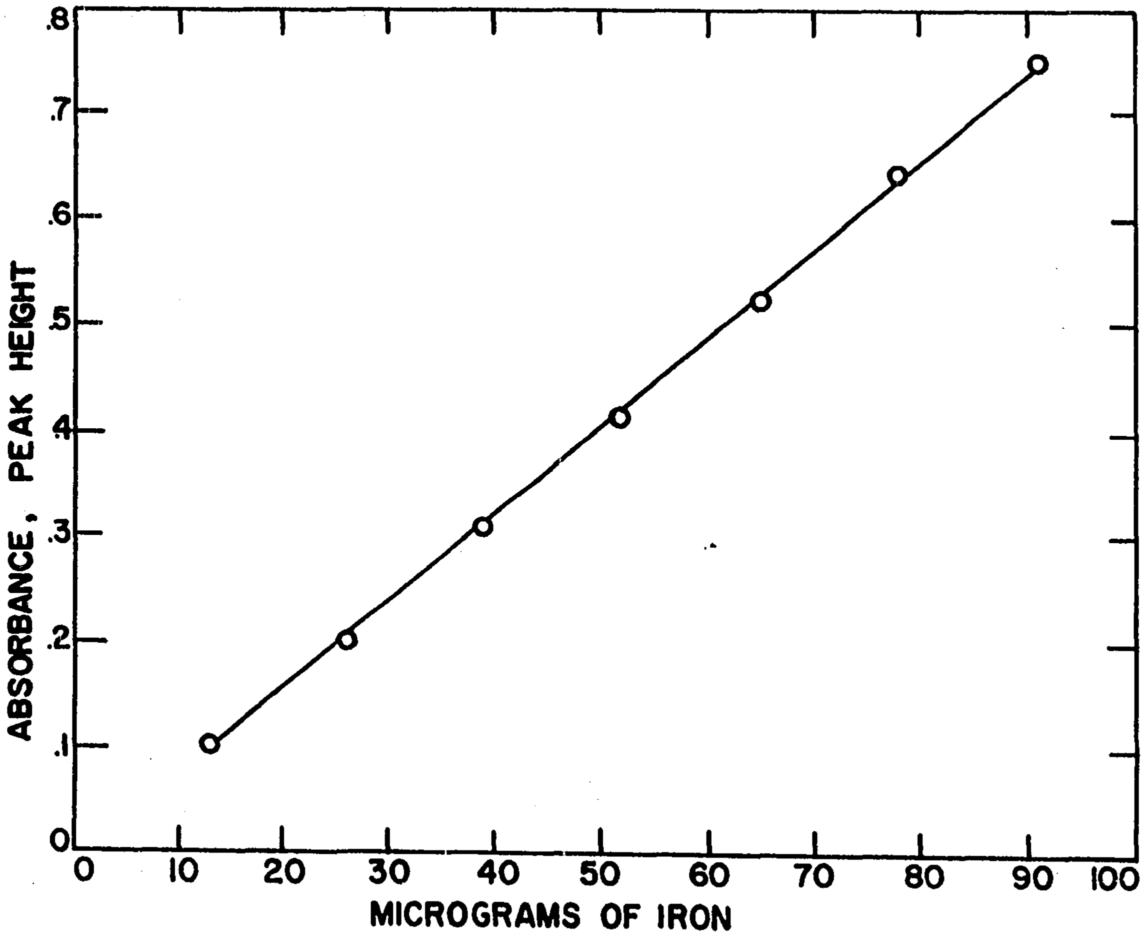


Figure 27. Calibration plot for iron

Results for analysis of five National Bureau of Standards samples for iron are summarized in Table 5. In each case the size of sample taken for analysis was varied so that the iron content would be within the range of the calibration curve. Out analyses show less deviation than indicated by the significant figures or deviation on the Certificates of Analysis. The accuracy is within the instrumental error with the exception of NBS 81, Glass Sand. The Certificant of Analysis and our experience indicates that this error probably occurred in the sample dissolution step.

Combining the use of anion exchange and spectrophotometry in hydrochloric acid gives exceptional specificity for iron. However, chemical interference may occur from column overloading. This is illustrated by our attempted analysis of NBS 94a (zinc matrix, 0.015% iron). To obtain enough iron for analysis the sample size was so large that the zinc in the sample exceeded the resin capacity. Zinc(II) is strongly sorbed by the resin over a wide range of hydrochloric acid concentrations. However, NBS 124d (copper matrix, 0.18% iron), which contains 5.06% zinc, presented no difficulty because a smaller sample could be used.

Instrumental interference occurs only if the sorption from 6 $\underline{M}$ hydrochloric acid, desorption into 2 $\underline{M}$ hydrochloric acid and photometric absorption at 355 nm occur sequentially.

The spectra of metal ions in hydrochloric acid (15) and

Table 5. Results for analysis of National Bureau of Standards iron samples

Sample type	NBS No.	NBS analysis	Our analysis	s	rsd
Argillaceous Limestone	1b	0.75% Fe_2O_3	0.756% Fe_2O_3	0.006	0.8 pph
Glass Sand	81	0.0724% Fe_2O_3	0.0704% Fe_2O_3	0.00286	4.06 pph
Aluminum Alloy	85A	0.208% Fe	0.212% Fe	0.002	0.9 pph
Ounce Metal	124d	0.18% Fe	0.186% Fe	0.0084	4.5 pph
Portland Cement	177	2.38% Fe_2O_3	2.36% Fe_2O_3	0.05	2.1 pph

the chart of Kraus and Nelson (38) indicate only a few interferences. Of 65 ions tested only ruthenium(III), molybdenum (VI), uranium(VI), palladium(II), and bismuth(III) fall into this category. The metal/iron ratios that will cause a 1% error in the analysis are 1.3, 4.4, 5.5, 13 and 22 respectively. Interference from ruthenium(III), palladium(II), and bismuth (III) can be eliminated by prolonged elution in the sorption step.

The specificity of this sequence is apparent; each step limits the number of interferences. The NBS samples include many metal ions which would interfere by other methods (see Table 6).

Determination of Chromium

Choice of separation method

Although many chemical methods are available for determination of chromium, few are useful in the presence of large amounts of other elements and at the same time adaptable to semi-automated analysis. Other colorimetric methods are not comparable in sensitivity to the established diphenylcarbazide reaction. However, interference from other metals and colored ions limits its use (33).

Preliminary concentration and separation by an extraction has been used including direct colorimetric measurement of the extract (50). An extraction lacks adaptability to semi-auto-

Table 6. National Bureau of Standards Certificates of Analysis for iron samples

Element	NBS 1b	NBS 81	NBS 85A	NBS 124d	NBS 177
Fe	0.75% Fe_2O_3	0.0724% Fe_2O_3	0.208% Fe	0.18% Fe	2.38% Fe_2O_3
Al	1.12% Al_2O_3	0.265% Al_2O_3	Matrix		5.28% Al_2O_3
Sb				0.17% Sb	
As				0.02% As	
Ca	50.9% CaO	0.029% CaO	0.01% Ca		64.25% CaO
Cr			0.231% Cr		
Cu			2.48% Cu	83.60% Cu	
Ga			0.01% Ga		
Pb			0.002% Pb	5.20% Pb	
Li					0.005% Li
Mg	0.36% MgO	0.016% MgO	1.58% Mg		2.42% MgO
Mn	0.20% MnO		0.66% Mn		0.05% Mn_2O_3
Ni			0.41% Ni	0.99% Ni	
P	0.08% P_2O_5			0.02% P	0.05% P_2O_5
K	0.25% K_2O				0.56% K_2O
Si	4.29% SiO_2	Matrix	0.114% Si		21.90% SiO_2
Ag				0.02% Ag	
Na	0.04% Na_2O				0.14% Na_2O
Sr	0.14% SrO				0.05% SrO
S				0.093%	1.59% SO_3

Table 6. (Continued)

Element	NBS 1b	NBS 81	NBS 85A	NBS 124d	NBS 177
Sn			0.001% Sn	4.56% Sn	
Ti	0.046% TiO_2	0.095% TiO_2	0.016% Ti		0.27% TiO_2
V			0.001% V		
Zn			0.019% Zn	5.06% Zn	
Zr		0.031% ZrO_2			

mated analysis cycles.

Present anion exchange separations of chromium(III) are not easily adapted to a short cycle since the resin is not maintained in a single form or the chromium is not retained completely (51). Chromium(VI) separations (52) require solution conditions for sorption which are difficult to attain after sample dissolution and oxidation.

The method described uses strong-base anion exchange resin in only the perchlorate form to achieve a short (ten minute) analysis cycle for chromium(VI). Solution conditions for sorption and concentration permit use of standard persulfate (53), periodate or cerium(IV) oxidation (54) procedures; 0.5 to 1 M nitric acid with other acids in moderate amounts do not interfere. Selectivity of the method for chromium is shown by analysis of standard samples containing many other metal ions. Detection uses the chromium(VI) visible color at 360 nm to achieve sensitivity in the 1 to 10 microgram range for sample volumes from 40 µl to 5 ml, without amplification of the spectrophotometer signal.

Experimental

Resin Amberlyst A-26 macroreticular, strong-base anion exchange resin was used as in the determination of iron with these changes. The resin was ground wet as received and sieved while moist to collect a 140-160 mesh fraction. Approximately 0.5 g of this was slurried with 25 ml of the pH 3.75, 0.4 M

$NaClO_4$ solution described below. After 10 minutes equilibration time the resin was slurried again, settled 3 minutes and fines decanted. The decantation of fines cycle was repeated until the supernatant was clear.

Standard reference materials   The treatment of each sample is described below.

Acid Open-Hearth Steel 20F. The sample was dissolved according to a standard procedure (53) using minimum amounts of acid. Twenty milliliters of sulfuric-phosphoric acid mix ture (160 ml H_2SO_4 + 80 ml H_3PO_4/1) was added and heated. After dissolution, 5 ml of HNO_3 was added cautiously and boiled to remove most of the excess water and HNO_3. The salts were taken up in 50 ml of 1 $\underline{M}$ HNO_3. Silver nitrate solution (2 ml of 10 g/1) and 20 ml of freshly prepared ammonium persulfate solution (150 g/1) was added, then the solution was boiled for 20 minutes, maintaining the volume with water. This was cooled, diluted to volume with 1 $\underline{M}$ HNO_3 and analyzed within 20 minutes.

Aluminum Alloy 85A. The sample was dissolved according to a standard procedure for nonferrous alloys (53). Ten milliliters of HNO_3 and 2 drops of HF were added to a sample. Dissolution was slow so 1 ml of 30% H_2O_2 was added dropwise. After complete dissolution, 3 ml of H_2SO_4 was added and the sample taken to fumes. Twenty-five milliliters of 1 M HNO_3, 2 ml of silver nitrate solution (10 g/1) and 4 g of ammonium persulfate were added. The solution was boiled 20 minutes, maintaining the volume with water. It was cooled, diluted to volume with 1 m HNO_3 and analyzed within 20 minutes.

Waspaloy (Ni, Cr, Co) 349. Dissolution followed the same procedure as for 85A above. The cooled solution was transferred to a volumetric flask, 50 ml of 0.018 M Ce(IV) in 1 M HNO_3 added and diluted to volume. Oxidation was done 15-20 minutes prior to analysis.

Standard chromium A 0.05298 M standard chromium(III) stock solution was prepared by dissolving Baker Analytical Reagent $CrCl_3 \cdot 6H_2O$, assay 99.0% in water and 10 ml of HNO_3. This was taken to dryness, redissolved in HNO_3 and taken to dryness two more times to remove chloride. The salts were redissolved and made to volume in 1 M HNO_3. Analysis followed the standard persulfate oxidation method (53) except that a primary standard potassium dichromate solution and diphenylamine sulfonate indicator were substituted for the permanganate titrant.

Cerium(IV) oxidant Primary standard ceric ammonium nitrate from G. Frederick Smith Chemical Company was dissolved and diluted to volume with 1 M HNO_3 to give a 0.018 M Ce(IV) solution.

Eluents A solution of acidic 0.4 M sodium perchlorate was prepared by dissolution of reagent salt from G. Frederick Smith Chemical Company. The pH was adjusted to 3.75 with B & A perchloric acid using a pH meter. This solution was filtered through paper to remove numerous particulates from the perchlorate salt before use on the chromatograph.

The pH 3.75 eluent was prepared by adding perchloric acid to distilled water while monitoring with a pH meter. No filtration was necessary.

Procedure

The 6.3 mm i.d. analytical column was filled with 0.25 g (weighed dry after use) of 140-160 mesh perchlorate form Amberlyst A-26 slurried in pH 3.75, 0.4 M sodium perchlorate. This gave a column bed 1.8 cm long having a total capacity of approximately 1.5 meq. A typical chromium sample would contain 1×10^{-4} meq of chromium(VI).

The detector was set at 360 nm using a straylight filter and a tungsten source. The pressure was set to give a flow rate of 3.0 ml/min for the acidic 0.4 M sodium perchlorate eluent used to strip Cr(VI) from the column. The sorbing eluent (pH 3.75 perchloric acid) was allowed to pre-equilibrate the column for two minutes prior to injection of a 0.514 ml sample in approximately 0.5 to 1 M nitric acid. After 5.5 min the sample injection valve was returned to the bypass loop mode for another aliquot in the sample loop. At minute 6 the acidic 0.4 M sodium perchlorate was started and chromium was eluted; the peak obtained within 7 minutes from the beginning of analysis. At minute 10 equilibration was started for the next sample.

Using the peak height obtained, micrograms of chromium(VI) in the sample were read from a calibration curve prepared with standard solutions using the same procedure.

Results and discussion

A typical chromatogram is shown in Figure 28. This is the aluminum matrix as in the determination of iron. The matrix peak is due to copper and iron.

The system to use in the separation of chromium was determined by three factors: 1. The chromium must not be reduced by the resin and thus prevent quantitative recovery or limit column life. 2. The solution should be easily prepared for sorption from sample dissolution through oxidation of chromium. 3. Sorption, desorption and detection should be selective for chromium(VI) in the presence of large amounts of other metal ions.

1. Poor resin stability toward alkaline oxidation by chromium(VI) was shown in initial work using Amberlyst A-21, macroreticular, weak base anion exchange resin. This confirmed the results of Sussman *et al*. (55) on recovery of chromium(VI) with a weak base anion exchanger.

Amines in the protonated form are very stable to oxidation whereas under alkaline conditions oxidation of the free amine occurs (56). On this basis, the strong base ion exchanger was selected and the eluents both for sorbing and stripping main-

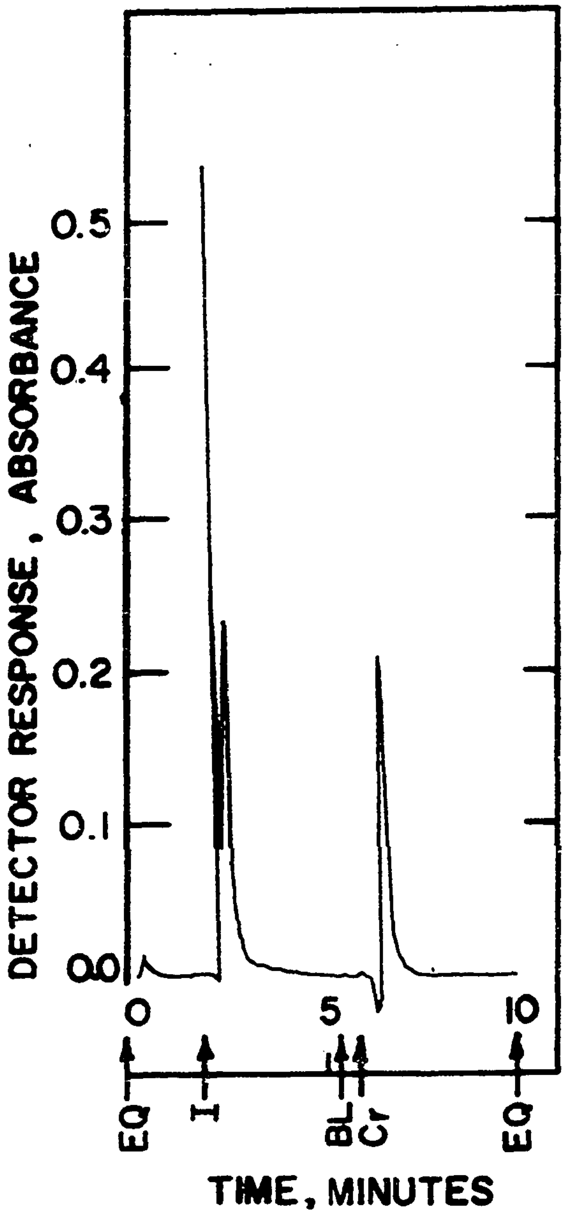


Figure 28. Typical chromatogram of chromium analysis Conditions: sample NBS 85A, 157.0 mg/50 ml; 0.514 ml loop; detection at 360 nm

tained at least slightly acidic. These criteria prevent reduction of chromium(VI) even if some traces of tertiary amine are in the resin matrix.

2. Sample dissolution and oxidation of chromium(III) to chromium(VI) has been studied and standard methods proven. Many different oxidation procedures have been used including alkaline hypobromite (33), ammonium persulfate (53), cerium(IV) (54), and argentic oxide (57). Only those reacting in acidic solution were considered.

Cerium(IV) was chosen initially as the oxident for its rapid reaction rate at room temperature. The rate of reaction in perchloric or nitric acid is rapid but slower in sulfuric acid (58). Because chromium(VI) is not retained on the resin from a solution containing enough perchloric acid for the oxidation, nitric acid was chosen for the sample dissolution and oxidation step.

Injection of 0.514 ml chromium(VI) samples dissolved in 0.5 to 1.0 M nitric acid showed no change in detector response. At 2 M nitric acid, the detector response decreased although no chromium(VI) peak was detected immediately after injection. This is an equilibrium phenomenon (59).

A simple experiment to determine the time required for the oxidation of chromium(III) by cerium(IV) was set up as in the schematic diagram of Figure 29. Time of mixing was from contact of the solutions in the mixing chamber to sample

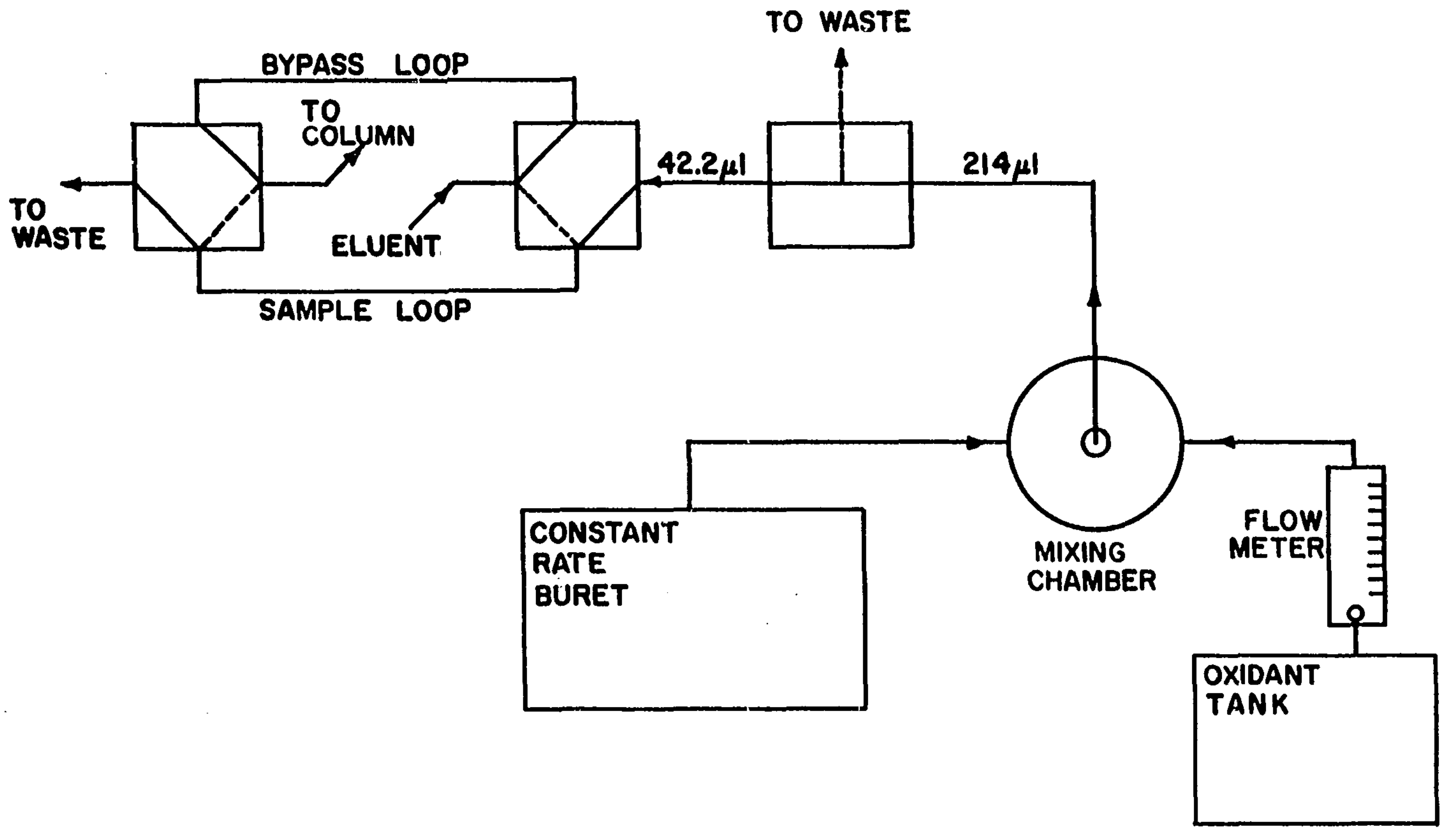


Figure 29. Schematic diagram for determining time required for oxidation of chromium(III) by cerium(IV)

injection. The sequence of analysis given in the procedure was followed on the final mixture containing 2.08 x 10^{-3} M Ce(III) and (IV) and 2.97 x 10^{-4} M Cr(III) and (VI) in 0.75 M nitric acid.

A plot of the results in Figure 30 shows that although the rate is rapid, it cannot be used for in-stream oxidation unless a tubing coil requiring two minutes for the mixed solution to traverse it is inserted ahead of the sample loop.

In the presence of large amounts of manganese(II), cerium (IV) oxidation produces a manganese dioxide precipitate. So, other oxidents were checked for use as discussed below under interference studies. Ammonium persulfate was found satisfactory and periodate may be used if a large excess is destroyed.

3. Selectivity of the anion exchange resin in the perchlorate form for chromium(VI) is an important consideration. Except in large concentrations where mass action becomes important, chemical interference is limited to those anions whose parent acid is stronger than H_2CrO_4 (pK 0.75) (60).

The only common mineral acid anion which will rapidly displace chromium(VI) from a strong-base anion exchange resin in low concentrations of acidic solution is perchlorate. Reasons for this extremely high selectivity coefficient of perchlorate have been discussed (60).

The equilibria of chromium(VI) in solutions of various

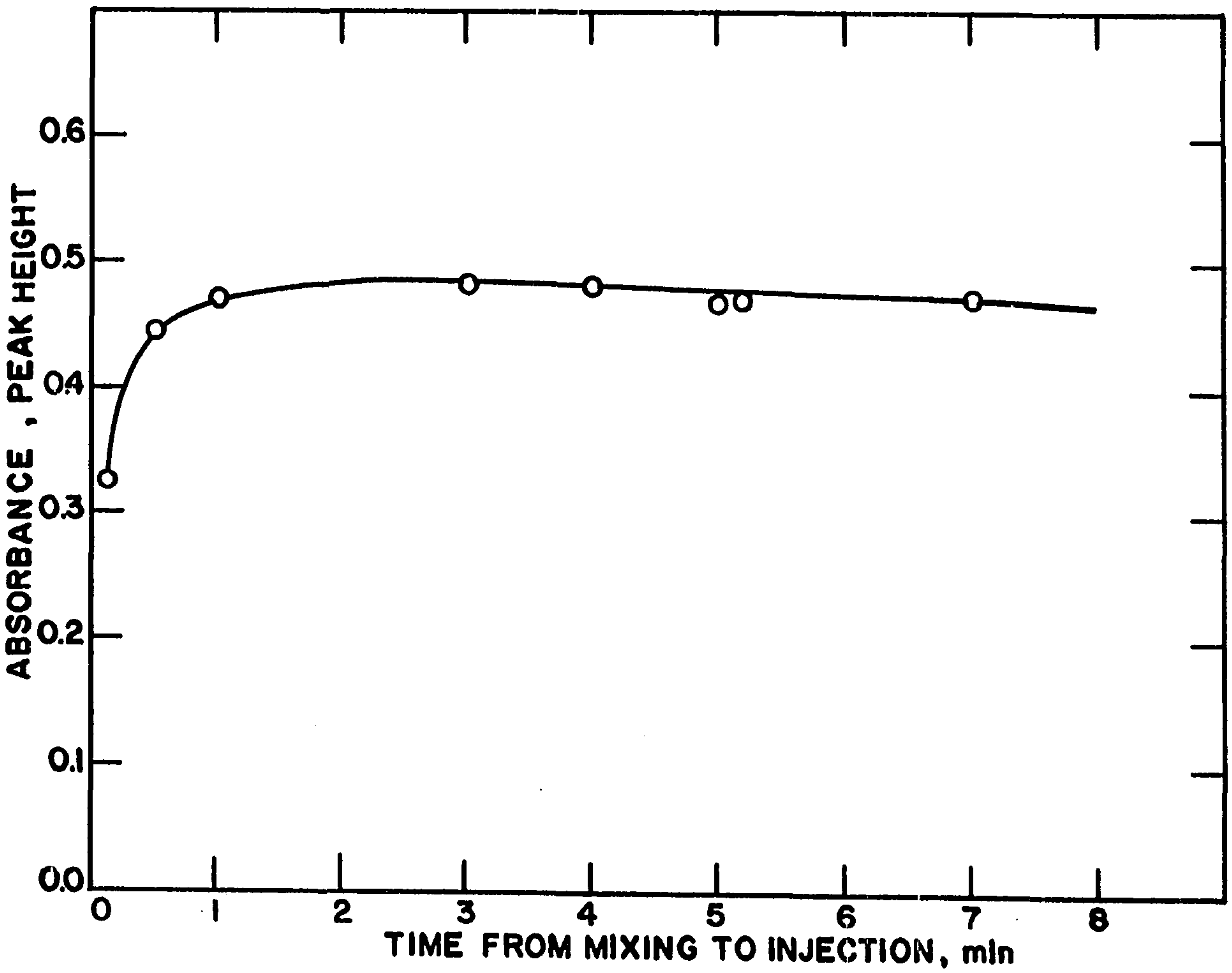


Figure 30. Time required for oxidation of chromium(III) by cerium(IV) Conditions: 2.08×10^{-3} M cerium(III) and (IV); 2.97×10^{-4} M chromium(III) and (VI) in 0.75 M nitric acid

acids cause severe changes in the UV spectrum, indicating that a variety of species is present. In perchlorate media between pH 2 and 4, the spectrum is due solely to $HCrO_4$ (59). A 0.4 M sodium perchlorate solution at pH 3.75 with perchloric acid was selected as the lowest concentration to rapidly strip chromium(VI) from the resin and produce a single colored species for detection.

Because of its high selectivity coefficient, perchlorate must also be used to equilibrate the column and wash matrix element through after sample injection. Other anionic species would take prohibitively long times or high concentrations to convert the resin. A pH 3.75 solution was selected to correlate with the stripping eluent.

Spectra of the various species of chromium(VI) in acid are given by Haight *et al.* (59). The $HCrO_4^-$ species has maxima at 260 nm ($\varepsilon = 2200$) and 355 nm ($\varepsilon = 1600$). Initial work was done at 254 nm using the Chromatronix detector. Samples (0.514 ml) of 0.5 *M* nitric and sulfuric acids each interfered in the detection step using the PROCEDURE described.

Because most use of the method would require large amounts of nitric or sulfuric acid in the sample, the longer wavelength was selected for standard sample analysis. At 360 nm neither interference is detected. This also allows detection in the visible region with a tungsten source.

The effect of varying the analysis cycle times versus peak height response was studied to determine optimum conditions. Each was varied while the remaining two were held constant. Plots of peak height versus the time varied for the parameter are in Figure 31.

The time from column equilibration to injection, EQ to I-curve A, shows little effect because the pH 3.75 perchloric acid is simply washing interstitial stripping eluent from the column.

Changing the time from sample injection to stripping eluent, I to Cr-curve B, shows the greatest effect. Peak height decreases as the band spreads by diffusion. The initial decrease in peak height reflects slow conversion of various chromium(VI) species to a single one on the resin sites. Sample injected in pH 3.75 perchloric acid contains traces of CrO_4^{2-}. The latter has a 50% larger molar absorptivity at 254 nm than $HCrO_4^-$, thus incomplete conversion would produce greater response.

Stripping time, Cr to EQ-curve C, produces little variation in response. A minimum of four minutes is necessary to return to baseline after a full scale peak.

The optimum cycle selected was two minutes for equilibration EQ to I, four minutes for injection and matrix separation, I to Cr and four minutes for stripping the chromium, Cr to EQ resulting in a ten minute cycle. Changing the sample injection valve from the inject position to the bypass mode, BL, prior to

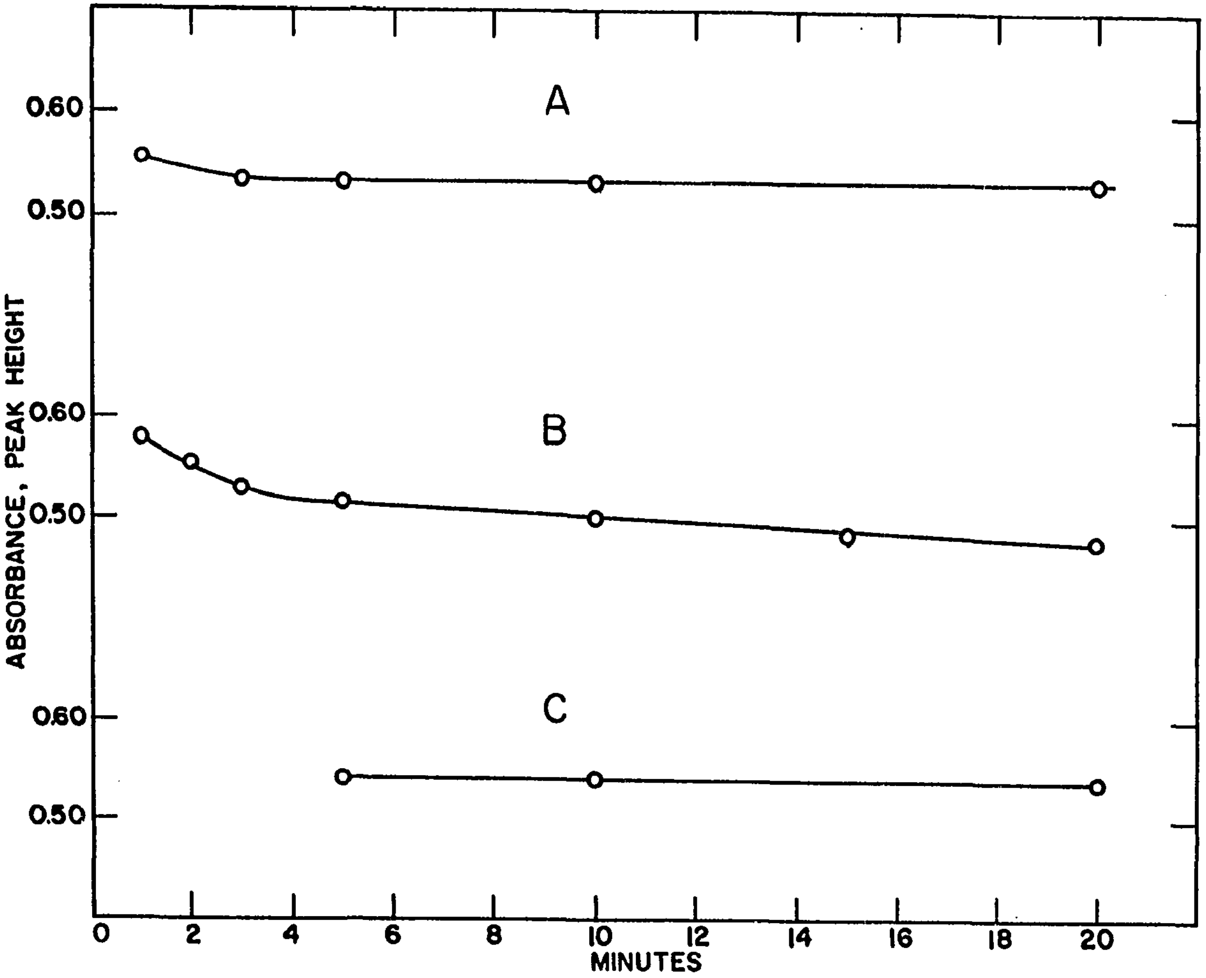


Figure 31. Effect of varying chromium analysis cycle times on peak height response
A - Varying time from column equilibration to injection
B - Varying time from injection to stripping eluent
C - Varying stripping time

stripping allows interchange of widely varying sample loop volumes without change of flow rate and the consequent detector response during stripping.

Calibration curves were prepared at both wavelengths. At 254 nm, Figures 32 and 33, the usefulness is limited with nitrate present, however the curve of Figure 32 demonstrates the potential of signal amplification with a photomultiplier since none could be used at 360 nm. Standard potassium dichromate solution was diluted with pH 3.75 perchloric acid for sample preparation and injected from a 0.514 ml loop. At 254 nm the detection limit, where signal/noise is two, was reached at 25 nanograms of chromium(VI) with 0.01 absorbance unit expanded full scale. A 5.13 ml loop was inserted and a sample containing 25 nonograms (5 ppb Cr(VI)) injected. The peak was within 7% of the smaller, more concentrated sample. A solvent change blank produced a peak height of 0.00038 absorbance unit above noise.

A calibration curve for 360 nm prepared by oxidizing standard chromium(III) solution to chromium(VI) with cerium(IV) in five-fold stoichiometric excess is shown in Figure 34. The upper portion of the curve deviates from a straight line as the Beer's Law region of concentration (59) is exceeded.

Chemically this deviation may be due to the monomer-dimer equilibrium of chromium(VI) (61) or its loss in a weak cationic complex of chromium and cerium (62) which would not be retained

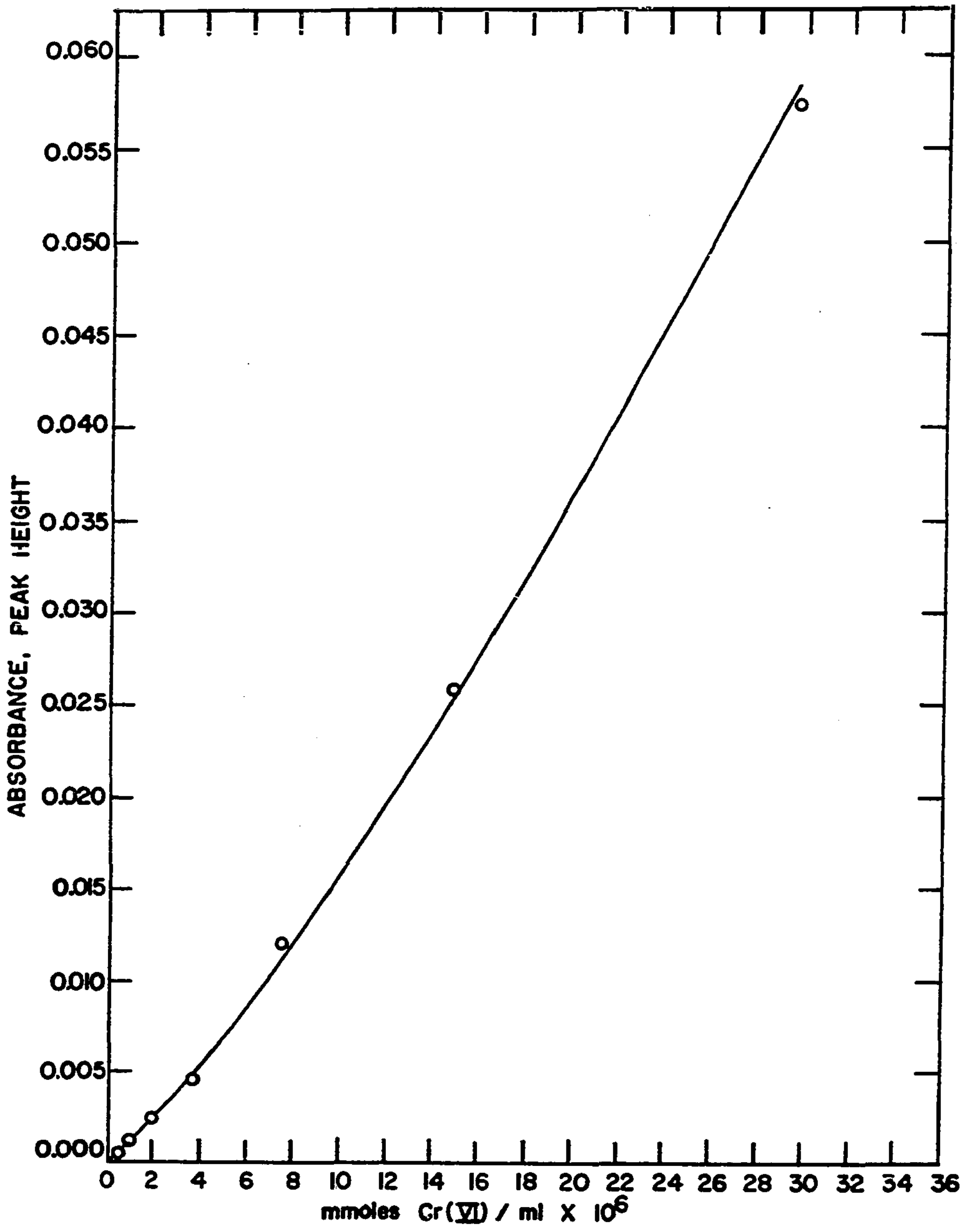


Figure 32. Calibration curve for chromium(VI) at 254 nm with photomultiplier amplification

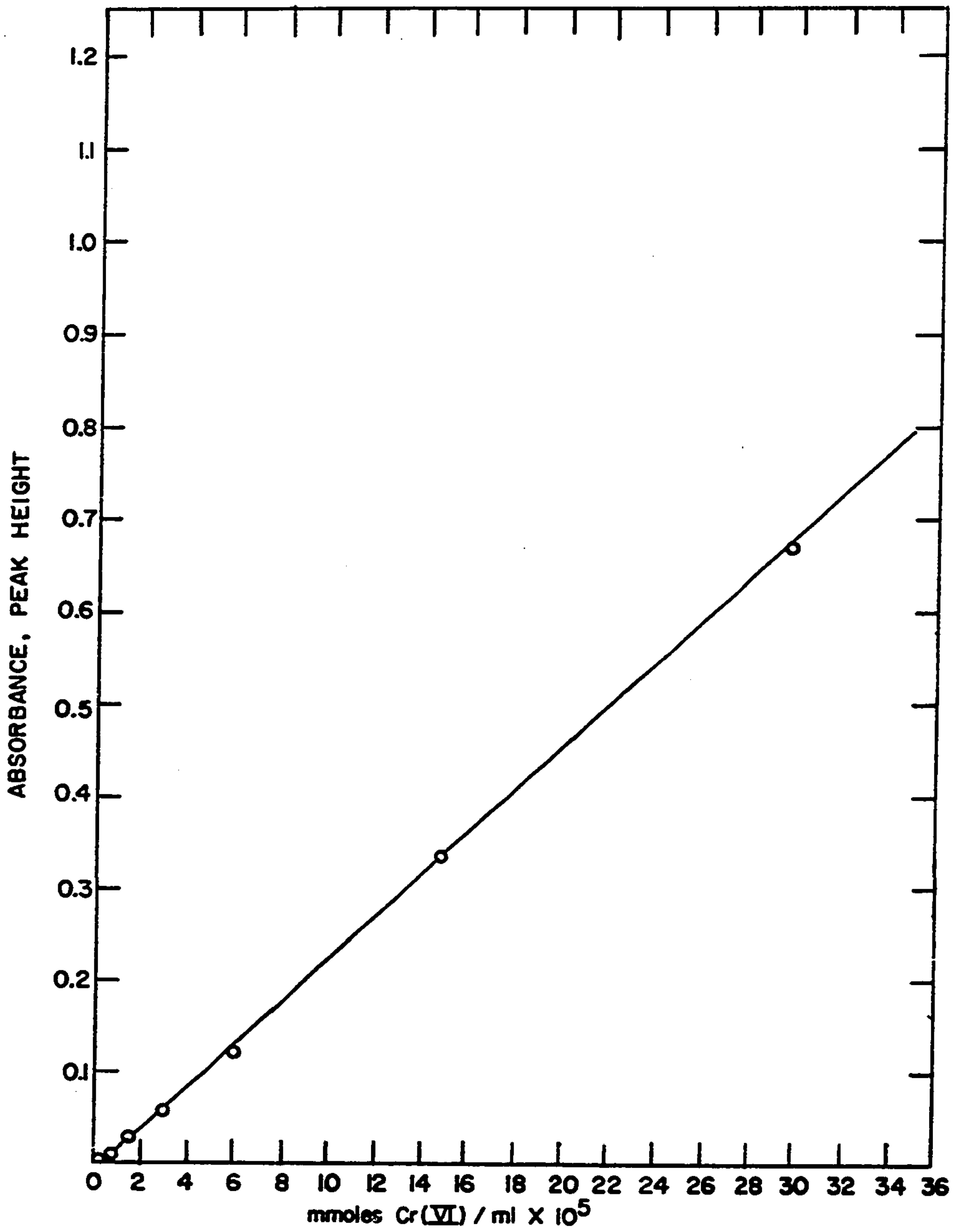


Figure 33. Calibration curve for chromium(VI) at 254 nm

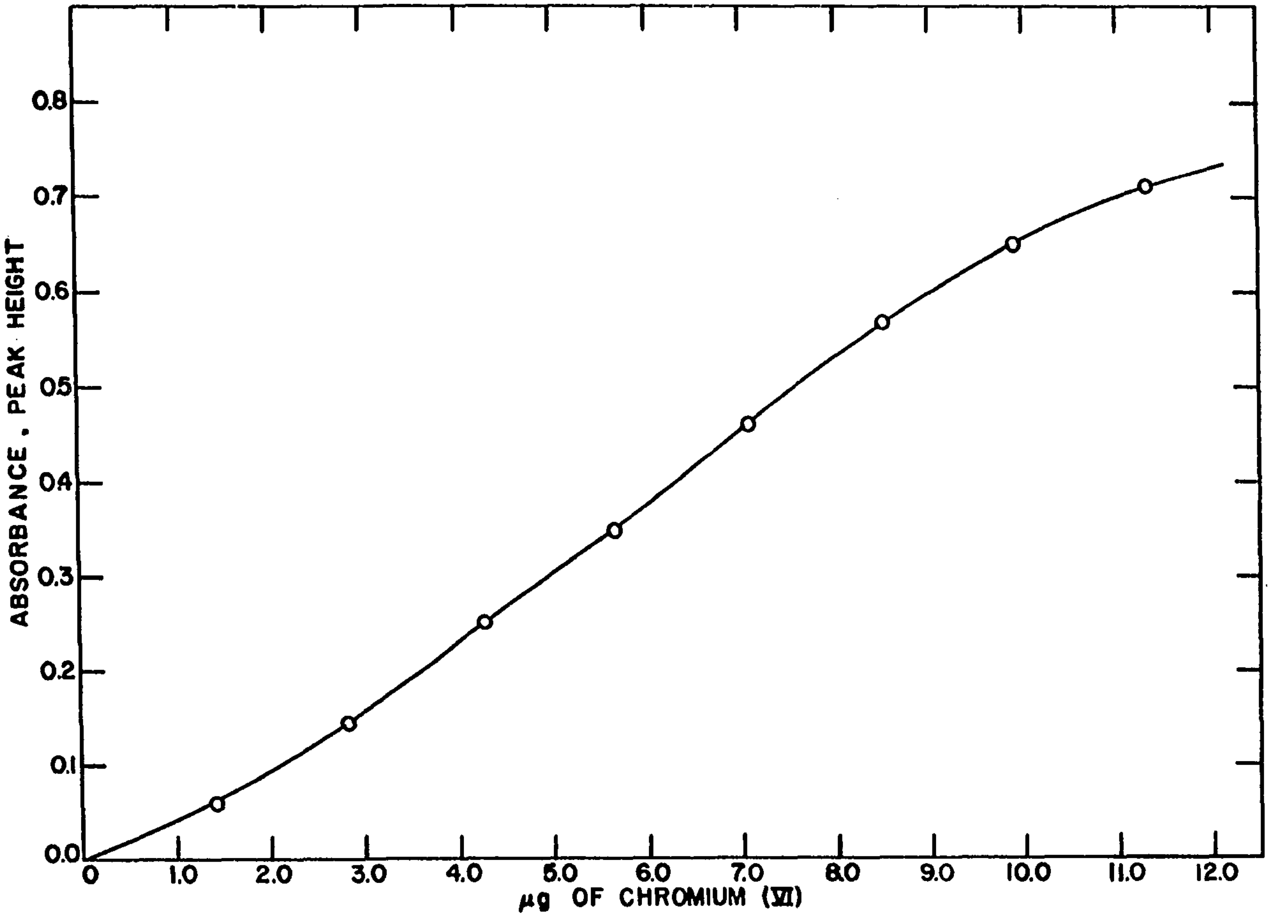


Figure 34. Calibration curve for chromium(VI) at 360 nm

by the resin. Standard sample analysis indicates the former cause with the latter effect unlikely. Curvature at lower concentration is a phenomenon caused by using peak height rather than area (63) since the base width remains nearly constant.

The optimum range for chromium detection is 1 to 10 μg without amplification at 360 nm.

Results for analysis of three National Bureau of Standards samples containing chromium are summarized in Table 7. In each case the weight of sample taken for analysis was varied to use a 0.514 ml loop containing the appropriate μg of chromium for the calibration curve. Three replicates of each were analyzed.

For Aluminum Alloy 85A and Acid Open-Hearth Steel 20F the precision is comparable to that shown on the Certificate of Analysis. Precision for Waspaloy 349 was not indicated by the Provisional Certificate of Analysis with the sample. For steel analysis the result was 4 pph higher than that of the Certificate or 2 pph higher than the NBS analyst. Interference studies below indicate that this is due to the seven and one-half times excess permanganate over chromium(VI) concentration. No attempt was made to selectively reduce this with hydrochloric acid as in the titrimetric procedure (53).

Oxidation of chromium(III) with cerium(IV) in standard sample preparation was used only with Waspaloy 349. For the other samples containing manganese in much larger amounts than

Table 7. Results for analysis of National Bureau of Standards chromium samples

Sample type	NBS No.	Oxidant	NBS analysis	Our analysis	s	rsd
Acid-Open-Hearth Steel	20F	$(NH_4)_2S_2O_8$	0.097% Cr	0.101% Cr	0.0021	2.1 pph
Aluminum Alloy	85A	$(NH_4)_2S_2O_8$	0.231	0.232	0.0055	2.4
Waspalow (Ni,Cr,Co)	349	Ce(IV)	19.50	19.47	0.308	1.6

chromium, a precipitate of manganese dioxide formed. Although the sample was filtered, chromium results were low. Oxidation with ammonium persulfate proved satisfactory.

Specificity of this procedure for chromium(VI) is indicated by the elemental analysis of the NBS samples shown in Table 8 and interference tests in Table 9. In a nitric acid sample, elements forming strong nitrate complexes could show chemical (column overloading) interference. Below 1 M nitric acid these are limited to molybdenum(VI), paladium(II), rhenium(VII), gold(III), mercury(II), thallium(III), bismuth (III), neptunium(IV) and plutonium(IV) (40). Another anion interfering chemically is MnO_4^-. Permanganate was retained in a highly colored band by the column in standard sample analysis and was desorbed only by repeated injections of 1:1 perchloric acid into the stripping eluent. No detrimental effect on the resin was apparent.

Determination of Zinc and Cadmium

Choice of separation method and detection

The separation and determination of zinc and cadmium was chosen to demonstrate the use of in-stream reagent addition with the mixing chamber. A large amount of published survey work for separations on ion exchange resins may be the basis for outlining a method when no specific one is given for the metal(s) of interest. From published data one may predict the

Table 8. National Bureau of Standards Certificate of Analysis for chromium samples

Element	NBS 20F	NBS 85A	NBS 349
Cr	0.097%	0.231%	19.50%
Al		Matrix	1.23%
B			0.0046%
Ca		0.01%	
Co			13.95%
Cu	0.238%	2.48%	0.006%
Fe	Matrix	0.208%	0.13%
Ga		0.01%	
Pb		0.002%	
Mg		1.58%	
Mn	0.754%	0.66%	0.43%
Mo	0.058%		4.04%
Ni	0.243%	0.41%	57.15%
Nb			<0.01%
P	0.028%		0.002%
Si	0.299%	0.114%	0.29%
S	0.034%		
Sn	0.021%	<0.001%	
Ta			<0.01%
Ti		0.016%	3.05%
V	0.007%	0.001%	
W			<0.01%
Zn		0.019%	
Zr			0.081%

Table 9. Interference tests for chromium analysis

Interference	Concentration in 1 M HNO_3	Absorbance, peak height	Injection peak seen?
Ce(IV)	10^{-2} M	0.000	yes
Hg(II)	10^{-3} M + xs Ce(IV)	0.003	yes,Ce(IV)
Mo(VI)	10^{-2} M	0.002	yes
Mn(VII)	10^{-3} M	0.002	no
Ti(IV)	10^{-3} M	0.002	yes
V(V)	10^{-3} M + xs Ce(IV)	0.001	yes,Ce(IV)
Na_3PO_4	1g/100ml	0.004	yes
$Na_3P_5O_{10}$	1g/100ml	0.004	yes
KIO_4	1g/100ml	0.011	yes
$(NH_4)_2S_2O_8$	3g/100ml	0.000	yes

appropriate resin, acid system, concentration and times for the separation of matrix and individual metal ions.

When an ion is directly detectable in the eluate either by UV or visible photometry this is preferable. If direct detection is not possible, a color-forming reagent may be chosen either from color tests in this work, books on photometric procedures (33) or Annual Reviews in Analytical Chemistry.

The choice of separation system here was based entirely on the survey work of Kraus and Nelson in hydrochloric acid

(38) and Faris and Buchanan in nitric acid (40). Each used strong base anion exchange resin.

The color reagent of choice was 4-(2-pyridylazo)resorcinol, PAR, as used in the color test section. It was used in ammonium hydroxide-ammonium chloride buffer to give more intense colors and both at the same wavelength of maximum absorbance.

Experimental

Resin Dowex 1 x 8 gel type strong-base anion exchange resin was received as water-moist, 20-50 mesh beads in the chloride form. The capacity is 1.4 meq/g of dry resin or 4.0 meq/ml as received. It was washed with methanol, dilute hydrochloric acid then water before grinding and sieving wet to obtain a 150-200 mesh fraction. Fines were removed by decantation after settling from a methanol slurry. The bed after use was 3.0 cm long containing 0.43 g of dry chloride form resin.

Standard solutions Zinc. A sample of high purity, 99.999% assay zinc was weighed into a 250 ml tall form beaker which had been thoroughly cleaned with alkaline EDTA solution. Dissolution was rapid with 1:1 hydrochloric acid. The solution was cooled then transferred to a 1 ℓ flask which had been cleaned as above. It was diluted almost to volume with 0.1 M hydrochloric acid made from deionized water and equilibrated to room temperature overnight before diluting to volume. The stock solution of 0.8091×10^{-2} M zinc was diluted in the sorbing eluent described below before use.

Cadmium. A sample of Fisher Certified Reagent stick cadmium assay 99.9% cadmium, 0.02% zinc was cut with a carborundum wheel. This piece was washed three times with 6 M hydrochloric acid, rinsed with deionized, distilled water and air dried to constant weight. The weighed sample was dissolved and diluted as above except that three 1 ml increments of 30% hydrogen peroxide were added to facilitate dissolution. Prolonged boiling removed the excess peroxide. The stock solution of 1.164×10^{-2} M cadmium was diluted in sorbing eluent described below before use.

Mercury. A sample of Baker Analyzed Reagent mercuric chloride, assay 99.5% as $HgCl_2$, was weighed and dissolved with 0.1 M hydrochloric acid. The calculated stock solution concentration was 1.00×10^{-2} M but no attempt was made to analyze it more accurately. Aliquots were diluted to volume in the sorbing eluent for studies.

Other metal ions. The stock metal solutions described in the color test section (see Figure 5) were diluted in sorbing eluent to the appropriate concentrations for interference tests.

PAR color reagent. A 215 mg sample of Eastman number 7714 4-(2-pyridylozo)resorcinal was mixed with 50 ml of 1.0 M ammonium hydroxide, 1.0 M ammonium chloride buffer in a beaker, crushing lumps with a stirring rod. This was transferred to a

2 ℓ volumetric flask and diluted to volume with the same buffer solution. A Teflon stirring bar was added and the solution agitated overnight. The solution was transferred to a graduated cylinder and covered to allow insoluble particles to settle overnight. After settling, the supernatant clear solution was siphoned off with tubing while observing with a light behind the graduated cylinder. The final solution was approximately 5×10^{-4} $\underline{M}$ indicator in ammonia buffer.

Eluents Equilibrating acid was 150 ml of concentrated hydrochloric acid diluted to 1 ℓ with distilled, deionized water. The sorbing eluent was prepared by diluting 25 ml of concentrated hydrochloric acid to 1 ℓ with distilled, deionized water to give approximately 0.3 $\underline{M}$ acid.

Eluent for stripping zinc from the column was prepared by diluting 50 ml of the sorbing eluent above to 1 ℓ with deionized, distilled water to give approximately 0.015 $\underline{M}$ acid.

Stripping and separating eluent for cadmium and mercury was prepared by dissolving 80 g of Baker Analyzed Reagent potassium nitrate and 1 ml of B & A nitric acid in 1 ℓ of distilled, deionized water.

Procedure

The 6.3 mm i.d. analytical column was filled with 0.43 g (dry weight) of 150-200 mesh, chloride form Dowex 1 x 8 anion exchange resin slurried in sorbing eluent. This gave a column bed 3.0 cm long having a total capacity of approximately 1.4

meq. A typical sample contained 10^{-4} meq of zinc plus cadmium.

The detector was set at 525 nm using a tungsten source. The pressure was set to give a flow rate of 3.0 ml/min for the sorbing eluent. Color reagent flow was set at 2.0 ml/min.

Equilibrating acid (150 ml HCl/l) washed the column for two minutes before sample injection. The sample injection valve was put into the bypass loop mode at minute 5.5 prior to stripping zinc with 0.015 M HCl at minute 6. Cadmium was then stripped at minute 9 with nitrate eluent (80 g KNO_3, 1 ml HNO_3 ℓ). The equilibrating acid was started again at minute 12, or in the presence of mercury at minute 19. The zinc peak was obtained within 7 minutes and cadmium within 11 minutes. A solvent change blank was run by the same procedure.

Using the peak heights above the blanks, the micrograms of zinc and cadmium were read. A calibration curve was prepared from standard solutions containing only zinc and cadmium following the same procedure.

Results and discussion

A typical chromatogram of zinc, cadmium and mercury separation is shown in Figure 35. This is a synthetic sample containing only those three ions with mercury in large excess over zinc and cadmium. The solvent blank baseline is displaced downward on the same time scale to differentiate it from the chromatogram baseline and show solvent blank peaks. The shoulder peak between the analytical peaks is due to a very

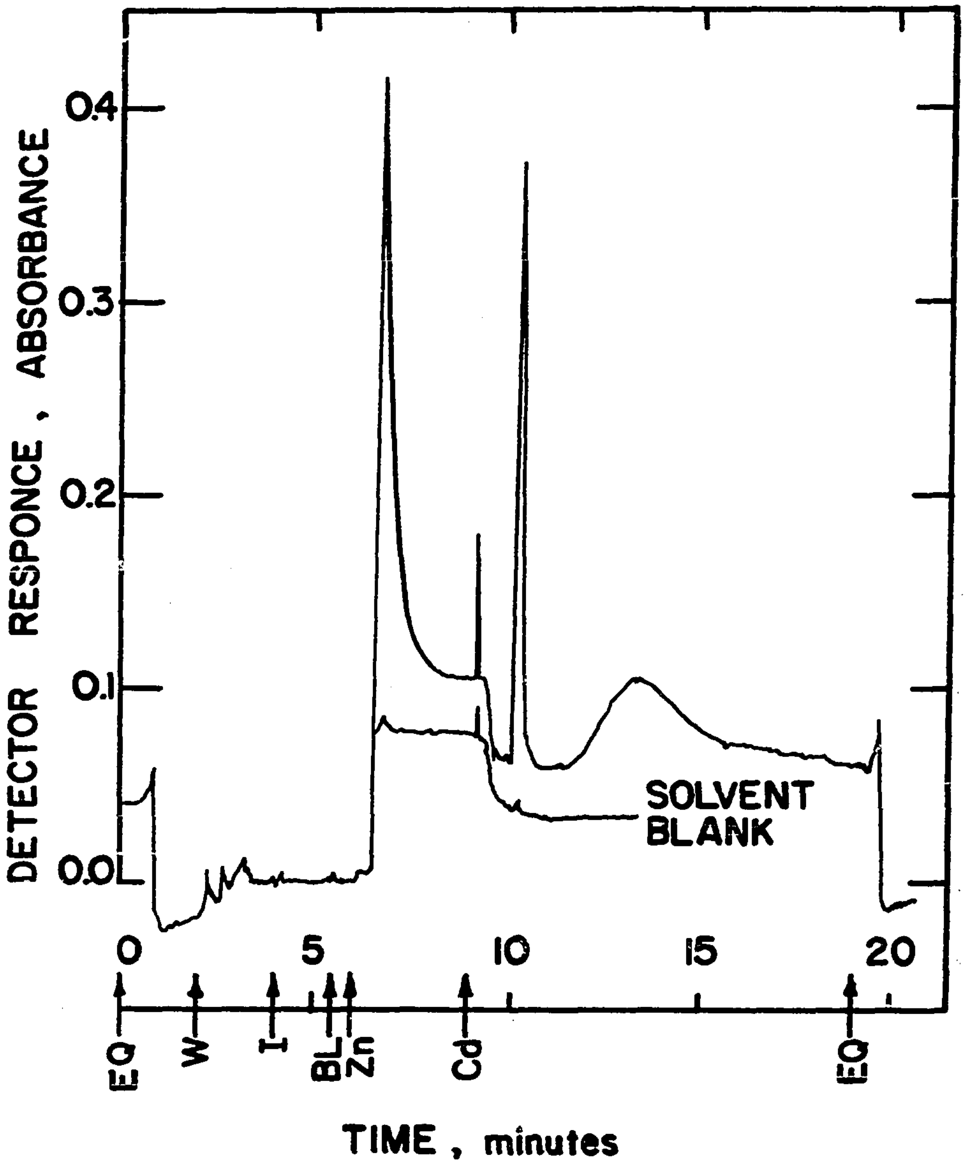


Figure 35. Typical chromatogram of zinc and cadmium analysis Conditions: synthetic sample containing 2.50 μg zinc, 6.72 μg cadmium, 85.3 μg mercury in a 51.4 μl loop; detection at 525 nm with PAR color reagent

short eluent flow stoppage during the valve change. Frequently there is no such peak but it interferes in no way.

Kraus and Nelson (38) suggest the separation of zinc and cadmium from most other elements by first sorbing each from any hydrochloric acid concentration greater than 0.1 M. Zinc is then eluted with 0.01 M acid and cadmium with 0.001 M acid. Mention was not made of mercury however which desorbed with cadmium in a range of hydrochloric acid concentrations tried in the work.

Separation of cadmium from mercury follows the survey work (40) in a nitrate system where mercury is weakly retained by the resin while cadmium immediately elutes. A concentration of approximately 2 M nitrate would give maximum separation of the two peaks, but a lower concentration of approximately 0.8 M was selected to minimize the time between peaks without overlap.

Changing the anionic form of the resin from chloride to nitrate required insertion of an extra two minute step to equilibrate the resin with chloride before repeating the analysis cycle.

Minimum cycle times were not experimentally determined. An estimation of the time required for converting the resin back to chloride form was based on selectivity coefficients (64) and the flow rates used. Two minutes at 3 ml/min proved adequate; no attempt was made to reduce this. Wash time could be reduced or eliminated but lowering the interstitial hydrochloric acid concentration prevents chemical interference by overloading with matrix elements which would be sorbed from higher acid but are eluted in the given procedure. Matrix separation occurs between injection and stripping time so this can be varied as necessary. Elements partitioning such as lead(II) may be eliminated as interferences in this manner. Elution of the analytical peaks requires only enough time to establish a baseline on either side of the peak. Elements partitioning must be allowed to appear in the eluate or they will interfere with a subsequent peak or on the following cycle.

PAR was chosen for color formation and detection because it had been studied as a part of the color test work. Other reagents may be more specific with greater molar absorptivities and formation constants. The acetate buffer system gave less intensely colored zinc and cadmium PAR complexes than in an ammonium hydroxide, ammonium chloride buffer. The wavelength of absorbance maxima were different in acetate but both appeared at 500 nm in ammonia buffer. Molar absorptivity and formation constants were not measured.

For actual use a wavelength of 525 nm was selected to avoid the indicator background absorbance which tails from a maximum at 420 nm.

Calibration curves for quantitative determination of zinc and cadmium are shown in Figures 36 and 37 respectively. The

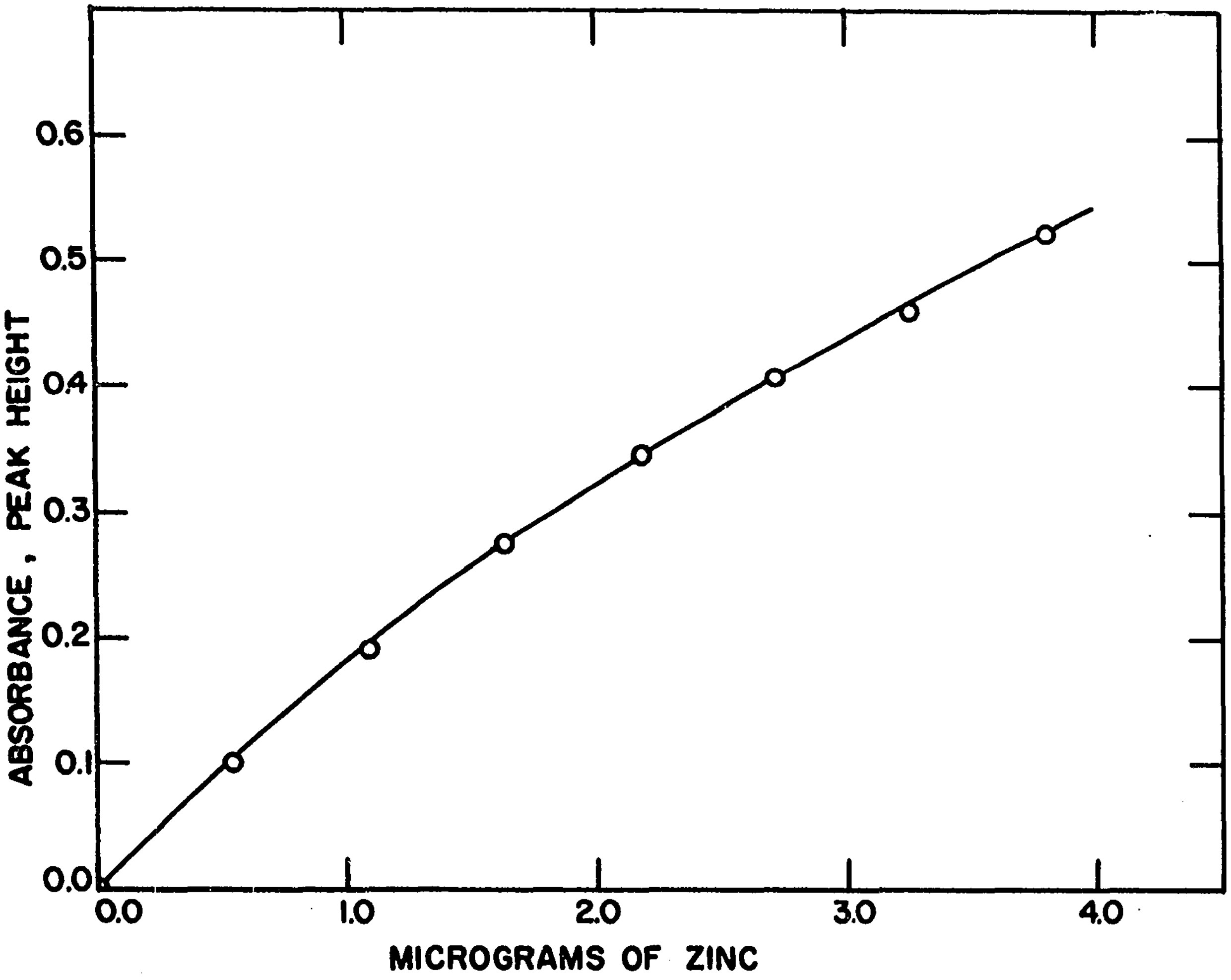


Figure 36. Calibration curve for zinc

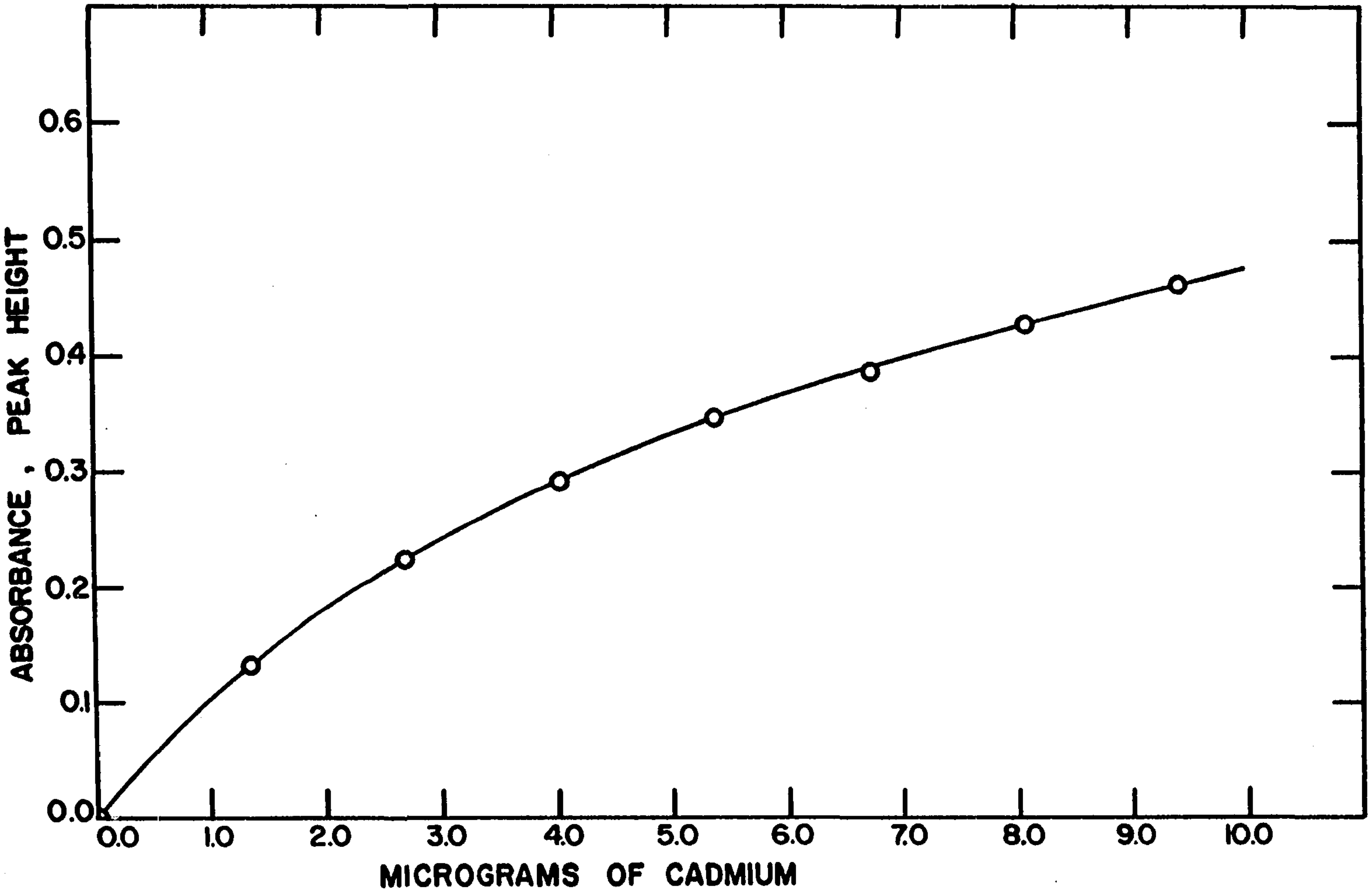


Figure 37. Calibration curve for cadmium

curves were generated by running seven chromatograms, each containing zinc and cadmium stock solution aliquots in varying ratios diluted to 100 ml as shown by Table 10.

Table 10. Calibration curve preparation data for zinc and cadmium determination

Flask No.	ml Zn stock	g Zn in sample	Absorbance peak height	ml Cd stock	g Cd in sample	Absorbance, peak height
1	14.00	3.81	0.525	2.00	1.35	0.132
2	12.00	3.26	0.462	4.00	2.69	0.219
3	10.00	2.72	0.408	6.00	4.04	0.291
4	8.00	2.18	0.346	8.00	5.38	0.348
5	6.00	1.63	0.275	10.00	6.73	0.386
6	4.00	1.09	0.192	12.00	8.07	0.428
7	2.00	0.54	0.101	14.00	9.42	0.463

A solvent change blank peak must be subtracted from the analytical peak for quantitative work. It is doubtful that these are actually due to traces of metal ions in the eluents since no change was seen after prolonged elution with the wash solvent. The phenomenon is not due to refractive index change but possibly the abrupt elution of interstitial eluent gives this effect, since others have reported such peak artifacts (65). A viscosity change lowering flow rate of effluent briefly might show the same effect as valve change when flow slows temporarily. The reason for such peaks was not elucidated experimentally however.

Curvature of the plots can be explained by looking at equilibria involved in the colored species formation and a color forming reagent which also has absorbance at the same wavelength.

For more than one colored species in solution

absorbance, $A = \Sigma\varepsilon_i[i]$ (1)

so $$A = \varepsilon_I[I]_{free} + \varepsilon_{MI}[MI] \quad (2)$$

where $[I]_{free}$ is free indicator concentration and [MI] is the metal-indicator complex concentration, ε_I and ε_{MI} are molar absorptivities for these species respectively.

Making the assumption of only one metal-indicator species,

$$[I]_{total} = [I]_{free} + [MI] \,. \quad (3)$$

Rearranging Equation 3 and substituting into Equation 2 gives

$$A = \varepsilon_I([I]_{total} - [MI]) + \varepsilon_{MI}[MI] \quad (4)$$

or

$$A = \varepsilon_I[I]_{total} + (\varepsilon_{MI} - \varepsilon_I)[MI]$$

This predicts a non-zero intercept at $A = \varepsilon_I[I]_{total}$ but a straight line plot when $[I]_{total} >> [MI]$ and ε_I is significant.

If not all the metal ion is complexed then the formation constant for the species becomes important.

$$K_f = \frac{[MI]}{[M][I]_{free}} \quad (5)$$

since [MI] is not >>[M].

The expression thus becomes

$$A = \varepsilon_I[I]_{total} + (\varepsilon_{MI} - \varepsilon_I)(K_f)[M][I]_{free} \quad (6)$$

or

$$A = \text{constant} + \text{constant}' \, [M][I]_{\text{free}} \qquad (7)$$

The last equation does not give a straight line plot.

For zinc the formation constant is high enough to show only slight curvature. Cadmium shows more severe deviation from linearity. As shown in the interference studies below, mercury and thallium are complexed by nitrate in the eluent so sensitivity falls severely as expected with competing equilibria.

Tap water containing approximately 350 ppm total hardness was analyzed for zinc after acidification. Duplicate flasks prepared at the same time gave 0.126 and 0.109 ppm zinc respectively with no cadmium in 5.13 ml sample loops.

The typical chromatogram (Figure 35) shows analysis of a synthetic sample of zinc, cadmium and mercury. The results are in Table 11 below.

Table 11. Results for analysis of a synthetic zinc, cadmium and mercury sample

	Zinc	Cadmium	Mercury
μg added	2.50	6.72	85.3
μg found	2.52	6.69	
% recovery	100.8	99.6	

Interference studies were limited by evaluating the survey work for hydrochloric acid (38) and nitric acid (40) and performing PAR color tests in ammonia buffer. Table 12 summarizes the evaluation.

Data from the table suggest four metal ions, bismuth(III), mercury(II), lead(II) and thallium(III), that should be checked as interferences which follow all three steps in the analysis-sorption, desorption and reaction with PAR for detection. Nine elements would cause chemical interference by column overloading, silver(I), gold(III), chromium(VI), molybdenum (VI), paladium(II), platinum(IV), rhenium(VII), antimony(III) and (V) and tin(II) and (IV). Of these molybdenum(VI) could be separated by prolonged elution with sorbing eluent, silver (I) by eluting with a higher acid concentration. Eight other elements would interfere chemically but their occurrence is rare and they were not checked.

Samples containing 2.18 μg zinc(II) and 5.38 μg cadmium (II) plus 26.9 μg bismuth(III), 86.6 μg mercury(II), 26.6 μg lead(II) and 21.0 μg thallium(III), respectively, were injected to test for interference. Bismuth(III) interferes with the cadmium(II) elution to broaden the band and give high results. Increasing either the column length or nitrate concentration to 3 <u>M</u> should permit separation (40). Mercury does not interfere and could be determined by the same procedure with less sensitivity. Lead(II) interferes with the zinc(II)

Table 12. Interference evaluation for determination of zinc and cadmium

Element	Retained in sorbing eluent?	Stripped with zinc	Stripped with cadmium	Detected with PAR	Remarks
Zn(II)	yes	yes		yes	
Cd(II)	yes	no	yes	yes	
Ag(I)	yes	?	yes	no	chemical interference
Au(III)	yes	no	no		chemical interference
Bi(III)	yes	?	partitions	yes	eluted with Cd
Cr(VI)	yes	no	no		chemical interference
Cu(I)	?	?	?	?	not checked
Fe(III)	no			no	no interference
Hg(II)	yes	no	partitions	yes	eluted after Cd
Ir(IV)	yes	?	yes		not checked
Mo(VI)	partitions	yes		no	chemical interference
Nb(V)	hydrolyzes	no	no		not checked
Os(IV)	yes	?	?		not checked
Pb(II)	partitions	yes		yes	eluted with Zn
Pd(II)	yes	?	no		chemical interference
Pt(IV)	yes	?	?		chemical interference
Re(VII)	yes	no	no		chemical interference
Rh(IV)	yes	?	yes		not checked

Table 12. (Continued)

Element	Retained in sorbing eluent?	Stripped with zinc	Stripped with cadmium	Detected with PAR	Remarks
Ru(IV)	?	no	no		not checked
Sb(III),(V)	hydrolyzes	no	no	no,(V) only	chemical interference
Sn(II),(IV)	hydrolyzes	no	no	no,(IV) only	chemical interference
Ta(V)	hydrolyzes	?	?		not checked
Tc(VII)	yes	no	no		not checked
Tl(III)	yes	no	partitions	yes	see discussion

band to give high results. It may be removed by prolonged elution with sorbing eluent or eluting with a higher acid concentration such as 6 M before stripping zinc(II). Thallium (III) does not interfere and could be determined with zinc, cadmium and mercury. It elutes in a broad band after mercury with low sensitivity.

Common acids were checked for interference by addition to solutions containing 2.18 μg zinc(II) and 5.38 μg cadmium(II) in 51.4 μl of 0.5 M hydrochloric acid. With 0.5 M nitric acid added, about 1% of the cadmium(II) eluted with zinc(II). Both zinc and cadmium were low by about 3% in the presence of 0.5 N sulfuric acid. Phosphoric acid at 0.1 M lowered zinc by 3% and cadmium by 7%; 0.1 M perchloric lowered results by 6% and 12% respectively. Hydrobromic and hydriodic each lowered the zinc by 3 and 4% respectively and cadmium by 31 and 85% respectively. Addition of much higher hydrochloric acid to the samples when these acids are present should prevent interference by mass action to form the chloro complexes. Other complexing agents or anions may be expected to interfere similarly.

The purpose of this determination was to demonstrate the use of in-stream reagent addition with survey data in the literature to outline a method of analysis where no adequate one is available. A rigorous, all-inclusive method for zinc(II) and cadmium(II) was not shown, but the principles may certainly be adapted to a large number of analyses.

CONCLUSIONS

A number of liquid chromatographs are now available for the rapid separation of organic compounds. Most of these instruments will not tolerate the highly corrosive eluents often needed for inorganic separations, and they lack provision for rapid changing of eluents. The liquid chromatograph described here incorporates these features, is simple in design and inexpensive to construct. Gas pressurization provides a simple method of forcing eluent flow up to a pressure drop of approximately 70 psi. Rapid flow rates are possible with fine mesh ion exchange resin to increase separation efficiency and reproducibility compared to classical gravity flow techniques.

A spectrophotometer mounted with a micro volume flow-through cell monitors the eluate continuously. The analytical species is detected by its own absorbance or that of its complex. For metal ions without color in the eluate, a buffer plus color-forming reagent is mixed in-stream prior to detection.

The device experimentally selected from various designs has a 16 μl dead volume, divided tangential entry chamber for mixing the eluate and color-forming reagent streams. Parameters important for use are shown experimentally. 1. Flow rate of color reagent, 2. total flow rate, 3. flow rate of analytical species and 4. total amount of analytical species are the principle variables. Each of these is interrelated but can be easily controlled. The mixing chamber permits stoichiometric color development at a total flow rate of 1 ml/min or greater with excess reagent. Reaction rate must be rapid or time increased between mixing and detection (60-100 msec for 100 μl tubing volume). A linear plot of μg metal vs. peak height absorbance is possible when the complex formation constant is high and reagent is colorless at the complex λ max.

Experimental work did not show the limit of resolution due to bandspreading in terms of peak width. However, a 1 ml peak base width was demonstrated in the analysis of cadmium. The method makes continuous monitoring of metal ion separations by liquid chromatography simple, sensitive and inexpensive.

Three analysis systems on the liquid chromatograph demonstrate its potential use for rapid determination of metal ions. Short columns, small amounts of metal ion and high eluent flow rates decreased the time. Selective sorption and desorption on anion exchange resin was used because numerous systems have been characterized and the large differences in selectivity coefficients are helpful.

Determination of iron in the hydrochloric acid system demonstrates the adaptation of a well known separation to rapid analysis by the liquid chromatograph. The importance of stripping eluent flow rate control was shown as well as reproducibility of peak height for quantitative analysis. Five National Bureau of Standards samples were analyzed with excellent precision and accuracy in the 10 to 100 μg range. Interferences were determined in addition to those present in the samples. Sensitivity for subsequent work was improved by use of a 32 μl "Z" flow-through cell.

A new separation method for chromium(VI) in complex matrices was devised. Under the conditions used chromium(VI) does not oxidize the resin; the sample solution is easily prepared for sorption after oxidation and the retention of chromium(VI) is selective. Accepted dissolution and oxidation procedures were used in the analysis of three National Bureau of Standards samples. The precision and accuracy again were reasonable. Some interferences were studies in addition to those levels found in the samples. A non-linear calibration curve in the 1 to 10 μg range was discussed. To demonstrate the sensitivity limit as a function of signal/noise, a Chromatronix detector was used. Twenty-five nanograms were detected after sorption from a 5 ml sample (∿5 ppb Cr(VI)).

Analysis of zinc and cadmium demonstrated use of in-stream reagent addition with the mixing chamber. The method of separation was outlined from survey data in the literature. The color-forming reagent PAR, 4-(2-pyridylazo)resorcinol, in ammonia buffer was added in-stream for detection. PAR gave positive spot tests for 34 metal ions in acetate buffer; in ammonia buffer others are detectable. It was chosen over other reagents tested because of its general use with a large number of metal ions.

Zinc and cadmium were separated and quantitatively determined in a synthetic sample and zinc only in a tap water sample. Interferences were predicted from their published distribution coefficients and simple spot tests with PAR-buffer solution. Mercury(II) and thallium(III) may be determined in the same procedure with less sensitivity. Calibration curves in the range of 1 to 4 μg of zinc and 1 to 10 μg of cadmium were shown and discussed.

Specificity of the method is a function of the ion exchange-eluent system chosen and the species in the eluate which absorb. Interference may be either chemical or instrumental. Chemical interference from column overloading occurs when the matrix is strongly sorbed by the resin. Instrumental interference occurs only if the sorption, desorption and photometric absorption steps follow sequentially those of the analytical species. Careful choice of eluents and/or color-forming reagent may be used to eliminate an interference in this group.

Sensitivity is a function of sample loop size and the molar absorptivity of the analytical species or the detectable complex.

Time of the analysis was considerably reduced compared to classical procedures. The total analysis cycles were 7 minutes for iron(III), 10 minutes for chromium(VI) and 12 minutes for zinc(II) plus cadmium(II). A concerted effort was not made to further minimize these times.

SUGGESTIONS FOR FUTURE WORK

The instrument described here is crude, built to prove the value of rapid inorganic separations inexpensively. It was changed continuously to improve design and since this experimental work it has been modified. A second generation inorganic liquid chromatograph has been built also.

Some of the suggestions included here have been incorporated already, others are planned soon. These and more sophisticated ones are included to show logical development.

Instrumental Considerations

Organic liquid chromatography has used positive displacement pumps almost exclusively. Inorganic work would benefit from this also for two reasons. 1. Peak artifacts are accentuated by a flow rate change as well as the abrupt solvent change. 2. Where the mixing chamber is to be used for color-forming reagent addition, baseline shifts are pronounced on eluent change. When color forming reagent has some color of its own at the wavelength of detection the effect is increased.

Peak artifacts are much more serious in the UV than visible but have not been explained satisfactorily. The phenomenon may be due to light scattering at the mixing interface or even the slight temperature change (heat of mixing) involved. Studying this problem may lead to a detector principle more sensitive and general than the one with which it interferes.

Gradient elution capability was suggested at the inception of this project but was not incorporated. Its use in organic separations has been studied and is frequently used. For inorganic as well, it offers increased resolution, decreased times and minimization of baseline shifts. The multi-entrance, microvolume device described by Efremov (23) would be particularly useful.

Detectors with single wavelength sources and photomultipliers are available. This instrument used a monochrometer without amplification. Combining the monochrometer and photomultiplier offers a significant increase in sensitivity.

The mixing device needs more studies to show the actual limits of resolution in terms of bandspreading. This experimentation will require an oscilloscope for readout.

The most important possibility for improving the capability of liquid chromatography is in the use of very high flow rates of eluent. Decreasing the column diameter to less than 0.5 mm is not uncommon in organic liquid chromatography. However, the flow rate usually is decreased concurrently. From a plot of plate height vs. linear velocity of eluent (7), it is apparent that longitudinal diffusion is negligible compared to gas chromatography and the effect of non-equilibrium less significant. Velocities of 1 m/sec or more should be practiceable and useful in ion exchange if diffusion into the bead is minimized as described below.

Chemical Considerations

Ion exchange resins with functional groups throughout a bead have been shown less useful than surface sites on an inert bead. For use with very high velocities of eluent, a minimum thickness of sites on the bead is important. Diffusion on or into the bead are the limiting factors in non-equilibrium. Fusing commercially available beads into a glass column as a frit before chemically bonding the ion exchange capacity may be particularly attractive for high velocity work.

A general system of color-forming reagents for detection of a large number of metal ions with the same system would be particularly helpful.

Survey work in separations used acid systems at high concentrations. Use of salts for ionic strength offers the potential of minimum corrosion and less need for high capacity buffers in the color reagent stream.

Uses in Teaching

This simple pressure system and a flow through cell in a spectrophotometer offer a valuable and inexpensive teaching instrument. Survey work in many systems is extensive enough to allow a student to predict the conditions of a separation then prove it in the laboratory along with minimal interference tests.

Kinetic studies with the mixing device may be simplified by using the length of tubing between it and the detector as a direct method of changing the time available for reactions to occur.

Studies with the mixing chamber also suggest its use in determining combining ratios of complexes rapidly. Where the molar ratio is known, the indicator purity may be considered as in experiment 1. These assume a high formation constant and reasonably rapid kinetics. The former may also be studied with this instrument.

Systems for Analysis

A rapid method of screening new chelating or solvent extraction reagents could be developed with liquid-liquid partition chromatography on this type instrument. The use of acyclic imide extractants as proposed by this author is only one example.

A large number of metals can be maintained in solution easily with a fluoride system. The corrosion problem has largely prevented its use to now. A simple test has shown that polyethylene flow-through cell windows have sufficient transparency for use to 220 nm. By machining a Kel-F analytical column, the fluoride system could easily be handled. A specific element of interest is arsenic for this system.

Fluoride provides one of the few systems from which it can be concentrated readily. The UV absorption of the arsenic(III) bromide or iodide complex were shown very sensitive for its detection.

LITERATURE CITED

(1) O. Samuelson, "Ion Exchange Separations in Analytical Chemistry", John Wiley & Sons, Inc., New York, N.Y., 1963.

(2) J. S. Fritz and G. L. Latwesen, Talanta, 17, 81 (1970).

(3) Chem. Eng. News, 47 (7), 38 (1969).

(4) Anal. Chem., 43, 110LG (1971).

(5) J. L. Waters, J. N. Little and D. F. Horgan, J. Chromatog. Sci., 7, 293 (1969).

(6) C. A. Burtis, Abstracts 160th National Meeting of the American Chemical Society, Chicago, Illinois, Sept. 1970, No. ANAL 55.

(7) J. J. Kirkland, Editor, "Modern Practice of Liquid Chromatography", Wiley-Interscience, New York, 1971.

(8) R. D. Conlon, Anal. Chem., 41, 107A (1969).

(9) R. H. Muller, Anal. Chem., 41, 113A (1969).

(10) R. D. Conlon, "Current Criteria for Liquid Chromatograph Design", Presented at the Pittsburgh Conference, March 1971.

(11) Chromatronix, Incorporated, Berkeley, California, "High Speed, High Resolution Liquid Chromatography", Lab Notes 3, Oct. 1968.

(12) J. C. Giddings, "Dynamics of Chromatography, Part I; Principles and Theory", Marcel Dekker, Inc., New York, 1965.

(13) P. R. Rony, Sep. Sci., 3, 239 (1968).

(14) F. W. Sears and M. W. Zemansky, "College Physics", Third edition, Addison-Wesley Publishing Co., Reading, Mass., 1960.

(15) M. D. Seymour, unpublished work, 1971.

(16) D. H. Spackman, W. H. Stein and S. Moore, Anal. Chem., 30, 1190 (1958).

(17) D. B. Hamilton, Anal. Chem., 35, 2055 (1963).

(18) H. Hartridge and F. W. Roughton, Proc. Roy. Soc., 104A. 376 (1923).

(19) B. Chance, Discussions Faraday Soc., 17, 123 (1954).

(20) Yu. B. Kletenik, Zh. Fiz. Khim., 37, 1193 (1963). Russian J. Phys. Chem., 37, 638 (1963).

(21) R. L. Berger, Proc. Intern. Colloq. Rapid Mixing Sampling Tech. Appl. Study Biochem. Reactions, 1st, Philadelphia, 1964, discussion 38. Chem. Abstr., 64, 13775c (1966).

(22) G. W. Moskowitz and R. L. Bowman, Science, 153, 428 (1966).

(23) Yu. V. Efremov, Zh. Fiz. Khim., 40, 1413 (1966). Russian J. Phys. Chem., 40, 767 (1966).

(24) H. L. Toor, A.I.Ch.E. Journal, 8, 70 (1962).

(25) H. L. Toor, Ind. Eng. Chem. Fund., 8, 655 (1969).

(26) C. Chu, Chem. Eng. Sci., 24, 1187 (1969).

(27) G. Vassilatos and H. L. Toor, A.I.Ch.E. Journal, 11, 666 (1965).

(28) J. W. Ferguson, et al., Anal. Chem., 36, 796 (1964).

(29) S. B. Savvin, Talanta, 8, 673 (1961).

(30) S. B. Savvin, Talanta, 11, 7 (1964).

(31) M. A. Desea and L. B. Rogers, Anal. Chim. Acta, 6, 534 (1952).

(32) C. Merritt, Jr., H. M. Hershenson and L. B. Rogers, Anal. Chem., 25, 572 (1953).

(33) E. B. Sandell, "Colorimetric Determination of Traces of Metals", Third Edition, Interscience Publishers, Inc., New York, N.Y., 1959. p. 196.

(34) W. F. Rieman III and H. F. Walton, "Ion Exchange in Analytical Chemistry", Pergamon Press, New York, N.Y., 1970.

(35) J. Kerkisch, "Modern Methods for the Separation of Rarer Metal Ions", Pergamon Press, New York, N.Y., 1969.

(36) G. Goldstein, "Equilibrium Distribution of Metal-Ion Complexes", ORNL-3620, November 1964.

(37) J. P. Faris, *Anal. Chem.*, *32*, 520 (1960).

(38) K. A. Kraus and F. Nelson, "Metal Separations by Anion Exchange", ASTM Special Technical Publication No. 195, 1958.

(39) F. W. E. Strelow and C. J. C. Bothma, *Anal. Chem.*, *39*, 595 (1967).

(40) J. P. Faris and R. F. Buchanan, *Anal. Chem.*, *36*, 1158 (1964).

(41) F. Nelson, T. Murase, K. A. Kraus, *J. Chromatog.*, *13*, 503 (1964).

(42) F. W. E. Strelow, *Anal. Chem.*, *32*, 1185 (1960).

(43) F. Nelson and D. C. Michelson, *J. Chromatog.*, *25*, 414 (1966).

(44) F. W. E. Strelow, R. Rethemeyer and C. J. C. Bothma, *Anal. Chem.*, *37*, 106 (1965).

(45) D. J. Pietrzyk and D. L. Kiser, *Anal. Chem.*, *37*, 233 (1965).

(46) F. W. E. Strelow, *et al.*, *Anal. Chem.*, *43*, 870 (1971).

(47) E. R. Wright and M. G. Mellon, *Ind. Eng. Chem.*, *9*, 375 (1935).

(48) G. Telep, and D. F. Boltz, *Anal. Chem.*, *22*, 1030 (1950).

(49) J. S. Fritz and G. L. Latwesen, *Talanta*, *14*, 529 (1967).

(50) J. Adam and R. Pribil, *Talanta*, *18*, 91 (1971).

(51) O. Samuelson, *Svensk Kem. Tidskr.*, *52*, 115 (1940).

(52) A. Iguchi, *Sci. Papers Coll. Gen. Educ., Univ. Tokyo*, *8*, 145 (1958). *Chem. Abstr.*, *53*, 16644 (1959).

(53) F. J. Welcher, Editor, "Standard Methods of Chemical Analysis," Sixth Edition, Vol. II, Part A, D. Van Nostrand Co., Inc., New York, N.Y., 1963.

(54) H. H. Willard and P. Young, J. Am. Chem. Soc., 51, 139 (1929).

(55) S. Sussman, F. C. Machod and W. Wood, Ind. Eng. Chem., 37, 618 (1945).

(56) F. W. Neumann and C. W. Gould, Anal. Chem. 25, 751 (1953).

(57) J. J. Lingane and D. G. Davis, Anal. Chim. Acta, 15, 201 (1956).

(58) J. Y. Tong and E. L. King, J. Am. Chem. Soc., 82, 3805 (1960).

(59) G. P. Haight, Jr., D. C. Richardson and N. H. Coburn, Inorg. Chem., 3, 1777 (1964).

(60) B. Chu, D. C. Whitney and R. M. Diamond, J. Inorg. Nucl. Chem., 24, 1405 (1962).

(61) J. Y. Tong and E. L. King, J. Am. Chem. Soc., 75, 6180 (1953).

(62) J. Y. Tong and E. L. King, J. Am. Chem. Soc., 76, 2132 (1954).

(63) H. M. McNair and E. J. Bonelli, "Basic Gas Chromatography," Varian Aerograph, Walnut Creek, California, 1968.

(64) H. P. Gregor, J. Belle and R. A. Marcus, J. Am. Chem. Soc., 77, 2713 (1955).

(65) R. W. Yost, "A New Concept in Solvent Transport for Liquid Chromatography," Presented at the Pittsburgh Conference, March 1971.

ACKNOWLEDGEMENTS

The help and patience of Professor James S. Fritz in this work is gratefully acknowledged.

This chromatograph was built by fusing the author's ideas with those of Mark D. Seymour. The determination of iron was a joint project and is presently in press for publication in Analytical Chemistry.

Ames Laboratory personnel deserve special thanks for their help. James F. Jenson designed and tested the pressure system; Dean D. Woods machined all special parts and suggested design simplifications.

The efficient typing and format advice of Verna J. Thompson was sincerely appreciated.

The love and understanding of my wife Catherine has been a continuous inspiration to me.